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(71) Demandeur/Applicant:
PRAXIS PRECISION MEDICINES, INC., US
(72) Inventeurs/Inventors:
PETROU, STEVEN, AU;
KAHLIG, MICHAEL KRISTOPHER, MATHIEU, US;
REDDY, KIRAN, US
(74) Agent: SMART & BIGGAR LLP

(54) Titre : COMPOSITIONS ET METHODES POUR LE TRAITEMENT DES MALADIES ASSOCIES A KCNT1
(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF KCNT1 RELATED DISORDERS

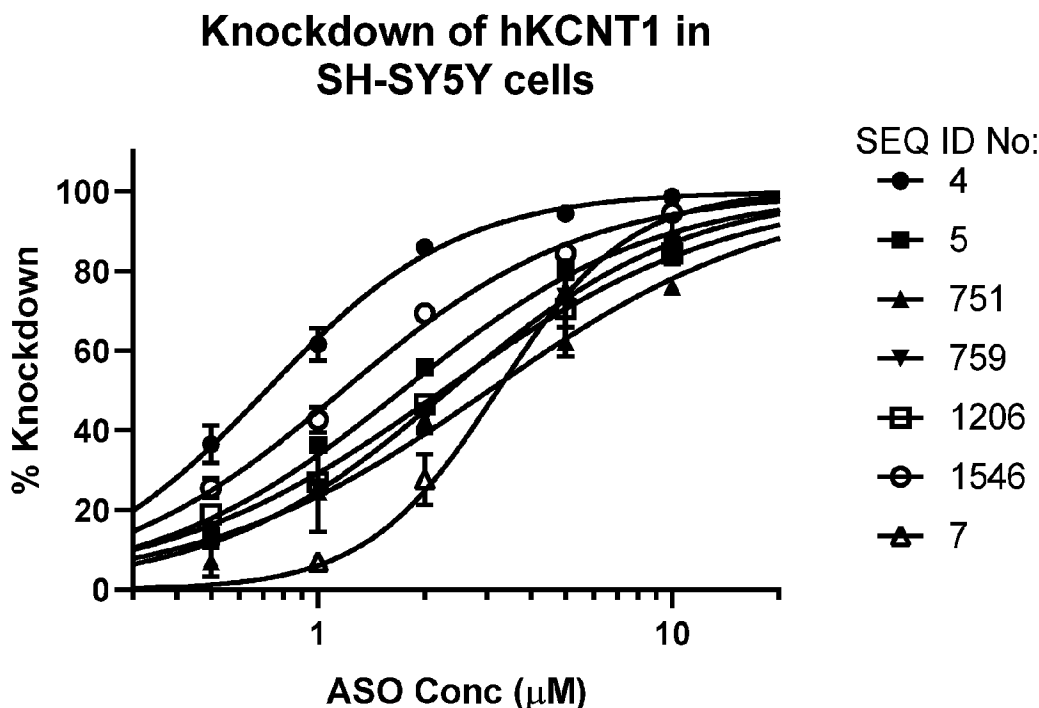


FIG. 1

(57) Abrégé/Abstract:

The present disclosure features useful compositions and methods to treat KCNT1 related disorders, e.g., in a subject in need thereof.

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(71) Applicant: PRAXIS PRECISION MEDICINES, INC.

[US/US]; One Broadway, 16th Floor, Cambridge, MA 02142 (US).

(72) Inventors: PETROU, Steven; 24 Hartland Way, Eltham,

3095 (AU). KAHLIG, Michael Kristopher, Mathieu;

1130 James Avenue, Redwood City, CA 94062 (US). RED-

DY, Kiran; 14 9th Street, Unit 14, Boston, MA 02129 (US).

(74) Agent: ASHRAF, Shovon et al.; Goodwin Procter LLP,

100 Northern Avenue, Boston, MA 02210 (US).

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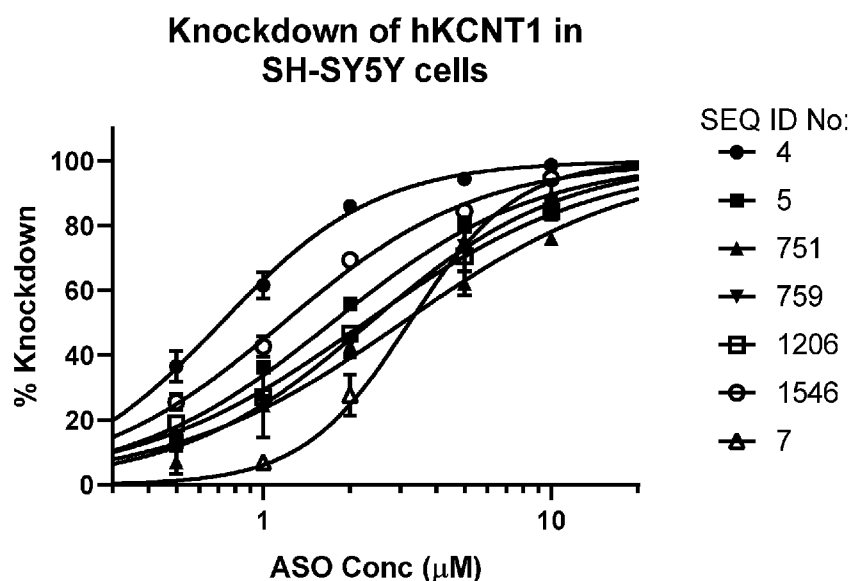


FIG. 1

(57) **Abstract:** The present disclosure features useful compositions and methods to treat KCNT1 related disorders, e.g., in a subject in need thereof.

[Continued on next page]

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COMPOSITIONS AND METHODS FOR THE TREATMENT OF KCNT1 RELATED DISORDERS

CROSS REFERENCE

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/782,877 filed December 20, 2018; U.S. Provisional Patent Application No. 62/862,328 filed on June 17, 2019; and U.S. Provisional Patent Application No. 62/884,567 filed on August 8, 2019, the entire disclosure of each of which is hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 19, 2019, is named PRX-039WO_SL.txt and is 1,005,552 bytes in size.

BACKGROUND

KCNT1 encodes a sodium-activated potassium channel (intracellular sodium-activated channel, subfamily T member 1) that is expressed in the central nervous system. Also known as Slack and $K_{Na}1.1$, *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 (I_{KNa}), which is triggered by an influx of sodium channels ions through sodium channels or neurotransmitter receptors. It is thought that this delayed outward current is involved in regulating neuronal excitability.

Mutations in *KCNT1* (e.g., gain-of-function mutations) have been associated with particular forms of epilepsy, including epilepsy of infancy with migrating focal seizures (EIMFS), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome. Currently, no cures exist for these diseases. Accordingly, new compositions and methods of treating these diseases are needed.

SUMMARY

In one aspect, provided herein are compounds comprising an oligonucleotide comprising a nucleobase sequence at least 90% complementary to at least 10 contiguous

nucleobases of a transcript comprising a sequence at least 90% identity to SEQ ID NO: 3526, or a contiguous 15 to 50 nucleobase portion of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the present disclosure provides compounds comprising an oligonucleotide comprising a nucleobase sequence 100% complementary to at least 10 contiguous nucleobases of a transcript comprising a sequence at least 90% identity to SEQ ID NO: 3526, or a contiguous 15 to 50 nucleobase portion of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In another aspect, provided herein are oligonucleotides comprising a nucleobase sequence at least 90% complementary to at least 10 contiguous nucleobases of a transcript comprising a sequence at least 90% identity to SEQ ID NO: 3526, or a contiguous 15 to 50 nucleobase portion of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In some embodiments, the oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares 90% identity with an equal length portion of any one of SEQ ID NOs: 1-3525. In some embodiments, the present disclosure provides an oligonucleotide comprising at least a contiguous 10 nucleobase sequence that shares 100% identity with an equal length portion of any one of SEQ ID NOs: 1-3525.

In some embodiments, the oligonucleotide comprises at least a contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 1-3525. In some embodiments, the present disclosure provides an oligonucleotide comprising at least a contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares 100% identity with an equal length portion of any one of SEQ ID NOs: 1-3525.

In some embodiments, the oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares 90% identity with an equal length portion of any one of SEQ ID NOs: 1-116. In some embodiments, the present disclosure provides an oligonucleotide comprising at least a contiguous 10 nucleobase sequence that shares 100% identity with an equal length portion of any one of SEQ ID NOs: 1-116.

In some embodiments, the oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the

oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the present disclosure provides an
5 oligonucleotide comprising at least a contiguous 10 nucleobase sequence that shares 100% identity with an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

10 In some embodiments, the oligonucleotide comprises at least a contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some
15 embodiments, the oligonucleotide comprises at least a contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some
20 embodiments, the present disclosure provides an oligonucleotide comprising at least a contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares 100% identity with an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

25 In another aspect, provided herein are compounds comprising an oligonucleotide comprising at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some
30 embodiments, provided herein are compounds comprising an oligonucleotide comprising at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the

oligonucleotide comprises at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

5 In another aspect, provided herein are oligonucleotides comprising at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the
10 oligonucleotide comprises at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In some embodiments, the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16,
15 17, 18, or 19 contiguous nucleobases of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleobases of any one of SEQ ID NOs: 4, 1046,
20 1071, 1388, 1551, 1546, or 2595, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In another aspect, provided herein are compounds comprising an oligonucleotide comprising at least 10 contiguous nucleobases which is at least 90% complementary to an equal length portion of nucleobases within a 10 nucleobase range of any one of positions 374,
25 661, 765, 837, 1347, 1629, 2879, 3008, 3168, 1760, 1752, 1795, 1775, 665-680, 1340-1370, 1740-1815, or 3110-3171 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In another aspect, provided herein are oligonucleotides comprising at least 10 contiguous nucleobases which is at least 90% complementary to an equal length portion of
30 nucleobases within a 10 nucleobase range of positions 374, 661, 765, 837, 1347, 1629, 2879, 3008, 3168, 1760, 1752, 1795, 1775, 665-680, 1340-1370, 1740-1815, or 3110-3171 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 655-680, 1340-137, 1740-1815, or 3110-3175 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside

5 linkage.

In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 655-665, 660-670, 665-675, or 670-680 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

10 In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 1340-1350, 1345-1355, 1350-1360, 1355-1365, or 1360-1370 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage. In some embodiments, the oligonucleotide comprises at least 10
15 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 1740-1750, 1745-1755, 1750-1760, 1755-1765, 1760-1770, 1765-1775, 1770-1780, 1775-1785, 1780-1790, 1785-1795, 1790-1800, 1795-1805, 1800-1810, or 1805-1815 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

20 In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 3110-3120, 3115-3125, 3120-3130, 3125-3135, 3130-3140, 3135-3145, 3140-3150, 3145-3155, 3150-3160, 3155-3165, 3160-3170, 3165-3175, 3170-3180 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified
25 internucleoside linkage.

In some embodiments, the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleobases complementary to an equal length portion of nucleobases within any one of positions 374, 661, 765, 837, 1347, 1629, 2879, 3008, 3168, 1760, 1752, 1795, 1775, 655-680, 1340-1370, 1740-1815, or 3110-3171 of SEQ ID NO:
30 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

In some embodiments, the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleobases complementary to an equal length portion of

nucleobases within any one of 655-680, 1340-137, 1740-1815, or 3110-3175 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

5 In some embodiments, the oligonucleotide is between 12 and 40 nucleobases in length.

10 In some embodiments, the oligonucleotide comprises: a gap segment comprising one or more of linked deoxyribonucleosides, 2'-Fluoro Arabino Nucleic Acids (FANA), and Fluoro Cyclohexenyl nucleic acid (F-CeNA); a 5' wing segment comprising linked nucleosides; and a 3' wing segment comprising linked nucleosides; wherein the gap segment comprises a region of at least 8 contiguous nucleobases having at least 80% identity to an equal length portion of any one of SEQ ID NOs: 1-3525 positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment each comprises at least two linked nucleosides; and wherein at least one nucleoside of each wing segment comprises a modified sugar.

15 In some embodiments, the oligonucleotide comprises; a gap segment comprising one or more of linked deoxyribonucleosides, 2'-Fluoro Arabino Nucleic Acids (FANA), and Fluoro Cyclohexenyl nucleic acid (F-CeNA); a 5' wing segment comprising linked nucleosides; and a 3' wing segment comprising linked nucleosides; wherein the gap segment comprises a region of at least 8 contiguous nucleobases having at least 80% identity to an equal length portion of any one of SEQ ID NOs: 1-3525 positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment each comprises at least two linked nucleosides; and wherein at least one nucleoside of each wing segment comprises a modified sugar.

20

25 In some embodiments, the oligonucleotide comprises at least 13, 14, 15, 16, 17, 18, 19, or 20 linked nucleosides.

30 In some embodiments, at least one nucleoside linkage of the nucleobase sequence is selected from the group consisting of a phosphodiester linkage, a phosphorothioate linkage, a 2'-alkoxy linkage, an alkyl phosphate linkage, alkyl phosphonate linkage, a phosphorodithioate linkage, a phosphotriester linkage, an alkylphosphonate linkage, a methylphosphonate linkage, a dimethylphosphonate linkage, an aminoalkylphosphotriester linkage, an alkylene phosphonate linkage, a phosphinate linkage, a phosphoramidate linkage, a phosphorodiamidate linkage, an aminoalkylphosphoramidate linkage, a thiophosphoramidate linkage, a thionoalkylphosphonate linkage, a thionoalkylphosphotriester linkage, a thiophosphate linkage, a selenophosphate linkage, and a boranophosphate linkage.

In some embodiments, the at least two linked nucleosides of the 5' wing segment are linked through a phosphodiester internucleoside linkage and wherein the at least two linked nucleosides of the 3' wing segment are linked through a phosphodiester internucleoside linkage, and wherein at least one of the internucleoside linkages of the gap segment is a modified internucleoside linkage.

In some embodiments, at least two, three, or four internucleoside linkages of the nucleobase sequence are phosphodiester internucleoside linkages.

In some embodiments, at least one, two, three, or four internucleoside linkages between nucleoside bases of the gap segment are phosphodiester internucleoside linkages.

In some embodiments, at least two internucleoside linkages of the nucleobase sequence is a modified internucleoside linkage.

In some embodiments, the modified internucleoside linkage of the nucleobase sequence is a phosphorothioate linkage.

In some embodiments, all internucleoside linkages of the nucleobase sequence are phosphorothioate linkages.

In some embodiments, the at least two linked nucleosides of the 5' wing segment are linked through a modified internucleoside linkage.

In some embodiments, the at least two linked nucleosides of the 3' wing segment are linked through a modified internucleoside linkage.

In some embodiments, the at least two linked nucleosides of the 5' wing segment are linked through a phosphorothioate internucleoside linkage and wherein the at least two linked nucleosides of the 3' wing segment are linked through a phosphorothioate internucleoside linkage, and wherein at least one of the internucleoside linkages of the gap segment is a modified internucleoside linkage.

In some embodiments, at least two, three, or four internucleoside linkages of the nucleobase sequence are phosphorothioate internucleoside linkages.

In some embodiments, at least one, two, three, or four internucleoside linkages between nucleoside bases of the gap segment are phosphorothioate internucleoside linkages.

In some embodiments, the phosphorothioate internucleoside linkage is in one of a *Rp* configuration or a *Sp* configuration. In some embodiments the phosphorothioate linkages are mixed stereo-enriched (*e.g.*, *Sp-Rp-Sp* or *Rp-Sp-Rp*) phosphorothioate linkages.

In some embodiments, the oligonucleotide comprises at least one modified nucleobase.

In some embodiments, the at least one modified nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine.

In some embodiments, the oligonucleotide comprises at least one modified sugar moiety.

5 In some embodiments, the at least one modified sugar is a bicyclic sugar. In some embodiments, the bicyclic sugar comprises a 4'-CH(R)—O-2' bridge wherein R is, independently, H, C₁-C₁₂ alkyl, or a protecting group. In some embodiments, R is methyl. In some embodiments, R is H.

10 In some embodiments, the modified sugar moiety is one of a 2'-OMe modified sugar moiety, bicyclic sugar moiety, 2'-O-methoxyethyl (MOE), 2'-deoxy-2'-fluoro nucleoside, 2'-fluoro-β-D-arabinonucleoside, locked nucleic acid (LNA), constrained ethyl 2'-4'-bridged nucleic acid (cEt), S-cEt, tcDNA, hexitol nucleic acids (HNA), and tricyclic analog (*e.g.*, tcDNA). In some embodiments, the modified sugar moiety is constrained ethyl 2'-4'-bridged nucleic acid (cEt), for example, S-cEt.

15 In some embodiments, the oligonucleotide comprises one or more 2'-O-methoxyethyl nucleosides that are linked through phosphorothioate internucleoside linkages.

In some embodiments, the oligonucleotide comprises three contiguous nucleoside bases that are linked through phosphorothioate internucleoside linkages at the 5' end and three contiguous nucleoside bases that are linked through phosphorothioate internucleoside linkages at the 3' end.

20 In some embodiments, the oligonucleotide comprises five contiguous nucleoside bases that are linked through phosphorothioate internucleoside linkages.

In some embodiments, each of the five contiguous nucleoside bases are 2'-O-methoxyethyl nucleosides. In some embodiments, each of the nucleoside bases of the oligonucleotide are 2'-O-methoxyethyl nucleosides. In some embodiments, the gap segment comprises one or more 2'-O-methoxyethyl nucleosides.

25 In some embodiments, the gap segment comprises phosphorothioate internucleoside linkages, wherein the 5' wing segment comprises two contiguous nucleoside bases that are linked through phosphodiester internucleoside linkages, and wherein the 3' wing segment comprises two contiguous nucleoside bases that are linked through phosphodiester internucleoside linkages.

In some embodiments, five contiguous nucleoside bases in the gap segment are linked through phosphorothioate internucleoside linkages, wherein the 5' wing segment comprises

at least one phosphorothioate internucleoside linkage, and wherein the 3' wing segment comprises at least one phosphorothioate internucleoside linkage.

In some embodiments, the oligonucleotide comprises one or more chiral centers and/or double bonds. In some embodiments, the oligonucleotide exist as stereoisomers

5 selected from geometric isomers, enantiomers, and diastereomers.

In some embodiments, the oligonucleotide comprises sugar modifications in any of the following patterns: eeeee-d10-eeee, d20, eeeee-d12-eeee, eeeee-d8-eeee, and eekk-d8-kkeee, wherein e = 2'-O-methoxyethyl nucleoside; d = a 2'-deoxynucleoside; k = a locked nucleic acid (LNA), constrained methoxyethyl (cMOE) nucleoside, constrained ethyl (cET) nucleoside, or peptide nucleic acid (PNA).

In some embodiments, the oligonucleotide comprises internucleoside linkages in any of the following patterns: sssssssssssssssss; sssssssssssssssss; soossssssssooss; and soossssssssooss; wherein s = a phosphorothioate linkage, and o = a phosphodiester linkage.

In some embodiments, the the oligonucleotide comprises sugar modification and internucleoside linkage combinations, respectively, in any of the following patterns: a) d20 and sssssssssssssssss; b) eeeee-d10-eeee and sssssssssssssssss; c) eeeee-d12-eeee and sssssssssssssssss; d) eeeee-d8-eeee and soossssssssooss; and e) eekk-d8-kkeee and soossssssssooss.

In some embodiments, the the oligonucleotide comprises a modified cytosine.

In some embodiments, the the modified cytosine is 5-methyl-dexocytosine (5-methyl-dC).

In some embodiments, the the oligonucleotide comprises sugar modification and internucleoside linkage combinations eeeee-d10-eeee and sssssssssssssssss, and the cytosines are modified as 5-methyl-dC.

In some embodiments, the the oligonucleotide comprises sugar modification and internucleoside linkage combinations, respectively, in any of the following patterns: a) d20 and sssssssssssssssss; b) eeeee-d12-eeee and sssssssssssssssss; c) eeeee-d8-eeee and soossssssssooss; and d) eekk-d8-kkeee and soossssssssooss; and the any cytosine in the oligonucleotide is an unmodified cytosine.

In some embodiments, the oligonucleotide is complementary to a nucleobase sequence of a target region of a target nucleic acid sequence, wherein the nucleobase sequence of the target region of the target nucleic acid differs from the nucleobase sequence of at least one non-target nucleic acid sequence by 1-3 differentiating nucleobases, and wherein the non-target nucleic acid comprises a sequence of SEQ ID NO: 3526. In some

embodiments, the 1-3 differentiating nucleobases comprises a single-nucleotide polymorphism (SNP). In some embodiments, the SNP present in the target region is a SNP compared to an equal length portion of SEQ ID NO: 3526. In some embodiments, the single nucleotide polymorphism is selected from the group consisting of: rs397515403,
5 rs397515402, rs587777264, rs397515404, rs866242631, rs886043455, rs397515407, and rs397515406. In some embodiments, the single nucleotide polymorphism is selected from the group consisting of: a C to a G at position 1112 of the sequence shown in SEQ ID NO: 3526, a C to a T at position 2845 of the sequence shown in SEQ ID NO: 3526, and a G to a T at position 885 of the sequence shown in SEQ ID NO: 3526.

10 In another aspect, provided herein are pharmaceutical compositions comprising the compound or oligonucleotide of any one of the above claims and a pharmaceutically acceptable carrier or excipient.

In some embodiments, the pharmaceutical composition is suitable for topical, intrathecal, intracisternal, parenteral, oral, pulmonary, intratracheal, intranasal, transdermal,
15 rectal, buccal, sublingual, vaginal, or intraduodenal administration.

In another aspect, provided herein are compositions comprising the compound or oligonucleotide of any one of the above claims and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome.

In another aspect, provided herein are methods of reducing a level and/or activity of
20 KCNT1 in a cell of a subject having a KCNT1 related disorder, the method comprising contacting the cell with the compound as described herein, the oligonucleotide as described herein or the pharmaceutical composition as described herein in an amount and for a duration sufficient to reduce the level and/or activity of KCNT1 in the cell.

In some embodiments, the cell is a cell of the central nervous system.

25 In another aspect, provided herein are methods of treating a neurological disease in a subject in need thereof, the method comprising administering to the patient an inhibitor of a transcript, wherein the transcript shares at least 90% identity with SEQ ID NO: 3526.

In some embodiments, the inhibitor is the oligonucleotide as described herein or the pharmaceutical composition as described herein.

30 In another aspect, provided herein are methods of treating, preventing, or delaying the progression of a KCNT1 related disorder in a subject in need thereof, the method comprising administering to the subject the compound as described herein, the oligonucleotide as described herein or the pharmaceutical composition as described herein in an amount and for a duration sufficient to treat, prevent, or delay the progression of the KCNT1 related disorder.

In some embodiments, the KCNT1 related disorder is selected from the group consisting of epilepsy of infancy with migrating focal seizures, autosomal dominant nocturnal frontal lobe epilepsy, West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome.

In some embodiments, the subject has a gain-of-function mutation in KCNT1.

In some embodiments, the gain-of-function mutation is selected from the group consisting of V271F, L274I, G288S, F346L, R398Q, R428Q, R474H, F502V, M516V, K629N, I760M, Y796H, E893K, M896I, M896K, P924L, R928C, F932I, A934T, A966T, H257D, R262Q, Q270E, V340M, C377S, P409S, L437F, R474C, A477T, R565H, K629E, G652V, I760F, Q906H, R933G, A934T, R950Q, R961H, R1106Q, K1154Q, R474Q, Y1903C, H469L, M896R, K946E, and R950L.

In some embodiments, the gain-of-function mutation is G288S, R398Q, R428Q, R928C, or A934T.

In some embodiments, the method reduces one or more symptoms of the KCNT1 related disorder.

In some embodiments, the one or more symptoms of the KCNT1 related disorder is selected from the group consisting of prolonged seizures, frequent seizures, behavioral and developmental delays, movement and balance issues, orthopedic conditions, delayed language and speech issues, growth and nutrition issues, sleeping difficulties, chronic infection, sensory integration disorder, disruption of the autonomic nervous system, and sweating.

In some embodiments, the oligonucleotide or pharmaceutical composition is administered topically, parenterally, intrathecally, intracisternally, orally, rectally, buccally, sublingually, vaginally, pulmonarily, intratracheally, intranasally, transdermally, or intraduodenally.

In some embodiments, the patient is a human.

In another aspect, provided herein are compounds comprising a modified oligonucleotide of 18-22 linked nucleosides in length and having at least 85% sequence complementarity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1 transcript.

In another aspect, provided herein are compounds comprising a modified oligonucleotide of 18-22 linked nucleosides in length and having at least 85% sequence

complementarity to an equal length portion of *H. sapiens* KCNT1 and *M. fascicularis* KCNT1 transcript.

In another aspect, provided herein are compounds comprising a modified oligonucleotide of 18-22 linked nucleosides in length and having at least 85% sequence complementarity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and/or *M. fascicularis* KCNT1 transcript.

In some embodiments, the oligonucleotide comprises a GC content from 40% to 70%.

In some embodiments, the oligonucleotide comprises no more than 2 mismatches to *H. sapiens* KCNT1 transcript. In some embodiments, the oligonucleotide comprises at least 3 mismatches to any non KCNT1 transcript.

In some embodiments, the oligonucleotide lacks a GGGG tetrad.

In some embodiments, the oligonucleotide is not any one of SEQ ID NOs: 3512-3525.

In some embodiments, the oligonucleotide is not any one of SEQ ID NOs: 3512-3525.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

FIG. 1 is a plot demonstrating percentage knockdown of hKCNT1 in response to antisense oligonucleotide treatments.

DETAILED DESCRIPTION

Definitions

For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed technology, because the scope of the technology is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”; and (iii) the terms “including” and “comprising” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more

5 additional components or steps.

As used herein, the terms “about” and “approximately” refer to a value that is within 10% above or below the value being described. For example, the term “about 5 nM” indicates a range of from 4.5 to 5.5 nM.

The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term “at least”, and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, “at least 18 nucleotides of a 21-nucleotide nucleic acid molecule” means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that

10 “at least” can modify each of the numbers in the series or range.

As used herein, “no more than” or “less than” is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, an oligonucleotide with “no more than 3 mismatches to a target sequence” has 3, 2, 1, or 0 mismatches to a target sequence. When “no more than” is present before a series of numbers or a range, it is understood that “no more than” can modify each of the numbers in the series or range.

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As used herein, the term “administration” refers to the administration of a composition (*e.g.*, a compound or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (*e.g.*, to a human) may be by any appropriate route, such as one described herein.

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As used herein, a “combination therapy” or “administered in combination” means that two (or more) different agents or treatments are administered to a subject as part of a defined treatment regimen for a particular disease or condition. The treatment regimen defines the doses and periodicity of administration of each agent such that the effects of the separate agents on the subject overlap. In some embodiments, the delivery of the two or more agents is simultaneous or concurrent and the agents may be co-formulated. In some embodiments, the two or more agents are not co-formulated and are administered in a sequential manner as part of a prescribed regimen. In some embodiments, administration of two or more agents or treatments in combination is such that the reduction in a symptom, or other parameter related

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to the disorder is greater than what would be observed with one agent or treatment delivered alone or in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive (*e.g.*, synergistic). Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination may be administered by intravenous injection while a second therapeutic agent of the combination may be administered orally.

As used herein, the term "KCNT1" refers potassium sodium-activated channel subfamily T member 1, having an amino acid sequence from any vertebrate or mammalian source, including, but not limited to, human, bovine, chicken, rodent, mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise. The term also refers to fragments and variants of native KCNT1 that maintain at least one *in vivo* or *in vitro* activity of a native KCNT1. The term encompasses full-length unprocessed precursor forms of KCNT1 as well as mature forms resulting from post-translational cleavage of the signal peptide. KCNT1 is encoded by the KCNT1 gene. The nucleic acid sequence of an exemplary *Homo sapien* (human) KCNT1 gene is set forth in NCBI Reference No. NG_033070.1. The nucleic acid sequence of an exemplary *Homo sapien* (human) KCNT1 transcript is set forth in NCBI Reference No. NM_020822.2 and NM_001272003.1. The term "KCNT1" also refers to natural variants of the wild-type KCNT1 protein, such as proteins having at least 85% identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% identity, or more) to the amino acid sequence of wild-type human KCNT1. The nucleic acid sequence of an exemplary *Mus musculus* (mouse) KCNT1 transcript is set forth in NCBI Reference No. NM_175462.4 and NM_001145403.2, and NM_01302351.1. The nucleic acid sequence of an exemplary *Macaca fascicularis* (cyno) KCNT1 transcript is set forth in NCBI Reference No. XM_015436456.1.

The term "KCNT1" as used herein also refers to a particular polypeptide expressed in a cell by naturally occurring DNA sequence variations of the KCNT1 gene, such as a single nucleotide polymorphism in the KCNT1 gene. Numerous SNPs within the KCNT1 transcript have been identified (*see, e.g.*, Table 1).

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a KCNT1 gene, including

mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to serve as a substrate for oligonucleotide-directed (*e.g.*, antisense oligonucleotide (ASO)-directed) cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a KCNT1 gene. The target sequence may be, for example, from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length, *e.g.*, about 18-22 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

“G,” “C,” “A,” “T,” and “U” each generally stand for a naturally-occurring nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term “nucleotide” can also refer to an alternative nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of oligonucleotides featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms “nucleobase” and “base” include the purine (*e.g.* adenine and guanine) and pyrimidine (*e.g.* uracil, thymine, and cytosine) moiety present in nucleosides and nucleotides which form hydrogen bonds in nucleic acid hybridization. In the context of the present invention, the term nucleobase also encompasses alternative nucleobases which may differ from naturally-occurring nucleobases, but are functional during nucleic acid hybridization. In this context “nucleobase” refers to both naturally occurring nucleobases such as adenine, guanine, cytosine, thymidine, uracil, xanthine, and hypoxanthine, as well as alternative

nucleobases. Such variants are for example described in Hirao et al (2012) Accounts of Chemical Research vol 45 page 2055 and Bergstrom (2009) Current Protocols in Nucleic Acid Chemistry Suppl. 37 1.4.1.

The term “nucleoside” refers to a monomeric unit of an oligonucleotide or a polynucleotide having a nucleobase and a sugar moiety. A nucleoside may include those that are naturally-occurring as well as alternative nucleosides, such as those described herein. The nucleobase of a nucleoside may be a naturally-occurring nucleobase or an alternative nucleobase. Similarly, the sugar moiety of a nucleoside may be a naturally-occurring sugar or an alternative sugar.

The term “alternative nucleoside” or “modified nucleoside” refers to a nucleoside having an alternative sugar or an alternative nucleobase, such as those described herein.

In some embodiments the nucleobase moiety is modified by changing the purine or pyrimidine into a modified purine or pyrimidine, such as substituted purine or substituted pyrimidine, such as an “alternative nucleobase” selected from isocytosine, pseudoisocytosine, 5-methyl cytosine, 5-thiazolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uridine, 5-bromouridine 5-thiazolo-uridine, 2-thio-uridine, pseudouridine, 1-methylpseudouridine, 5-methoxyuridine, 2'-thio-thymine, inosine, diaminopurine, 6-aminopurine, 2-aminopurine, 2,6-diaminopurine, and 2-chloro-6-aminopurine.

The nucleobase moieties may be indicated by the letter code for each corresponding nucleobase, *e.g.* A, T, G, C, or U, wherein each letter may optionally include alternative nucleobases of equivalent function. In some embodiments, *e.g.*, for gapmers, 5-methyl cytosine LNA nucleosides may be used.

A “sugar” or “sugar moiety” includes naturally occurring sugars having a furanose ring. A sugar also includes an “alternative sugar,” defined as a structure that is capable of replacing the furanose ring of a nucleoside. In certain embodiments, alternative sugars are non-furanose (or 4'-substituted furanose) rings or ring systems or open systems. Such structures include simple changes relative to the natural furanose ring, such as a six-membered ring, or may be more complicated as is the case with the non-ring system used in peptide nucleic acid. Alternative sugars may also include sugar surrogates wherein the furanose ring has been replaced with another ring system such as, for example, a morpholino or hexitol ring system. Sugar moieties useful in the preparation of oligonucleotides having motifs include, without limitation, β -D-ribose, β -D-2'-deoxyribose, substituted sugars (such as 2', 5' and bis substituted sugars), 4'-S-sugars (such as 4'-S-ribose, 4'-S-2'-deoxyribose and 4'-S-2'-substituted ribose), bicyclic alternative sugars (such as the 2'-O—CH₂-4' or 2'-O—

(CH₂)₂-4' bridged ribose derived bicyclic sugars) and sugar surrogates (such as when the ribose ring has been replaced with a morpholino or a hexitol ring system). The type of heterocyclic base and internucleoside linkage used at each position is variable and is not a factor in determining the motif. In most nucleosides having an alternative sugar moiety, the heterocyclic nucleobase is generally maintained to permit hybridization.

A "nucleotide," as used herein, refers to a monomeric unit of an oligonucleotide or polynucleotide that comprises a nucleoside and an internucleosidic linkage. The internucleosidic linkage may or may not include a phosphate linkage. Similarly, "linked nucleosides" may or may not be linked by phosphate linkages. Many "alternative internucleosidic linkages" are known in the art, including, but not limited to, phosphate, phosphorothioate, and boranophosphate linkages. Alternative nucleosides include bicyclic nucleosides (BNAs) (*e.g.*, locked nucleosides (LNAs) and constrained ethyl (cEt) nucleosides), peptide nucleosides (PNAs), phosphotriesters, phosphorothionates, phosphoramidates, and other variants of the phosphate backbone of native nucleoside, including those described herein.

An "alternative nucleotide," as used herein, refers to a nucleotide having an alternative nucleoside or an alternative sugar, and an internucleoside linkage, which may include alternative nucleoside linkages.

The terms "oligonucleotide" and "polynucleotide" as used herein are defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers. Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The oligonucleotide of the invention may be man-made, is chemically synthesized, and is typically purified or isolated. Oligonucleotide is also intended to include (i) compounds that have one or more furanose moieties that are replaced by furanose derivatives or by any structure, cyclic or acyclic, that may be used as a point of covalent attachment for the base moiety, (ii) compounds that have one or more phosphodiester linkages that are either modified, as in the case of phosphoramidate or phosphorothioate linkages, or completely replaced by a suitable linking moiety as in the case of formacetal or riboacetal linkages, and/or (iii) compounds that have one or more linked furanose-phosphodiester linkage moieties replaced by any structure, cyclic or acyclic, that may be used as a point of covalent

attachment for the base moiety. The oligonucleotide of the invention may comprise one or more alternative nucleosides or nucleotides (*e.g.*, including those described herein). It is also understood that oligonucleotide includes compositions lacking a sugar moiety or nucleobase but is still capable of forming a pairing with or hybridizing to a target sequence.

5 “Oligonucleotide” refers to a short polynucleotide (*e.g.*, of 100 or fewer linked nucleosides).

 “Chimeric” oligonucleotides or “chimeras,” in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide or nucleoside in the case of an oligonucleotide.

10 Chimeric oligonucleotides also include “gapmers.”

 The oligonucleotide may be of any length that permits specific degradation of a desired target RNA through an RNase H-mediated pathway, and may range from about 10-30 base pairs in length, *e.g.*, about 15-30 base pairs in length or about 18-22 (*e.g.*, 18-20) base pairs in length, for example, about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,
 15 25, 26, 27, 28, 29, or 30 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs
 20 in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

 As used herein, the term “oligonucleotide comprising a nucleobase sequence” refers to an oligonucleotide comprising a chain of nucleotides or nucleosides that is described by the sequence referred to using the standard nucleotide nomenclature.

25 The term “contiguous nucleobase region” refers to the region of the oligonucleotide which is complementary to the target nucleic acid. The term may be used interchangeably herein with the term “contiguous nucleotide sequence” or “contiguous nucleobase sequence.” In some embodiments all the nucleotides of the oligonucleotide are present in the contiguous nucleotide or nucleoside region. In some embodiments the oligonucleotide comprises the
 30 contiguous nucleotide region and may optionally comprise further nucleotide(s) or nucleoside(s), for example a nucleotide linker region which may be used to attach a functional group to the contiguous nucleotide sequence. The nucleotide linker region may or may not be complementary to the target nucleic acid. In some embodiments the internucleoside linkages present between the nucleotides of the contiguous nucleotide region

are all phosphorothioate internucleoside linkages. In some embodiments, the contiguous nucleotide region comprises one or more sugar-modified nucleosides.

The term "gapmer" as used herein refers to an oligonucleotide which comprises a region of RNase H recruiting oligonucleotides (gap) which is flanked 5' and 3' by regions which comprise one or more affinity enhancing alternative nucleosides (wings or flanks). Various gapmer designs are described herein. Headmers and tailmers are oligonucleotides capable of recruiting RNase H where one of the wings is missing, *e.g.*, only one of the ends of the oligonucleotide comprises affinity enhancing alternative nucleosides. For headmers, the 3' wing is missing (*e.g.*, the 5' wing comprises affinity enhancing alternative nucleosides) and for tailmers the 5' wing is missing (*e.g.*, the 3' wing comprises affinity enhancing alternative nucleosides). A "mixed wing gapmer" refers to a gapmer wherein the wing regions comprise at least one alternative nucleoside, such as at least one DNA nucleoside or at least one 2' substituted alternative nucleoside, such as, for example, 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, 2'-F-ANA nucleoside(s), or bicyclic nucleosides (*e.g.*, locked nucleosides or constrained ethyl (cEt) nucleosides). In some embodiments the mixed wing gapmer has one wing which comprises alternative nucleosides (*e.g.*, 5' or 3') and the other wing (3' or 5' respectfully) comprises 2' substituted alternative nucleoside(s).

The term "linker" or "linking group" is a connection between two atoms that links one chemical group or segment of interest to another chemical group or segment of interest via one or more covalent bonds. Conjugate moieties can be attached to the oligonucleotide directly or through a linking moiety (*e.g.*, linker or tether). Linkers serve to covalently connect a third region, *e.g.*, a conjugate moiety to an oligonucleotide (*e.g.*, the termini of region A or C). In some embodiments of the invention the conjugate or oligonucleotide conjugate of the invention may optionally, comprise a linker region which is positioned between the oligonucleotide and the conjugate moiety. In some embodiments, the linker between the conjugate and oligonucleotide is biocleavable. Phosphodiester containing biocleavable linkers are described in more detail in International Publication No. WO 2014/076195 (herein incorporated by reference).

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide or nucleoside sequence in relation to a second nucleotide or nucleoside sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide or nucleoside sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second

nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 °C, or 70 °C, for 12-16 hours followed by washing (see, *e.g.*, "Molecular Cloning: A Laboratory Manual, Sambrook, et al. (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides or nucleosides.

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and alternative nucleotides or nucleosides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing. Complementary sequences between an oligonucleotide and a target sequence as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide or nucleoside sequence to an oligonucleotide or polynucleotide comprising a second nucleotide or nucleoside sequence over the entire length of one or both nucleotide or nucleoside sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via an RNase H-mediated pathway. "Substantially complementary" can also refer to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding KCNT1). For example, a polynucleotide is complementary to at least a part of a KCNT1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding KCNT1.

As used herein, the term "region of complementarity" refers to the region on the oligonucleotide that is substantially complementary to all or a portion of a gene, primary transcript, a sequence (*e.g.*, a target sequence, *e.g.*, a KCNT1 mRNA nucleotide sequence or a KCNT1 mRNA transcript variant), or processed mRNA, so as to interfere with expression of the endogenous gene (*e.g.*, KCNT1). Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal

regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the oligonucleotide.

As used herein, an "agent that reduces the level and/or activity of KCNT1" refers to any polynucleotide agent (*e.g.*, an oligonucleotide, *e.g.*, an ASO) that reduces the level of or inhibits expression of KCNT1 in a cell or subject. The phrase "inhibiting expression of KCNT1," as used herein, includes inhibition of expression of any KCNT1 gene (such as, *e.g.*, a mouse KCNT1 gene, a rat KCNT1 gene, a monkey KCNT1 gene, or a human KCNT1 gene) as well as variants or mutants of a KCNT1 gene that encode a KCNT1 protein. Thus, the KCNT1 gene may be a wild-type KCNT1 gene, a mutant KCNT1 gene, or a transgenic KCNT1 gene in the context of a genetically manipulated cell, group of cells, or organism.

By "reducing the activity of KCNT1" is meant decreasing the level of an activity related to KCNT1 (*e.g.*, an ion channel function). The activity level of KCNT1 may be measured using any method known in the art (*e.g.*, using standard biophysical methods).

By "reducing the level of KCNT1" is meant decreasing the amount of KCNT1 in a cell or subject, *e.g.*, by administering an oligonucleotide to the cell or subject. The level of KCNT1 may be measured using any method known in the art (*e.g.*, by measuring the levels of KCNT1 mRNA or levels of KCNT1 protein in a cell or a subject).

As used herein, the term "inhibitor" refers to any agent which reduces the level and/or activity of a protein (*e.g.*, KCNT1). Non-limiting examples of inhibitors include polynucleotides (*e.g.*, oligonucleotide, *e.g.*, ASOs). The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating," "suppressing," and other similar terms, and includes any level of inhibition.

The phrase "contacting a cell with an oligonucleotide," such as an oligonucleotide, as used herein, includes contacting a cell by any possible means. Contacting a cell with an oligonucleotide includes contacting a cell *in vitro* with the oligonucleotide or contacting a cell *in vivo* with the oligonucleotide. The contacting may be done directly or indirectly. Thus, for example, the oligonucleotide may be put into physical contact with the cell by the individual performing the method, or alternatively, the oligonucleotide agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the oligonucleotide. Contacting a cell *in vivo* may be done, for example, by injecting the oligonucleotide into or near the tissue where the cell is located, or by injecting the oligonucleotide agent into another area, *e.g.*, the bloodstream or the subcutaneous space, such

that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the oligonucleotide may contain and/or be coupled to a ligand, *e.g.*, GalNAc3, that directs the oligonucleotide to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an oligonucleotide and subsequently transplanted into a subject.

In one embodiment, contacting a cell with an oligonucleotide includes "introducing" or "delivering the oligonucleotide into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an ASO can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing an oligonucleotide into a cell may be *in vitro* and/or *in vivo*. For example, for *in vivo* introduction, oligonucleotides can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

As used herein, "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.*, an oligonucleotide. LNP refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). LNPs are described in, for example, U.S. Pat. Nos. 6,858,225; 6,815,432; 8,158,601; and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the oligonucleotide composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the oligonucleotide composition, although in some examples, it may. Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids.

"Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with

the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

The term “antisense,” as used herein, refers to a nucleic acid comprising an oligonucleotide or polynucleotide that is sufficiently complementary to all or a portion of a gene, primary transcript, or processed mRNA, so as to interfere with expression of the endogenous gene (*e.g.*, KCNT1). “Complementary” polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other.

As used herein, the terms “effective amount,” “therapeutically effective amount,” and “a sufficient amount” of an agent that reduces the level and/or activity of KCNT1 (*e.g.*, in a cell or a subject) described herein refer to a quantity sufficient to, when administered to the subject, including a human, effect beneficial or desired results, including clinical results, and, as such, an “effective amount” or synonym thereto depends on the context in which it is being applied. For example, in the context of treating a KCNT1 related disorder, it is an amount of the agent that reduces the level and/or activity of KCNT1 sufficient to achieve a treatment response as compared to the response obtained without administration of the agent that reduces the level and/or activity of KCNT1. The amount of a given agent that reduces the level and/or activity of KCNT1 described herein that will correspond to such an amount will vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (*e.g.*, age, sex, and/or weight) or host being treated, and the like, but can nevertheless be routinely determined by one of skill in the art. Also, as used herein, a “therapeutically effective amount” of an agent that reduces the level and/or activity of KCNT1 of the present disclosure is an amount which results in a beneficial or desired result in a subject as compared to a control. As defined herein, a therapeutically effective amount of an agent that reduces the level and/or activity of KCNT1 of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art. Dosage regimen may be adjusted to provide the optimum therapeutic response.

“Prophylactically effective amount,” as used herein, is intended to include the amount of an oligonucleotide that, when administered to a subject having or predisposed to having a KCNT1 related disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The “prophylactically effective amount” may vary depending on the oligonucleotide, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated. A prophylactically effective amount also refer to, for example, an amount of the agent reduces the level and/or activity of KCNT1 (*e.g.*, in a cell or a subject) described herein refer to a quantity sufficient to, when administered to the subject, including a human, to delay the onset of a KCNT1 related disorder, as described herein, by at least 120 days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with the predicted onset.

A “therapeutically-effective amount” or “prophylactically effective amount” also includes an amount (either administered in a single or in multiple doses) of an oligonucleotide that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. Oligonucleotides employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

As used herein, the term “region of complementarity” refers to the region on the oligonucleotide that is substantially complementary to all or a portion of a gene, primary transcript, a sequence (*e.g.*, a target sequence, *e.g.*, a KCNT1 mRNA nucleotide sequence or KCNT1 mRNA transcript variant), or processed mRNA, so as to interfere with expression of the endogenous gene (*e.g.*, KCNT1). Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the oligonucleotide.

As used herein, the term “a subject identified as having a KCNT1 related disorder” refers to a subject identified as having a molecular or pathological state, disease or condition of or associated with a KCNT1 related disorder, such as the identification of a KCNT1 related disorder or symptoms thereof, or to refer to identification of a subject having or

suspected of having a KCNT1 related disorder who may benefit from a particular treatment regimen.

As used herein, “KCNT1 related disorder,” refers to a class of genetic diseases or disorders characterized by aberrant function of KCNT1. KCNT1 related disorders include, for example, epilepsy of infancy with migrating focal seizures (EIMFS), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome

By “determining the level of a protein” is meant the detection of a protein, or an mRNA encoding the protein, by methods known in the art either directly or indirectly. “Directly determining” means performing a process (*e.g.*, performing an assay or test on a sample or “analyzing a sample” as that term is defined herein) to obtain the physical entity or value. “Indirectly determining” refers to receiving the physical entity or value from another party or source (*e.g.*, a third-party laboratory that directly acquired the physical entity or value). Methods to measure protein level generally include, but are not limited to, western blotting, immunoblotting, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, liquid chromatography (LC)-mass spectrometry, microcytometry, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry, as well as assays based on a property of a protein including, but not limited to, enzymatic activity or interaction with other protein partners. Methods to measure mRNA levels are known in the art.

“Percent (%) sequence identity” with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the

sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a

5 given nucleic acid or amino acid sequence, B) is calculated as follows:

$$100 \text{ multiplied by (the fraction } X/Y)$$

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (*e.g.*, BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length
10 of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

By "level" is meant a level or activity of a protein, or mRNA encoding the protein (*e.g.*, KCNT1), optionally as compared to a reference. The reference can be any useful
15 reference, as defined herein. By a "decreased level" or an "increased level" of a protein is meant a decrease or increase in protein level, as compared to a reference (*e.g.*, a decrease or an increase by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about
20 200%, about 300%, about 400%, about 500%, or more; a decrease or an increase of more than about 10%, about 15%, about 20%, about 50%, about 75%, about 100%, or about 200%, as compared to a reference; a decrease or an increase by less than about 0.01-fold, about 0.02-fold, about 0.1-fold, about 0.3-fold, about 0.5-fold, about 0.8-fold, or less; or an increase by more than about 1.2-fold, about 1.4-fold, about 1.5-fold, about 1.8-fold, about 2.0-fold,
25 about 3.0-fold, about 3.5-fold, about 4.5-fold, about 5.0-fold, about 10-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 100-fold, about 1000-fold, or more). A level of a protein may be expressed in mass/vol (*e.g.*, g/dL, mg/mL, µg/mL, and ng/mL) or percentage relative to total protein or mRNA in a sample.

The term "pharmaceutical composition," as used herein, represents a composition
30 containing a compound described herein formulated with a pharmaceutically acceptable excipient, and preferably manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (*e.g.*, a tablet, capsule, caplet, gelcap, or syrup); for topical administration (*e.g.*,

as a cream, gel, lotion, or ointment); for intravenous administration (*e.g.*, as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); for intrathecal injection; for intracerebroventricular injections; for intraparenchymal injection; or in any other pharmaceutically acceptable formulation.

5 A “pharmaceutically acceptable excipient,” as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a subject. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, 10 emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, 15 cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), 20 stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

As used herein, the term “pharmaceutically acceptable salt” means any pharmaceutically acceptable salt of the compound of any of the compounds described herein. For example, pharmaceutically acceptable salts of any of the compounds described herein include those that are within the scope of sound medical judgment, suitable for use in contact 25 with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The 30 salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting a free base group with a suitable organic acid.

The compounds described herein may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the

compounds described herein, be prepared from inorganic or organic bases. Frequently, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases and methods for preparation of the appropriate salts are well-known in the

5 art. Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, 10 heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, and valerate salts.

15 Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, and ethylamine.

20 By a “reference” is meant any useful reference used to compare protein or mRNA levels or activity. The reference can be any sample, standard, standard curve, or level that is used for comparison purposes. The reference can be a normal reference sample or a reference standard or level. A “reference sample” can be, for example, a control, *e.g.*, a predetermined negative control value such as a “normal control” or a prior sample taken from 25 the same subject; a sample from a normal healthy subject, such as a normal cell or normal tissue; a sample (*e.g.*, a cell or tissue) from a subject not having a disease; a sample from a subject that is diagnosed with a disease, but not yet treated with a compound described herein; a sample from a subject that has been treated by a compound described herein; or a sample of a purified protein (*e.g.*, any described herein) at a known normal concentration. By 30 “reference standard or level” is meant a value or number derived from a reference sample. A “normal control value” is a pre-determined value indicative of non-disease state, *e.g.*, a value expected in a healthy control subject. Typically, a normal control value is expressed as a range (“between X and Y”), a high threshold (“no higher than X”), or a low threshold (“no lower than X”). A subject having a measured value within the normal control value for a

particular biomarker is typically referred to as “within normal limits” for that biomarker. A normal reference standard or level can be a value or number derived from a normal subject not having a disease or disorder (*e.g.*, a KCNT1 related disorder); a subject that has been treated with a compound described herein. In preferred embodiments, the reference sample, standard, or level is matched to the sample subject sample by at least one of the following criteria: age, weight, sex, disease stage, and overall health. A standard curve of levels of a purified protein, *e.g.*, any described herein, within the normal reference range can also be used as a reference.

As used herein, the term “subject” refers to any organism to which a composition in accordance with the invention may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include any animal (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans). A subject may seek or be in need of treatment, require treatment, be receiving treatment, be receiving treatment in the future, or be a human or animal who is under care by a trained professional for a particular disease or condition.

As used herein, the terms “treat,” “treated,” or “treating” mean both therapeutic treatment and prophylactic or preventative measures wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder, or disease, or obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of a condition, disorder, or disease; stabilized (*i.e.*, not worsening) state of condition, disorder, or disease; delay in onset or slowing of condition, disorder, or disease progression; amelioration of the condition, disorder, or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the subject; or enhancement or improvement of condition, disorder, or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

As used herein, the term “derivative” refers to naturally-occurring, synthetic, and semi-synthetic analogues of a compound, peptide, protein, or other substance described herein. A derivative of a compound, peptide, protein, or other substance described herein may retain or improve upon the biological activity of the original material.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

5 KCNT1 RELATED DISORDERS

The present inventors have found that inhibition or depletion of KCNT1 level and/or activity in a cell is effective in the treatment of a KCNT1 related disorder. Accordingly, the invention features useful compositions and methods to treat KCNT1 related disorders, *e.g.*, in a subject in need thereof. The invention features single-stranded oligonucleotides that

10 include 18-22 (*e.g.*, 18, 19, 20, 21, and 22) linked nucleosides in length having a region of at least 18 (*e.g.*, 18, 19, 20, 21, and 22) contiguous nucleobases of any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525). Also featured are oligonucleotides that cross-hybridize between human and mouse, human and monkey, or,

15 human, mouse, and monkey KCNT1. The sequence of human KCNT1 mRNA transcript (NCBI NM_020822.2) is provided as SEQ ID NO: 3526. The sequence of mouse KCNT1 mRNA transcript is provided as SEQ ID NO: 3533. The sequence of cynomolgous monkey (*Macaca fascicularis*) KCNT1 mRNA transcript is provided as SEQ ID NO: 3534. The oligonucleotides (*e.g.*, chemically modified oligonucleotides) may be administered to a

20 subject with a KCNT1 related disorder (*e.g.*, epilepsy) in order to treat, reduce the symptoms of, or prevent the KCNT1 related disorder. The oligonucleotides are antisense (*e.g.*, at least partially complementary) to a target region of KCNT1 (*e.g.*, KCNT1 mRNA, including pre-mRNA and processed mRNA). Following administration, the oligonucleotides reduce the level, expression, and/or activity of KCNT1 (*e.g.*, KCNT1 mRNA and/or protein), thereby

25 providing a therapeutic effect to the subject with a KCNT1 related disorder.

KCNT1 encodes an intracellular sodium-activated potassium channel (potassium sodium-activated channel subfamily T member 1 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

30 mediate a sodium-sensitive potassium current (*IKNa*), which is triggered by an influx of sodium channels ions through sodium channels or neurotransmitter receptors. Delayed outward current may be involved in regulating neuronal excitability. The amino acid sequence of wild type KCNT1 (UNIPROT ID Q5JUK3-3) is provided as SEQ ID NO: 3527.

The amino acid sequence of G288S KCNT1 is provided as SEQ ID NO: 3528. The amino acid sequence of R398Q KCNT1 is provided as SEQ ID NO: 3529. The amino acid sequence of R428Q KCNT1 is provided as SEQ ID NO: 3530. The amino acid sequence of R928C KCNT1 is provided as SEQ ID NO: 3531. The amino acid sequence of A934T KCNT1 is provided as SEQ ID NO: 3532.

Mutations in *KCNT1* (*e.g.*, gain-of-function mutations) have been associated with particular forms of epilepsy, including epilepsy of infancy with migrating focal seizures (EIMFS), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome.

EIMFS is a rare and debilitating genetic condition characterized by an early onset (before 6 months of age) of almost continuous heterogeneous focal seizures, where seizures appear to migrate from one brain region and hemisphere to another. Subjects with EIMFS may be intellectually impaired, non-verbal and non-ambulatory. Subjects with EIMFS may have a mutation (*e.g.*, gain-of-function mutation) in KCNT1, such as V271F, G288S, R428Q, R474Q, R474H, R474C, I760M, A934T and P924L.

ADNFLE has a later onset than EIMFS, generally in mid-childhood, and is generally a less severe condition. It is characterized by nocturnal frontal lobe seizures and can result in psychiatric, behavioral and cognitive disabilities in subjects with the condition. Subjects with ADNFLE may have a mutation (*e.g.*, gain-of-function mutation) in KCNT1, such as M896I, R398Q, Y796H and R928C.

West syndrome is a severe form of epilepsy in which subjects exhibit one or more of infantile spasms, an interictal electroencephalogram (EEG) pattern termed hypsarrhythmia, and mental retardation. Subjects with West syndrome may have a mutation (*e.g.*, gain-of-function mutation) in KCNT1, such as G652V and R474H.

Any of the KCNT1 related disorders described herein may be treated by administering the oligonucleotides (*e.g.*, chemically modified oligonucleotides) of any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525) or oligonucleotides that are 18-22 nucleosides in length and have a region of at least 18 contiguous nucleosides, at least 19 contiguous nucleosides, at least 20 contiguous nucleosides, at least 21 contiguous nucleosides, or at least 22 contiguous nucleosides from any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525). In some embodiments, any of the

KCNT1 related disorders described herein may be treated by administering the oligonucleotides (*e.g.*, chemically modified oligonucleotides) of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595.

The subject to be treated may have a gain-of-function mutation in KCNT1. The gain-of-function mutation may be one or more of V271F, L274I, G288S, F346L, R398Q, R428Q, R474H, F502V, M516V, K629N, I760M, Y796H, E893K, M896I, M896K, P924L, R928C, F932I, A934T, A966T, H257D, R262Q, Q270E, V340M, C377S, P409S, L437F, R474C, A477T, R565H, K629E, G652V, I760F, Q906H, R933G, R950Q, R961H, R1106Q, K1154Q, R474Q, Y1903C, H469L, M896R, K946E, and R950L.

Table 1 below shows single nucleotide polymorphisms (SNPs) in *KCNT1* transcript and the position of each SNP in comparison to the KCNT1 transcript (*e.g.*, SEQ ID NO: 3526). The phrase “KCNT1 transcript variant” or “KCNT1 mRNA transcript variant” refers to a KCNT1 transcript that differs from the wild type KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) by at least one nucleotide (*e.g.*, two, three, four, or five nucleotides).

Table 1: SNPs in *KCNT1*. Further shown is the corresponding Amino Acid Mutation due to each SNP. RS No. refers to the dbSNP ID reference number, if the SNP has been entered into the dbSNP database.

Single Nucleotide Polymorphisms (SNPs) in KCNT1 transcript (<i>e.g.</i> , KCNT1 transcript variant) and their positions in SEQ ID NO: 3526				
RS No.	HGVS Nomenclature	Polymorphism	Position in SEQ ID NO: 3526	Amino Acid Mutation
rs397515403	NM_020822.2 c.2800 G>A	G/A	2874	A934T
rs397515402	NM_020822.2 c.1283 G>A	G/A	1357	R428Q
rs587777264	NM_020822.2 c.862 G>A	G/A	936	G288S
rs397515404	NM_020822.2 c.1421 G>A	G/A	1495	R474H
rs866242631	NM_020822.2 c.1420 C>T	C/T	1494	R474C
rs886043455	NM_020822.2 c.2849 G>A	G/A	2923	R950Q
rs397515407	NM_020822.2 c.1193 G>A	G/A	1267	R398Q
rs397515406	NM_020822.2 c.2386 T>C	T/C	2460	Y796H
	NM_020822.2 c.1038 C>G	C/G	1112	F346L
	NM_020822.2 c.2771 C>T	C/T	2845	P924L
	NM_020822.2 c.811 G>T	G/T	885	V271F

Oligonucleotide Agents

Agents described herein that reduce the level and/or activity of KCNT1 in a cell may be, for example, a polynucleotide, *e.g.*, an oligonucleotide. These agents reduce the level of an activity related to KCNT1, or a related downstream effect, or reduce the level of KCNT1 in a cell or subject.

In some embodiments, the agent that reduces the level and/or activity of KCNT1 is a polynucleotide. In some embodiments, the polynucleotide is a single-stranded oligonucleotide (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)), *e.g.*, that acts by way of an RNase H-mediated pathway. Oligonucleotides include DNA and DNA/RNA chimeric molecules, typically about 10 to 30 nucleotides in length, which recognize polynucleotide target sequences or sequence portions through hydrogen bonding interactions with the nucleotide bases of the target sequence (*e.g.*, KCNT1 mRNA transcript or KCNT1 mRNA transcript variant). An oligonucleotide molecule can decrease the expression level (*e.g.*, protein level or mRNA level) of KCNT1. For example, an oligonucleotide includes oligonucleotides that targets full-length KCNT1. In some embodiments, the oligonucleotide molecule recruits an RNase H enzyme, leading to target mRNA degradation. In various embodiments, the oligonucleotide may be at least 16 nucleobases in length. In various embodiments, the oligonucleotide may be 17, 18, 19, 20, 21, or 22 nucleobases in length. In various embodiments, the oligonucleotide may be at least 17, at least 18, at least 19, at least 20, at least 21, or at least 22 nucleobases in length. In various embodiments, the oligonucleotide may be between 17-22, 18-21, or 19-20 nucleobases in length.

In some embodiments, the oligonucleotide decreases the level and/or activity of a positive regulator of function. In other embodiments, the oligonucleotide increases the level and/or activity of an inhibitor of a positive regulator of function. In some embodiments, the oligonucleotide increases the level and/or activity of a negative regulator of function.

In some embodiments, the oligonucleotide (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)) decreases the level and/or activity or function of KCNT1. In some embodiments, the oligonucleotide (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)) inhibits expression of KCNT1. In

other embodiments, the oligonucleotide (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)) increases degradation of KCNT1 and/or decreases the stability (*e.g.*, half-life) of KCNT1. In some embodiments, an oligonucleotide (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)) can be chemically synthesized.

In some embodiments, an oligonucleotide (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)) includes an oligonucleotide having a region of complementarity (*e.g.*, a contiguous nucleobase region) which is complementary to at least a part of an mRNA formed in the expression of a KCNT1 gene. In some embodiments, the region of complementarity may be about 30 nucleotides or less in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). In some embodiments, upon contact with a cell expressing the KCNT1 gene, the oligonucleotide may inhibit the expression of the KCNT1 gene (*e.g.*, a human, a primate, a non-primate, or a bird KCNT1 gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

In some embodiments, the region of complementarity to the target sequence may be between 10 and 30 linked nucleosides in length, *e.g.*, between 10-29, 10-28, 10-27, 10-26, 10-25, 10-24, 10-23, 10-22, 10-21, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 linked nucleosides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, an oligonucleotide can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

In some embodiments, an oligonucleotide compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide comprising unnatural or alternative nucleotides can be easily prepared.

Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In some embodiments, an oligonucleotide of the invention includes a region of at least 10 contiguous nucleobases having at least 80% (*e.g.*, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementary to at least 10 contiguous nucleotides of a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 transcript variant. In one aspect, an oligonucleotide of the invention includes a region of at least 10 contiguous nucleobases that are complementary to 10 contiguous nucleotides of a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 transcript variant. In some embodiments, the oligonucleotide comprises a sequence complementary to at least 10 contiguous nucleotides, 11 contiguous nucleotides, 12 contiguous nucleotides, 13 contiguous nucleotides, 14 contiguous nucleotides, 15 contiguous nucleotides, 16 contiguous nucleotides, 17 contiguous nucleotides, 18 contiguous nucleotides, 19 contiguous nucleotides, or 20 contiguous nucleotides of a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 transcript variant. In some embodiments, the oligonucleotide comprises a sequence complementary to between 19-23 contiguous nucleotides, the oligonucleotide sequence may be selected from any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525). In some embodiments, an oligonucleotides (*e.g.*, chemically modified oligonucleotide) is selected from any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, and 2595. In this aspect, the sequence is substantially complementary to a sequence of an mRNA generated in the expression of a KCNT1 gene.

In some embodiments, an oligonucleotide has a nucleic acid sequence with at least 50% (*e.g.*, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) sequence identity to the nucleic acid sequence any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525). In some embodiments, an oligonucleotide has a nucleic acid sequence with at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525). In some embodiments, an oligonucleotide has a nucleic acid sequence of any one of SEQ ID

NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525).

In some embodiments, an oligonucleotide has a nucleic acid sequence with at least 50% (*e.g.*, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) sequence identity to the nucleic acid sequence any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595.

It will be understood that, although, in some embodiments, the sequences in SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525) are described as unmodified and/or un-conjugated sequences, the nucleosides of the oligonucleotide of the invention *e.g.*, an oligonucleotide of the invention, may comprise any one of the sequences set forth in any one of SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525) that is an alternative nucleoside and/or conjugated as described in detail below. In some embodiments, an oligonucleotide comprising any of the sequences shown in SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525) can be unmodified or modified ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or a mixture of RNA and DNA.

The skilled person is well aware that oligonucleotides having a structure of between about 18-20 base pairs may be particularly effective in inducing RNase H-mediated degradation. However, one can appreciate that shorter or longer oligonucleotides can also be effective. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, oligonucleotides described herein can include. It can be reasonably expected that shorter oligonucleotides minus only a few linked nucleosides on one or both ends can be similarly effective as compared to the oligonucleotides described above. Hence, oligonucleotides having a sequence of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous linked nucleosides derived from one of the sequences provided herein (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)), and differing in their ability to inhibit the expression of a KCNT1 gene by not more

than about 5, 10, 15, 20, 25, or 30% inhibition from an oligonucleotide comprising the full sequence, are contemplated to be within the scope of the present invention.

In some embodiments, the oligonucleotides described herein may function via nuclease-mediated degradation of the target nucleic acid, where the oligonucleotides of the invention are capable of recruiting a nuclease, particularly an endonuclease, preferably endoribonuclease (RNase) such as RNase H. Examples of oligonucleotide designs which operate via nuclease-mediated mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 DNA nucleosides and are flanked on one side or both sides by affinity enhancing alternative nucleosides, for example gapmers, headmers, and tailmers.

The RNase H activity of an oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule. International application publication number WO 01/23613 (incorporated by reference herein) provides *in vitro* methods for determining RNase H activity, which may be used to determine the ability to recruit RNase H. Typically an oligonucleotide is deemed capable of recruiting RNase H if it, when provided with a complementary target nucleic acid sequence, has an initial rate, as measured in pmol/l/min, of at least 5%, such as at least 10% or more than 20% of the of the initial rate determined when using an oligonucleotide having the same base sequence as the modified oligonucleotide being tested, but containing only DNA monomers, with phosphorothioate linkages between all monomers in the oligonucleotide, and using the methodology provided by Example 91-95 of WO01/23613 (hereby incorporated by reference).

In some embodiments, the oligonucleotides described herein (*e.g.*, SEQ ID NOs: 1-3525 (*e.g.*, SEQ ID NOs: 1-116 or 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525)) identify a site(s) in a KCNT1 transcript that is susceptible to RNase H-mediated cleavage. As such, the present invention further features oligonucleotides that target within this site(s). As used herein, an oligonucleotide is said to target within a particular site of an RNA transcript if the oligonucleotide promotes cleavage of the transcript anywhere within that particular site. Such an oligonucleotide will generally include at least about 5-10 contiguous linked nucleosides from one of the sequences provided herein coupled to additional linked nucleoside sequences taken from the region contiguous to the selected sequence in a KCNT1 gene.

Inhibitory oligonucleotides can be designed by methods well known in the art. While a target sequence is generally about 10-30 linked nucleosides in length, there is wide

variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA.

Oligonucleotides with homology sufficient to provide sequence specificity required to uniquely degrade any RNA can be designed using programs known in the art

5 Systematic testing of several designed species for optimization of the inhibitory oligonucleotide sequence can also be undertaken in accordance with the teachings provided herein. Considerations when designing interfering oligonucleotides include, but are not limited to, biophysical, thermodynamic, and structural considerations, base preferences at specific positions, and homology. The making and use of inhibitory therapeutic agents based
10 on non-coding oligonucleotides are also known in the art.

Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, *in silico*) placed on the
15 target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified
20 sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an oligonucleotide agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified herein represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the
25 window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified herein, further optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences and testing those sequences generated by
30 walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of oligonucleotides based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition.

Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of alternative nucleosides, alternative sugar moieties, and/or alternative internucleosidic linkages as described herein or as known in the art, including alternative nucleosides, alternative sugar moieties, and/or alternative internucleosidic linkages as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor. An oligonucleotide agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an oligonucleotide as described herein contains no more than 3 mismatches. If the oligonucleotide contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the oligonucleotide contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 30-linked nucleoside oligonucleotide agent, the contiguous nucleobase region which is complementary to a region of a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 mRNA transcript variant, generally does not contain any mismatch within the central 5-10 linked nucleosides. The methods described herein or methods known in the art can be used to determine whether an oligonucleotide containing a mismatch to a target sequence is effective in inhibiting the expression of a KCNT1 gene. Consideration of the efficacy of oligonucleotides with mismatches in inhibiting expression of a KCNT1 gene is important, especially if the particular region of complementarity in a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 mRNA transcript variant is known to have polymorphic sequence variation within the population.

Construction of vectors for expression of polynucleotides for use in the invention may be accomplished using conventional techniques which do not require detailed explanation to one of ordinary skill in the art. For generation of efficient expression vectors, it is necessary to have regulatory sequences that control the expression of the polynucleotide. These regulatory sequences include promoter and enhancer sequences and are influenced by specific cellular factors that interact with these sequences, and are well known in the art.

Alternative Oligonucleosides

In one embodiment, one or more of the linked nucleosides or internucleosidic linkages of the oligonucleotide of the invention, is naturally occurring, and does not

comprise, *e.g.*, chemical modifications and/or conjugations known in the art and described herein. In another embodiment, one or more of the linked nucleosides or internucleosidic linkages of an oligonucleotide of the invention, is chemically modified to enhance stability or other beneficial characteristics. Without being bound by theory, it is believed that certain modifications can increase nuclease resistance and/or serum stability or decrease immunogenicity. For example, oligonucleotides of the invention may contain nucleotides found to occur naturally in DNA or RNA (*e.g.*, adenine, thymidine, guanosine, cytidine, uridine, or inosine) or may contain alternative nucleosides or internucleosidic linkages which have one or more chemical modifications to one or more components of the nucleotide (*e.g.*, the nucleobase, sugar, or phospho-linker moiety). Oligonucleotides of the invention may be linked to one another through naturally occurring phosphodiester bonds, or may contain alternative linkages (*e.g.*, covalently linked through phosphorothioate (*e.g.*, Sp phosphorothioate or Rp phosphorothioate), 3'-methylenephosphonate, 5'-methylenephosphonate, 3'-phosphoamidate, 2'-5' phosphodiester, guanidinium, S-methylthiourea, 2'-alkoxy, alkyl phosphate, or peptide bonds).

In certain embodiments of the invention, substantially all of the nucleosides or internucleosidic linkages of an oligonucleotide of the invention are alternative nucleosides. In other embodiments of the invention, all of the nucleosides or internucleosidic linkages of an oligonucleotide of the invention are alternative nucleosides. Oligonucleotides of the invention in which "substantially all of the nucleosides are alternative nucleosides" are largely but not wholly modified and can include not more than five, four, three, two, or one naturally occurring nucleosides. In still other embodiments of the invention, oligonucleotides of the invention can include not more than five, four, three, two, or one alternative nucleosides.

The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S. L. et al. (Eds.), John Wiley & Sons, Inc., New York, N.Y., USA, which is hereby incorporated herein by reference.

Alternative nucleotides and nucleosides include those with modifications including, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position)

or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. The nucleobase may also be an isonucleoside in which the nucleobase is moved from the C1 position of the sugar moiety to a different position (*e.g.* C2, C3, C4, or C5). Specific examples of oligonucleotide compounds useful in the embodiments described herein include, but are not limited to, alternative nucleosides containing modified backbones or no natural internucleoside linkages. Nucleotides and nucleosides having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, alternative RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, an oligonucleotide will have a phosphorus atom in its internucleoside backbone.

Alternative Internucleoside Linkages

Alternative internucleoside linkages, also referred to as modified internucleoside linkages, include, for example, phosphorothioates, chiral phosphorothioates, 2'-alkoxy internucleoside linkages, alkyl phosphate internucleoside linkages, methylphosphonates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, morpholinos, PNAs, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, alkylphosphonates, methylphosphonates, dimethylphosphonates, thionoalkylphosphonates, thionoalkylphosphotriesters, phosphorodiamidates, thiophosphoramidates, thiophosphates, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts, and free acid forms are also included.

In various embodiments, oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiester, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat. RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Alternative internucleoside linkages that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH₂ component parts.

In some embodiments, the oligonucleotide may be defined by its pattern of backbone chiral centers. For example, a phosphorothioate internucleoside linkage may be an R or S enantiomer. Each internucleoside linkage may thus be defined as R_p or S_p such that the entire stereochemistry of the backbone is chirally defined, *e.g.*, as described in the International Publication No. WO 2015/107425, which is hereby incorporated by reference in its entirety. In some embodiments, only specific internucleoside linkages of the oligonucleotide (*e.g.*, a plurality of the oligonucleotides) contain a chiral center. In other embodiments, the oligonucleotide (*e.g.*, a plurality of the oligonucleotides) includes a mix of stereorandom and stereospecific chiral centers.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

In other embodiments, suitable oligonucleotides include those in which both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, a mimetic that has been shown to have excellent

5 hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar of a nucleoside is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby
10 incorporated herein by reference. Additional PNA compounds suitable for use in the oligonucleotides of the invention are described in, for example, in Nielsen *et al.*, Science, 1991, 254, 1497-1500.

Some embodiments featured in the invention include oligonucleotides with
15 phosphorothioate backbones and oligonucleotides with heteroatom backbones, and in particular -CH₂-NH-CH₂-, -CH₂-N(CH₃)-O-CH₂-[known as a methylene (methylimino) or MMI backbone], -CH₂-O-N(CH₃)-CH₂-, -CH₂-N(CH₃)-N(CH₃)-CH₂- and -N(CH₃)-CH₂-CH₂-[wherein the native phosphodiester backbone is represented as -O-P-O-CH₂-] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced
20 U.S. Pat. No. 5,602,240. In some embodiments, the oligonucleotides featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506. In other embodiments, the oligonucleotides described herein include phosphorodiamidate morpholino oligomers (PMO), in which the deoxyribose moiety is replaced by a morpholine ring, and the charged phosphodiester inter-subunit linkage is replaced by an uncharged phosphorodiamidate
25 linkage, as described in Summerton, et al., Antisense Nucleic Acid Drug Dev. 1997, 7:63-70.

Alternative Sugar Moieties

Alternative nucleosides and nucleotides can also contain one or more substituted and/or modified sugar moieties. In some embodiments, oligonucleotides comprise modified sugar moieties, such as any one of a 2'-O-methyl (2'OMe) moiety, a 2'-O-methoxyethyl
30 moiety, a bicyclic sugar moiety, PNA (*e.g.*, an oligonucleotide comprising one or more *N*-(2-aminoethyl)-glycine units linked by amide bonds or carbonyl methylene linkage as repeating units in place of a sugar-phosphate backbone), locked nucleoside (LNA) (*e.g.*, an oligonucleotide comprising one or more locked ribose, and can be a mixture of 2'-deoxy

nucleotides or 2'OMe nucleotides), c-ET (*e.g.*, an oligonucleotide comprising one or more cET sugar), cMOE (*e.g.*, an oligonucleotide comprising one or more cMOE sugar), morpholino oligomer (*e.g.*, an oligonucleotide comprising a backbone comprising one or more phosphorodiamidate morpholino oligomers), 2'-deoxy-2'-fluoro nucleoside (*e.g.*, an oligonucleotide comprising one or more 2'-fluoro- β -D-arabinonucleoside), tcDNA (*e.g.*, an oligonucleotide comprising one or more tcDNA modified sugar), constrained ethyl 2'-4'-bridged nucleic acid (cEt), S-cEt, ethylene bridged nucleic acid (ENA) (*e.g.*, an oligonucleotide comprising one or more ENA modified sugar), hexitol nucleic acids (HNA) (*e.g.*, an oligonucleotide comprising one or more HNA modified sugar), or tricyclic analog (tcDNA) (*e.g.*, an oligonucleotide comprising one or more tcDNA modified sugar).

The oligonucleotides, *e.g.*, oligonucleotides, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary suitable modifications include -O[(CH₂)_nO]_mCH₃, -O(CH₂)_nOCH₃, -O(CH₂)_nNH₂, -O(CH₂)_nCH₃, -O(CH₂)_nONH₂, and -O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, oligonucleotides include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78:486-504) i.e., an alkoxy-alkoxy group. MOE nucleosides confer several beneficial properties to oligonucleotides including, but not limited to, increased nuclease resistance, improved pharmacokinetic properties, reduced non-specific protein binding, reduced toxicity, reduced immunostimulatory properties, and enhanced target affinity as compared to unmodified oligonucleotides.

Another exemplary alternative contains 2'-dimethylaminoethoxy, i.e., a -O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-(CH₂)₂-O-(CH₂)₂-N(CH₃)₂. Further exemplary alternatives

include: 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other alternatives include 2'-methoxy (2'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the nucleosides and nucleotides of an oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

In some embodiments, the sugar moiety in the nucleotide may be a ribose molecule, optionally having a 2'-O-methyl, 2'-O-MOE, 2'-F, 2'-amino, 2'-O-propyl, 2'-aminopropyl, or 2'-OH modification.

An oligonucleotide of the invention can include one or more bicyclic sugar moieties. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. In some embodiments, the bicyclic sugar comprises a 4'-CH(R)—O-2' bridge wherein R is, independently, H, C₁-C₁₂ alkyl, or a protecting group. In some embodiments, R is methyl. In some embodiments, R is H.

In some embodiments an agent of the invention may include one or more locked nucleosides. A locked nucleoside is a nucleoside having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, a locked nucleoside is a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleosides to oligonucleotides has been shown to increase oligonucleotide stability in serum, and to reduce off-target effects (Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising

a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof; see *e.g.*, U.S. Pat. No. 8,278,283); 4'-CH₂-N(OCH₃)-2' (and analogs thereof; see *e.g.*, U.S. Pat. No. 8,278,425); 4'-CH₂-O-N(CH₃)₂-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4'-CH₂-C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof; see, *e.g.*, U.S. Pat. No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (*see* International Publication No. WO 99/14226, contents of which are incorporated by reference herein).

An oligonucleotide of the invention can also be modified to include one or more constrained ethyl nucleosides. As used herein, a "constrained ethyl nucleoside" or "cEt" is a locked nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge. In one embodiment, a constrained ethyl nucleoside is in the *S* conformation referred to herein as "S-cEt."

An oligonucleotide of the invention may also include one or more "conformationally restricted nucleosides" ("CRN"). CRN are nucleoside analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and --C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

5 In some embodiments, an oligonucleotide of the invention comprises one or more monomers that are UNA (unlocked nucleoside) nucleosides. UNA is unlocked acyclic nucleoside, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (i.e., the covalent carbon-oxygen-carbon bond between the C1' and
10 C4' carbons). In another example, the C2'-C3' bond (i.e., the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see Nuc. Acids Symp. Series, 52, 133-134 (2008) and Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are
15 not limited to, U.S. Pat. No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

The ribose molecule may also be modified with a cyclopropane ring to produce a tricyclodeoxynucleic acid (tricyclo DNA). The ribose moiety may be substituted for another
20 sugar such as 1,5,-anhydrohexitol, threose to produce a threose nucleoside (TNA), or arabinose to produce an arabino nucleoside. The ribose molecule can also be replaced with non-sugars such as cyclohexene to produce cyclohexene nucleoside or glycol to produce glycol nucleosides.

Potentially stabilizing modifications to the ends of nucleoside molecules can include
25 N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

30 Other alternatives chemistries of an oligonucleotide of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.*, a 5'-terminal phosphate or phosphate mimic of an oligonucleotide. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

Alternative Nucleobases

An oligonucleotide of the invention can also include nucleobase (often referred to in the art simply as "base") alternatives (*e.g.*, modifications or substitutions). Unmodified or natural nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Alternative nucleobases, also referred to as modified nucleobases, include other synthetic and natural nucleobases such as 5-methylcytosine, pseudouridine, 5-methoxyuridine, 5-methylcytidine, 5-hydroxymethylcytidine, 5-formylcytidine, 5-carboxycytidine, pyrrolocytidine, dideoxycytidine, uridine, 5-methoxyuridine, 5-hydroxydeoxyuridine, dihydrouridine, 4-thiouridine, pseudouridine, 1-methyl-pseudouridine, deoxyuridine, 5-hydroxybutynl-2'-deoxyuridine, xanthine, hypoxanthine, 7-deaza-xanthine, thienoguanine, 8-aza-7-deazaguanosine, 7-methylguanosine, 7-deazaguanosine, 6-aminomethyl-7-deazaguanosine, 8-aminoguanine, 2,2,7-trimethylguanosine, 8-methyladenine, 8-azidoadenine, 7-methyladenine, 7-deazaadenine, 3-deazaadenine, 2,6-diaminopurine, 2-aminopurine, 7-deaza-8-aza-adenine, 8-amino-adenine, thymine, dideoxythymine, 5-nitroindole, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouridine, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uridine and cytidine, 6-azo uridine, cytidine and thymine, 4-thiouridine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uridines and cytidines, 8-azaguanine and 8-azaadenine, and 3-deazaguanine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., (1991) *Angewandte Chemie, International Edition*, 30:613, and those disclosed by Sanghvi, Y S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines, and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil, and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., *Antisense Research and*

Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications. Examples of 5-methylcytosine substitutions include 5-Methyl-2'-deoxycytosine (5-Methyl-dC) or 5-Methyl-2'-cytosine (5-Methyl-C).

5 Representative U.S. patents that teach the preparation of certain of the above noted alternative nucleobases as well as other alternative nucleobases include, but are not limited to, the above noted U.S. Pat. Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 10 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

Exemplary Oligonucleotides Embodiments

Exemplary oligonucleotides of the invention comprise nucleosides with alternative 15 sugar moieties and may also comprise DNA or RNA nucleosides. In some embodiments, the oligonucleotide comprises nucleosides comprising alternative sugar moieties and DNA nucleosides. Incorporation of alternative nucleosides into the oligonucleotide of the invention may enhance the affinity of the oligonucleotide for the target nucleic acid. In that case, the alternative nucleosides can be referred to as affinity enhancing alternative 20 nucleotides.

In some embodiments, the oligonucleotide comprises at least one alternative nucleoside, such as at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 alternative nucleosides. 25 In other embodiments, the oligonucleotides comprise from one to ten alternative nucleosides, from two to nine alternative nucleosides, from three to eight alternative nucleosides, from four to seven alternative nucleosides (*e.g.*, 6 or 7 alternative nucleosides). In an embodiment, the oligonucleotide of the invention may comprise alternatives, which are independently selected from these three types of alternative (alternative sugar moiety, alternative 30 nucleobase, and alternative internucleoside linkage), or a combination thereof. In some embodiments, the oligonucleotide comprises one or more nucleosides comprising alternative sugar moieties, *e.g.*, 2' sugar alternative nucleosides. In some embodiments, the oligonucleotide of the invention comprise the one or more 2' sugar alternative nucleoside

independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA, and BNA (*e.g.*, LNA) nucleosides. In some embodiments, the one or more alternative nucleoside is a BNA.

5 In some embodiments, at least one of the alternative nucleosides is a BNA (*e.g.*, an LNA), such as at least two, such as at least three, at least four, at least five, at least six, at least seven, or at least eight of the alternative nucleosides are BNAs. In a still further embodiment, all the alternative nucleosides are BNAs.

In a further embodiment the oligonucleotide comprises at least one alternative
10 internucleoside linkage. In some embodiments, the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate or boranophosphate internucleoside linkages. In some embodiments, all the internucleotide linkages in the contiguous sequence of the oligonucleotide are phosphorothioate linkages. In some embodiments the phosphorothioate linkages are stereochemically pure phosphorothioate linkages. In some
15 embodiments, the phosphorothioate linkages are Sp phosphorothioate linkages. In other embodiments, the phosphorothioate linkages are Rp phosphorothioate linkages.

In some embodiments, the oligonucleotide of the invention comprises at least one alternative nucleoside which is a 2'-MOE-RNA, such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 2'-MOE-RNA nucleoside units. In some embodiments, the 2'-MOE-RNA nucleoside units are
20 connected by phosphorothioate linkages. In some embodiments, at least one of said alternative nucleoside is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 2'-fluoro-DNA nucleoside units. In some embodiments, the oligonucleotide of the invention comprises at least one BNA unit and at least one 2' substituted modified nucleoside. In some embodiments of the invention, the oligonucleotide comprises both 2' sugar modified
25 nucleosides and DNA units. In some embodiments, the oligonucleotide of the invention or contiguous nucleotide region thereof is a gapmer oligonucleotide.

Additional Gapmer Oligonucleotide Embodiments

In some embodiments the oligonucleotide of the invention, or contiguous nucleotide region thereof, has a gapmer design or structure also referred herein merely as "gapmer." In
30 a gapmer structure the oligonucleotide comprises at least three distinct structural regions a 5'-wing, a gap and a 3'-wing, in '5->3' orientation. In this design, the 5' and 3' wing regions (also termed flanking regions) comprise at least one alternative nucleoside which is adjacent to a gap region, and may in some embodiments comprise a contiguous stretch of 2, 3, 4, 5, 6,

7, 8, 9, 10, 11, or 12 alternative nucleosides, or a contiguous stretch of alternative and DNA nucleosides (mixed wings comprising both alternative and DNA nucleosides). The length of the 5'- wing region may be at least two nucleosides in length (*e.g.*, at least at least 2, at least 3, at least 4, at least 5, or more nucleosides in length). The length of the 3'- wing region may be at least two nucleosides in length (*e.g.*, at least 2, at least 3, at least at least 4, at least 5, or more nucleosides in length). The 5' and 3' wing regions may be symmetrical or asymmetrical with respect to the number of nucleosides they comprise. In some embodiments, the gap region comprises about 10 nucleosides flanked by a 5' and a 3' wing region each comprising about 5 nucleosides, also referred to as a 5-10-5 gapmer.

Consequently, the nucleosides of the 5' wing region and the 3' wing region which are adjacent to the gap region are alternative nucleosides, such as 2' alternative nucleosides. The gap region comprises a contiguous stretch of nucleotides which are capable of recruiting RNase H, when the oligonucleotide is in duplex with the KCNT1 target nucleic acid. In some embodiments, the gap segment comprising one or more of linked deoxyribonucleosides, 2'-Fluoro Arabino Nucleic Acids (FANA), and Fluoro Cyclohexenyl nucleic acid (F-CeNA). In some embodiments, the gap region comprises a contiguous stretch of 5-16 DNA nucleosides. In some embodiments, the gap region comprises a contiguous stretch of 6-15, 7-14, 8-13, or 9-11 DNA nucleosides. In some embodiments, the gap region comprises a contiguous stretch of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 DNA nucleosides. In some embodiments, the gap region comprises a region of at least at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases having at least 80% (*e.g.*, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) complementarity to a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 transcript variant. In some embodiments, the gapmer comprises a region complementary to at least 17 contiguous nucleotides, 19-23 contiguous nucleotides, or 19 contiguous nucleotides of a KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 transcript variant. The gapmer is complementary to the KCNT1 target nucleic acid (KCNT1 transcript (*e.g.*, SEQ ID NO: 3526) or KCNT1 transcript variant), and may therefore be the contiguous nucleoside region of the oligonucleotide. In some embodiments, the gap region comprises a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases having

at least 80% (*e.g.*, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity to an equal length portion of any one of SEQ ID NOs: 1-3525.

5 The 5' and 3' wing regions, flanking the 5' and 3' ends of the gap region, may comprise one or more affinity enhancing alternative nucleosides. In some embodiments, the 5' and/or 3' wing comprises at least one 2'-O-methoxyethyl (MOE) nucleoside, for example at least two MOE nucleosides. In some embodiments, the 5' wing comprises at least one MOE nucleoside. In some embodiments both the 5' and 3' wing regions comprise a MOE
10 nucleoside. In some embodiments all the nucleosides in the wing regions are MOE nucleosides. In other embodiments, the wing regions may comprise both MOE nucleosides and other nucleosides (mixed wings), such as DNA nucleosides and/or non-MOE alternative nucleosides, such as bicyclic nucleosides (BNAs) (*e.g.*, LNA nucleosides or cET nucleosides), or other 2' substituted nucleosides. In this case the gap is defined as a
15 contiguous sequence of at least 5 RNase H recruiting nucleosides (such as 5-16 DNA nucleosides) flanked at the 5' and 3' end by an affinity enhancing alternative nucleoside, such as an MOE nucleoside.

 In other embodiments, the 5' and/or 3' wing comprises at least one BNA (*e.g.*, at least one LNA nucleoside or cET nucleoside), for example at least 2 bicyclic nucleosides. In some
20 embodiments, the 5' wing comprises at least one BNA. In some embodiments both the 5' and 3' wing regions comprise a BNA. In some embodiments all the nucleosides in the wing regions are BNAs. In other embodiments, the wing regions may comprise both BNAs and other nucleosides (mixed wings), such as DNA nucleosides and/or non-BNA alternative nucleosides, such as 2' substituted nucleosides. In this case the gap is defined as a contiguous
25 sequence of at least five RNase H recruiting nucleosides (such as 5-16 DNA nucleosides) flanked at the 5' and 3' end by an affinity enhancing alternative nucleoside, such as a BNA, such as an LNA, such as beta-D-oxy-LNA.

 The 5' flank or 5' wing attached to the 5' end of the gap region comprises, contains, or consists of at least one alternative sugar moiety (*e.g.*, at least three, at least four, at least five,
30 at least six, at least seven, or more alternative sugar moieties). In some embodiments the wing region comprises or consists of from 1 to 7 alternative nucleobases, such as from two to six alternative nucleobases, from two to five alternative nucleobases, from two to four alternative nucleobases, or from one to three alternative nucleobases (*e.g.*, one, two, three or four alternative nucleobases). In some embodiments, the wing region comprises or consists

of at least one alternative internucleoside linkage (*e.g.*, at least three, at least four, at least five, at least six, at least seven, or more alternative internucleoside linkages).

In some embodiments, the 3' flank or 3' wing attached to the 3' end of the gap region comprises, contains, or consists of at least one alternative sugar moiety (*e.g.*, at least three, at least four, at least five, at least six, at least seven, or more alternative sugar moieties). In some embodiments the wing region comprises or consists of from one to seven alternative nucleobases, such as from two to six alternative nucleobases, from two to five alternative nucleobases, from two to four alternative nucleobases, or from one to three alternative nucleobases (*e.g.*, two, three, or four alternative nucleobases). In some embodiments, the wing region comprises or consists of at least one alternative internucleoside linkage (*e.g.*, at least three, at least four, at least five, at least six, at least seven, or more alternative internucleoside linkages).

In an embodiment, one or more or all of the alternative sugar moieties in the wing regions are 2' alternative sugar moieties.

In a further embodiment, one or more of the 2' alternative sugar moieties in the wing regions are selected from 2'-O-alkyl-sugar moieties, 2'-O-methyl-sugar moieties, 2'-amino-sugar moieties, 2'-fluoro-sugar moieties, 2'-alkoxy-sugar moieties, MOE sugar moieties, LNA sugar moieties, arabino nucleic acid (ANA) sugar moieties, and 2'-fluoro-ANA sugar moieties.

In one embodiment of the invention all the alternative nucleosides in the wing regions are bicyclic nucleosides. In a further embodiment the bicyclic nucleosides in the wing regions are independently selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA, cET, and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof.

In some embodiments, the one or more alternative internucleoside linkages in the wing regions are phosphorothioate internucleoside linkages. In some embodiments, the phosphorothioate linkages are stereochemically pure phosphorothioate linkages. In some embodiments the phosphorothioate linkages are *Sp* phosphorothioate linkages. In other embodiments, the phosphorothioate linkages are *Rp* phosphorothioate linkages. In some embodiments the phosphorothioate linkages are mixed stereo-enriched (*e.g.*, *Sp-Rp-Sp* or *Rp-Sp-Rp*) phosphorothioate linkages. In some embodiments, the alternative internucleoside linkages are 2'-alkoxy internucleoside linkages. In other embodiments, the alternative internucleoside linkages are alkyl phosphate internucleoside linkages.

The gap region may comprise, contain, or consist of at least 5-16 consecutive DNA nucleosides capable of recruiting RNase H. In some embodiments, all of the nucleosides of the gap region are DNA units. In further embodiments the gap region may consist of a mixture of DNA and other nucleosides capable of mediating RNase H cleavage. In some
5 embodiments, at least 50% of the nucleosides of the gap region are DNA, such as at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% DNA.

The oligonucleotide of the invention comprises a contiguous region which is complementary to the target nucleic acid. In some embodiments, the oligonucleotide may
10 further comprise additional linked nucleosides positioned 5' and/or 3' to either the 5' and 3' wing regions. These additional linked nucleosides can be attached to the 5' end of the 5' wing region or the 3' end of the 3' wing region, respectively. The additional nucleosides may, in some embodiments, form part of the contiguous sequence, which is complementary to the target nucleic acid, or in other embodiments, may be non-complementary to the target
15 nucleic acid.

The inclusion of the additional nucleosides at either, or both of the 5' and 3' wing regions may independently comprise one, two, three, four, or five additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. In this respect the oligonucleotide of the invention, may in some embodiments comprise a
20 contiguous sequence capable of modulating the target which is flanked at the 5' and/or 3' end by additional nucleotides. Such additional nucleosides may serve as a nuclease susceptible biocleavable linker and may therefore be used to attach a functional group such as a conjugate moiety to the oligonucleotide of the invention. In some embodiments the additional 5' and/or 3' end nucleosides are linked with phosphodiester linkages and may be
25 DNA or RNA. In another embodiment, the additional 5' and/or 3' end nucleosides are alternative nucleosides which may for example be included to enhance nuclease stability or for ease of synthesis.

In other embodiments, the oligonucleotides of the invention utilize "altimer" design and comprise alternating 2'-fluoro-ANA and DNA regions that are alternated every three
30 nucleosides. Altimer oligonucleotides are discussed in more detail in Min, et al., Bioorganic & Medicinal Chemistry Letters, 2002, 12(18): 2651-2654 and Kalota, et al., Nuc. Acid Res. 2006, 34(2): 451-61 (herein incorporated by reference).

In other embodiments, the oligonucleotides of the invention utilize "hemimer" design and comprise a single 2'-modified wing segment adjacent to (on either side of the 5' or the 3'

side of) a gap region. Hemimer oligonucleotides are discussed in more detail in Geary et al., 2001, J. Pharm. Exp. Therap., 296: 898-904 (herein incorporated by reference).

In various embodiments, the oligonucleotide comprises a 5' wing region, a 3' wing region, and a gap region between the 5' and 3' wing regions. In some embodiments, the gap region comprises a contiguous stretch of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 DNA nucleosides. In some embodiments, the gap region comprises a contiguous stretch of 8, 10, or 12 DNA nucleosides. In various embodiments, the 5' and 3' wing regions comprise one or more affinity enhancing alternative nucleosides, such as one or more 2'-O-methoxyethyl (MOE) nucleosides. In some embodiments, the 5' wing region comprises one, two, three, four, five, or six 2'-O-MOE nucleosides. In particular embodiments, the 5' wing region comprises either two or five 2'-O-MOE nucleosides. In some embodiments, the 5' wing region comprises one, two, three, four, five, or six locked nucleosides (LNAs). In particular embodiments, the 5' wing region comprises two LNAs. In some embodiments, the 5' wing region comprises two 2'-O-MOE nucleosides and two LNAs.

In some embodiments, the 3' wing region comprises one, two, three, four, five, or six 2'-O-MOE nucleosides. In particular embodiments, the 3' wing region comprises either three or five MOE nucleosides. In some embodiments, the 3' wing region comprises one, two, three, four, five, or six locked nucleosides (LNAs). In particular embodiments, the 3' wing region comprises two LNAs. In some embodiments, the 3' wing region comprises three MOE nucleosides and two LNAs.

In various embodiments, one or more internucleoside linkages of the oligonucleotide are naturally occurring linkages (e.g., phosphodiester bonds). In some embodiments, all of the internucleoside linkages of the oligonucleotide are naturally occurring linkages (e.g., phosphodiester bonds). In various embodiments, one or more internucleoside linkages of the oligonucleotide are alternative linkages (e.g., phosphorothioate linkages). In some embodiments, at least one, two, three, four, five, six, seven, eight, nine, or ten internucleoside linkages are phosphorothioate linkages. In various embodiments, the oligonucleotide includes both phosphodiester bonds and phosphorothioate linkages. In some embodiments, the gap region of the oligonucleotide comprises phosphodiester bonds and the 5' wing region and 3' wing region each comprises one or more phosphorothioate linkages.

In various embodiments, the oligonucleotide includes one or more unmodified cytosines. In some embodiments, all of the cytosines in the oligonucleotide are unmodified. In various embodiments, the oligonucleotide includes one or more modified cytosines. An

example of a modified cytosine is a 5-Methyl-2'-deoxycytosine (5-Methyl-dC) or 5-Methyl-2'-cytosine (5-Methyl-C). In some embodiments, all cytosines of the oligonucleotide are 5-Methyl-2'-deoxycytosine. In some embodiments, all cytosines in the gap region of the oligonucleotide are 5-Methyl-2'-deoxycytosine and all cytosines in the 5' wing region and 3' wing region are 5-Methyl-C.

In one embodiment, the oligonucleotide has a chemically modified nucleobase sequence of eeeee-d10-eeee (where "e" denotes a 2'-O-MOE modified nucleoside and where "d10" denotes a contiguous 10 DNA nucleobase sequence). In this embodiment, the 5' wing region includes five 2'-O-MOE modified nucleosides, the gap region includes 10 contiguous DNA nucleobases, and the 3' wing region includes five 2'-O-MOE modified nucleosides. The internucleoside linkages connecting the nucleobases can be phosphodiester bonds. In one embodiment, the oligonucleotide includes unmodified cytidines. In another embodiment, the oligonucleotide includes modified cytidines (*e.g.*, 5-Methyl-dC and/or 5-Methyl-C).

In one embodiment, the oligonucleotide has a chemically modified nucleobase sequence of eeeee-d12-eeee. In this embodiment, the 5' wing region includes five 2'-O-MOE modified nucleosides, the gap region includes 12 contiguous DNA nucleobases, and the 3' wing region includes five 2'-O-MOE modified nucleosides. The internucleoside linkages connecting the nucleobases can be phosphodiester bonds. The oligonucleotide includes unmodified cytidines.

In one embodiment, the oligonucleotide has a chemically modified nucleobase sequence of eeeee-d8-eeee. In this embodiment, the 5' wing region includes five 2'-O-MOE modified nucleosides, the gap region includes 8 contiguous DNA nucleobases, and the 3' wing region includes five 2'-O-MOE modified nucleosides. The internucleoside linkages connecting the nucleobases can be as follows: sooooooooooooo (where "s" refers to a phosphorothiorate bond and "o" refers to a phosphodiester bond). The oligonucleotide includes unmodified cytidines.

In one embodiment, the oligonucleotide has a chemically modified nucleobase sequence of eekk-d8-kkee (where "e" denotes a 2'-O-MOE modified nucleoside, "d8" denotes a contiguous 8 DNA nucleobase sequence, and "k" denotes a locked nucleic acid (LNA), constrained methoxyethyl (cMOE) nucleoside, constrained ethyl (cET) nucleoside, or peptide nucleic acid (PNA). In this embodiment, the 5' wing region includes two 2'-O-MOE modified nucleosides and two LNAs, the gap region includes 8 contiguous DNA nucleobases, and the 3' wing region includes two LNAs and three 2'-O-MOE modified nucleosides. The

internucleoside linkages connecting the nucleobases can be as follows: soossssssssooss (where “s” refers to a phosphorothiorate bond and “o” refers to a phosphodiester bond). The oligonucleotide includes unmodified cytidines.

Oligonucleotides Conjugated to Ligands

5 Oligonucleotides of the invention may be chemically linked to one or more ligands, moieties, or conjugates that enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., (1989) Proc. Natl. Acad. Sci. USA, 86: 6553-6556), cholic acid (Manoharan et al., (1994) Biorg. Med. Chem. Lett., 4:1053-1060), a thioether, *e.g.*,
 10 beryl-S-tritylthiol (Manoharan et al., (1992) Ann. N.Y. Acad. Sci., 660:306-309; Manoharan et al., (1993) Biorg. Med. Chem. Lett., 3:2765-2770), a thiocholesterol (Oberhauser et al., (1992) Nucl. Acids Res., 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras et al., (1991) EMBO J, 10:1111-1118; Kabanov et al., (1990) FEBS Lett., 259:327-330; Svinarchuk et al., (1993) Biochimie, 75:49-54), a phospholipid,
 15 *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan et al., (1995) Tetrahedron Lett., 36:3651-3654; Shea et al., (1990) Nucl. Acids Res., 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., (1995) Nucleosides & Nucleotides, 14:969-973), or adamantane acetic acid (Manoharan et al., (1995) Tetrahedron Lett., 36:3651-3654), a palmityl moiety (Mishra et al., (1995)
 20 Biochim. Biophys. Acta, 1264:229-237), or an octadecylamine or hexylamino-carboxycholesterol moiety (Crooke et al., (1996) J. Pharmacol. Exp. Ther., 277:923-937).

In one embodiment, a ligand alters the distribution, targeting, or lifetime of an oligonucleotide agent into which it is incorporated. In some embodiments, a ligand provides
 25 an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ, or region of the body, as, *e.g.*, compared to a species absent such a ligand.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a
 30 dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly

L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralen, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu³⁺ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins,

cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the oligonucleotide agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an oligonucleotide as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins etc. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin etc. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides of the present invention, such as the ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer

utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

5 When using conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-
10 nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

Lipid Conjugates

15 In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule may bind a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

20 In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. Exemplary vitamins include vitamin A, E, and K.

Cell Permeation Agents

25 In another aspect, the ligand is a cell-permeation agent, for example a helical cell-permeation agent. In some embodiments, the cell permeation agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. In some embodiments, the helical agent is an alpha-helical agent, which may have a lipophilic and a lipophobic phase.

30 The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to oligonucleotide agents can affect pharmacokinetic distribution of the

oligonucleotide, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 3535). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 3536) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ) (SEQ ID NO: 3537) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK) (SEQ ID NO: 3538) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam et al., Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to an oligonucleotide agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Some conjugates of this ligand target PECAM-1 or VEGF.

A cell permeation peptide is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α -helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β -defensin, or bactenecin), or a

peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni et al., Nucl. Acids Res. 31:2717-2724, 2003).

Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated oligonucleotides are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate per se made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a cell permeation peptide.

Additional carbohydrate conjugates (and linkers) suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an oligonucleotide with various linkers that can be cleavable or non-cleavable.

Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR⁸, C(O), C(O)NH, SO, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R⁸), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R⁸ is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential, or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selective for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable

linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

5 A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the
10 cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore
15 the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by
20 testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be
25 indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50,
30 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

Redox Cleavable Linking Groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group ($--S--S--$). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular oligonucleotide moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents known in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one embodiment, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

Phosphate-Based Cleavable Linking Groups

In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are $-O-P(O)(OR^k)-O-$,

$-O-P(S)(OR^k)-O-$, $-O-P(S)(SR^k)-O-$, $-S-P(O)(OR^k)-O-$, $-O-P(O)(OR^k)-S-$, $-S-P(O)(OR^k)-S-$, $-O-P(S)(OR^k)-S-$, $-S-P(S)(OR^k)-O-$, $-O-P(O)(R^k)-O-$, $-O-P(S)(R^k)-O-$, $-S-P(O)(R^k)-O-$, $-S-P(S)(R^k)-O-$, $-S-P(O)(R^k)-S-$, $-O-P(S)(R^k)-S-$. These candidates can be evaluated using methods analogous to those described above.

Acid Cleavable Linking Groups

In another embodiment, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula – C=NN–, C(O)O, or –OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

Ester-Based Linking Groups

In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula –C(O)O–, or –OC(O)–. These candidates can be evaluated using methods analogous to those described above.

Peptide-Based Cleaving Groups

In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (–C(O)NH–). The amide group can be formed between any alkylene, alkenylene, or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include

the entire amide functional group. Peptide-based cleavable linking groups have the general formula

-NHCHR^AC(O)NHCHR^BC(O)--, where R^A and R^B are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

In one embodiment, an oligonucleotide of the invention is conjugated to a carbohydrate through a linker. Linkers include bivalent and trivalent branched linker groups. Linkers for oligonucleotide carbohydrate conjugates include, but are not limited to, those described in formulas 24-35 of PCT Publication No. WO 2018/195165.

Representative U.S. patents that teach the preparation of oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes oligonucleotide compounds that are chimeric compounds. Chimeric oligonucleotides typically contain at least one region wherein the RNA is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide can serve as a substrate for enzymes capable of cleaving RNA:DNA. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxy oligonucleotides hybridizing to the same target region. Cleavage of the RNA target

can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the nucleotides of an oligonucleotide can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. et al., Biochem. Biophys. Res. Comm, 2007, 365(1):54-61; Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al., FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, *e.g.*, dihexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such oligonucleotide conjugates have been listed above. Typical conjugation protocols involve the synthesis of an oligonucleotide bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide, in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate.

Pharmaceutical Uses

The oligonucleotide compositions described herein are useful in the methods of the invention and, while not bound by theory, are believed to exert their desirable effects through their ability to modulate the level, status, and/or activity of KCNT1, *e.g.*, by inhibiting the activity or level of the KCNT1 protein in a cell in a mammal.

An aspect of the present invention relates to methods of treating disorders (*e.g.*, epilepsy) related to KCNT1 in a subject in need thereof. Another aspect of the invention includes reducing the level of KCNT1 in a cell of a subject identified as having a KCNT1 related disorder. Still another aspect includes a method of inhibiting expression of KCNT1 in a cell in a subject. The methods may include contacting a cell with an oligonucleotide, in an amount effective to inhibit expression of KCNT1 in the cell, thereby inhibiting expression of KCNT1 in the cell.

Based on the above methods, further aspects of the present invention include an oligonucleotide of the invention, or a composition comprising such an oligonucleotide, for use in therapy, or for use as a medicament, or for use in treating KCNT1 related disorders in a subject in need thereof, or for use in reducing the level of KCNT1 in a cell of a subject identified as having a KCNT1 related disorder, or for use in inhibiting expression of KCNT1 in a cell in a subject. The uses include the contacting of a cell with the oligonucleotide, in an amount effective to inhibit expression of KCNT1 in the cell, thereby inhibiting expression of KCNT1 in the cell. Embodiments described below in relation to the methods of the invention are also applicable to these further aspects.

Contacting of a cell with an oligonucleotide may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the oligonucleotide includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the oligonucleotide. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc₃ ligand, or any other ligand that directs the oligonucleotide to a site of interest. Cells can include those of the central nervous system, or muscle cells.

Inhibiting expression of a KCNT1 gene includes any level of inhibition of a KCNT1 gene, *e.g.*, at least partial suppression of the expression of a KCNT1 gene, such as an inhibition by at least about 20%. In certain embodiments, inhibition is by at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

The expression of a KCNT1 gene may be assessed based on the level of any variable associated with KCNT1 gene expression, *e.g.*, KCNT1 mRNA level or KCNT1 protein level.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In certain embodiments, surrogate markers can be used to detect inhibition of KCNT1. For example, effective treatment of a KCNT1 related disorder, as demonstrated by acceptable diagnostic and monitoring criteria with an agent to reduce KCNT1 expression can be understood to demonstrate a clinically relevant reduction in KCNT1.

In some embodiments of the methods of the invention, expression of a KCNT1 gene is inhibited by at least 20%, a 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In certain embodiments, the methods include a clinically relevant inhibition of expression of KCNT1, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of KCNT1.

Inhibition of the expression of a KCNT1 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a KCNT1 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an oligonucleotide of the invention, or by administering an oligonucleotide of the invention to a subject in which the cells are or were present) such that the expression of a KCNT1 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with an oligonucleotide or not treated with an oligonucleotide targeted to the gene of interest). The degree of inhibition may be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \times 100\%$$

In other embodiments, inhibition of the expression of a KCNT1 gene may be assessed in terms of a reduction of a parameter that is functionally linked to KCNT1 gene expression, *e.g.*, KCNT1 protein expression or KCNT1 activity. KCNT1 gene silencing may be

determined in any cell expressing KCNT1, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a KCNT1 protein may be manifested by a reduction in the level of the KCNT1 protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of a KCNT1 gene includes a cell or group of cells that has not yet been contacted with an oligonucleotide of the invention. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an oligonucleotide.

The level of KCNT1 mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of KCNT1 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the KCNT1 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNEASY™ RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, in situ hybridization, and microarray analysis.

Circulating KCNT1 mRNA may be detected using methods the described in PCT Publication WO 2012/177906, the entire contents of which are hereby incorporated herein by reference.

In some embodiments, the level of expression of KCNT1 is determined using a nucleic acid probe. The term "probe," as used herein, refers to any molecule that is capable of selectively binding to a specific KCNT1 sequence, *e.g.* to an mRNA or polypeptide. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses, and probe arrays. One method for the determination of mRNA levels involves contacting the

isolated mRNA with a nucleic acid molecule (probe) that can hybridize to KCNT1 mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an AFFYMETRIX gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of KCNT1 mRNA.

An alternative method for determining the level of expression of KCNT1 in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88:189-193), self-sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of KCNT1 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TAQMANTM System) or the DUAL-GLO® Luciferase assay.

The expression levels of KCNT1 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722; 5,874,219; 5,744,305; 5,677,195; and 5,445,934, which are incorporated herein by reference. The determination of KCNT1 expression level may also comprise using nucleic acid probes in solution.

In some embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of this PCR method is described and exemplified in the Examples presented herein. Such methods can also be used for the detection of KCNT1 nucleic acids.

The level of KCNT1 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC),

thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs),

5 immunofluorescent assays, electrochemiluminescence assays, and the like. Such assays can also be used for the detection of proteins indicative of the presence or replication of KCNT1 proteins.

In some embodiments of the methods of the invention, the oligonucleotide is administered to a subject such that the oligonucleotide is delivered to a specific site within
10 the subject. The inhibition of expression of KCNT1 may be assessed using measurements of the level or change in the level of KCNT1 mRNA or KCNT1 protein in a sample derived from a specific site within the subject. In certain embodiments, the methods include a clinically relevant inhibition of expression of KCNT1, *e.g.*, as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of
15 KCNT1.

In other embodiments, the oligonucleotide is administered in an amount and for a time effective to result in reduction (*e.g.*, by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) of one or more symptoms of a KCNT1 disorder. Such symptoms include, but are not limited to, prolonged seizures, frequent seizures, behavioral and developmental delays,
20 movement and balance issues, orthopedic conditions, delayed language and speech issues, growth and nutrition issues, sleeping difficulties, chronic infection, sensory integration disorder, disruption of the autonomic nervous system, and sweating.

Treating KCNT1 related disorders can result in an increase in average survival time of an individual or a population of subjects treated according to the present invention in
25 comparison to a population of untreated subjects. For example, the survival time is of an individual or average survival time of a population is increased by more than 30 days (more than 60 days, 90 days, or 120 days). An increase in survival time of an individual or in average survival time of a population may be measured by any reproducible means. An increase in survival time of an individual may be measured, for example, by calculating for
30 an individual the length of survival time following the initiation of treatment with the compound described herein. An increase in average survival time of a population may be measured, for example, by calculating for the average length of survival time following initiation of treatment with the compound described herein. An increase in survival time of an individual may also be measured, for example, by calculating for an individual length of

survival time following completion of a first round of treatment with a compound or pharmaceutically acceptable salt of a compound described herein. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival time following completion of a first round of treatment with a compound or pharmaceutically acceptable salt of a compound described herein.

Treating KCNT1 related disorders can also result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. For example, the mortality rate is decreased by more than 2% (*e.g.*, more than 5%, 10%, or 25%). A decrease in the mortality rate of a population of treated subjects may be measured by any reproducible means, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with a compound or pharmaceutically acceptable salt of a compound described herein. A decrease in the mortality rate of a population may also be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with a compound or pharmaceutically acceptable salt of a compound described herein.

Delivery of Oligonucleotides

The delivery of an oligonucleotide of the invention to a cell *e.g.*, a cell within a subject, such as a human subject *e.g.*, a subject in need thereof, such as a subject having a KCNT1 related disorder can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an oligonucleotide of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an oligonucleotide to a subject. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an oligonucleotide of the invention (see *e.g.*, Akhtar S. and Julian R L., (1992) Trends Cell. Biol. 2(5):139-144 and WO 94/02595, which are incorporated herein by reference in their entireties). *For in vivo* delivery, factors to consider in order to deliver an oligonucleotide molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an oligonucleotide can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically

administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the oligonucleotide molecule to be administered.

5 For administering an oligonucleotide systemically for the treatment of a disease, the oligonucleotide can include alternative nucleobases, alternative sugar moieties, and/or alternative internucleoside linkages, or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the oligonucleotide by endo- and exo-nucleases *in vivo*. Modification of the oligonucleotide or the pharmaceutical carrier can also
 10 permit targeting of the oligonucleotide composition to the target tissue and avoid undesirable off-target effects. Oligonucleotide molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. In an alternative embodiment, the oligonucleotide can be delivered using drug delivery systems such as a nanoparticle, a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, a
 15 dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an oligonucleotide molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an oligonucleotide by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an oligonucleotide, or induced to form a vesicle or micelle that encases an
 20 oligonucleotide. The formation of vesicles or micelles further prevents degradation of the oligonucleotide when administered systemically. In general, any methods of delivery of nucleic acids known in the art may be adaptable to the delivery of the oligonucleotides of the invention. Methods for making and administering cationic oligonucleotide complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, D R., et al. (2003) J. Mol.
 25 Biol 327:761-766; Verma, U N. et al., (2003) Clin. Cancer Res. 9:1291-1300; Arnold, A S et al., (2007) J. Hypertens. 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of oligonucleotides include DOTAP (Sorensen, D R., et al (2003), *supra*; Verma, U N. et al., (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, T S. et al.,
 30 (2006) Nature 441:111-114), cardiolipin (Chien, P Y. et al., (2005) Cancer Gene Ther. 12:321-328; Pal, A. et al., (2005) Int J. Oncol. 26:1087-1091), polyethyleneimine (Bonnet M E. et al., (2008) Pharm. Res. Aug 16 Epub ahead of print; Aigner, A. (2006) J. Biomed. Biotechnol. 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) Mol. Pharm. 3:472-487), and polyamidoamines (Tomalia, D A. et al., (2007) Biochem. Soc. Trans. 35:61-67; Yoo, H.

et al., (1999) Pharm. Res. 16:1799-1804). In some embodiments, an oligonucleotide forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of oligonucleotides and cyclodextrins can be found in U.S. Pat. No. 7,427,605, which is herein incorporated by reference in its entirety. In some
5 embodiments the oligonucleotides of the invention are delivered by polyplex or lipoplex nanoparticles. Methods for administration and pharmaceutical compositions of oligonucleotides and polyplex nanoparticles and lipoplex nanoparticles can be found in U.S. Patent

Application Nos. 2017/0121454; 2016/0369269; 2016/0279256; 2016/0251478;
10 2016/0230189; 2015/0335764; 2015/0307554; 2015/0174549; 2014/0342003; 2014/0135376; and 2013/0317086, which are herein incorporated by reference in their entirety.

In some embodiments, the compounds described herein may be administered in combination with additional therapeutics. Examples of additional therapeutics include standard of care anti-epilepsy medications such as quinidine and/or sodium channel blockers.
15 Additionally, the compounds described herein may be administered in combination with recommended lifestyle changes such as a ketogenic diet.

Membranous Molecular Assembly Delivery Methods

Oligonucleotides of the invention can also be delivered using a variety of membranous molecular assembly delivery methods including polymeric, biodegradable
20 microparticle, or microcapsule delivery devices known in the art. For example, a colloidal dispersion system may be used for targeted delivery of an oligonucleotide agent described herein. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vesicles that are useful as
25 delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the
30 liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the oligonucleotide are delivered into the cell where the oligonucleotide can specifically bind to a target RNA and can mediate RNase H-mediated gene silencing. In some cases, the liposomes are also

specifically targeted, *e.g.*, to direct the oligonucleotide to particular cell types. The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

A liposome containing an oligonucleotide can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The oligonucleotide preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the oligonucleotide and condense around the oligonucleotide to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of oligonucleotide.

If necessary, a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). The pH can also be adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as a structural component of the delivery vehicle, are further described in, *e.g.*, WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Feigner, P. L. et al., (1987) Proc. Natl. Acad. Sci. USA 8:7413-7417; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; Bangham et al., (1965) M. Mol. Biol. 23:238; Olson et al., (1979) Biochim. Biophys. Acta 557:9; Szoka et al., (1978) Proc. Natl. Acad. Sci. 75: 4194; Mayhew et al., (1984) Biochim. Biophys. Acta 775:169; Kim et al., (1983) Biochim. Biophys. Acta 728:339; and Fukunaga et al., (1984) Endocrinol. 115:757. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.*, Mayer et al., (1986) Biochim. Biophys. Acta 858:161. Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew et al., (1984) Biochim. Biophys. Acta 775:169). These methods are readily adapted to packaging oligonucleotide preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al. (1987) Biochem. Biophys. Res. Commun., 147:980-985).

Liposomes, which are pH-sensitive or negatively charged, entrap nucleic acids rather than complex with them. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al. (1992) Journal of Controlled Release, 19:269-274).

One major type of liposomal composition includes phospholipids other than naturally derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Feigner, (1994) J. Biol. Chem. 269:2550; Nabel, (1993) Proc. Natl. Acad. Sci. 90:11307; Nabel, (1992) Human Gene Ther. 3:649; Gershon, (1993) Biochem. 32:7143; and Strauss, (1992) EMBO J. 11:417.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising NOVASOME™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and NOVASOME™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu et al., (1994) S.T.P.Pharma. Sci., 4(6):466).

Liposomes may also be sterically stabilized liposomes, comprising one or more specialized lipids that result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., (1987) FEBS Letters, 223:42; Wu et al., (1993) Cancer Research, 53:3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (Ann. N.Y. Acad. Sci., (1987), 507:64) reported the ability of monosialoganglioside G^{M1}, galactocerebroside sulfate, and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (Proc. Natl. Acad. Sci. U.S.A., (1988), 85:6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al).

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages *in vivo* and can be used to deliver oligonucleotides to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated oligonucleotides in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of

fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of oligonucleotide (see, *e.g.*, Feigner, P. L. et al., (1987) Proc. Natl. Acad. Sci. USA 8:7413-7417, and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

5 A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. LIPOFECTINTM (Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged
10 polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-
15 (trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Ind.) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine
20 dioctaoyleamide ("DOGS") (TRANSFECTAMTM, Promega, Madison, Wis.) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPE") (see, *e.g.*, U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See,
25 Gao, X. and Huang, L., (1991) Biochim. Biophys. Res. Commun. 179:280). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., (1991) Biochim. Biophys. Acta 1065:8). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing
30 compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, Calif.) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Md.). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer oligonucleotide into the skin. In some implementations, liposomes are used for delivering oligonucleotide to epidermal cells and also to enhance the penetration of oligonucleotide into dermal tissues, *e.g.*, into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner et al., (1992) *Journal of Drug Targeting*, vol. 2,405-410 and du Plessis et al., (1992) *Antiviral Research*, 18:259-265; Mannino, R. J. and Fould-Fogerite, S., (1998) *Biotechniques* 6:682-690; Itani, T. et al., (1987) *Gene* 56:267-276; Nicolau, C. et al. (1987) *Meth. Enzymol.* 149:157-176; Straubinger, R. M. and Papahadjopoulos, D. (1983) *Meth. Enzymol.* 101:512-527; Wang, C. Y. and Huang, L., (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising NOVASOME I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and NOVASOME II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with oligonucleotides are useful for treating a dermatological disorder.

The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand. Additional methods are known in the art and are described, for example in U.S. Patent Application Publication No. 20060058255, the linking groups of which are herein incorporated by reference.

Liposomes that include oligonucleotides can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the

droplet. Transfersomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include oligonucleotides can be delivered, for example, subcutaneously by infection in order to deliver oligonucleotides to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transfersomes can be self-optimizing (adaptive to the shape of pores, *e.g.*, in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Other formulations amenable to the present invention are described in PCT Publication Nos. WO 2009/088891, WO 2009/132131, and WO 2008/042973, which are hereby incorporated by reference in their entirety.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general, their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl

isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines, and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The oligonucleotides for use in the methods of the invention can also be provided as micellar formulations. Micelles are a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

Lipid Nanoparticle-Based Delivery Methods

Oligonucleotides of in the invention may be fully encapsulated in a lipid formulation, *e.g.*, a lipid nanoparticle (LNP), or other nucleic acid-lipid particle. LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Pat. Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to oligonucleotide ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

Non-limiting examples of cationic lipids include N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N--(I-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N--(I-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleoyloxy)propylamine (DODMA), 1,2-DiLinoleoyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleoyloxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-Dilinoleoyloxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleoyloxy-3-dimethylaminopropane (DLin-2-DMA), 1,2-Dilinoleoyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleoyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyetetrahydro-- 3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yeethylazanediyl)dodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise, for example, from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoylloleoylphosphatidylcholine (POPC), palmitoylloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-

carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be, for example, from about 5 mol % to about 90 mol %, about 10 mol %, or about 60 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkylxyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C₁₂), a PEG-dimyristyloxypropyl (C₁₄), a PEG-dipalmitoyloxypropyl (C₁₆), or a PEG-distearoyloxypropyl (C₁₈). The conjugated lipid that prevents aggregation of particles can be, for example, from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 50 mol % of the total lipid present in the particle.

Combination Therapies

A method of the invention can be used alone or in combination with an additional therapeutic agent, *e.g.*, other agents that treat KCNT1 related disorders or symptoms associated therewith, or in combination with other types of therapies to treat KCNT1 related disorders. In combination treatments, the dosages of one or more of the therapeutic compounds may be reduced from standard dosages when administered alone. For example, doses may be determined empirically from drug combinations and permutations or may be deduced by isobolographic analysis (*e.g.*, Black et al., *Neurology* 65:S3-S6 (2005)). In this case, dosages of the compounds when combined should provide a therapeutic effect.

In some embodiments, the oligonucleotide agents described herein may be used in combination with an additional therapeutic agent to treat a KCNT1 related disorder. In some embodiments, the additional therapeutic agent may be an oligonucleotide (*e.g.*, an ASO) that hybridizes with the mRNA of gene associated with a KCNT1 related disorder.

In some embodiments, the second therapeutic agent is a chemotherapeutic agent (*e.g.*, a cytotoxic agent or other chemical compound useful in the treatment of a KCNT1 related disorder).

The second agent may be a therapeutic agent which is a non-drug treatment. For example, the second therapeutic agent is physical therapy.

In any of the combination embodiments described herein, the first and second therapeutic agents may be administered simultaneously or sequentially, in either order. The first therapeutic agent may be administered immediately, up to 1 hour, up to 2 hours, up to 3 hours, up to 4 hours, up to 5 hours, up to 6 hours, up to 7 hours, up to 8 hours, up to 9 hours, up to 10 hours, up to 11 hours, up to 12 hours, up to 13 hours, 14 hours, up to hours 16, up to 17 hours, up to 18 hours, up to 19 hours up to 20 hours, up to 21 hours, up to 22 hours, up to 23 hours up to 24 hours or up to 1-7, 1-14, 1-21 or 1-30 days before or after the second therapeutic agent.

Pharmaceutical Compositions

In some embodiments, the oligonucleotides described herein are formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration *in vivo*.

The compounds described herein may be used in the form of the free base, in the form of salts, solvates, and as prodrugs. All forms are within the methods described herein. In accordance with the methods of the invention, the described compounds or salts, solvates, or prodrugs thereof may be administered to a subject in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compounds described herein may be administered, for example, by oral, parenteral, intrathecal, intracerebroventricular, intraparenchymal, buccal, sublingual, nasal, rectal, patch, pump, or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, intracerebroventricular, intraparenchymal, rectal, and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

A compound described herein may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, a compound described herein may be incorporated with an excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, and wafers. A compound described herein may also be administered parenterally. Solutions of a compound described herein can be

prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO, and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of

5 microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2012, 22nd ed.) and in The United States Pharmacopeia: The National Formulary (USP 41 NF 36), published in 2018. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous
10 preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that may be easily administered via syringe. Compositions for nasal administration may conveniently be formulated as aerosols, drops, gels, and powders. Aerosol formulations typically include a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in
15 single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container may be a unitary dispensing device, such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form includes an aerosol dispenser, it will contain a propellant, which can be a compressed gas,
20 such as compressed air or an organic propellant, such as fluorochlorohydrocarbon. The aerosol dosage forms can also take the form of a pump-atomizer. Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, where the active ingredient is formulated with a carrier, such as sugar, acacia, tragacanth, gelatin, and glycerine. Compositions for rectal administration are conveniently in the form of
25 suppositories containing a conventional suppository base, such as cocoa butter

The compounds described herein may be administered to an animal, *e.g.*, a human, alone or in combination with pharmaceutically acceptable carriers, as noted herein, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration, and standard pharmaceutical practice.

30 *Dosages*

The dosage of the compositions (*e.g.*, a composition including an oligonucleotide) described herein, can vary depending on many factors, such as the pharmacodynamic properties of the compound; the mode of administration; the age, health,

and weight of the recipient; the nature and extent of the symptoms; the frequency of the treatment, and the type of concurrent treatment, if any; and the clearance rate of the compound in the animal to be treated. The compositions described herein may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. In some embodiments, the dosage of a composition (*e.g.*, a composition including an oligonucleotide) is a prophylactically or a therapeutically effective amount.

Kits

The invention also features kits including (a) a pharmaceutical composition including an oligonucleotide agent that reduces the level and/or activity of KCNT1 in a cell or subject described herein, and (b) a package insert with instructions to perform any of the methods described herein. In some embodiments, the kit includes (a) a pharmaceutical composition including an oligonucleotide agent that reduces the level and/or activity of KCNT1 in a cell or subject described herein, (b) an additional therapeutic agent, and (c) a package insert with instructions to perform any of the methods described herein.

Methods of selecting ASOs

Oligonucleotides suitable for use in ASO treatment may be selected using bioinformatic methods. Oligonucleotides may be from 18-22 nucleotides in length. The oligonucleotides may have a GC content of from about 40% to about 70% (*e.g.*, 45%, 50%, 55%, 60%, 65%, or 70%). The oligonucleotides may include 3 or fewer (*e.g.*, 2, 1, or 0) mismatches to human KCNT1. In some embodiments, the oligonucleotide may include at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an equal length target of mouse KCNT1. In some embodiments, the oligonucleotide may include a sequence with 100% sequence identity to an equal length target of mouse KCNT1. In some embodiments, the oligonucleotide may include at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an equal length target of cynomolgus monkey KCNT1. In some embodiments, the oligonucleotide may include a sequence with 100% sequence identity to an equal length target of cynomolgus monkey KCNT1. The oligonucleotide may include 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at

least 99% sequence identity to an equal length target of mouse and cynomolgus monkey KCNT1. In some embodiments, the oligonucleotide may include a sequence with 100% sequence identity to an equal length target of mouse and cynomolgus monkey KCNT1. The oligonucleotides may include at least 3 (*e.g.*, 4, 5, 6, 7, 8, 9, 10, or more) mismatches to non
 5 KCNT1 transcripts. The oligonucleotides may not form dimers. The oligonucleotides may not form hairpins. The oligonucleotides may lack polyG runs, such as GGGG.

In some embodiments, an oligonucleotide comprises at least 10 contiguous nucleobases which is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an
 10 equal length portion of nucleobases within a 10 nucleobase range of any one of positions 1-4770 or SED ID NO: 3526. In some embodiments, an oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases which is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of nucleobases within a
 15 nucleobase range of any one of positions 1-4770 or SED ID NO: 3526. In some embodiments, an oligonucleotide comprises at least 10 contiguous nucleobases which is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 complementary to an equal length portion of nucleobases within a 10 nucleobase range of any one of positions 374, 661, 655-680, 765,
 20 837, 1347, 1340-1370, 1629, 1760, 1752, 1795, 1775, 1740-1815, 2879, 3008, 3168, or 3110-3171 of SEQ ID NO: 3526. In some embodiments, an oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases which are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of nucleobases of any one of
 25 positions 374, 661, 655-680, 765, 837, 1347, 1340-1370, 1629, 1760, 1752, 1795, 1775, 1740-1815, 2879, 3008, 3168, or 3110-3171 of SEQ ID NO: 3526. In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of nucleobases within
 30 any one of positions 655-680, 1340-137, 1740-1815, or 3110-3175 of SEQ ID NO: 3526. In some embodiments, an oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of nucleobases within any one of positions

655-680, 1340-137, 1740-1815, or 3110-3175 of SEQ ID NO: 3526. In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 655-665, 660-670, 665-675, 670-680, 1340-1350, 1345-1355, 1350-1360, 1355-1365, 1360-1370, 1740-1750, 1745-1755, 1750-1760, 1755-1765, 1760-1770, 1765-1775, 1770-1780, 1775-1785, 1780-1790, 1785-1795, 1790-1800, 1795-1805, 1800-1810, 1805-1815, 3110-3120, 3115-3125, 3120-3130, 3125-3135, 3130-3140, 3135-3145, 3140-3150, 3145-3155, 3150-3160, 3155-3165, 3160-3170, 3165-3175, or 3170-3180 of SEQ ID NO: 3526. In some embodiments, an oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 655-665, 660-670, 665-675, 670-680, 1340-1350, 1345-1355, 1350-1360, 1355-1365, 1360-1370, 1740-1750, 1745-1755, 1750-1760, 1755-1765, 1760-1770, 1765-1775, 1770-1780, 1775-1785, 1780-1790, 1785-1795, 1790-1800, 1795-1805, 1800-1810, 1805-1815, 3110-3120, 3115-3125, 3120-3130, 3125-3135, 3130-3140, 3135-3145, 3140-3150, 3145-3155, 3150-3160, 3155-3165, 3160-3170, 3165-3175, or 3170-3180 of SEQ ID NO: 3526.

The position of SEQ ID NO: 3526 refers to the nucleotide position of the KCNT1 transcript. For instance, the nucleotide at position 1261 of KCNT1 transcript (SEQ ID NO: 3526) is an adenine. Any of the antisense oligonucleotides described herein can bind to at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 contiguous nucleobases of any position of KCNT1 transcript or KCNT1 transcript variant. The oligonucleotide can comprise at least 10 contiguous nucleobases which are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of nucleobases within a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 nucleobase range of any position of KCNT1 transcript or KCNT1 transcript variant. In some embodiments, the oligonucleotide comprises at least 10 contiguous nucleobases, at least 11 contiguous nucleobases, at least 12 contiguous nucleobases, at least 13 contiguous nucleobases, at least 14 contiguous nucleobases, at least 15 contiguous nucleobases, at least 16 contiguous nucleobases, at least 17 contiguous nucleobases, at least 18 contiguous nucleobases, at least 19 contiguous nucleobases, or at least 20 contiguous nucleobases, which are at least 90% complementary to an equal length portion of nucleobases within a 10 nucleobase range of any position of KCNT1 transcript or KCNT1 transcript variant. For example, a 20 nucleobase oligonucleotide that is at least 90% complementary to the 10 nucleobases at position 220-230 of KCNT1 transcript or transcript

variant is within a 10 nucleobase range of positions 211-239 of KCNT1 transcript or KCNT1 transcript variant. In some embodiments, the oligonucleotide binding overlaps with the KCNT1 transcript or KCNT1 transcript variant nucleobase position. For example, a 20 nucleobase oligonucleotide that is complementary to position 500 of KCNT1 transcript or
5 KCNT1 transcript variant can hybridize to the nucleobases 481-500, 483-503, 490-510, 497-517, or 500-519, or any range therein of the KCNT1 transcript or KCNT1 transcript variant nucleotide positions.

Assessment of ASOs

The activity of the antisense oligonucleotides of the present disclosure can be assessed
10 and confirmed using various techniques known in the art. For example, the ability of the antisense oligonucleotides to inhibit *KCNT1* expression and/or whole cell current can be assessed in *in vitro* assays to confirm that the antisense oligonucleotides are suitable for use in treating a disease or condition associated with a gain-of-function mutation in *KCNT1* and/or excessive neuronal excitability. Mouse models can be used to not only assess the
15 ability of the antisense oligonucleotides to inhibit *KCNT1* expression or whole cell current, but to also ameliorate symptoms associated with gain-of-function *KCNT1* mutations and/or excessive neuronal excitability.

In one example, cells such as mammalian cells (*e.g.* CHO cells) that are transfected with *KCNT1* and express this gene are also transfected with an antisense oligonucleotide of
20 the present disclosure. Typically, the *KCNT1* contains a gain-of-function mutation. In another example, a human neuronal cell line (*e.g.* SH-SY5Y) that naturally expresses native wild type *KCNT1* is used. Optionally, the genome of this cell is edited so as to contain a gain-of-function mutation, such that the resulting *KCNT1* is a disease-causing variant. The levels of *KCNT1* mRNA can be assessed using qRT-PCR or Northern blot as is well known
25 in the art. The level of expression of protein from *KCNT1* can be assessed by Western blot on total cell lysates or fractions as described in Rizzo et al. (Mol Cell Neurosci. 72:54-63, 2016). Residual function of the *KCNT1*-encoded channels can also be assessed using electrophysiology or ion flux assay.

In a particular examples, the activity of the antisense oligonucleotides of the present
30 disclosure are assessed and confirmed using stem cell modelling (for review, see *e.g.* Tidball and Parent Stem Cells 34:27-33, 2016; Parent and Anderson Nature Neuroscience 18:360-366, 2015). For example, human induced pluripotent stem cells (iPSCs) can be produced from somatic cells (*e.g.* dermal fibroblasts or blood-derived hematopoietic cells) derived

from a patient with a *KCNT1* gain-of-function mutation and presenting with an associated disease or condition (*e.g.* EIMFS, ADNFLE or West syndrome). Optionally, genome editing can be used to revert the gain-of-function mutation to wild-type to produce an isogenic control cell line (Gaj et al. Trends Biotechnol 31, 397–405, 2013), which can also be used to determine desirable wild-type levels of activity for subsequent assessment and comparison of oligonucleotides. Alternatively, genome editing can be used to introduce a gain-of-function mutation into the *KCNT1* gene of wild-type, control iPSCs (*e.g.* a reference iPSC line). The iPSCs containing the gain-of-function mutation, and optionally the isogenic control, can then be differentiated into neurons, including excitatory neurons, using known techniques (see *e.g.* Kim et al. Front Cell Neurosci 8:109, 2014; Zhang et al. 2013, Chambers et al. Nat Biotechnol 27, 275–280, 2009). The effect of the antisense oligonucleotides of the present invention on *KCNT1* expression (as assessed by *KCNT1* mRNA or protein levels) and/or activity (as assessed by ion flux assay and/or electrophysiology, *e.g.* using the whole cell patch clamp technique, the single electrode voltage clamp technique or the two-electrode voltage clamp (TEVC) technique) can then be assessed following exposure of the iPSCs to the antisense oligonucleotides of the present invention.

The levels of *KCNT1* expression (mRNA or protein) or whole cell current observed when cells expressing *KCNT1* are exposed to an antisense oligonucleotide of the present disclosure are compared to the respective levels observed when cells expressing *KCNT1* are exposed with a negative control antisense oligonucleotide, so as to determine the level of inhibition resulting from the antisense oligonucleotide of the present disclosure. Typically, expression levels of *KCNT1* or whole cell current levels are reduced by at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more. Accordingly, the antisense oligonucleotides of the present disclosure can be used for treating a disease or condition associated with a gain-of-function mutation in *KCNT1*.

Mouse models can also be used to assess and confirm the activity of the antisense oligonucleotides of the present disclosure. For example, knock-in or transgenic mouse models can be generated using *KCNT1* genes containing a gain-of-function mutation in a similar manner to that described for *SCN1A* and *SCN2A* knock-in and transgenic mouse models (see *e.g.* Kearney et al. Neuroscience 102, 307–317, 2001; Ogiwara et al. J Neurosci 27:5903–5914, 2007; Yu et al. Nat Neurosci 9:1142–1149, 2006). In particular examples, a *KCNT1* gene that matches the particular antisense oligonucleotide (*e.g.* an allele-specific oligonucleotide) is used to produce the knock-in or transgenic mouse. The gain-of-function *KCNT1* knock-in or transgenic mice may present with a phenotype similar to EIMFS,

ADNFLE and/or West syndrome, including, for example, increased neuronal activity, spontaneous seizures, and heterogeneous focal seizure activity on electroencephalogram (EEG). In other examples, *SCN1A* and *SCN2A* knock-in and transgenic mouse models may be used for models exhibiting excessive neuronal excitability. The ability of the antisense oligonucleotides of the present invention to inhibit expression of *KCNT1* in these mice and to ameliorate any symptoms associated with the gain-of-function *KCNT1* mutations and/or excessive neuronal excitability in the mice, can then be assessed.

For example, the levels of *KCNT1* mRNA and/or protein can be assessed following administration of an antisense oligonucleotide of the present disclosure or a negative control antisense oligonucleotide to the mice. In a particular example, *KCNT1* mRNA and/or protein levels in the brain, and in particular the neurons, are assessed. The levels of *KCNT1* expression following administration of an antisense oligonucleotide of the present disclosure are compared to the respective levels observed when a negative control antisense oligonucleotide is administered, so as to determine the level of inhibition resulting from the antisense oligonucleotide of the present disclosure. Typically, expression levels of *KCNT1* in the mice (*e.g.* in the brains of the mice) are reduced by at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more.

In another example, the functional effect of administration of an antisense oligonucleotide of the present disclosure is assessed. For example, the number, severity and/or type of seizures can be assessed visually and/or by EEG. Neuronal excitability can also be assessed, such as by excising brain slices from mice administered an antisense oligonucleotide of the present disclosure or a negative control antisense oligonucleotide and assessing whole cell current (*e.g.* using the whole cell patch clamp technique). Similar neuronal excitability analyses can be performed using neurons isolated from the mice and then cultured. Additionally, mouse behavior, including gait characteristics, can be assessed to determine the functional effect of administration of an antisense oligonucleotide of the present disclosure.

Additional Embodiments

Disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85%

sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. cynomolgus* KCNT1.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and *M. cynomolgus* KCNT1.

In one aspect, the oligonucleotide comprises no more than 2 mismatches to *H. sapiens* KCNT1. In one aspect, the oligonucleotide comprises at least 3 mismatches to any non KCNT1 transcript. In one aspect, the oligonucleotide lacks a GGGG tetrad.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 1-3409. In one aspect, the region of at least 10 nucleobases has at least 90% complementary to an equal length portion of any one of SEQ ID NOs: 1-3409. In one aspect, the region of at least 10 nucleobases has at least 95% complementary to an equal length portion of any one of SEQ ID NOs: 1-3409. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 1-3409. In one aspect, the nucleobase sequence of the oligonucleotide consists of any one of SEQ ID NOs: 1-3409.

In one aspect, the oligonucleotide comprises: (a) a gap segment comprising linked deoxyribonucleosides; (b) a 5' wing segment comprising linked nucleosides; and (c) a 3' wing segment comprising linked nucleosides; wherein the gap segment comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an equal length portion of any one of SEQ ID NOs: 1-3409 positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment each comprises at least two linked nucleosides; and wherein at least one nucleoside of each wing segment comprises an alternative nucleoside.

In one aspect, the oligonucleotide comprises at least one alternative internucleoside linkage. In one aspect, the at least one alternative internucleoside linkage is a phosphorothioate internucleoside linkage. In one aspect, the at least one alternative internucleoside linkage is a 2'-alkoxy internucleoside linkage. In one aspect, the at least one alternative internucleoside linkage is an alkyl phosphate internucleoside linkage. In one aspect, the oligonucleotide comprises at least one alternative nucleobase. In one aspect, the alternative nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine. In one aspect, the oligonucleotide comprises at least one alternative sugar moiety. In one aspect, the alternative sugar moiety is 2'-OMe or a bicyclic nucleic acid. In one aspect, the

oligonucleotide further comprises a ligand conjugated to the 5' end or the 3' end of the oligonucleotide through a monovalent or branched bivalent or trivalent linker.

In one aspect, the oligonucleotide comprises a region complementary to at least 17 contiguous nucleotides of a KCNT1 gene. In one aspect, the oligonucleotide comprises a region complementary to at least 19 contiguous nucleotides of a KCNT1 gene. In one aspect, the oligonucleotide comprises a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 1-17 and 19-50. In one aspect, the oligonucleotide comprises a region of at least 18 contiguous nucleobases of SEQ ID NO: 18. In one aspect, the oligonucleotide comprises a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 51-81, 83-86, and 88-96. In one aspect, the oligonucleotide comprises a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 82 and 87. In one aspect, the oligonucleotide comprises a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 97-116.

Additionally disclosed herein is a pharmaceutical composition comprising the oligonucleotide and a pharmaceutically acceptable carrier or excipient. Additionally disclosed herein is a composition comprising the oligonucleotide of any one of claims 1-28 and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome. Additionally disclosed herein is a method of treating, preventing, or delaying the progression of a KCNT1 related disorder in a subject in need thereof, the method comprising administering to the subject the oligonucleotide, the pharmaceutical composition, or the composition in an amount and for a duration sufficient to treat, prevent, or delay the progression of the KCNT1 related disorder.

Additionally disclosed herein is a method of treating, preventing, or delaying the progression of a KCNT1 related disorder in a subject comprising: (a) selecting a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70%, wherein the oligonucleotide: (i) has at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1; (ii) has at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. cynomolgus* KCNT1; or (iii) has at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and *M. cynomolgus* KCNT1; and (b) administering the oligonucleotide to the subject in an amount and for a duration sufficient to treat, prevent, or delay the progression of the KCNT1 related disorder.

In one aspect, the oligonucleotide comprises no more than 2 mismatches to *H. sapiens* KCNT1. In one aspect, the oligonucleotide comprises at least 3 mismatches to any non KCNT1 transcript. In one aspect, the oligonucleotide lacks a GGGG tetrad.

Additionally disclosed herein is a method of inhibiting transcription of KCNT1 in a cell, the method comprising contacting the cell with the oligonucleotide, the pharmaceutical composition, or the composition in an amount and for a duration sufficient to obtain degradation of an mRNA transcript of the KCNT1 gene, wherein the oligonucleotide inhibits expression of the KCNT1 gene in the cell.

Additionally disclosed herein is a method of reducing a level and/or activity of KCNT1 in a cell of a subject having a KCNT1 related disorder, the method comprising contacting the cell with the oligonucleotide, the pharmaceutical composition, or the composition in an amount and for a duration sufficient to reduce the level and/or activity of KCNT1 in the cell. In one aspect, the subject is a human. In one aspect, the cell is a cell of the central nervous system. In one aspect, the KCNT1 related disorder is selected from the group consisting of epilepsy of infancy with migrating focal seizures, autosomal dominant nocturnal frontal lobe epilepsy, West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome. In one aspect, the subject has a gain-of-function mutation in KCNT1. In one aspect, the gain-of-function mutation is selected from the group consisting of V271F, L274I, G288S, F346L, R398Q, R428Q, R474H, F502V, M516V, K629N, I760M, Y796H, E893K, M896I, M896K, P924L, R928C, F932I, A934T, A966T, H257D, R262Q, Q270E, V340M, C377S, P409S, L437F, R474C, A477T, R565H, K629E, G652V, I760F, Q906H, R933G, R950Q, R961H, R1106Q, K1154Q, R474Q, Y1903C, H469L, M896R, K946E, and R950L. In one aspect, the method reduces one or more symptoms of the KCNT1 related disorder. In one aspect, the one or more symptoms of the KCNT1 related disorder is selected from the group consisting of prolonged seizures, frequent seizures, behavioral and developmental delays, movement and balance issues, orthopedic conditions, delayed language and speech issues, growth and nutrition issues, sleeping difficulties, chronic infection, sensory integration disorder, disruption of the autonomic nervous system, and sweating.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1. Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides

in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. cynomolgus* KCNT1. Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and *M. cynomolgus* KCNT1.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. cynomolgus* KCNT1.

Additionally disclosed herein is a single-stranded oligonucleotide of 18-22 linked nucleosides in length comprising a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and *M. cynomolgus* KCNT1.

In one aspect, the invention features a single-stranded oligonucleotide of 18-22 linked nucleosides in length including a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of both *H. sapiens* KCNT1 and *M. musculus* KCNT1.

In another aspect, the invention features a single-stranded oligonucleotide of 18-22 linked nucleosides in length including a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. cynomolgus* KCNT1.

In another aspect, the invention features a single-stranded oligonucleotide of 18-22 linked nucleosides in length including a GC content from 40% to 70% and having at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and *M. cynomolgus* KCNT1.

In some embodiments, the oligonucleotide includes no more than 2 mismatches to *H. sapiens* KCNT1.

In some embodiments, the oligonucleotide includes at least 3 mismatches to any non KCNT1 transcript.

In some embodiments, the oligonucleotide lacks a GGGG tetrad.

In another aspect, the invention features a single-stranded oligonucleotide of 18-22 linked nucleosides in length including a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 1-3409 (*e.g.*, SEQ ID NOs: 1-116 or 1-3384).

5 In some embodiments, the oligonucleotide includes a region having at least 85%, 90%, or 95% sequence identity to at least 18 contiguous nucleobases of any one of SEQ ID NOs: 1-3409 (*e.g.*, SEQ ID NOs: 1-116 or 1-3384).

10 In some embodiments, the oligonucleotide includes a gap segment including linked deoxyribonucleosides; a 5' wing segment including linked nucleosides; and a 3' wing segment including linked nucleosides. The gap segment may include a region of at least 10 contiguous nucleobases having at least 80% complementarity to an equal length portion of any one of SEQ ID NOs: 1-3409 (*e.g.*, SEQ ID NOs: 1-116 or 1-3384) positioned between the 5' wing segment and the 3' wing segment. The 5' wing segment and the 3' wing segment may each include at least two linked nucleosides, and at least one nucleoside of each wing segment may include an alternative nucleoside.

15 In some embodiments, the region of at least 10 nucleobases has at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) complementarity to an equal length portion of any one of SEQ ID NOs: 1-3409 (*e.g.*, SEQ ID NOs: 1-116 or 1-3384).

In some embodiments, the oligonucleotide includes the nucleobase sequence of any one of SEQ ID NOs: 1-3409 (*e.g.*, SEQ ID NOs: 1-116 or 1-3384).

20 In some embodiments, the nucleobase sequence of the oligonucleotide consists of any one of SEQ ID NOs: 1-3409 (*e.g.*, SEQ ID NOs: 1-116 or 1-3384).

In some embodiments, the oligonucleotide includes at least one alternative internucleoside linkage.

25 In some embodiments, the at least one alternative internucleoside linkage is a phosphorothioate internucleoside linkage.

In some embodiments, the at least one alternative internucleoside linkage is a 2'-alkoxy internucleoside linkage.

In some embodiments, the at least one alternative internucleoside linkage is an alkyl phosphate internucleoside linkage.

30 In some embodiments, the oligonucleotide includes at least one alternative nucleobase.

In some embodiments, the alternative nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine.

In some embodiments, the oligonucleotide includes at least one alternative sugar moiety.

In some embodiments, the alternative sugar moiety is 2'-OMe or a bicyclic nucleic acid.

5 In some embodiments, the oligonucleotide further includes a ligand conjugated to the 5' end or the 3' end of the oligonucleotide through a monovalent or branched bivalent or trivalent linker.

In some embodiments, the oligonucleotide includes a region complementary to at least 17 contiguous nucleotides of a KCNT1 gene.

10 In some embodiments, the oligonucleotide includes a region complementary to at least 19 contiguous nucleotides of a KCNT1 gene.

In some embodiments, the oligonucleotide includes a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 1-116.

15 In some embodiments, the oligonucleotide includes a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 1-17 and 19-50.

In some embodiments, the oligonucleotide includes a region of at least 18 contiguous nucleobases of SEQ ID NO: 18.

In some embodiments, the oligonucleotide includes a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 51-81, 83-86, and 88-96.

20 In some embodiments, the oligonucleotide includes a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 82 and 87.

In some embodiments, the oligonucleotide includes a region of at least 18 contiguous nucleobases of any one of SEQ ID NOs: 97-116.

25 In another aspect, the invention features a pharmaceutical composition including the oligonucleotide of any of the above embodiments and a pharmaceutically acceptable carrier or excipient.

In another aspect, the invention features a composition including the oligonucleotide of any of the above aspects and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome.

30 In another aspect, the invention features method of treating, preventing, or delaying the progression of a KCNT1 related disorder in a subject in need thereof, by administering to the subject the oligonucleotide, the pharmaceutical composition, or the composition any of the above aspects in an amount and for a duration sufficient to treat, prevent, or delay the progression of the KCNT1 related disorder.

In another aspect, the invention features method of treating, preventing, or delaying the progression of a KCNT1 related disorder in a subject by:

(a) selecting a single-stranded oligonucleotide of 18-22 linked nucleosides in length including a GC content from 40% to 70%, wherein the oligonucleotide:

5 (i) has at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1;

(ii) has at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1 and *M. cynomolgus* KCNT1; or

10 (iii) has at least 85% sequence identity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and *M. cynomolgus* KCNT1; and

(b) administering the oligonucleotide to the subject in an amount and for a duration sufficient to treat, prevent, or delay the progression of the KCNT1 related disorder.

In some embodiment, the oligonucleotide includes no more than 2 mismatches to *H. sapiens* KCNT1.

15 In some embodiment, the oligonucleotide includes at least 3 mismatches to any non KCNT1 transcript.

In some embodiment, the oligonucleotide lacks a GGGG tetrad.

In another aspect, the invention features a method of inhibiting transcription of KCNT1 in a cell, by contacting the cell with the oligonucleotide, the pharmaceutical
20 composition, or the composition of any of the above aspects in an amount and for a duration sufficient to obtain degradation of an mRNA transcript of the KCNT1 gene, wherein the oligonucleotide inhibits expression of the KCNT1 gene in the cell.

In another aspect, the invention features a method of reducing a level and/or activity of KCNT1 in a cell of a subject having a KCNT1 related disorder, by contacting the cell with
25 the oligonucleotide, the pharmaceutical composition, or the composition of any of the above aspects in an amount and for a duration sufficient to reduce the level and/or activity of KCNT1 in the cell.

In some embodiments, the subject is a human.

In some embodiments, the cell is a cell of the central nervous system.

30 In some embodiments, the KCNT1 related disorder is selected from the group consisting of epilepsy of infancy with migrating focal seizures, autosomal dominant nocturnal frontal lobe epilepsy, West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome.

In some embodiments, the subject has a gain-of-function mutation in KCNT1.

In some embodiments, the gain-of-function mutation is selected from the group consisting of V271F, L274I, G288S, F346L, R398Q, R428Q, R474H, F502V, M516V, K629N, I760M, Y796H, E893K, M896I, M896K, P924L, R928C, F932I, A934T, A966T, H257D, R262Q, Q270E, V340M, C377S, P409S, L437F, R474C, A477T, R565H, K629E, G652V, I760F, Q906H, R933G, R950Q, R961H, R1106Q, K1154Q, R474Q, Y1903C, H469L, M896R, K946E, and R950L.

In some embodiments, the method reduces one or more symptoms of the KCNT1 related disorder.

In some embodiments, the one or more symptoms of the KCNT1 related disorder is selected from the group consisting of prolonged seizures, frequent seizures, behavioral and developmental delays, movement and balance issues, orthopedic conditions, delayed language and speech issues, growth and nutrition issues, sleeping difficulties, chronic infection, sensory integration disorder, disruption of the autonomic nervous system, and sweating.

EXAMPLES

Example 1. Design, Selection, and Testing of Antisense Oligonucleotides

A bioinformatic analysis was performed to identify regions of human, mouse, and monkey KCNT1 genes with 20 base pair regions having pairwise homology. For example, 20 bp regions having at least 17 bp overlap between human and monkey KCNT1, human and mouse KCNT1, or human, monkey, and mouse KCNT1 were identified. Target sequences that only bind human KCNT1 were also identified. The ASO sequences, positions in the specified human transcript, and number of mismatches are shown in Table 1 of U.S. Provisional Application 62/782,877 filed December 20, 2018, hereby incorporated by reference in its entirety. Furthermore, intronic target sequences in the human KCNT1 gene were identified. These ASO sequences are in Table 2 of U.S. Provisional Application 62/782,877. MM indicates the number of mismatches to either NM_020822.2 or NG_033070.1, for exonic or intronic directed ASOs, respectively.

For the ASO sequences, homology to non-KCNT1 spliced (secondary) mRNA transcripts was also determined using the NCBI RefSeq R92 (January 2019) and Ensemble R94 (October 2018) databases. Table 3 of U.S. Provisional Application 62/862,328 filed June 17, 2019, hereby incorporated by reference in its entirety, lists the number of non-

KCNT1 transcripts identified with increasing number of mismatches (MM) from 0MM to 4MM.

For the ASO sequences, homology to non-KCNT1 non-spliced (primary) pre-mRNA transcripts was also determined using the Ensemble R94 (October 2018) databases. Table 4 of U.S. Provisional Application 62/862,328 filed on June 17, 2019, lists the number of non-KCNT1 transcripts identified with increasing number of mismatches (MM) from 0MM to 4MM.

For the ASO sequences, the position of reported single nucleotide polymorphisms (SNPs) within the ASO sequence was also determined using the NCBI dbSNP Build 151 (October 2017, downloaded January 2019). Table 5 of U.S. Provisional Application 62/862,328 filed on June 17, 2019, lists the position of each SNP and the associated SNP ID.

Table 2 shows the SEQ ID NOs of the ASO sequences, the position in the specified human transcript of those ASO sequences, and the number of mismatches (MM). Of note, number of mismatches for SEQ ID NOs: 1-96 and 117-3525 was determined in comparison to NM_020822.2 (SEQ ID NO: 3526). The number of mismatches for SEQ ID NOs: 97-116 is determined in comparison to NG_033070.1.

Table 2. Exemplary ASOs, KCNT1 positions, and mismatches

SEQ ID NO:	Position	MM	SEQ ID NO:	Position	MM	SEQ ID NO:	Position	MM	SEQ ID NO:	Position	MM	SEQ ID NO:	Position	MM	SEQ ID NO:	Position	MM
1	374	0	706	739	0	1411	1622	0	2116	2539	0	2821	3530	0	2821	3530	0
2	420	0	707	740	0	1412	1623	0	2117	2540	0	2822	3531	0	2822	3531	0
3	497	0	708	741	0	1413	1624	0	2118	2542	0	2823	3532	0	2823	3532	0
4	661	0	709	742	0	1414	1625	0	2119	2543	0	2824	3533	0	2824	3533	0
5	765	0	710	743	0	1415	1626	0	2120	2544	0	2825	3534	0	2825	3534	0
6	837	0	711	744	0	1416	1627	0	2121	2545	0	2826	3535	0	2826	3535	0
7	979	0	712	745	0	1417	1628	0	2122	2546	0	2827	3536	0	2827	3536	0
8	1347	0	713	746	0	1418	1630	0	2123	2547	0	2828	3537	0	2828	3537	0
9	1399	0	714	747	0	1419	1631	0	2124	2548	0	2829	3538	0	2829	3538	0
10	1629	0	715	748	0	1420	1632	0	2125	2549	0	2830	3539	0	2830	3539	0
11	1667	0	716	749	0	1421	1633	0	2126	2550	0	2831	3540	0	2831	3540	0
12	2879	0	717	750	0	1422	1634	0	2127	2551	0	2832	3541	0	2832	3541	0
13	3008	0	718	751	0	1423	1635	0	2128	2552	0	2833	3542	0	2833	3542	0
14	3029	0	719	752	0	1424	1636	0	2129	2553	0	2834	3543	0	2834	3543	0
15	3168	0	720	753	0	1425	1637	0	2130	2571	0	2835	3544	0	2835	3544	0
16	2417	0	721	754	0	1426	1638	0	2131	2572	0	2836	3545	0	2836	3545	0
17	1760	0	722	756	0	1427	1639	0	2132	2573	0	2837	3546	0	2837	3546	0
18	2068	0	723	758	0	1428	1641	0	2133	2574	0	2838	3547	0	2838	3547	0
19	332	0	724	759	0	1429	1643	0	2134	2575	0	2839	3548	0	2839	3548	0
20	391	0	725	760	0	1430	1644	0	2135	2576	0	2840	3549	0	2840	3549	0
21	790	0	726	761	0	1431	1645	0	2136	2577	0	2841	3550	0	2841	3550	0
22	1032	0	727	762	0	1432	1646	0	2137	2578	0	2842	3555	0	2842	3555	0
23	1185	0	728	763	0	1433	1647	0	2138	2579	0	2843	3556	0	2843	3556	0
24	1232	0	729	764	0	1434	1648	0	2139	2580	0	2844	3557	0	2844	3557	0
25	1271	0	730	783	0	1435	1649	0	2140	2581	0	2845	3558	0	2845	3558	0

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26	1440	0	731	784	0	1436	1650	0	2141	2582	0	2846	3559	0
27	1532	0	732	785	0	1437	1651	0	2142	2583	0	2847	3560	0
28	1752	0	733	786	0	1438	1652	0	2143	2584	0	2848	3561	0
29	1795	0	734	787	0	1439	1653	0	2144	2585	0	2849	3562	0
30	1933	0	735	788	0	1440	1654	0	2145	2586	0	2850	3563	0
31	2457	0	736	789	0	1441	1655	0	2146	2587	0	2851	3564	0
32	2777	0	737	791	0	1442	1656	0	2147	2588	0	2852	3565	0
33	2796	0	738	792	0	1443	1657	0	2148	2589	0	2853	3566	0
34	2835	0	739	793	0	1444	1658	0	2149	2590	0	2854	3567	0
35	2859	0	740	794	0	1445	1659	0	2150	2591	0	2855	3568	0
36	3241	0	741	795	0	1446	1660	0	2151	2592	0	2856	3569	0
37	1864	0	742	796	0	1447	1661	0	2152	2593	0	2857	3570	0
38	2758	0	743	797	0	1448	1662	0	2153	2594	0	2858	3571	0
39	579	0	744	798	0	1449	1663	0	2154	2595	0	2859	3572	0
40	702	0	745	799	0	1450	1664	0	2155	2596	0	2860	3573	0
41	731	0	746	800	0	1451	1665	0	2156	2597	0	2861	3574	0
42	877	0	747	801	0	1452	1666	0	2157	2598	0	2862	3575	0
43	903	0	748	802	0	1453	1668	0	2158	2599	0	2863	3576	0
44	1879	0	749	803	0	1454	1669	0	2159	2600	0	2864	3577	0
45	1954	0	750	804	0	1455	1670	0	2160	2601	0	2865	3578	0
46	1983	0	751	805	0	1456	1686	0	2161	2602	0	2866	3579	0
47	2322	0	752	806	0	1457	1687	0	2162	2603	0	2867	3580	0
48	3087	0	753	807	0	1458	1688	0	2163	2604	0	2868	3581	0
49	3520	0	754	808	0	1459	1689	0	2164	2605	0	2869	3582	0
50	3654	0	755	809	0	1460	1690	0	2165	2606	0	2870	3583	0
51	631	0	756	810	0	1461	1691	0	2166	2607	0	2871	3584	0
52	1718	0	757	811	0	1462	1692	0	2167	2608	0	2872	3585	0
53	625	0	758	812	0	1463	1693	0	2168	2609	0	2873	3586	0

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54	1207	0	759	813	0	1464	1694	0	2169	2610	0	2874	3587	0
55	1595	0	760	814	0	1465	1695	0	2170	2611	0	2875	3588	0
56	1826	0	761	815	0	1466	1709	0	2171	2612	0	2876	3589	0
57	2433	0	762	816	0	1467	1710	0	2172	2613	0	2877	3590	0
58	539	0	763	817	0	1468	1711	0	2173	2614	0	2878	3591	0
59	999	0	764	818	0	1469	1712	0	2174	2615	0	2879	3592	0
60	1107	0	765	826	0	1470	1713	0	2175	2616	0	2880	3593	0
61	1324	0	766	827	0	1471	1714	0	2176	2634	0	2881	3594	0
62	1775	0	767	828	0	1472	1715	0	2177	2635	0	2882	3596	0
63	2480	0	768	829	0	1473	1716	0	2178	2636	0	2883	3597	0
64	2541	0	769	830	0	1474	1717	0	2179	2637	0	2884	3598	0
65	3107	0	770	831	0	1475	1719	0	2180	2638	0	2885	3599	0
66	3277	0	771	832	0	1476	1720	0	2181	2639	0	2886	3600	0
67	1074	0	772	833	0	1477	1721	0	2182	2640	0	2887	3601	0
68	2904	0	773	834	0	1478	1722	0	2183	2641	0	2888	3602	0
69	3190	0	774	835	0	1479	1723	0	2184	2642	0	2889	3603	0
70	3595	0	775	836	0	1480	1724	0	2185	2643	0	2890	3604	0
71	519	0	776	838	0	1481	1725	0	2186	2644	0	2891	3605	0
72	964	0	777	839	0	1482	1726	0	2187	2645	0	2892	3606	0
73	1476	0	778	840	0	1483	1727	0	2188	2646	0	2893	3607	0
74	3210	0	779	841	0	1484	1728	0	2189	2647	0	2894	3608	0
75	3908	0	780	842	0	1485	1729	0	2190	2648	0	2895	3609	0
76	3974	0	781	843	0	1486	1730	0	2191	2649	0	2896	3610	0
77	1601	0	782	844	0	1487	1731	0	2192	2650	0	2897	3611	0
78	3847	0	783	845	0	1488	1732	0	2193	2651	0	2898	3612	0
79	4360	0	784	846	0	1489	1733	0	2194	2652	0	2899	3613	0
80	1449	0	785	847	0	1490	1734	0	2195	2653	0	2900	3614	0
81	1988	0	786	848	0	1491	1735	0	2196	2654	0	2901	3615	0

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82	4429	0	787	849	0	1492	1736	0	2197	2655	0	2902	3616	0
83	4499	0	788	850	0	1493	1737	0	2198	2656	0	2903	3617	0
84	3876	0	789	851	0	1494	1738	0	2199	2657	0	2904	3618	0
85	2442	0	790	852	0	1495	1739	0	2200	2658	0	2905	3619	0
86	2026	0	791	853	0	1496	1740	0	2201	2659	0	2906	3620	0
87	1012	0	792	854	0	1497	1741	0	2202	2660	0	2907	3621	0
88	496	0	793	855	0	1498	1742	0	2203	2661	0	2908	3622	0
89	4183	0	794	856	0	1499	1743	0	2204	2662	0	2909	3624	0
90	4434	0	795	857	0	1500	1744	0	2205	2663	0	2910	3625	0
91	4524	0	796	858	0	1501	1745	0	2206	2664	0	2911	3626	0
92	4581	0	797	859	0	1502	1746	0	2207	2665	0	2912	3627	0
93	4630	0	798	860	0	1503	1747	0	2208	2666	0	2913	3628	0
94	4680	0	799	861	0	1504	1748	0	2209	2667	0	2914	3629	0
95	4747	0	800	862	0	1505	1749	0	2210	2668	0	2915	3630	0
96	312	0	801	863	0	1506	1750	0	2211	2669	0	2916	3631	0
97	3527	0	802	864	0	1507	1751	0	2212	2670	0	2917	3632	0
98	8995	0	803	865	0	1508	1753	0	2213	2671	0	2918	3633	0
99	671	0	804	866	0	1509	1754	0	2214	2672	0	2919	3634	0
100	1379	0	805	867	0	1510	1755	0	2215	2673	0	2920	3635	0
101	5358	0	806	868	0	1511	1756	0	2216	2674	0	2921	3636	0
102	9844	0	807	869	0	1512	1757	0	2217	2675	0	2922	3637	0
103	8176	0	808	870	0	1513	1758	0	2218	2676	0	2923	3638	0
104	2942	0	809	871	0	1514	1759	0	2219	2677	0	2924	3639	0
105	3523	0	810	872	0	1515	1761	0	2220	2678	0	2925	3640	0
106	4677	0	811	873	0	1516	1762	0	2221	2679	0	2926	3641	0
107	18797	0	812	874	0	1517	1763	0	2222	2680	0	2927	3642	0
108	18207	0	813	875	0	1518	1764	0	2223	2681	0	2928	3643	0
109	28183	0	814	876	0	1519	1765	0	2224	2682	0	2929	3644	0

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110	14791	0	815	878	0	1520	1766	0	2225	2683	0	2930	3645	0
111	34987	0	816	879	0	1521	1767	0	2226	2684	0	2931	3646	0
112	16368	0	817	880	0	1522	1768	0	2227	2685	0	2932	3647	0
113	35212	0	818	881	0	1523	1769	0	2228	2686	0	2933	3648	0
114	45235	0	819	882	0	1524	1770	0	2229	2687	0	2934	3649	0
115	40062	0	820	883	0	1525	1771	0	2230	2688	0	2935	3650	0
116	27111	0	821	884	0	1526	1772	0	2231	2689	0	2936	3651	0
117	1	0	822	885	0	1527	1773	0	2232	2690	0	2937	3652	0
118	2	0	823	886	0	1528	1774	0	2233	2691	0	2938	3653	0
119	3	0	824	887	0	1529	1776	0	2234	2692	0	2939	3655	0
120	4	0	825	888	0	1530	1777	0	2235	2693	0	2940	3656	0
121	5	0	826	889	0	1531	1778	0	2236	2694	0	2941	3657	0
122	6	0	827	890	0	1532	1779	0	2237	2695	0	2942	3658	0
123	7	0	828	891	0	1533	1780	0	2238	2696	0	2943	3659	0
124	8	0	829	892	0	1534	1781	0	2239	2697	0	2944	3660	0
125	9	0	830	893	0	1535	1782	0	2240	2698	0	2945	3661	0
126	10	0	831	894	0	1536	1783	0	2241	2699	0	2946	3662	0
127	11	0	832	895	0	1537	1784	0	2242	2700	0	2947	3663	0
128	12	0	833	896	0	1538	1785	0	2243	2701	0	2948	3682	0
129	13	0	834	897	0	1539	1786	0	2244	2702	0	2949	3683	0
130	14	0	835	898	0	1540	1787	0	2245	2703	0	2950	3684	0
131	15	0	836	899	0	1541	1788	0	2246	2704	0	2951	3685	0
132	16	0	837	900	0	1542	1789	0	2247	2705	0	2952	3686	0
133	17	0	838	901	0	1543	1790	0	2248	2706	0	2953	3687	0
134	18	0	839	902	0	1544	1791	0	2249	2707	0	2954	3688	0
135	19	0	840	904	0	1545	1792	0	2250	2708	0	2955	3689	0
136	20	0	841	905	0	1546	1793	0	2251	2709	0	2956	3690	0
137	21	0	842	906	0	1547	1794	0	2252	2710	0	2957	3691	0

138	22	0	843	907	0	1548	1796	0	2253	2711	0	2958	3692	0
139	23	0	844	908	0	1549	1797	0	2254	2712	0	2959	3693	0
140	24	0	845	909	0	1550	1798	0	2255	2713	0	2960	3694	0
141	25	0	846	910	0	1551	1799	0	2256	2714	0	2961	3695	0
142	26	0	847	911	0	1552	1800	0	2257	2715	0	2962	3696	0
143	27	0	848	912	0	1553	1801	0	2258	2716	0	2963	3697	0
144	28	0	849	913	0	1554	1802	0	2259	2717	0	2964	3698	0
145	29	0	850	914	0	1555	1803	0	2260	2718	0	2965	3699	0
146	30	0	851	915	0	1556	1804	0	2261	2719	0	2966	3700	0
147	31	0	852	916	0	1557	1805	0	2262	2720	0	2967	3701	0
148	32	0	853	917	0	1558	1806	0	2263	2721	0	2968	3702	0
149	33	0	854	918	0	1559	1807	0	2264	2722	0	2969	3703	0
150	34	0	855	919	0	1560	1808	0	2265	2723	0	2970	3704	0
151	35	0	856	920	0	1561	1809	0	2266	2724	0	2971	3705	0
152	36	0	857	921	0	1562	1810	0	2267	2725	0	2972	3706	0
153	37	0	858	922	0	1563	1811	0	2268	2726	0	2973	3707	0
154	38	0	859	923	0	1564	1812	0	2269	2727	0	2974	3708	0
155	39	0	860	924	0	1565	1813	0	2270	2728	0	2975	3725	0
156	40	0	861	925	0	1566	1814	0	2271	2729	0	2976	3726	0
157	41	0	862	926	0	1567	1815	0	2272	2730	0	2977	3727	0
158	42	0	863	927	0	1568	1816	0	2273	2731	0	2978	3728	0
159	43	0	864	928	0	1569	1817	0	2274	2732	0	2979	3729	0
160	45	0	865	929	0	1570	1818	0	2275	2733	0	2980	3730	0
161	46	0	866	930	0	1571	1819	0	2276	2734	0	2981	3731	0
162	47	0	867	931	0	1572	1820	0	2277	2735	0	2982	3732	0
163	49	0	868	932	0	1573	1821	0	2278	2736	0	2983	3733	0
164	50	0	869	933	0	1574	1822	0	2279	2737	0	2984	3734	0
165	51	0	870	934	0	1575	1823	0	2280	2738	0	2985	3735	0

166	52	0	871	935	0	1576	1824	0	2281	2739	0	2986	3753	0
167	56	0	872	936	0	1577	1825	0	2282	2740	0	2987	3754	0
168	57	0	873	937	0	1578	1827	0	2283	2741	0	2988	3755	0
169	58	0	874	938	0	1579	1828	0	2284	2742	0	2989	3756	0
170	59	0	875	939	0	1580	1829	0	2285	2743	0	2990	3757	0
171	60	0	876	940	0	1581	1830	0	2286	2744	0	2991	3758	0
172	61	0	877	948	0	1582	1831	0	2287	2745	0	2992	3759	0
173	62	0	878	949	0	1583	1832	0	2288	2746	0	2993	3760	0
174	63	0	879	951	0	1584	1833	0	2289	2747	0	2994	3761	0
175	64	0	880	952	0	1585	1834	0	2290	2748	0	2995	3762	0
176	65	0	881	953	0	1586	1835	0	2291	2749	0	2996	3763	0
177	66	0	882	954	0	1587	1836	0	2292	2750	0	2997	3764	0
178	67	0	883	955	0	1588	1837	0	2293	2751	0	2998	3765	0
179	68	0	884	956	0	1589	1838	0	2294	2752	0	2999	3766	0
180	69	0	885	957	0	1590	1839	0	2295	2753	0	3000	3767	0
181	70	0	886	958	0	1591	1840	0	2296	2754	0	3001	3768	0
182	71	0	887	959	0	1592	1841	0	2297	2755	0	3002	3769	0
183	72	0	888	960	0	1593	1842	0	2298	2756	0	3003	3770	0
184	73	0	889	961	0	1594	1843	0	2299	2757	0	3004	3771	0
185	74	0	890	962	0	1595	1844	0	2300	2759	0	3005	3772	0
186	75	0	891	963	0	1596	1845	0	2301	2760	0	3006	3773	0
187	76	0	892	965	0	1597	1846	0	2302	2761	0	3007	3774	0
188	80	0	893	966	0	1598	1847	0	2303	2762	0	3008	3775	0
189	126	0	894	967	0	1599	1848	0	2304	2763	0	3009	3776	0
190	127	0	895	968	0	1600	1849	0	2305	2764	0	3010	3777	0
191	128	0	896	969	0	1601	1850	0	2306	2765	0	3011	3778	0
192	129	0	897	970	0	1602	1851	0	2307	2766	0	3012	3779	0
193	130	0	898	971	0	1603	1852	0	2308	2767	0	3013	3780	0

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194	131	0	899	972	0	1604	1853	0	2309	2768	0	3014	3781	0
195	132	0	900	973	0	1605	1854	0	2310	2769	0	3015	3782	0
196	133	0	901	974	0	1606	1855	0	2311	2770	0	3016	3783	0
197	134	0	902	975	0	1607	1856	0	2312	2771	0	3017	3784	0
198	135	0	903	976	0	1608	1857	0	2313	2772	0	3018	3785	0
199	136	0	904	977	0	1609	1858	0	2314	2773	0	3019	3786	0
200	137	0	905	978	0	1610	1859	0	2315	2774	0	3020	3787	0
201	138	0	906	980	0	1611	1860	0	2316	2775	0	3021	3788	0
202	139	0	907	981	0	1612	1861	0	2317	2776	0	3022	3789	0
203	140	0	908	982	0	1613	1862	0	2318	2778	0	3023	3790	0
204	141	0	909	983	0	1614	1863	0	2319	2779	0	3024	3791	0
205	142	0	910	984	0	1615	1865	0	2320	2780	0	3025	3792	0
206	143	0	911	985	0	1616	1866	0	2321	2781	0	3026	3793	0
207	144	0	912	986	0	1617	1867	0	2322	2782	0	3027	3794	0
208	145	0	913	987	0	1618	1868	0	2323	2783	0	3028	3795	0
209	146	0	914	988	0	1619	1869	0	2324	2784	0	3029	3796	0
210	147	0	915	989	0	1620	1870	0	2325	2785	0	3030	3797	0
211	148	0	916	990	0	1621	1871	0	2326	2786	0	3031	3798	0
212	149	0	917	991	0	1622	1872	0	2327	2787	0	3032	3799	0
213	150	0	918	992	0	1623	1873	0	2328	2788	0	3033	3815	0
214	151	0	919	993	0	1624	1874	0	2329	2789	0	3034	3816	0
215	152	0	920	994	0	1625	1875	0	2330	2790	0	3035	3817	0
216	153	0	921	995	0	1626	1876	0	2331	2791	0	3036	3818	0
217	154	0	922	996	0	1627	1877	0	2332	2792	0	3037	3819	0
218	155	0	923	997	0	1628	1878	0	2333	2793	0	3038	3820	0
219	156	0	924	998	0	1629	1880	0	2334	2811	0	3039	3821	0
220	157	0	925	1000	0	1630	1881	0	2335	2812	0	3040	3822	0
221	158	0	926	1001	0	1631	1882	0	2336	2813	0	3041	3823	0

222	159	0	927	1002	0	1632	1883	0	2337	2814	0	3042	3824	0
223	160	0	928	1003	0	1633	1884	0	2338	2815	0	3043	3825	0
224	161	0	929	1004	0	1634	1885	0	2339	2816	0	3044	3826	0
225	223	0	930	1005	0	1635	1886	0	2340	2817	0	3045	3827	0
226	224	0	931	1006	0	1636	1910	0	2341	2818	0	3046	3828	0
227	225	0	932	1007	0	1637	1911	0	2342	2819	0	3047	3829	0
228	226	0	933	1008	0	1638	1912	0	2343	2820	0	3048	3830	0
229	227	0	934	1009	0	1639	1913	0	2344	2821	0	3049	3831	0
230	228	0	935	1010	0	1640	1914	0	2345	2822	0	3050	3832	0
231	229	0	936	1011	0	1641	1915	0	2346	2823	0	3051	3833	0
232	230	0	937	1013	0	1642	1916	0	2347	2824	0	3052	3834	0
233	231	0	938	1014	0	1643	1917	0	2348	2825	0	3053	3835	0
234	232	0	939	1015	0	1644	1918	0	2349	2826	0	3054	3836	0
235	233	0	940	1016	0	1645	1919	0	2350	2827	0	3055	3837	0
236	234	0	941	1028	0	1646	1920	0	2351	2828	0	3056	3838	0
237	235	0	942	1029	0	1647	1921	0	2352	2829	0	3057	3839	0
238	236	0	943	1030	0	1648	1922	0	2353	2830	0	3058	3840	0
239	237	0	944	1031	0	1649	1923	0	2354	2831	0	3059	3841	0
240	238	0	945	1033	0	1650	1924	0	2355	2832	0	3060	3842	0
241	239	0	946	1034	0	1651	1925	0	2356	2833	0	3061	3843	0
242	240	0	947	1035	0	1652	1926	0	2357	2834	0	3062	3844	0
243	241	0	948	1036	0	1653	1927	0	2358	2836	0	3063	3845	0
244	242	0	949	1037	0	1654	1928	0	2359	2837	0	3064	3846	0
245	243	0	950	1038	0	1655	1929	0	2360	2838	0	3065	3848	0
246	244	0	951	1039	0	1656	1930	0	2361	2839	0	3066	3849	0
247	245	0	952	1040	0	1657	1931	0	2362	2840	0	3067	3850	0
248	246	0	953	1041	0	1658	1932	0	2363	2841	0	3068	3851	0
249	247	0	954	1042	0	1659	1934	0	2364	2842	0	3069	3852	0

250	248	0	955	1056	0	1660	1935	0	2365	2843	0	3070	3853	0
251	249	0	956	1057	0	1661	1936	0	2366	2844	0	3071	3854	0
252	250	0	957	1058	0	1662	1937	0	2367	2845	0	3072	3855	0
253	251	0	958	1059	0	1663	1938	0	2368	2846	0	3073	3856	0
254	252	0	959	1060	0	1664	1939	0	2369	2847	0	3074	3857	0
255	253	0	960	1061	0	1665	1940	0	2370	2848	0	3075	3858	0
256	258	0	961	1062	0	1666	1941	0	2371	2849	0	3076	3859	0
257	259	0	962	1063	0	1667	1942	0	2372	2850	0	3077	3860	0
258	263	0	963	1064	0	1668	1943	0	2373	2851	0	3078	3861	0
259	264	0	964	1065	0	1669	1944	0	2374	2852	0	3079	3862	0
260	265	0	965	1066	0	1670	1945	0	2375	2853	0	3080	3863	0
261	270	0	966	1067	0	1671	1946	0	2376	2854	0	3081	3864	0
262	271	0	967	1068	0	1672	1947	0	2377	2855	0	3082	3865	0
263	272	0	968	1069	0	1673	1948	0	2378	2856	0	3083	3866	0
264	273	0	969	1070	0	1674	1949	0	2379	2857	0	3084	3867	0
265	274	0	970	1071	0	1675	1950	0	2380	2858	0	3085	3868	0
266	275	0	971	1072	0	1676	1951	0	2381	2860	0	3086	3869	0
267	276	0	972	1073	0	1677	1952	0	2382	2861	0	3087	3870	0
268	277	0	973	1075	0	1678	1953	0	2383	2862	0	3088	3871	0
269	278	0	974	1076	0	1679	1955	0	2384	2863	0	3089	3872	0
270	279	0	975	1077	0	1680	1956	0	2385	2864	0	3090	3873	0
271	280	0	976	1078	0	1681	1957	0	2386	2865	0	3091	3874	0
272	281	0	977	1079	0	1682	1958	0	2387	2866	0	3092	3875	0
273	282	0	978	1080	0	1683	1959	0	2388	2867	0	3093	3877	0
274	283	0	979	1081	0	1684	1960	0	2389	2868	0	3094	3878	0
275	285	0	980	1082	0	1685	1961	0	2390	2869	0	3095	3879	0
276	286	0	981	1083	0	1686	1962	0	2391	2870	0	3096	3880	0
277	287	0	982	1084	0	1687	1963	0	2392	2871	0	3097	3881	0

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278	288	0	983	1085	0	1688	1964	0	2393	2872	0	3098	3882	0
279	289	0	984	1086	0	1689	1965	0	2394	2873	0	3099	3883	0
280	290	0	985	1087	0	1690	1966	0	2395	2874	0	3100	3884	0
281	291	0	986	1088	0	1691	1967	0	2396	2875	0	3101	3885	0
282	292	0	987	1089	0	1692	1968	0	2397	2876	0	3102	3886	0
283	293	0	988	1090	0	1693	1969	0	2398	2877	0	3103	3887	0
284	294	0	989	1091	0	1694	1970	0	2399	2878	0	3104	3888	0
285	295	0	990	1092	0	1695	1971	0	2400	2880	0	3105	3889	0
286	296	0	991	1093	0	1696	1972	0	2401	2881	0	3106	3890	0
287	297	0	992	1094	0	1697	1973	0	2402	2882	0	3107	3891	0
288	298	0	993	1095	0	1698	1974	0	2403	2883	0	3108	3892	0
289	299	0	994	1096	0	1699	1975	0	2404	2884	0	3109	3893	0
290	300	0	995	1097	0	1700	1976	0	2405	2885	0	3110	3894	0
291	301	0	996	1098	0	1701	1977	0	2406	2886	0	3111	3895	0
292	302	0	997	1099	0	1702	1978	0	2407	2887	0	3112	3896	0
293	303	0	998	1100	0	1703	1979	0	2408	2888	0	3113	3897	0
294	304	0	999	1101	0	1704	1980	0	2409	2889	0	3114	3898	0
295	305	0	1000	1102	0	1705	1981	0	2410	2890	0	3115	3899	0
296	306	0	1001	1103	0	1706	1982	0	2411	2891	0	3116	3900	0
297	307	0	1002	1104	0	1707	1984	0	2412	2892	0	3117	3901	0
298	308	0	1003	1105	0	1708	1985	0	2413	2893	0	3118	3902	0
299	309	0	1004	1106	0	1709	1986	0	2414	2894	0	3119	3903	0
300	310	0	1005	1108	0	1710	1987	0	2415	2895	0	3120	3904	0
301	311	0	1006	1109	0	1711	1989	0	2416	2896	0	3121	3905	0
302	313	0	1007	1110	0	1712	1990	0	2417	2898	0	3122	3906	0
303	314	0	1008	1111	0	1713	1991	0	2418	2899	0	3123	3907	0
304	315	0	1009	1112	0	1714	1992	0	2419	2900	0	3124	3909	0
305	316	0	1010	1113	0	1715	1993	0	2420	2901	0	3125	3910	0

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306	317	0	1011	1114	0	1716	1994	0	2421	2902	0	3126	3911	0
307	318	0	1012	1115	0	1717	1995	0	2422	2903	0	3127	3912	0
308	319	0	1013	1116	0	1718	1996	0	2423	2905	0	3128	3913	0
309	320	0	1014	1117	0	1719	1997	0	2424	2906	0	3129	3914	0
310	321	0	1015	1118	0	1720	1998	0	2425	2907	0	3130	3934	0
311	322	0	1016	1119	0	1721	1999	0	2426	2908	0	3131	3935	0
312	323	0	1017	1120	0	1722	2000	0	2427	2909	0	3132	3937	0
313	324	0	1018	1121	0	1723	2001	0	2428	2910	0	3133	3970	0
314	325	0	1019	1122	0	1724	2002	0	2429	2911	0	3134	3971	0
315	326	0	1020	1123	0	1725	2003	0	2430	2912	0	3135	3972	0
316	327	0	1021	1124	0	1726	2004	0	2431	2913	0	3136	3973	0
317	328	0	1022	1125	0	1727	2005	0	2432	2914	0	3137	3975	0
318	329	0	1023	1126	0	1728	2006	0	2433	2915	0	3138	3976	0
319	330	0	1024	1127	0	1729	2007	0	2434	2916	0	3139	3977	0
320	331	0	1025	1128	0	1730	2008	0	2435	2917	0	3140	3979	0
321	333	0	1026	1129	0	1731	2009	0	2436	2918	0	3141	3980	0
322	334	0	1027	1130	0	1732	2010	0	2437	2919	0	3142	3981	0
323	335	0	1028	1131	0	1733	2013	0	2438	2920	0	3143	3982	0
324	336	0	1029	1132	0	1734	2014	0	2439	2921	0	3144	3995	0
325	337	0	1030	1133	0	1735	2015	0	2440	2922	0	3145	3996	0
326	338	0	1031	1134	0	1736	2016	0	2441	2923	0	3146	3997	0
327	339	0	1032	1135	0	1737	2017	0	2442	2924	0	3147	3998	0
328	340	0	1033	1136	0	1738	2019	0	2443	2925	0	3148	3999	0
329	341	0	1034	1137	0	1739	2023	0	2444	2926	0	3149	4000	0
330	342	0	1035	1138	0	1740	2024	0	2445	2927	0	3150	4001	0
331	343	0	1036	1139	0	1741	2025	0	2446	2928	0	3151	4002	0
332	344	0	1037	1140	0	1742	2048	0	2447	2929	0	3152	4003	0
333	345	0	1038	1141	0	1743	2049	0	2448	2930	0	3153	4004	0

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334	346	0	1039	1142	0	1744	2050	0	2449	2931	0	3154	4005	0
335	347	0	1040	1143	0	1745	2051	0	2450	2932	0	3155	4006	0
336	348	0	1041	1144	0	1746	2052	0	2451	2933	0	3156	4007	0
337	349	0	1042	1145	0	1747	2053	0	2452	2934	0	3157	4008	0
338	350	0	1043	1146	0	1748	2054	0	2453	2935	0	3158	4009	0
339	351	0	1044	1147	0	1749	2055	0	2454	2936	0	3159	4010	0
340	352	0	1045	1148	0	1750	2056	0	2455	2937	0	3160	4011	0
341	353	0	1046	1149	0	1751	2057	0	2456	2938	0	3161	4012	0
342	354	0	1047	1150	0	1752	2058	0	2457	2939	0	3162	4013	0
343	355	0	1048	1151	0	1753	2059	0	2458	2940	0	3163	4014	0
344	356	0	1049	1152	0	1754	2060	0	2459	2941	0	3164	4015	0
345	357	0	1050	1153	0	1755	2061	0	2460	2942	0	3165	4016	0
346	358	0	1051	1154	0	1756	2062	0	2461	2943	0	3166	4017	0
347	359	0	1052	1155	0	1757	2063	0	2462	2944	0	3167	4018	0
348	360	0	1053	1156	0	1758	2064	0	2463	2945	0	3168	4019	0
349	361	0	1054	1157	0	1759	2065	0	2464	2946	0	3169	4020	0
350	362	0	1055	1158	0	1760	2066	0	2465	2947	0	3170	4021	0
351	363	0	1056	1159	0	1761	2067	0	2466	2948	0	3171	4022	0
352	364	0	1057	1160	0	1762	2069	0	2467	2949	0	3172	4023	0
353	365	0	1058	1161	0	1763	2070	0	2468	2950	0	3173	4024	0
354	366	0	1059	1162	0	1764	2071	0	2469	2951	0	3174	4025	0
355	367	0	1060	1163	0	1765	2072	0	2470	2952	0	3175	4026	0
356	368	0	1061	1164	0	1766	2073	0	2471	2973	0	3176	4027	0
357	369	0	1062	1167	0	1767	2074	0	2472	2974	0	3177	4028	0
358	370	0	1063	1168	0	1768	2075	0	2473	2975	0	3178	4029	0
359	371	0	1064	1169	0	1769	2076	0	2474	2976	0	3179	4030	0
360	372	0	1065	1170	0	1770	2077	0	2475	2977	0	3180	4031	0
361	373	0	1066	1171	0	1771	2078	0	2476	2978	0	3181	4032	0

362	375	0	1067	1172	0	1772	2079	0	2477	2979	0	3182	4033	0
363	376	0	1068	1173	0	1773	2080	0	2478	2980	0	3183	4034	0
364	377	0	1069	1174	0	1774	2081	0	2479	2981	0	3184	4035	0
365	379	0	1070	1175	0	1775	2082	0	2480	2982	0	3185	4036	0
366	380	0	1071	1176	0	1776	2083	0	2481	2983	0	3186	4037	0
367	381	0	1072	1177	0	1777	2084	0	2482	2984	0	3187	4038	0
368	388	0	1073	1178	0	1778	2085	0	2483	2985	0	3188	4039	0
369	389	0	1074	1179	0	1779	2086	0	2484	2986	0	3189	4040	0
370	390	0	1075	1180	0	1780	2100	0	2485	2987	0	3190	4041	0
371	392	0	1076	1181	0	1781	2101	0	2486	2988	0	3191	4042	0
372	393	0	1077	1182	0	1782	2102	0	2487	2989	0	3192	4043	0
373	394	0	1078	1183	0	1783	2103	0	2488	2990	0	3193	4044	0
374	395	0	1079	1184	0	1784	2104	0	2489	2991	0	3194	4045	0
375	396	0	1080	1186	0	1785	2105	0	2490	2992	0	3195	4046	0
376	397	0	1081	1187	0	1786	2106	0	2491	2993	0	3196	4047	0
377	398	0	1082	1188	0	1787	2107	0	2492	2994	0	3197	4048	0
378	399	0	1083	1189	0	1788	2108	0	2493	2995	0	3198	4049	0
379	400	0	1084	1190	0	1789	2109	0	2494	2996	0	3199	4050	0
380	401	0	1085	1191	0	1790	2110	0	2495	2997	0	3200	4051	0
381	402	0	1086	1192	0	1791	2111	0	2496	2998	0	3201	4052	0
382	403	0	1087	1193	0	1792	2112	0	2497	2999	0	3202	4053	0
383	404	0	1088	1194	0	1793	2113	0	2498	3000	0	3203	4054	0
384	405	0	1089	1195	0	1794	2114	0	2499	3001	0	3204	4055	0
385	406	0	1090	1196	0	1795	2115	0	2500	3002	0	3205	4056	0
386	407	0	1091	1197	0	1796	2116	0	2501	3003	0	3206	4057	0
387	408	0	1092	1198	0	1797	2119	0	2502	3004	0	3207	4059	0
388	409	0	1093	1199	0	1798	2120	0	2503	3005	0	3208	4060	0
389	410	0	1094	1200	0	1799	2121	0	2504	3006	0	3209	4061	0

390	411	0	1095	1201	0	1800	2122	0	2505	3007	0	3210	4062	0
391	412	0	1096	1202	0	1801	2123	0	2506	3009	0	3211	4063	0
392	413	0	1097	1203	0	1802	2143	0	2507	3010	0	3212	4064	0
393	414	0	1098	1204	0	1803	2144	0	2508	3011	0	3213	4065	0
394	415	0	1099	1205	0	1804	2145	0	2509	3012	0	3214	4066	0
395	416	0	1100	1206	0	1805	2146	0	2510	3013	0	3215	4067	0
396	417	0	1101	1208	0	1806	2147	0	2511	3014	0	3216	4068	0
397	418	0	1102	1209	0	1807	2148	0	2512	3015	0	3217	4069	0
398	419	0	1103	1210	0	1808	2149	0	2513	3016	0	3218	4070	0
399	421	0	1104	1211	0	1809	2150	0	2514	3017	0	3219	4071	0
400	422	0	1105	1212	0	1810	2151	0	2515	3018	0	3220	4072	0
401	423	0	1106	1213	0	1811	2152	0	2516	3019	0	3221	4073	0
402	424	0	1107	1214	0	1812	2153	0	2517	3020	0	3222	4074	0
403	425	0	1108	1215	0	1813	2154	0	2518	3021	0	3223	4075	0
404	426	0	1109	1216	0	1814	2155	0	2519	3022	0	3224	4076	0
405	427	0	1110	1217	0	1815	2156	0	2520	3023	0	3225	4077	0
406	428	0	1111	1218	0	1816	2157	0	2521	3024	0	3226	4078	0
407	429	0	1112	1219	0	1817	2158	0	2522	3025	0	3227	4079	0
408	430	0	1113	1220	0	1818	2159	0	2523	3026	0	3228	4080	0
409	431	0	1114	1221	0	1819	2160	0	2524	3027	0	3229	4081	0
410	432	0	1115	1222	0	1820	2161	0	2525	3028	0	3230	4082	0
411	433	0	1116	1223	0	1821	2162	0	2526	3030	0	3231	4083	0
412	434	0	1117	1224	0	1822	2163	0	2527	3031	0	3232	4084	0
413	435	0	1118	1225	0	1823	2164	0	2528	3032	0	3233	4085	0
414	436	0	1119	1226	0	1824	2165	0	2529	3033	0	3234	4086	0
415	437	0	1120	1227	0	1825	2166	0	2530	3034	0	3235	4087	0
416	438	0	1121	1228	0	1826	2167	0	2531	3035	0	3236	4088	0
417	439	0	1122	1229	0	1827	2168	0	2532	3036	0	3237	4089	0

418	440	0	1123	1230	0	1828	2169	0	2533	3037	0	3238	4090	0
419	441	0	1124	1231	0	1829	2170	0	2534	3038	0	3239	4091	0
420	442	0	1125	1233	0	1830	2171	0	2535	3039	0	3240	4092	0
421	443	0	1126	1234	0	1831	2172	0	2536	3040	0	3241	4094	0
422	444	0	1127	1235	0	1832	2173	0	2537	3041	0	3242	4095	0
423	445	0	1128	1236	0	1833	2196	0	2538	3042	0	3243	4096	0
424	446	0	1129	1237	0	1834	2197	0	2539	3043	0	3244	4097	0
425	447	0	1130	1238	0	1835	2198	0	2540	3044	0	3245	4098	0
426	448	0	1131	1239	0	1836	2199	0	2541	3045	0	3246	4099	0
427	449	0	1132	1240	0	1837	2200	0	2542	3046	0	3247	4100	0
428	450	0	1133	1241	0	1838	2201	0	2543	3048	0	3248	4101	0
429	451	0	1134	1242	0	1839	2202	0	2544	3051	0	3249	4103	0
430	452	0	1135	1243	0	1840	2203	0	2545	3052	0	3250	4104	0
431	453	0	1136	1244	0	1841	2204	0	2546	3053	0	3251	4105	0
432	454	0	1137	1245	0	1842	2205	0	2547	3054	0	3252	4106	0
433	455	0	1138	1264	0	1843	2206	0	2548	3055	0	3253	4107	0
434	456	0	1139	1265	0	1844	2207	0	2549	3056	0	3254	4108	0
435	457	0	1140	1266	0	1845	2208	0	2550	3057	0	3255	4109	0
436	458	0	1141	1267	0	1846	2209	0	2551	3058	0	3256	4110	0
437	459	0	1142	1268	0	1847	2210	0	2552	3059	0	3257	4111	0
438	460	0	1143	1269	0	1848	2211	0	2553	3060	0	3258	4112	0
439	461	0	1144	1270	0	1849	2212	0	2554	3061	0	3259	4113	0
440	462	0	1145	1272	0	1850	2213	0	2555	3065	0	3260	4114	0
441	463	0	1146	1273	0	1851	2214	0	2556	3066	0	3261	4115	0
442	464	0	1147	1274	0	1852	2215	0	2557	3067	0	3262	4116	0
443	465	0	1148	1275	0	1853	2216	0	2558	3072	0	3263	4117	0
444	466	0	1149	1276	0	1854	2217	0	2559	3075	0	3264	4118	0
445	467	0	1150	1277	0	1855	2218	0	2560	3077	0	3265	4119	0

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446	468	0	1151	1278	0	1856	2219	0	2561	3078	0	3266	4121	0
447	469	0	1152	1279	0	1857	2220	0	2562	3079	0	3267	4122	0
448	470	0	1153	1280	0	1858	2221	0	2563	3080	0	3268	4124	0
449	471	0	1154	1281	0	1859	2222	0	2564	3081	0	3269	4125	0
450	472	0	1155	1299	0	1860	2223	0	2565	3082	0	3270	4126	0
451	473	0	1156	1300	0	1861	2224	0	2566	3083	0	3271	4127	0
452	474	0	1157	1301	0	1862	2225	0	2567	3084	0	3272	4128	0
453	475	0	1158	1302	0	1863	2226	0	2568	3085	0	3273	4129	0
454	476	0	1159	1303	0	1864	2227	0	2569	3086	0	3274	4130	0
455	477	0	1160	1304	0	1865	2228	0	2570	3088	0	3275	4131	0
456	478	0	1161	1305	0	1866	2229	0	2571	3089	0	3276	4132	0
457	479	0	1162	1306	0	1867	2230	0	2572	3090	0	3277	4133	0
458	480	0	1163	1307	0	1868	2231	0	2573	3091	0	3278	4134	0
459	481	0	1164	1308	0	1869	2232	0	2574	3092	0	3279	4135	0
460	482	0	1165	1309	0	1870	2233	0	2575	3093	0	3280	4136	0
461	483	0	1166	1310	0	1871	2234	0	2576	3094	0	3281	4137	0
462	484	0	1167	1311	0	1872	2235	0	2577	3095	0	3282	4138	0
463	485	0	1168	1312	0	1873	2236	0	2578	3096	0	3283	4139	0
464	487	0	1169	1313	0	1874	2237	0	2579	3097	0	3284	4140	0
465	488	0	1170	1314	0	1875	2238	0	2580	3098	0	3285	4141	0
466	489	0	1171	1315	0	1876	2239	0	2581	3099	0	3286	4142	0
467	490	0	1172	1316	0	1877	2240	0	2582	3100	0	3287	4143	0
468	491	0	1173	1317	0	1878	2241	0	2583	3101	0	3288	4144	0
469	492	0	1174	1318	0	1879	2242	0	2584	3102	0	3289	4145	0
470	493	0	1175	1319	0	1880	2243	0	2585	3103	0	3290	4146	0
471	494	0	1176	1320	0	1881	2244	0	2586	3104	0	3291	4147	0
472	495	0	1177	1321	0	1882	2245	0	2587	3105	0	3292	4148	0
473	498	0	1178	1322	0	1883	2246	0	2588	3106	0	3293	4149	0

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474	499	0	1179	1323	0	1884	2247	0	2589	3108	0	3294	4150	0
475	500	0	1180	1325	0	1885	2248	0	2590	3109	0	3295	4151	0
476	501	0	1181	1326	0	1886	2249	0	2591	3110	0	3296	4152	0
477	502	0	1182	1327	0	1887	2250	0	2592	3111	0	3297	4153	0
478	503	0	1183	1328	0	1888	2251	0	2593	3112	0	3298	4154	0
479	504	0	1184	1329	0	1889	2252	0	2594	3113	0	3299	4155	0
480	505	0	1185	1330	0	1890	2253	0	2595	3114	0	3300	4156	0
481	506	0	1186	1331	0	1891	2254	0	2596	3115	0	3301	4157	0
482	507	0	1187	1332	0	1892	2255	0	2597	3116	0	3302	4158	0
483	508	0	1188	1333	0	1893	2256	0	2598	3117	0	3303	4159	0
484	509	0	1189	1334	0	1894	2257	0	2599	3118	0	3304	4177	0
485	510	0	1190	1335	0	1895	2258	0	2600	3119	0	3305	4178	0
486	511	0	1191	1336	0	1896	2259	0	2601	3120	0	3306	4179	0
487	512	0	1192	1337	0	1897	2260	0	2602	3121	0	3307	4180	0
488	513	0	1193	1338	0	1898	2261	0	2603	3122	0	3308	4181	0
489	514	0	1194	1339	0	1899	2262	0	2604	3123	0	3309	4182	0
490	515	0	1195	1340	0	1900	2263	0	2605	3124	0	3310	4184	0
491	516	0	1196	1341	0	1901	2264	0	2606	3125	0	3311	4185	0
492	517	0	1197	1342	0	1902	2265	0	2607	3141	0	3312	4186	0
493	518	0	1198	1343	0	1903	2266	0	2608	3142	0	3313	4187	0
494	520	0	1199	1344	0	1904	2267	0	2609	3143	0	3314	4188	0
495	521	0	1200	1345	0	1905	2268	0	2610	3144	0	3315	4204	0
496	522	0	1201	1346	0	1906	2269	0	2611	3145	0	3316	4205	0
497	523	0	1202	1348	0	1907	2270	0	2612	3146	0	3317	4206	0
498	524	0	1203	1349	0	1908	2271	0	2613	3147	0	3318	4207	0
499	525	0	1204	1350	0	1909	2272	0	2614	3148	0	3319	4208	0
500	526	0	1205	1351	0	1910	2273	0	2615	3149	0	3320	4209	0
501	527	0	1206	1352	0	1911	2274	0	2616	3150	0	3321	4210	0

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502	528	0	1207	1353	0	1912	2275	0	2617	3151	0	3322	4211	0
503	529	0	1208	1354	0	1913	2276	0	2618	3152	0	3323	4212	0
504	530	0	1209	1355	0	1914	2277	0	2619	3153	0	3324	4213	0
505	531	0	1210	1356	0	1915	2278	0	2620	3154	0	3325	4214	0
506	532	0	1211	1357	0	1916	2279	0	2621	3155	0	3326	4215	0
507	533	0	1212	1358	0	1917	2280	0	2622	3156	0	3327	4216	0
508	534	0	1213	1359	0	1918	2281	0	2623	3157	0	3328	4217	0
509	535	0	1214	1360	0	1919	2282	0	2624	3158	0	3329	4218	0
510	536	0	1215	1361	0	1920	2283	0	2625	3159	0	3330	4219	0
511	537	0	1216	1362	0	1921	2286	0	2626	3160	0	3331	4220	0
512	538	0	1217	1363	0	1922	2287	0	2627	3161	0	3332	4221	0
513	540	0	1218	1364	0	1923	2288	0	2628	3162	0	3333	4222	0
514	541	0	1219	1365	0	1924	2289	0	2629	3163	0	3334	4223	0
515	542	0	1220	1366	0	1925	2290	0	2630	3164	0	3335	4224	0
516	543	0	1221	1367	0	1926	2291	0	2631	3165	0	3336	4225	0
517	544	0	1222	1368	0	1927	2292	0	2632	3183	0	3337	4226	0
518	545	0	1223	1369	0	1928	2293	0	2633	3184	0	3338	4227	0
519	546	0	1224	1370	0	1929	2294	0	2634	3185	0	3339	4228	0
520	547	0	1225	1371	0	1930	2295	0	2635	3186	0	3340	4229	0
521	548	0	1226	1372	0	1931	2296	0	2636	3187	0	3341	4230	0
522	549	0	1227	1373	0	1932	2297	0	2637	3188	0	3342	4231	0
523	550	0	1228	1374	0	1933	2298	0	2638	3189	0	3343	4232	0
524	551	0	1229	1375	0	1934	2299	0	2639	3191	0	3344	4233	0
525	552	0	1230	1376	0	1935	2300	0	2640	3192	0	3345	4234	0
526	553	0	1231	1377	0	1936	2301	0	2641	3193	0	3346	4235	0
527	554	0	1232	1378	0	1937	2302	0	2642	3194	0	3347	4236	0
528	555	0	1233	1379	0	1938	2303	0	2643	3195	0	3348	4237	0
529	556	0	1234	1380	0	1939	2304	0	2644	3196	0	3349	4238	0

530	557	0	1235	1381	0	1940	2305	0	2645	3197	0	3350	4239	0
531	558	0	1236	1382	0	1941	2306	0	2646	3198	0	3351	4240	0
532	559	0	1237	1383	0	1942	2307	0	2647	3199	0	3352	4241	0
533	560	0	1238	1384	0	1943	2308	0	2648	3200	0	3353	4242	0
534	561	0	1239	1385	0	1944	2309	0	2649	3201	0	3354	4244	0
535	562	0	1240	1386	0	1945	2310	0	2650	3202	0	3355	4245	0
536	563	0	1241	1387	0	1946	2311	0	2651	3203	0	3356	4249	0
537	564	0	1242	1388	0	1947	2312	0	2652	3204	0	3357	4254	0
538	565	0	1243	1389	0	1948	2313	0	2653	3205	0	3358	4255	0
539	566	0	1244	1390	0	1949	2314	0	2654	3206	0	3359	4256	0
540	567	0	1245	1391	0	1950	2315	0	2655	3207	0	3360	4257	0
541	568	0	1246	1392	0	1951	2316	0	2656	3208	0	3361	4258	0
542	569	0	1247	1393	0	1952	2317	0	2657	3209	0	3362	4259	0
543	570	0	1248	1394	0	1953	2318	0	2658	3211	0	3363	4260	0
544	571	0	1249	1395	0	1954	2319	0	2659	3212	0	3364	4261	0
545	572	0	1250	1396	0	1955	2320	0	2660	3213	0	3365	4262	0
546	573	0	1251	1397	0	1956	2321	0	2661	3214	0	3366	4263	0
547	574	0	1252	1398	0	1957	2323	0	2662	3232	0	3367	4264	0
548	575	0	1253	1400	0	1958	2324	0	2663	3233	0	3368	4265	0
549	576	0	1254	1401	0	1959	2325	0	2664	3234	0	3369	4266	0
550	577	0	1255	1402	0	1960	2326	0	2665	3235	0	3370	4267	0
551	578	0	1256	1403	0	1961	2327	0	2666	3236	0	3371	4268	0
552	580	0	1257	1404	0	1962	2328	0	2667	3237	0	3372	4269	0
553	581	0	1258	1405	0	1963	2329	0	2668	3238	0	3373	4270	0
554	582	0	1259	1406	0	1964	2330	0	2669	3239	0	3374	4271	0
555	583	0	1260	1407	0	1965	2331	0	2670	3240	0	3375	4272	0
556	584	0	1261	1408	0	1966	2332	0	2671	3242	0	3376	4273	0
557	585	0	1262	1409	0	1967	2333	0	2672	3243	0	3377	4274	0

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558	586	0	1263	1410	0	1968	2334	0	2673	3244	0	3378	4275	0
559	587	0	1264	1411	0	1969	2335	0	2674	3245	0	3379	4276	0
560	588	0	1265	1412	0	1970	2336	0	2675	3246	0	3380	4277	0
561	589	0	1266	1413	0	1971	2337	0	2676	3247	0	3381	4278	0
562	590	0	1267	1414	0	1972	2338	0	2677	3248	0	3382	4279	0
563	591	0	1268	1415	0	1973	2339	0	2678	3249	0	3383	4280	0
564	592	0	1269	1416	0	1974	2340	0	2679	3250	0	3384	4281	0
565	593	0	1270	1417	0	1975	2341	0	2680	3251	0	3385	1248	0
566	594	0	1271	1418	0	1976	2342	0	2681	3252	0	3386	1249	0
567	595	0	1272	1419	0	1977	2343	0	2682	3253	0	3387	1503	0
568	596	0	1273	1420	0	1978	2344	0	2683	3254	0	3388	1504	0
569	597	0	1274	1421	0	1979	2345	0	2684	3255	0	3389	1505	0
570	598	0	1275	1422	0	1980	2363	0	2685	3256	0	3390	1506	0
571	599	0	1276	1423	0	1981	2364	0	2686	3257	0	3391	1511	0
572	600	0	1277	1424	0	1982	2365	0	2687	3258	0	3392	1512	0
573	601	0	1278	1425	0	1983	2366	0	2688	3259	0	3393	1530	0
574	602	0	1279	1426	0	1984	2367	0	2689	3260	0	3394	2629	0
575	603	0	1280	1427	0	1985	2368	0	2690	3261	0	3395	3126	0
576	604	0	1281	1428	0	1986	2369	0	2691	3262	0	3396	3127	0
577	605	0	1282	1429	0	1987	2370	0	2692	3263	0	3397	3128	0
578	606	0	1283	1430	0	1988	2371	0	2693	3264	0	3398	3129	0
579	607	0	1284	1431	0	1989	2372	0	2694	3265	0	3399	3130	0
580	608	0	1285	1432	0	1990	2373	0	2695	3266	0	3400	3131	0
581	609	0	1286	1433	0	1991	2374	0	2696	3267	0	3401	3132	0
582	610	0	1287	1434	0	1992	2375	0	2697	3268	0	3402	3171	0
583	611	0	1288	1435	0	1993	2376	0	2698	3269	0	3403	3172	0
584	612	0	1289	1436	0	1994	2377	0	2699	3270	0	3404	3173	0
585	613	0	1290	1437	0	1995	2378	0	2700	3271	0	3405	3174	0

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586	614	0	1291	1438	0	1996	2397	0	2701	3272	0	3406	3175	0
587	615	0	1292	1439	0	1997	2398	0	2702	3273	0	3407	3182	0
588	616	0	1293	1441	0	1998	2399	0	2703	3274	0	3408	3664	0
589	617	0	1294	1442	0	1999	2400	0	2704	3275	0	3409	3665	0
590	618	0	1295	1443	0	2000	2401	0	2705	3276	0	3410	374	0
591	619	0	1296	1444	0	2001	2402	0	2706	3278	0	3411	661	0
592	620	0	1297	1445	0	2002	2403	0	2707	3279	0	3412	765	0
593	621	0	1298	1446	0	2003	2404	0	2708	3280	0	3413	837	0
594	622	0	1299	1447	0	2004	2405	0	2709	3281	0	3414	979	0
595	623	0	1300	1448	0	2005	2406	0	2710	3282	0	3415	1347	0
596	624	0	1301	1450	0	2006	2407	0	2711	3283	0	3416	1629	0
597	626	0	1302	1451	0	2007	2408	0	2712	3284	0	3417	2879	0
598	627	0	1303	1452	0	2008	2409	0	2713	3285	0	3418	3008	0
599	628	0	1304	1453	0	2009	2410	0	2714	3286	0	3419	3168	0
600	629	0	1305	1454	0	2010	2411	0	2715	3287	0	3420	661	0
601	630	0	1306	1455	0	2011	2412	0	2716	3288	0	3421	666	0
602	632	0	1307	1456	0	2012	2413	0	2717	3320	0	3422	1347	0
603	633	0	1308	1457	0	2013	2414	0	2718	3321	0	3423	1349	0
604	634	0	1309	1458	0	2014	2415	0	2719	3322	0	3424	1352	0
605	635	0	1310	1459	0	2015	2416	0	2720	3323	0	3425	1354	0
606	636	0	1311	1460	0	2016	2418	0	2721	3324	0	3426	1746	0
607	637	0	1312	1461	0	2017	2419	0	2722	3325	0	3427	1751	0
608	638	0	1313	1462	0	2018	2420	0	2723	3326	0	3428	1755	0
609	639	0	1314	1463	0	2019	2421	0	2724	3327	0	3429	1793	0
610	640	0	1315	1464	0	2020	2422	0	2725	3335	0	3430	1799	0
611	641	0	1316	1465	0	2021	2423	0	2726	3336	0	3431	1803	0
612	642	0	1317	1466	0	2022	2424	0	2727	3369	0	3432	1807	0
613	643	0	1318	1467	0	2023	2425	0	2728	3370	0	3433	1809	0

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614	644	0	1319	1468	0	2024	2426	0	2729	3371	0	3434	3112	0
615	645	0	1320	1469	0	2025	2427	0	2730	3372	0	3435	3114	0
616	646	0	1321	1470	0	2026	2428	0	2731	3373	0	3436	3125	0
617	647	0	1322	1471	0	2027	2429	0	2732	3374	0	3437	3127	0
618	648	0	1323	1472	0	2028	2430	0	2733	3375	0	3438	3162	0
619	649	0	1324	1473	0	2029	2431	0	2734	3376	0	3439	3171	0
620	650	0	1325	1474	0	2030	2432	0	2735	3377	0	3440	3133	0
621	651	0	1326	1475	0	2031	2434	0	2736	3378	0	3441	3134	0
622	652	0	1327	1477	0	2032	2435	0	2737	3379	0	3442	3135	0
623	653	0	1328	1478	0	2033	2436	0	2738	3380	0	3443	3136	0
624	654	0	1329	1494	0	2034	2437	0	2739	3381	0	3444	3137	0
625	655	0	1330	1495	0	2035	2438	0	2740	3382	0	3445	3138	0
626	656	0	1331	1496	0	2036	2439	0	2741	3383	0	3446	3139	0
627	657	0	1332	1497	0	2037	2440	0	2742	3384	0	3447	3140	0
628	658	0	1333	1498	0	2038	2441	0	2743	3385	0	3448	3166	0
629	659	0	1334	1499	0	2039	2443	0	2744	3386	0	3449	3167	0
630	660	0	1335	1500	0	2040	2444	0	2745	3387	0	3450	3169	0
631	662	0	1336	1501	0	2041	2445	0	2746	3388	0	3451	3170	0
632	663	0	1337	1502	0	2042	2446	0	2747	3389	0	3452	661	0
633	664	0	1338	1531	0	2043	2447	0	2748	3390	0	3453	666	0
634	665	0	1339	1533	0	2044	2448	0	2749	3391	0	3454	1347	0
635	666	0	1340	1534	0	2045	2449	0	2750	3392	0	3455	1349	0
636	667	0	1341	1535	0	2046	2450	0	2751	3393	0	3456	1352	0
637	668	0	1342	1536	0	2047	2451	0	2752	3394	0	3457	1354	0
638	669	0	1343	1537	0	2048	2452	0	2753	3411	0	3458	1746	0
639	670	0	1344	1538	0	2049	2453	0	2754	3412	0	3459	1751	0
640	671	0	1345	1539	0	2050	2454	0	2755	3413	0	3460	1755	0
641	672	0	1346	1540	0	2051	2455	0	2756	3414	0	3461	1793	0

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642	673	0	1347	1541	0	2052	2456	0	2757	3415	0	3462	1799	0
643	674	0	1348	1542	0	2053	2458	0	2758	3416	0	3463	1803	0
644	675	0	1349	1543	0	2054	2459	0	2759	3461	0	3464	1807	0
645	676	0	1350	1544	0	2055	2460	0	2760	3462	0	3465	1809	0
646	677	0	1351	1545	0	2056	2461	0	2761	3463	0	3466	3112	0
647	678	0	1352	1546	0	2057	2462	0	2762	3464	0	3467	3114	0
648	679	0	1353	1547	0	2058	2463	0	2763	3465	0	3468	3125	0
649	680	0	1354	1548	0	2059	2464	0	2764	3466	0	3469	3127	0
650	681	0	1355	1550	0	2060	2465	0	2765	3467	0	3470	3162	0
651	682	0	1356	1552	0	2061	2466	0	2766	3468	0	3471	3171	0
652	683	0	1357	1553	0	2062	2467	0	2767	3469	0	3472	661	0
653	684	0	1358	1554	0	2063	2468	0	2768	3470	0	3473	666	0
654	685	0	1359	1555	0	2064	2469	0	2769	3471	0	3474	1347	0
655	686	0	1360	1556	0	2065	2470	0	2770	3472	0	3475	1349	0
656	687	0	1361	1557	0	2066	2471	0	2771	3473	0	3476	1352	0
657	688	0	1362	1558	0	2067	2472	0	2772	3474	0	3477	1354	0
658	689	0	1363	1559	0	2068	2473	0	2773	3475	0	3478	1746	0
659	690	0	1364	1560	0	2069	2474	0	2774	3476	0	3479	1751	0
660	691	0	1365	1561	0	2070	2475	0	2775	3477	0	3480	1755	0
661	692	0	1366	1562	0	2071	2476	0	2776	3478	0	3481	1793	0
662	693	0	1367	1563	0	2072	2477	0	2777	3479	0	3482	1799	0
663	694	0	1368	1564	0	2073	2478	0	2778	3480	0	3483	1803	0
664	695	0	1369	1565	0	2074	2479	0	2779	3481	0	3484	1807	0
665	696	0	1370	1566	0	2075	2481	0	2780	3482	0	3485	1809	0
666	697	0	1371	1567	0	2076	2482	0	2781	3483	0	3486	3112	0
667	698	0	1372	1568	0	2077	2483	0	2782	3484	0	3487	3114	0
668	699	0	1373	1569	0	2078	2484	0	2783	3485	0	3488	3125	0
669	700	0	1374	1570	0	2079	2485	0	2784	3486	0	3489	3127	0

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670	701	0	1375	1571	0	2080	2503	0	2785	3487	0	3490	3162	0
671	703	0	1376	1572	0	2081	2504	0	2786	3488	0	3491	3171	0
672	704	0	1377	1573	0	2082	2505	0	2787	3489	0	3492	661	0
673	705	0	1378	1587	0	2083	2506	0	2788	3490	0	3493	666	0
674	706	0	1379	1588	0	2084	2507	0	2789	3491	0	3494	1347	0
675	707	0	1380	1589	0	2085	2508	0	2790	3492	0	3495	1349	0
676	708	0	1381	1590	0	2086	2509	0	2791	3493	0	3496	1352	0
677	709	0	1382	1591	0	2087	2510	0	2792	3500	0	3497	1354	0
678	710	0	1383	1592	0	2088	2511	0	2793	3501	0	3498	1746	0
679	711	0	1384	1593	0	2089	2512	0	2794	3502	0	3499	1751	0
680	712	0	1385	1594	0	2090	2513	0	2795	3503	0	3500	1755	0
681	713	0	1386	1596	0	2091	2514	0	2796	3504	0	3501	1793	0
682	714	0	1387	1597	0	2092	2515	0	2797	3505	0	3502	1799	0
683	715	0	1388	1598	0	2093	2516	0	2798	3506	0	3503	1803	0
684	716	0	1389	1599	0	2094	2517	0	2799	3507	0	3504	1807	0
685	717	0	1390	1600	0	2095	2518	0	2800	3508	0	3505	1809	0
686	718	0	1391	1602	0	2096	2519	0	2801	3509	0	3506	3112	0
687	719	0	1392	1603	0	2097	2520	0	2802	3510	0	3507	3114	0
688	720	0	1393	1604	0	2098	2521	0	2803	3511	0	3508	3125	0
689	721	0	1394	1605	0	2099	2522	0	2804	3512	0	3509	3127	0
690	722	0	1395	1606	0	2100	2523	0	2805	3513	0	3510	3162	0
691	723	0	1396	1607	0	2101	2524	0	2806	3514	0	3511	3171	0
692	724	0	1397	1608	0	2102	2525	0	2807	3515	0	3512	661	4
693	725	0	1398	1609	0	2103	2526	0	2808	3516	0	3513	661	10
694	726	0	1399	1610	0	2104	2527	0	2809	3517	0	3514	979	4
695	727	0	1400	1611	0	2105	2528	0	2810	3518	0	3515	979	18
696	728	0	1401	1612	0	2106	2529	0	2811	3519	0	3516	661	4
697	729	0	1402	1613	0	2107	2530	0	2812	3521	0	3517	1349	4

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698	730	0	1403	1614	0	2108	2531	0	2813	3522	0	3518	1352	4
699	732	0	1404	1615	0	2109	2532	0	2814	3523	0	3519	1746	4
700	733	0	1405	1616	0	2110	2533	0	2815	3524	0	3520	1755	4
701	734	0	1406	1617	0	2111	2534	0	2816	3525	0	3521	1803	4
702	735	0	1407	1618	0	2112	2535	0	2817	3526	0	3522	1807	4
703	736	0	1408	1619	0	2113	2536	0	2818	3527	0	3523	3127	4
704	737	0	1409	1620	0	2114	2537	0	2819	3528	0	3524	3162	4
705	738	0	1410	1621	0	2115	2538	0	2820	3529	0	3525	3171	4

For select ASOs, the degree of KCNT1 mRNA knock-down was determined using a taqman quantitative polymerase chain reaction (qPCR assay). Human (BE(2)-M17) or mouse (Neuro2a) neuronal cell lines were grown in 96 well plates and transfected with either 30 nM or 300 nM ASO using RNAiMAX transfection reagent (ThermoFisher Scientific). After 48 hour incubation at 37 °C, cDNA was prepared from each well using the Cell-to-Ct Kit (ThermoFisher Scientific). The expression level of KCNT1 was determined using taqman qPCR assays for either KCNT1 (human Hs01063050_m1 or mouse Mm01330638_g1) or the housekeeping gene HPRT1 (human Hs02800695_m1 or mouse Mm00446968_m1). All taqman assays were pre-designed by ThermoFisher Scientific. Human KCNT1 and HPRT1 detection were multiplexed in a single well. Mouse KCNT1 and HPRT1 detection were singleplexed in paired wells. The fold change in KCNT1 was calculated using the $\Delta\Delta C_p$ method whereby the expression of KCNT1 is first normalized to HPRT1 ($2^{-(C_p_{KCNT1} - C_p_{HPRT1})}$) in the same well followed by a secondary normalization to the vehicle, non-transfected control ($2^{-(C_p_{ASO} - C_p_{vehicle})}$). The assay was performed in biological duplicates and technical triplicates. **Table 3** lists the percent knock down of KCNT1 expressed in human (BE(2)-M17) or mouse (Neuro2a) cells.

Sequences with high homology to human KCNT1 and lower homology to cyno and mouse KCNT1 were identified. The ASO sequences, positions in the specified human transcript, and number of mismatches are shown in Table 7 of U.S. Provisional Application 62/862,328 and Table 11 of U.S. Provisional Application 62/884,567 filed August 8, 2019, hereby incorporated by reference in its entirety. MM indicates the number of mismatches.

For the ASO sequences, homology to non-KCNT1 spliced (secondary) mRNA transcripts were also determined using the NCBI RefSeq R92 (January 2019) and Ensemble R94 (October 2018) databases. Table 8 of U.S. Provisional Application 62/862,328, and Table 12 of U.S. Provisional Application 62/884,567 list the number of non-KCNT1 transcripts identified with increasing number of mismatches from 0MM to 4MM.

For the ASO sequences, homology to non-KCNT1 non-spliced (primary) pre-mRNA transcripts were also determined using the Ensemble R94 (October 2018) databases. Table 9 of U.S. Provisional Application 62/862,328 and Table 13 of U.S. Provisional Application 62/884,567 list the number of non-KCNT1 transcripts identified with increasing number of mismatches from 0MM to 4MM.

For the ASO sequences, the position of reported single nucleotide polymorphisms (SNPs) within the ASO sequence were also determined using the NCBI dbSNP Build 151

(October 2017, downloaded January 2019). Table 10 of U.S. Provisional Application 62/862,328 and Table 14 of U.S. Provisional Application 62/884,567 list the position of each SNP and the associated SNP ID.

5 For the ASO sequences, the level of KCNT1 knock-down were also determined using human (BE(2)-M17) or mouse (Neuro2a) neuronal cell lines . **Table 4** lists the data expressed as percent knock-down.

Table 3: Exemplary ASOs – Percent Knock down of KCNT1 expressed in human (BE(2)-M17) or mouse (Neuro2a) cells

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SEQ ID NO:	BE(2)-M17	BE(2)-M17	Neuro2a	Neuro2a
	3nM ASO	30nM ASO	3nM ASO	30nM ASO
	Mean	Mean	Mean	Mean
1	4	25	3	35
4	7	50	24	62
5	7	47	19	57
6	20	53	5	62
7	3	41	14	56
8	15	43	9	53
10	23	45	27	61
12	9	49	12	54
13	-1	25	-16	8
15	6	37	-2	37

Table 4: Exemplary ASOs – Percent Knock down of KCNT1 expressed in human (BE(2)-M17) or mouse (Neuro2a) cells

SEQ ID NO:	Neuro2a		SEQ ID NO:	BE(2)-M17		SEQ ID NO:	Neuro2a		SEQ ID NO:	BE(2)-M17	
	30nM ASO	Mean		30nM ASO	Mean		30nM ASO	Mean		30nM ASO	Mean
1	34.5		1201			1565	61.5		2530	80	15
4	69.5		1202			1566	51.5		2533	66.5	7
5	68	28	1203			1570	50.5		2534	49.5	
6	75		1205			1573	27.5		2592	32	
7	61	28.5	1206		32.5	1574	58.5		2593	52.5	
8	61.5		1207			1601	59.5		2594	30.5	
9	38.5		1208			1602	55		2595	67.5	29.5
10	69		1209			1968	37		2596	64.5	29
12	64		1210			1973	38		2597	54	
13	8		1243			1974	30.5		2598	52.5	
14	41		1253			1975	35		2606	74.5	
15	50		1255			1976	33		2628	54	
25	46		1302			1977	34.5		2679	64.5	
26	43		1303			2002	41.5		2680	63	
28	70.5		1304			2044	66.5		2681	65	39.5
35	45.5		1335		48.5	2046	60		2784	39.5	
38	67		1337			2050	72		2813	47	
50	28.5		1338			2052	76.5		2901	40.5	

77	67.5			1339	20.5			2053	71		2902	34.5	
80	47.5			1364	22.5			2054	74.5		2903	36.5	
584	22.5			1368	15.5			2055	72.5		2947	44	
587	5			1388	77	39		2236	29		3385	30	
588	52.5			1392	72.5			2294	51		3386	36	
672	23			1402	67			2303	57		3387	53	
675	47			1416	59.5			2304	54		3388	37	
751	69	33		1473	46			2305	53		3389	42.5	
753	34			1502	71	30.5		2307	3.5		3390	41	
756	39.5			1504	74.5			2310	56.5		3391	25	
758	70.5			1505	72			2311	50		3392	30	
759	76	43		1507	75			2319	26		3393	52.5	
988	77.5			1510	78			2320	24		3394	65	
1017	49.5			1538	31.5			2324	44.5		3395	75	42
1018	53.5			1541	49			2327	64		3396	87.5	37.5
1019	58			1542	61			2329	39		3397	79.5	
1021	50			1543	68.5			2346	61		3398	68.5	
1028	57			1544	62			2367	28		3399	70.5	
1045	58			1546	76.5	53.5		2375	69		3400	72.5	
1046	67.5	29		1548	69			2377	60.5		3401	59	
1047	66.5	27		1551	81.5	41		2378	66.5		3402	81.5	40
1071	73.5	48		1554	69.5			2379	67.5		3403	32.5	
1100	44.5			1555	75.5	37		2382	79.5		3404	18.5	
1130	6			1556	76.5			2383	70.5		3405	24.5	
1131	19.5			1558	76.5			2384	66		3406	27.5	
1132	39			1559	89.5	41.5		2392	71		3407	32.5	
1133	21			1560	80.5			2393	67		3408	35	
1134	4			1561	73.5			2394	64.5		3409	35	

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Example 2. Antisense Inhibition of KCNT1

Inhibition or knockdown of KCNT1 can be demonstrated using a cell-based assay. For example, neurons derived from iPSCs, SH-SY5Y cells, or another available mammalian cell line (*e.g.*, CHO cells) can be tested with oligonucleotides targeting KCNT1 identified above in Example 1 using at least five different dose levels, using transfection reagents such as lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. Cells are harvested at multiple time points up to 7 days post transfection for either mRNA or protein analyses. Knockdown of mRNA and protein are determined by RT-qPCR or western blot analyses respectively, using standard molecular biology techniques as previously described (see, for example, as described in Drouet *et al.*, 2014, PLOS One 9(6): e99341). The relative levels of the KCNT1 mRNA and protein at the different oligonucleotide levels are compared with a mock oligonucleotide control. The most potent oligonucleotides (for example, those which are capable of at least 90% reduction, for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% at least 97%, at least 98%, at least 99% or 100%, in protein levels when compared with controls) are selected for subsequent studies.

Example 3. Design, Selection, and Testing of Antisense Oligonucleotides with Modified Chemistries

Selected ASOs in tested in **Example 1** were synthesized with the sugar and linkage chemistries as shown in **Tables 4 and 5**.

For select ASOs, the degree of KCNT1 mRNA knock-down was determined using a taqman quantitative polymerase chain reaction (qPCR assay). Human (BE(2)-M17) neuronal cells line were grown in 96 well plates and transfected with 100 nM ASO using RNAiMAX transfection reagent (ThermoFisher Scientific). After 48 hour incubation at 37°C, cDNA was prepared from each well using the Cell-to-Ct Kit (ThermoFisher Scientific). The expression level of KCNT1 was determined using taqman qPCR assays for either KCNT1 (human Hs01063050_m1 or mouse Mm01330638_g1) or the housekeeping gene HPRT1 (human Hs02800695_m1 or mouse Mm00446968_m1). All taqman assays were predesigned by ThermoFisher Scientific. KCNT1 and HPRT1 detection were multiplexed in a single well. The fold change in KCNT1 was calculated using the $\Delta\Delta C_p$ method whereby the expression of KCNT1 is first normalized to HPRT1 ($2^{-(C_p_{KCNT1}-C_p_{HPRT1})}$) in the same well (multiplexed reaction) followed by a secondary normalization to the vehicle, non-transfected control ($2^{-(C_p_{ASO}-C_p_{vehicle})}$). The assay was performed in biological duplicates and technical triplicates.

Table 5 provides the oligonucleotide ASO sequences, positions in the KCNT1 transcript (NCBI NM_020822.2 (SEQ ID NO: 3526)), and chemistries used to modify the ASO. In the ASO Gap column, “d” is DNA, “e” indicates that ribonucleoside comprising a 2'-modified (*e.g.*, a 2'-O-(2-methoxyethyl) (2'MOE) modified) ribose, and “k” indicates a bicyclic sugar (*e.g.*, locked nucleoside (LNAs), or cET). In the ASO Linkages column, “s” indicates a phosphorothiate linkage and “o” indicates a phosphodiester linkage. In the “ASO Cytosines” column, “None” indicates that all cytosines are unmodified, while “Modified” indicates that all cytosines are 5-Methyl-2'-deoxycytosine (5-Methyl-dC). To create ASO specific negative controls, select ASOs (SEQ ID NOs: 3512-3525) were synthesized using either engineered mismatches (MM) at positions 5, 9, 13, 17 or a scrambled (SC) strategy whereby the original sequences were reordered in blocks of 5. These negative controls are organized by the original binding position on the NM_020822.2.

Table 5: Gap design, linkage chemistry, and cytosine modification of sequences.

SEQ ID NO:	Sequence (5' to 3')	Position	Mismatches to NM_020822.2	ASO Gap Design	ASO Linkages	ASO Cytosines
1	TGATGAAGAACAGCTTGAGC	374	0	eeee-d10-eeee	ssssssssssssssssss	None
4	GTTCGCTTTGTAGCTGAGGT	661	0	eeee-d10-eeee	ssssssssssssssssss	None
5	GGGATGAACAGGTTCCGCAG	765	0	eeee-d10-eeee	ssssssssssssssssss	None
6	AGGATGGCACGGTGGAAGTC	837	0	eeee-d10-eeee	ssssssssssssssssss	None
7	GCAGAAGTAGAAGGAGGTCA	979	0	eeee-d10-eeee	ssssssssssssssssss	None
8	TAGATGACCCGCTGGGACCA	1347	0	eeee-d10-eeee	ssssssssssssssssss	None
9	GTCCATCTTGCTCGCATGA	1399	0	eeee-d10-eeee	ssssssssssssssssss	None
10	GCCGGGCAGATGCAGTTCAG	1629	0	eeee-d10-eeee	ssssssssssssssssss	None
12	GAGCCAGAGAGTAGCTGTCC	2879	0	eeee-d10-eeee	ssssssssssssssssss	None
13	TCACGAAGGACTGGTAGAGC	3008	0	eeee-d10-eeee	ssssssssssssssssss	None
14	TGATGGTGATCATGTAGTCC	3029	0	eeee-d10-eeee	ssssssssssssssssss	None
15	ATGGGGATCTCGGCGCTGGA	3168	0	eeee-d10-eeee	ssssssssssssssssss	None
17	TGTCACCCATGCGGATGTGG	1760	0	eeee-d10-eeee	ssssssssssssssssss	None
25	TGACCACGTAATAGTCCTGG	1271	0	eeee-d10-eeee	ssssssssssssssssss	None

26	ACCTCGTTCCTGCTGCTGAG	1440	0	eeee-d10- eeee	ssssssssssss ssss	None
28	ATGCGGATGTGGTACACCTC	1752	0	eeee-d10- eeee	ssssssssssss ssss	None
29	GAAGCTCTTGCCCTCGTACT	1795	0	eeee-d10- eeee	ssssssssssss ssss	None
35	TTGGCGCGGAACCTGCATGAA	2859	0	eeee-d10- eeee	ssssssssssss ssss	None
38	GGTCTTGGCGTCCGCCATGT	2758	0	eeee-d10- eeee	ssssssssssss ssss	None
50	CGGATGAGATAGACAATGTC	3654	0	eeee-d10- eeee	ssssssssssss ssss	None
62	CGCGGAAGAACTTGCTGTCA	1775	0	eeee-d10- eeee	ssssssssssss ssss	None
77	TGGCGTACTTGCACTCCTCC	1601	0	eeee-d10- eeee	ssssssssssss ssss	None
80	GTGCGGTCCACCTCGTTCCT	1449	0	eeee-d10- eeee	ssssssssssss ssss	None
584	ATTATGGCCACGATGACCTG	612	0	eeee-d10- eeee	ssssssssssss ssss	None
587	CTTATTATGGCCACGATGAC	615	0	eeee-d10- eeee	ssssssssssss ssss	None
588	GCTTATTATGGCCACGATGA	616	0	eeee-d10- eeee	ssssssssssss ssss	None
625	TTTGTAGCTGAGGTAGATGA	655	0	eeee-d10- eeee	ssssssssssss ssss	None
626	CTTTGTAGCTGAGGTAGATG	656	0	eeee-d10- eeee	ssssssssssss ssss	None
627	CCTTTGTAGCTGAGGTAGAT	657	0	eeee-d10- eeee	ssssssssssss ssss	None
628	GCCTTTGTAGCTGAGGTAGA	658	0	eeee-d10- eeee	ssssssssssss ssss	None
629	TGCCTTTGTAGCTGAGGTAG	659	0	eeee-d10- eeee	ssssssssssss ssss	None
630	TTGCCTTTGTAGCTGAGGTA	660	0	eeee-d10- eeee	ssssssssssss ssss	None
631	TGTTCCTTTGTAGCTGAGG	662	0	eeee-d10- eeee	ssssssssssss ssss	None
632	ATGTTGCCTTTGTAGCTGAG	663	0	eeee-d10- eeee	ssssssssssss ssss	None
633	GATGTTGCCTTTGTAGCTGA	664	0	eeee-d10- eeee	ssssssssssss ssss	None
634	AGATGTTGCCTTTGTAGCTG	665	0	eeee-d10- eeee	ssssssssssss ssss	None
635	CAGATGTTGCCTTTGTAGCT	666	0	eeee-d10- eeee	ssssssssssss ssss	None
636	CCAGATGTTGCCTTTGTAGC	667	0	eeee-d10- eeee	ssssssssssss ssss	None
637	CCCAGATGTTGCCTTTGTAG	668	0	eeee-d10- eeee	ssssssssssss ssss	None
638	TCCCAGATGTTGCCTTTGTA	669	0	eeee-d10- eeee	ssssssssssss ssss	None
639	CTCCCAGATGTTGCCTTTGT	670	0	eeee-d10- eeee	ssssssssssss ssss	None
640	GCTCCCAGATGTTGCCTTTG	671	0	eeee-d10- eeee	ssssssssssss ssss	None
641	TGCTCCCAGATGTTGCCTTT	672	0	eeee-d10- eeee	ssssssssssss ssss	None

642	CTGCTCCCAGATGTTGCCTT	673	0	eeee-d10- eeee	ssssssssssss ssss	None
643	TCTGCTCCCAGATGTTGCCT	674	0	eeee-d10- eeee	ssssssssssss ssss	None
644	ATCTGCTCCCAGATGTTGCC	675	0	eeee-d10- eeee	ssssssssssss ssss	None
645	GATCTGCTCCCAGATGTTGC	676	0	eeee-d10- eeee	ssssssssssss ssss	None
646	AGATCTGCTCCCAGATGTTG	677	0	eeee-d10- eeee	ssssssssssss ssss	None
647	AAGATCTGCTCCCAGATGTT	678	0	eeee-d10- eeee	ssssssssssss ssss	None
648	GAAGATCTGCTCCCAGATGT	679	0	eeee-d10- eeee	ssssssssssss ssss	None
649	GGAAGATCTGCTCCCAGATG	680	0	eeee-d10- eeee	ssssssssssss ssss	None
672	TCATCTCCAGGACGAAGGAC	704	0	eeee-d10- eeee	ssssssssssss ssss	None
675	TGATCATCTCCAGGACGAAG	707	0	eeee-d10- eeee	ssssssssssss ssss	None
751	TTCCAGCGCGTGCTTGCCA	805	0	eeee-d10- eeee	ssssssssssss ssssss	None
753	TTTCCAGCGCGTGCTTGGC	807	0	eeee-d10- eeee	ssssssssssss ssssss	None
756	ATGTTTTCCAGCGCGTGCTT	810	0	eeee-d10- eeee	ssssssssssss ssssss	None
758	TCATGTTTTCCAGCGCGTGC	812	0	eeee-d10- eeee	ssssssssssss ssssss	None
759	ATCATGTTTTCCAGCGCGTG	813	0	eeee-d10- eeee	ssssssssssss ssssss	None
988	CTGCAGTGGGAGCACCACGA	1090	0	eeee-d10- eeee	ssssssssssss ssssss	None
1017	CTCCATCCAGAGGTAGACGA	1120	0	eeee-d10- eeee	ssssssssssss ssssss	None
1018	GCTCCATCCAGAGGTAGACG	1121	0	eeee-d10- eeee	ssssssssssss ssssss	None
1019	CGCTCCATCCAGAGGTAGAC	1122	0	eeee-d10- eeee	ssssssssssss ssssss	None
1021	GCCGCTCCATCCAGAGGTAG	1124	0	eeee-d10- eeee	ssssssssssss ssssss	None
1028	GACTTCTGCCGCTCCATCCA	1131	0	eeee-d10- eeee	ssssssssssss ssssss	None
1045	GGCTGTAGTTGCCCCCTGAC	1148	0	eeee-d10- eeee	ssssssssssss ssssss	None
1046	CGGCTGTAGTTGCCCCCTGA	1149	0	eeee-d10- eeee	ssssssssssss ssssss	None
1047	GCGGCTGTAGTTGCCCCCTG	1150	0	eeee-d10- eeee	ssssssssssss ssssss	None
1071	ACGTGCTTCTCCGTCTGCGC	1176	0	eeee-d10- eeee	ssssssssssss ssssss	None
1100	TCGATCTTGAGGGAGCTGAC	1206	0	eeee-d10- eeee	ssssssssssss ssssss	None
1130	CGTAGAACTCGTTCAGGAAG	1238	0	eeee-d10- eeee	ssssssssssss ssssss	None
1131	GCGTAGAACTCGTTCAGGAA	1239	0	eeee-d10- eeee	ssssssssssss ssssss	None
1132	GGCGTAGAACTCGTTCAGGA	1240	0	eeee-d10- eeee	ssssssssssss ssssss	None

1133	GGGCGTAGAACTCGTTCAGG	1241	0	eeee-d10- eeee	ssssssssssss ssssss	None
1134	TGGGCGTAGAACTCGTTCAG	1242	0	eeee-d10- eeee	ssssssssssss ssssss	None
1145	ATGACCACGTAATAGTCCTG	1272	0	eeee-d10- eeee	ssssssssssss ssssss	None
1146	GATGACCACGTAATAGTCCT	1273	0	eeee-d10- eeee	ssssssssssss ssssss	None
1154	GGGCACAGGATGACCACGTA	1281	0	eeee-d10- eeee	ssssssssssss ssssss	None
1195	CCCGCTGGGACCACAGAGGG	1340	0	eeee-d10- eeee	ssssssssssss ssss	None
1196	ACCCGCTGGGACCACAGAGG	1341	0	eeee-d10- eeee	ssssssssssss ssss	None
1197	GACCCGCTGGGACCACAGAG	1342	0	eeee-d10- eeee	ssssssssssss ssss	None
1198	TGACCCGCTGGGACCACAGA	1343	0	eeee-d10- eeee	ssssssssssss ssss	None
1199	ATGACCCGCTGGGACCACAG	1344	0	eeee-d10- eeee	ssssssssssss ssss	None
1200	GATGACCCGCTGGGACCACA	1345	0	eeee-d10- eeee	ssssssssssss ssss	None
1201	AGATGACCCGCTGGGACCAC	1346	0	eeee-d10- eeee	ssssssssssss ssss	None
1202	GATGATGACCCGCTGGGACC	1348	0	eeee-d10- eeee	ssssssssssss ssss	None
1203	GGTAGATGACCCGCTGGGAC	1349	0	eeee-d10- eeee	ssssssssssss ssss	None
1204	AGGTAGATGACCCGCTGGGA	1350	0	eeee-d10- eeee	ssssssssssss ssss	None
1205	GAGGTAGATGACCCGCTGGG	1351	0	eeee-d10- eeee	ssssssssssss ssss	None
1206	GGAGGTAGATGACCCGCTGG	1352	0	eeee-d10- eeee	ssssssssssss ssss	None
1207	TGGAGGTAGATGACCCGCTG	1353	0	eeee-d10- eeee	ssssssssssss ssss	None
1208	CTGGAGGTAGATGACCCGCT	1354	0	eeee-d10- eeee	ssssssssssss ssss	None
1209	CCTGGAGGTAGATGACCCGC	1355	0	eeee-d10- eeee	ssssssssssss ssss	None
1210	CCCTGGAGGTAGATGACCCG	1356	0	eeee-d10- eeee	ssssssssssss ssss	None
1211	GCCCTGGAGGTAGATGACCC	1357	0	eeee-d10- eeee	ssssssssssss ssss	None
1212	AGCCCTGGAGGTAGATGACC	1358	0	eeee-d10- eeee	ssssssssssss ssss	None
1213	GAGCCCTGGAGGTAGATGAC	1359	0	eeee-d10- eeee	ssssssssssss ssss	None
1214	AGAGCCCTGGAGGTAGATGA	1360	0	eeee-d10- eeee	ssssssssssss ssss	None
1215	CAGAGCCCTGGAGGTAGATG	1361	0	eeee-d10- eeee	ssssssssssss ssss	None
1216	GCAGAGCCCTGGAGGTAGAT	1362	0	eeee-d10- eeee	ssssssssssss ssss	None
1217	TGCAGAGCCCTGGAGGTAGA	1363	0	eeee-d10- eeee	ssssssssssss ssss	None
1218	GTGCAGAGCCCTGGAGGTAG	1364	0	eeee-d10- eeee	ssssssssssss ssss	None

1219	AGTGCAGAGCCCTGGAGGTA	1365	0	eeee-d10- eeee	ssssssssssss ssss	None
1220	GAGTGCAGAGCCCTGGAGGT	1366	0	eeee-d10- eeee	ssssssssssss ssss	None
1221	TGAGTGCAGAGCCCTGGAGG	1367	0	eeee-d10- eeee	ssssssssssss ssss	None
1222	TTGAGTGCAGAGCCCTGGAG	1368	0	eeee-d10- eeee	ssssssssssss ssss	None
1223	TTTGAGTGCAGAGCCCTGGA	1369	0	eeee-d10- eeee	ssssssssssss ssss	None
1224	CTTTGAGTGCAGAGCCCTGG	1370	0	eeee-d10- eeee	ssssssssssss ssss	None
1243	GCTCGCATGAGGTCCTGGTC	1389	0	eeee-d10- eeee	ssssssssssss ssssss	None
1253	TGTCCATCTTGGCTCGCATG	1400	0	eeee-d10- eeee	ssssssssssss ssssss	None
1255	ATTGTCCATCTTGGCTCGCA	1402	0	eeee-d10- eeee	ssssssssssss ssssss	None
1302	CCGTGCGGTCCACCTCGTTC	1451	0	eeee-d10- eeee	ssssssssssss ssssss	None
1303	GCCGTGCGGTCCACCTCGTT	1452	0	eeee-d10- eeee	ssssssssssss ssssss	None
1304	AGCCGTGCGGTCCACCTCGT	1453	0	eeee-d10- eeee	ssssssssssss ssssss	None
1335	GCGAAGTCCTTCACGGCCCA	1500	0	eeee-d10- eeee	ssssssssssss ssssss	None
1337	GGGCGAAGTCCTTCACGGCC	1502	0	eeee-d10- eeee	ssssssssssss ssssss	None
1338	GAGGATCTGGACGTAGAGGG	1531	0	eeee-d10- eeee	ssssssssssss ssssss	None
1339	TTGAGGATCTGGACGTAGAG	1533	0	eeee-d10- eeee	ssssssssssss ssssss	None
1364	AACTTGACGTGAACTTGTT	1560	0	eeee-d10- eeee	ssssssssssss ssssss	None
1368	AGCAAACCTTGACGTGAACT	1564	0	eeee-d10- eeee	ssssssssssss ssssss	None
1388	CGTACTTGCACTCCTCCTCA	1598	0	eeee-d10- eeee	ssssssssssss ssssss	None
1392	CATGGCGTACTTGCACTCCT	1603	0	eeee-d10- eeee	ssssssssssss ssssss	None
1402	TCAGCGCCAGCATGGCGTAC	1613	0	eeee-d10- eeee	ssssssssssss ssssss	None
1416	CGGGCAGATGCAGTTCAGCG	1627	0	eeee-d10- eeee	ssssssssssss ssssss	None
1473	CCATACATGCGCTGCCACTG	1716	0	eeee-d10- eeee	ssssssssssss ssssss	None
1496	TACACCTCGTTGCCGGAGCA	1740	0	eeee-d10- eeee	ssssssssssss ssss	None
1497	GTACACCTCGTTGCCGGAGC	1741	0	eeee-d10- eeee	ssssssssssss ssss	None
1498	GGTACACCTCGTTGCCGGAG	1742	0	eeee-d10- eeee	ssssssssssss ssss	None
1499	TGGTACACCTCGTTGCCGGA	1743	0	eeee-d10- eeee	ssssssssssss ssss	None
1500	GTGGTACACCTCGTTGCCGG	1744	0	eeee-d10- eeee	ssssssssssss ssss	None
1501	TGTGGTACACCTCGTTGCCG	1745	0	eeee-d10- eeee	ssssssssssss ssss	None

1502	ATGTGGTACACCTCGTTGCC	1746	0	eeee-d10- eeee	ssssssssssss ssss	None
1503	GATGTGGTACACCTCGTTGC	1747	0	eeee-d10- eeee	ssssssssssss ssss	None
1504	GGATGTGGTACACCTCGTTG	1748	0	eeee-d10- eeee	ssssssssssss ssss	None
1505	CGGATGTGGTACACCTCGTT	1749	0	eeee-d10- eeee	ssssssssssss ssss	None
1506	GCGGATGTGGTACACCTCGT	1750	0	eeee-d10- eeee	ssssssssssss ssss	None
1507	TGCGGATGTGGTACACCTCG	1751	0	eeee-d10- eeee	ssssssssssss ssss	None
1508	CATGCGGATGTGGTACACCT	1753	0	eeee-d10- eeee	ssssssssssss ssss	None
1509	CCATGCGGATGTGGTACACC	1754	0	eeee-d10- eeee	ssssssssssss ssss	None
1510	CCCATGCGGATGTGGTACAC	1755	0	eeee-d10- eeee	ssssssssssss ssss	None
1511	ACCCATGCGGATGTGGTACA	1756	0	eeee-d10- eeee	ssssssssssss ssss	None
1512	CACCCATGCGGATGTGGTAC	1757	0	eeee-d10- eeee	ssssssssssss ssss	None
1513	TCACCCATGCGGATGTGGTA	1758	0	eeee-d10- eeee	ssssssssssss ssss	None
1514	GTCACCCATGCGGATGTGGT	1759	0	eeee-d10- eeee	ssssssssssss ssss	None
1515	CTGTCACCCATGCGGATGTG	1761	0	eeee-d10- eeee	ssssssssssss ssss	None
1516	GCTGTCACCCATGCGGATGT	1762	0	eeee-d10- eeee	ssssssssssss ssss	None
1517	TGCTGTCACCCATGCGGATG	1763	0	eeee-d10- eeee	ssssssssssss ssss	None
1518	TTGCTGTCACCCATGCGGAT	1764	0	eeee-d10- eeee	ssssssssssss ssss	None
1519	CTTGCTGTCACCCATGCGGA	1765	0	eeee-d10- eeee	ssssssssssss ssss	None
1520	ACTTGCTGTCACCCATGCGG	1766	0	eeee-d10- eeee	ssssssssssss ssss	None
1521	AACTTGCTGTCACCCATGCG	1767	0	eeee-d10- eeee	ssssssssssss ssss	None
1522	GAAGCTTGCTGTCACCCATGC	1768	0	eeee-d10- eeee	ssssssssssss ssss	None
1523	AGAAGCTTGCTGTCACCCATG	1769	0	eeee-d10- eeee	ssssssssssss ssss	None
1524	AAGAAGCTTGCTGTCACCCAT	1770	0	eeee-d10- eeee	ssssssssssss ssss	None
1525	GAAGAAGCTTGCTGTCACCCA	1771	0	eeee-d10- eeee	ssssssssssss ssss	None
1526	GGAAGAAGCTTGCTGTCACCC	1772	0	eeee-d10- eeee	ssssssssssss ssss	None
1527	CGGAAGAAGCTTGCTGTCACC	1773	0	eeee-d10- eeee	ssssssssssss ssss	None
1528	GCGGAAGAAGCTTGCTGTCAC	1774	0	eeee-d10- eeee	ssssssssssss ssss	None
1529	TCGCGGAAGAAGCTTGCTGTC	1776	0	eeee-d10- eeee	ssssssssssss ssss	None
1530	CTCGCGGAAGAAGCTTGCTGT	1777	0	eeee-d10- eeee	ssssssssssss ssss	None

1531	ACTCGCGGAAGAACTTGCTG	1778	0	eeee-d10- eeee	ssssssssssss ssss	None
1532	TACTCGCGGAAGAACTTGCT	1779	0	eeee-d10- eeee	ssssssssssss ssss	None
1533	GTACTCGCGGAAGAACTTGC	1780	0	eeee-d10- eeee	ssssssssssss ssss	None
1534	CGTACTCGCGGAAGAACTTG	1781	0	eeee-d10- eeee	ssssssssssss ssss	None
1535	TCGTACTCGCGGAAGAACTT	1782	0	eeee-d10- eeee	ssssssssssss ssss	None
1536	CTCGTACTCGCGGAAGAACT	1783	0	eeee-d10- eeee	ssssssssssss ssss	None
1537	CCTCGTACTCGCGGAAGAAC	1784	0	eeee-d10- eeee	ssssssssssss ssss	None
1538	CCCTCGTACTCGCGGAAGAA	1785	0	eeee-d10- eeee	ssssssssssss ssss	None
1539	GCCCTCGTACTCGCGGAAGA	1786	0	eeee-d10- eeee	ssssssssssss ssss	None
1540	TGCCCTCGTACTCGCGGAAG	1787	0	eeee-d10- eeee	ssssssssssss ssss	None
1541	TTGCCCTCGTACTCGCGGAA	1788	0	eeee-d10- eeee	ssssssssssss ssss	None
1542	CTTGCCCTCGTACTCGCGGA	1789	0	eeee-d10- eeee	ssssssssssss ssss	None
1543	TCTTGCCCTCGTACTCGCGG	1790	0	eeee-d10- eeee	ssssssssssss ssss	None
1544	CTCTTGCCCTCGTACTCGCG	1791	0	eeee-d10- eeee	ssssssssssss ssss	None
1545	GCTCTTGCCCTCGTACTCGC	1792	0	eeee-d10- eeee	ssssssssssss ssss	None
1546	AGCTCTTGCCCTCGTACTCG	1793	0	eeee-d10- eeee	ssssssssssss ssss	None
1547	AAGCTCTTGCCCTCGTACTC	1794	0	eeee-d10- eeee	ssssssssssss ssss	None
1548	TGAAGCTCTTGCCCTCGTAC	1796	0	eeee-d10- eeee	ssssssssssss ssss	None
1549	GTGAAGCTCTTGCCCTCGTA	1797	0	eeee-d10- eeee	ssssssssssss ssss	None
1550	GGTGAAGCTCTTGCCCTCGT	1798	0	eeee-d10- eeee	ssssssssssss ssss	None
1551	AGGTGAAGCTCTTGCCCTCG	1799	0	eeee-d10- eeee	ssssssssssss ssss	None
1552	TAGGTGAAGCTCTTGCCCTC	1800	0	eeee-d10- eeee	ssssssssssss ssss	None
1553	GTAGGTGAAGCTCTTGCCCT	1801	0	eeee-d10- eeee	ssssssssssss ssss	None
1554	CGTAGGTGAAGCTCTTGCCC	1802	0	eeee-d10- eeee	ssssssssssss ssss	None
1555	GCGTAGGTGAAGCTCTTGCC	1803	0	eeee-d10- eeee	ssssssssssss ssss	None
1556	CGCGTAGGTGAAGCTCTTGC	1804	0	eeee-d10- eeee	ssssssssssss ssss	None
1557	CCGCGTAGGTGAAGCTCTTG	1805	0	eeee-d10- eeee	ssssssssssss ssss	None
1558	GCCGCGTAGGTGAAGCTCTT	1806	0	eeee-d10- eeee	ssssssssssss ssss	None
1559	GGCCGCGTAGGTGAAGCTCT	1807	0	eeee-d10- eeee	ssssssssssss ssss	None

1560	AGGCCGCGTAGGTGAAGCTC	1808	0	eeee-d10- eeee	ssssssssssss ssss	None
1561	AAGGCCGCGTAGGTGAAGCT	1809	0	eeee-d10- eeee	ssssssssssss ssss	None
1562	GAAGGCCGCGTAGGTGAAGC	1810	0	eeee-d10- eeee	ssssssssssss ssss	None
1563	GGAAGGCCGCGTAGGTGAAG	1811	0	eeee-d10- eeee	ssssssssssss ssss	None
1564	TGGAAGGCCGCGTAGGTGAA	1812	0	eeee-d10- eeee	ssssssssssss ssss	None
1565	GTGAAGGCCGCGTAGGTGA	1813	0	eeee-d10- eeee	ssssssssssss ssss	None
1566	CGTGGAAGGCCGCGTAGGTG	1814	0	eeee-d10- eeee	ssssssssssss ssss	None
1567	GCGTGGAAGGCCGCGTAGGT	1815	0	eeee-d10- eeee	ssssssssssss ssss	None
1570	TGGGCGTGGAAGGCCGCGTA	1818	0	eeee-d10- eeee	ssssssssssss ssssss	None
1573	TTGTGGGCGTGGAAGGCCGC	1821	0	eeee-d10- eeee	ssssssssssss ssssss	None
1574	CTTGTGGGCGTGGAAGGCCG	1822	0	eeee-d10- eeee	ssssssssssss ssssss	None
1601	TCAGCCCGATGAGGCACACG	1850	0	eeee-d10- eeee	ssssssssssss ssssss	None
1602	TTCAGCCCGATGAGGCACAC	1851	0	eeee-d10- eeee	ssssssssssss ssssss	None
1968	ATGTAGGGCGAGTTGGGAGG	2334	0	eeee-d10- eeee	ssssssssssss ssssss	None
1973	TGCCGATGTAGGGCGAGTTG	2339	0	eeee-d10- eeee	ssssssssssss ssssss	None
1974	CTGCCGATGTAGGGCGAGTT	2340	0	eeee-d10- eeee	ssssssssssss ssssss	None
1975	GCTGCCGATGTAGGGCGAGT	2341	0	eeee-d10- eeee	ssssssssssss ssssss	None
1976	AGCTGCCGATGTAGGGCGAG	2342	0	eeee-d10- eeee	ssssssssssss ssssss	None
1977	GAGCTGCCGATGTAGGGCGA	2343	0	eeee-d10- eeee	ssssssssssss ssssss	None
2002	TTGTCCAGCCGCAGGCAGCA	2403	0	eeee-d10- eeee	ssssssssssss ssssss	None
2044	AACCCGTAGGCCTTGCGTC	2448	0	eeee-d10- eeee	ssssssssssss ssssss	None
2046	TGAACCCGTAGGCCTTGCG	2450	0	eeee-d10- eeee	ssssssssssss ssssss	None
2050	TTCTTGAACCCGTAGGCCTT	2454	0	eeee-d10- eeee	ssssssssssss ssssss	None
2052	TGTTCTTGAACCCGTAGGCC	2456	0	eeee-d10- eeee	ssssssssssss ssssss	None
2053	CTTGTCTTGAACCCGTAGG	2458	0	eeee-d10- eeee	ssssssssssss ssssss	None
2054	GCTTGTCTTGAACCCGTAG	2459	0	eeee-d10- eeee	ssssssssssss ssssss	None
2055	AGCTTGTCTTGAACCCGTA	2460	0	eeee-d10- eeee	ssssssssssss ssssss	None
2236	AGGTTGTCCGCATAGATGAT	2694	0	eeee-d10- eeee	ssssssssssss ssssss	None
2294	GGCGTCCGCCATGTAGTCCT	2752	0	eeee-d10- eeee	ssssssssssss ssssss	None

2303	CGATGGTCTTGGCGTCCGCC	2762	0	eeee-d10- eeee	ssssssssssss ssssss	None
2304	ACGATGGTCTTGGCGTCCGC	2763	0	eeee-d10- eeee	ssssssssssss ssssss	None
2305	GACGATGGTCTTGGCGTCCG	2764	0	eeee-d10- eeee	ssssssssssss ssssss	None
2307	TTGACGATGGTCTTGGCGTC	2766	0	eeee-d10- eeee	ssssssssssss ssssss	None
2310	ACGTTGACGATGGTCTTGGC	2769	0	eeee-d10- eeee	ssssssssssss ssssss	None
2311	CACGTTGACGATGGTCTTGG	2770	0	eeee-d10- eeee	ssssssssssss ssssss	None
2319	CATGGTCTGCACGTTGACGA	2779	0	eeee-d10- eeee	ssssssssssss ssssss	None
2320	ACATGGTCTGCACGTTGACG	2780	0	eeee-d10- eeee	ssssssssssss ssssss	None
2324	CGGAACATGGTCTGCACGTT	2784	0	eeee-d10- eeee	ssssssssssss ssssss	None
2327	AGCCGGAACATGGTCTGCAC	2787	0	eeee-d10- eeee	ssssssssssss ssssss	None
2329	AGAGCCGGAACATGGTCTGC	2789	0	eeee-d10- eeee	ssssssssssss ssssss	None
2346	TGGGTGAGCTCCGTGGTGAT	2823	0	eeee-d10- eeee	ssssssssssss ssssss	None
2367	CATGAAGCGCATGTTGGAAG	2845	0	eeee-d10- eeee	ssssssssssss ssssss	None
2375	CGGAAGTGCATGAAGCGCAT	2853	0	eeee-d10- eeee	ssssssssssss ssssss	None
2377	CGCGGAAGTGCATGAAGCGC	2855	0	eeee-d10- eeee	ssssssssssss ssssss	None
2378	GCGCGGAAGTGCATGAAGCG	2856	0	eeee-d10- eeee	ssssssssssss ssssss	None
2379	GGCGCGGAAGTGCATGAAGC	2857	0	eeee-d10- eeee	ssssssssssss ssssss	None
2382	CCTTGGCGCGGAAGTGCATG	2861	0	eeee-d10- eeee	ssssssssssss ssssss	None
2383	TCCTTGGCGCGGAAGTGCAT	2862	0	eeee-d10- eeee	ssssssssssss ssssss	None
2384	GTCTTGGCGCGGAAGTGCA	2863	0	eeee-d10- eeee	ssssssssssss ssssss	None
2392	GAGTAGCTGTCCTTGGCGCG	2871	0	eeee-d10- eeee	ssssssssssss ssssss	None
2393	AGAGTAGCTGTCCTTGGCGC	2872	0	eeee-d10- eeee	ssssssssssss ssssss	None
2394	GAGAGTAGCTGTCCTTGGCG	2873	0	eeee-d10- eeee	ssssssssssss ssssss	None
2505	CACGAAGGACTGGTAGAGCA	3007	0	eeee-d10- eeee	ssssssssssss ssssss	None
2525	GATGGTGATCATGTAGTCCT	3028	0	eeee-d10- eeee	ssssssssssss ssssss	None
2529	CGGGTGATGGTGATCATGTA	3033	0	eeee-d10- eeee	ssssssssssss ssssss	None
2530	CCGGTGATGGTGATCATGT	3034	0	eeee-d10- eeee	ssssssssssss ssssss	None
2533	CAGCCGGGTGATGGTGATCA	3037	0	eeee-d10- eeee	ssssssssssss ssssss	None
2534	GCAGCCGGGTGATGGTGATC	3038	0	eeee-d10- eeee	ssssssssssss ssssss	None

2591	TCCACAGGTCGCCCTCGGTG	3110	0	eeee-d10- eeee	ssssssssssss ssss	None
2592	ATCCACAGGTCGCCCTCGGT	3111	0	eeee-d10- eeee	ssssssssssss ssss	None
2593	GATCCACAGGTCGCCCTCGG	3112	0	eeee-d10- eeee	ssssssssssss ssss	None
2594	GGATCCACAGGTCGCCCTCG	3113	0	eeee-d10- eeee	ssssssssssss ssss	None
2595	CGGATCCACAGGTCGCCCTC	3114	0	eeee-d10- eeee	ssssssssssss ssss	None
2596	GCGGATCCACAGGTCGCCCT	3115	0	eeee-d10- eeee	ssssssssssss ssss	None
2597	TGCGGATCCACAGGTCGCC	3116	0	eeee-d10- eeee	ssssssssssss ssss	None
2598	GTGCGGATCCACAGGTCGCC	3117	0	eeee-d10- eeee	ssssssssssss ssss	None
2599	CGTGCGGATCCACAGGTCGC	3118	0	eeee-d10- eeee	ssssssssssss ssss	None
2600	ACGTGCGGATCCACAGGTCG	3119	0	eeee-d10- eeee	ssssssssssss ssss	None
2601	TACGTGCGGATCCACAGGTC	3120	0	eeee-d10- eeee	ssssssssssss ssss	None
2602	GTACGTGCGGATCCACAGGT	3121	0	eeee-d10- eeee	ssssssssssss ssss	None
2603	CGTACGTGCGGATCCACAGG	3122	0	eeee-d10- eeee	ssssssssssss ssss	None
2604	CCGTACGTGCGGATCCACAG	3123	0	eeee-d10- eeee	ssssssssssss ssss	None
2605	GCCGTACGTGCGGATCCACA	3124	0	eeee-d10- eeee	ssssssssssss ssss	None
2606	GGCCGTACGTGCGGATCCAC	3125	0	eeee-d10- eeee	ssssssssssss ssss	None
2607	AGCTTCTGGAAGAGGCGGCC	3141	0	eeee-d10- eeee	ssssssssssss ssss	None
2608	GAGCTTCTGGAAGAGGCGGC	3142	0	eeee-d10- eeee	ssssssssssss ssss	None
2609	AGAGCTTCTGGAAGAGGCGG	3143	0	eeee-d10- eeee	ssssssssssss ssss	None
2610	CAGAGCTTCTGGAAGAGGCG	3144	0	eeee-d10- eeee	ssssssssssss ssss	None
2611	GCAGAGCTTCTGGAAGAGGC	3145	0	eeee-d10- eeee	ssssssssssss ssss	None
2612	AGCAGAGCTTCTGGAAGAGG	3146	0	eeee-d10- eeee	ssssssssssss ssss	None
2613	GAGCAGAGCTTCTGGAAGAG	3147	0	eeee-d10- eeee	ssssssssssss ssss	None
2614	GGAGCAGAGCTTCTGGAAGA	3148	0	eeee-d10- eeee	ssssssssssss ssss	None
2615	AGGAGCAGAGCTTCTGGAAG	3149	0	eeee-d10- eeee	ssssssssssss ssss	None
2616	GAGGAGCAGAGCTTCTGGA	3150	0	eeee-d10- eeee	ssssssssssss ssss	None
2617	GGAGGAGCAGAGCTTCTGGA	3151	0	eeee-d10- eeee	ssssssssssss ssss	None
2618	TGGAGGAGCAGAGCTTCTGG	3152	0	eeee-d10- eeee	ssssssssssss ssss	None
2619	CTGGAGGAGCAGAGCTTCTG	3153	0	eeee-d10- eeee	ssssssssssss ssss	None

2620	GCTGGAGGAGCAGAGCTTCT	3154	0	eeee-d10- eeee	ssssssssssss ssss	None
2621	CGCTGGAGGAGCAGAGCTTC	3155	0	eeee-d10- eeee	ssssssssssss ssss	None
2622	GCGCTGGAGGAGCAGAGCTT	3156	0	eeee-d10- eeee	ssssssssssss ssss	None
2623	GGCGCTGGAGGAGCAGAGCT	3157	0	eeee-d10- eeee	ssssssssssss ssss	None
2624	CGGCGCTGGAGGAGCAGAGC	3158	0	eeee-d10- eeee	ssssssssssss ssss	None
2625	TCGGCGCTGGAGGAGCAGAG	3159	0	eeee-d10- eeee	ssssssssssss ssss	None
2626	CTCGGCGCTGGAGGAGCAGA	3160	0	eeee-d10- eeee	ssssssssssss ssss	None
2627	TCTCGGCGCTGGAGGAGCAG	3161	0	eeee-d10- eeee	ssssssssssss ssss	None
2628	ATCTCGGCGCTGGAGGAGCA	3162	0	eeee-d10- eeee	ssssssssssss ssss	None
2629	GATCTCGGCGCTGGAGGAGC	3163	0	eeee-d10- eeee	ssssssssssss ssss	None
2630	GGATCTCGGCGCTGGAGGAG	3164	0	eeee-d10- eeee	ssssssssssss ssss	None
2631	GGGATCTCGGCGCTGGAGGA	3165	0	eeee-d10- eeee	ssssssssssss ssss	None
2679	GTTACCGAGATCTGGGACT	3250	0	eeee-d10- eeee	ssssssssssss ssssss	None
2680	CGTTCACCGAGATCTGGGAC	3251	0	eeee-d10- eeee	ssssssssssss ssssss	None
2681	ACGTTACCGAGATCTGGGA	3252	0	eeee-d10- eeee	ssssssssssss ssssss	None
2784	GAGCGCCGGTACAGGCTGAG	3486	0	eeee-d10- eeee	ssssssssssss ssssss	None
2813	CGGTTCTTACCGAGCTCGGA	3522	0	eeee-d10- eeee	ssssssssssss ssssss	None
2901	TCGGGCGGAGGGTTGATGAG	3615	0	eeee-d10- eeee	ssssssssssss ssssss	None
2902	GTCGGGCGGAGGGTTGATGA	3616	0	eeee-d10- eeee	ssssssssssss ssssss	None
2903	TGTCGGGCGGAGGGTTGATG	3617	0	eeee-d10- eeee	ssssssssssss ssssss	None
2947	GGGTCGGAGCGGATGAGATA	3663	0	eeee-d10- eeee	ssssssssssss ssssss	None
3385	CGGGGGTGGGCGTAGAACTC	1248	0	eeee-d10- eeee	ssssssssssss ssssss	None
3386	CCGGGGGTGGGCGTAGAACT	1249	0	eeee-d10- eeee	ssssssssssss ssssss	None
3387	GGGGCGAAGTCCTTCACGGC	1503	0	eeee-d10- eeee	ssssssssssss ssssss	None
3388	GGGGCGAAGTCCTTCACGG	1504	0	eeee-d10- eeee	ssssssssssss ssssss	None
3389	TGGGGCGAAGTCCTTCACG	1505	0	eeee-d10- eeee	ssssssssssss ssssss	None
3390	TTGGGGCGAAGTCCTTCAC	1506	0	eeee-d10- eeee	ssssssssssss ssssss	None
3391	GGCAGTTGGGGCGAAGTCC	1511	0	eeee-d10- eeee	ssssssssssss ssssss	None
3392	GGCAGTTGGGGCGAAGTC	1512	0	eeee-d10- eeee	ssssssssssss ssssss	None

3393	AGGATCTGGACGTAGAGGGG	1530	0	eeee-d10- eeee	ssssssssssss ssssss	None
3394	GTAGTAGACCATGGGGAAGC	2629	0	eeee-d10- eeee	ssssssssssss ssssss	None
3395	CGGCCGTACGTGCGGATCCA	3126	0	eeee-d10- eeee	ssssssssssss ssss	None
3396	GCGGCCGTACGTGCGGATCC	3127	0	eeee-d10- eeee	ssssssssssss ssss	None
3397	GGCGGCCGTACGTGCGGATC	3128	0	eeee-d10- eeee	ssssssssssss ssss	None
3398	AGGCGGCCGTACGTGCGGAT	3129	0	eeee-d10- eeee	ssssssssssss ssss	None
3399	GAGCGGCCGTACGTGCGGA	3130	0	eeee-d10- eeee	ssssssssssss ssss	None
3400	AGAGCGGCCGTACGTGCGG	3131	0	eeee-d10- eeee	ssssssssssss ssss	None
3401	AAGAGCGGCCGTACGTGCG	3132	0	eeee-d10- eeee	ssssssssssss ssss	None
3402	CCAATGGGGATCTCGGCGCT	3171	0	eeee-d10- eeee	ssssssssssss ssss	None
3403	GCCAATGGGGATCTCGGCGC	3172	0	eeee-d10- eeee	ssssssssssss ssssss	None
3404	TGCCAATGGGGATCTCGGCG	3173	0	eeee-d10- eeee	ssssssssssss ssssss	None
3405	ATGCCAATGGGGATCTCGGC	3174	0	eeee-d10- eeee	ssssssssssss ssssss	None
3406	GATGCCAATGGGGATCTCGG	3175	0	eeee-d10- eeee	ssssssssssss ssssss	None
3407	TCCGGTAGATGCCAATGGGG	3182	0	eeee-d10- eeee	ssssssssssss ssssss	None
3408	GGGGTCGGAGCGGATGAGAT	3664	0	eeee-d10- eeee	ssssssssssss ssssss	None
3409	GGGGTCGGAGCGGATGAGA	3665	0	eeee-d10- eeee	ssssssssssss ssssss	None
3410	TGATGAAGAACAGCTTGAGC	374	0	d20	ssssssssssss ssss	None
3411	GTTGCCTTTGTAGCTGAGGT	661	0	d20	ssssssssssss ssss	None
3412	GGGATGAACAGGTTCCGCAG	765	0	d20	ssssssssssss ssss	None
3413	AGGATGGCACGGTGGAAGTC	837	0	d20	ssssssssssss ssss	None
3414	GCAGAAGTAGAAGGAGGTCA	979	0	d20	ssssssssssss ssss	None
3415	TAGATGACCCGCTGGGACCA	1347	0	d20	ssssssssssss ssss	None
3416	GCCGGGCAGATGCAGTTCAG	1629	0	d20	ssssssssssss ssss	None
3417	GAGCCAGAGAGTAGCTGTCC	2879	0	d20	ssssssssssss ssss	None
3418	TCACGAAGGACTGGTAGAGC	3008	0	d20	ssssssssssss ssss	None
3419	ATGGGGATCTCGGCGCTGGA	3168	0	d20	ssssssssssss ssss	None
3420	GTTGCCTTTGTAGCTGAGGT	661	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3421	CAGATGTTGCCTTTGTAGCT	666	0	eeee-d10- eeee	ssssssssssss ssss	Modified

3422	TAGATGACCCGCTGGGACCA	1347	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3423	GGTAGATGACCCGCTGGGAC	1349	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3424	GGAGGTAGATGACCCGCTGG	1352	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3425	CTGGAGGTAGATGACCCGCT	1354	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3426	ATGTGGTACACCTCGTTGCC	1746	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3427	TGCGGATGTGGTACACCTCG	1751	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3428	CCCATGCGGATGTGGTACAC	1755	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3429	AGCTCTGCCCTCGTACTCG	1793	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3430	AGGTGAAGCTCTTGCCCTCG	1799	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3431	GCGTAGGTGAAGCTCTTGCC	1803	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3432	GGCCGCGTAGGTGAAGCTCT	1807	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3433	AAGGCCGCGTAGGTGAAGCT	1809	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3434	GATCCACAGGTCGCCCTCGG	3112	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3435	CGGATCCACAGGTCGCCCTC	3114	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3436	GGCCGTACGTGCGGATCCAC	3125	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3437	GCGGCCGTACGTGCGGATCC	3127	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3438	ATCTCGGCGCTGGAGGAGCA	3162	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3439	CCAATGGGGATCTCGGCGCT	3171	0	eeee-d10- eeee	ssssssssssss ssss	Modified
3440	GAAGAGGCGGCCGTACGTGC	3133	0	eeee-d10- eeee	ssssssssssss ssss	None
3441	GGAAGAGGCGGCCGTACGTG	3134	0	eeee-d10- eeee	ssssssssssss ssss	None
3442	TGGAAGAGGCGGCCGTACGT	3135	0	eeee-d10- eeee	ssssssssssss ssss	None
3443	CTGGAAGAGGCGGCCGTACG	3136	0	eeee-d10- eeee	ssssssssssss ssss	None
3444	TCTGGAAGAGGCGGCCGTAC	3137	0	eeee-d10- eeee	ssssssssssss ssss	None
3445	TTCTGGAAGAGGCGGCCGTA	3138	0	eeee-d10- eeee	ssssssssssss ssss	None
3446	CTTCTGGAAGAGGCGGCCGT	3139	0	eeee-d10- eeee	ssssssssssss ssss	None
3447	GCTTCTGGAAGAGGCGGCCG	3140	0	eeee-d10- eeee	ssssssssssss ssss	None
3448	GGGGATCTCGGCGCTGGAGG	3166	0	eeee-d10- eeee	ssssssssssss ssss	None
3449	TGGGGATCTCGGCGCTGGAG	3167	0	eeee-d10- eeee	ssssssssssss ssss	None
3450	AATGGGGATCTCGGCGCTGG	3169	0	eeee-d10- eeee	ssssssssssss ssss	None

3451	CAATGGGGATCTCGGCGCTG	3170	0	eeee-d10- eeee	ssssssssssss ssss	None
3452	ATGTTGCCTTTGTAGCTGAGGT	661	0	eeee-d12- eeee	ssssssssssss ssssss	None
3453	CCCAGATGTTGCCTTTGTAGCT	666	0	eeee-d12- eeee	ssssssssssss ssssss	None
3454	GGTAGATGACCCGCTGGGACCA	1347	0	eeee-d12- eeee	ssssssssssss ssssss	None
3455	GAGGTAGATGACCCGCTGGGAC	1349	0	eeee-d12- eeee	ssssssssssss ssssss	None
3456	CTGGAGGTAGATGACCCGCTGG	1352	0	eeee-d12- eeee	ssssssssssss ssssss	None
3457	CCCTGGAGGTAGATGACCCGCT	1354	0	eeee-d12- eeee	ssssssssssss ssssss	None
3458	GGATGTGGTACACCTCGTTGCC	1746	0	eeee-d12- eeee	ssssssssssss ssssss	None
3459	CATGCGGATGTGGTACACCTCG	1751	0	eeee-d12- eeee	ssssssssssss ssssss	None
3460	CACCCATGCGGATGTGGTACAC	1755	0	eeee-d12- eeee	ssssssssssss ssssss	None
3461	GAAGCTCTTGCCCTCGTACTCG	1793	0	eeee-d12- eeee	ssssssssssss ssssss	None
3462	GTAGGTGAAGCTCTTGCCCTCG	1799	0	eeee-d12- eeee	ssssssssssss ssssss	None
3463	CCGCGTAGGTGAAGCTCTTGCC	1803	0	eeee-d12- eeee	ssssssssssss ssssss	None
3464	AAGGCCGCGTAGGTGAAGCTCT	1807	0	eeee-d12- eeee	ssssssssssss ssssss	None
3465	GGAAGGCCGCGTAGGTGAAGCT	1809	0	eeee-d12- eeee	ssssssssssss ssssss	None
3466	CGGATCCACAGGTCGCCCTCGG	3112	0	eeee-d12- eeee	ssssssssssss ssssss	None
3467	TGCGGATCCACAGGTCGCCCTC	3114	0	eeee-d12- eeee	ssssssssssss ssssss	None
3468	GCGGCCGTACGTGCGGATCCAC	3125	0	eeee-d12- eeee	ssssssssssss ssssss	None
3469	AGGCGGCCGTACGTGCGGATCC	3127	0	eeee-d12- eeee	ssssssssssss ssssss	None
3470	GGATCTCGGCGCTGGAGGAGCA	3162	0	eeee-d12- eeee	ssssssssssss ssssss	None
3471	TGCCAATGGGGATCTCGGCGCT	3171	0	eeee-d12- eeee	ssssssssssss ssssss	None
3472	GTTGCCTTTGTAGCTGAG	661	0	eeee-d8- eeee	soooooooss ooss	None
3473	CAGATGTTGCCTTTGTAG	666	0	eeee-d8- eeee	soooooooss ooss	None
3474	TAGATGACCCGCTGGGAC	1347	0	eeee-d8- eeee	soooooooss ooss	None
3475	GGTAGATGACCCGCTGGG	1349	0	eeee-d8- eeee	soooooooss ooss	None
3476	GGAGGTAGATGACCCGCT	1352	0	eeee-d8- eeee	soooooooss ooss	None
3477	CTGGAGGTAGATGACCCG	1354	0	eeee-d8- eeee	soooooooss ooss	None
3478	ATGTGGTACACCTCGTTG	1746	0	eeee-d8- eeee	soooooooss ooss	None
3479	TGCGGATGTGGTACACCT	1751	0	eeee-d8- eeee	soooooooss ooss	None

3480	CCCATGCGGATGTGGTAC	1755	0	eeee-d8- eeee	sooooooooo ooss	None
3481	AGCTCTTGCCCTCGTACT	1793	0	eeee-d8- eeee	sooooooooo ooss	None
3482	AGGTGAAGCTCTTGCCCT	1799	0	eeee-d8- eeee	sooooooooo ooss	None
3483	GCGTAGGTGAAGCTCTTG	1803	0	eeee-d8- eeee	sooooooooo ooss	None
3484	GGCCGCGTAGGTGAAGCT	1807	0	eeee-d8- eeee	sooooooooo ooss	None
3485	AAGGCCGCGTAGGTGAAG	1809	0	eeee-d8- eeee	sooooooooo ooss	None
3486	GATCCACAGGTCGCCCTC	3112	0	eeee-d8- eeee	sooooooooo ooss	None
3487	CGGATCCACAGGTCGCCC	3114	0	eeee-d8- eeee	sooooooooo ooss	None
3488	GGCCGTACGTGCGGATCC	3125	0	eeee-d8- eeee	sooooooooo ooss	None
3489	GCGGCCGTACGTGCGGAT	3127	0	eeee-d8- eeee	sooooooooo ooss	None
3490	ATCTCGGCGCTGGAGGAG	3162	0	eeee-d8- eeee	sooooooooo ooss	None
3491	CCAATGGGGATCTCGGCG	3171	0	eeee-d8- eeee	sooooooooo ooss	None
3492	GTTGCCTTTGTAGCTGA	661	0	eekk-d8- kkeee	sooooooooo oss	None
3493	CAGATGTTGCCTTTGTA	666	0	eekk-d8- kkeee	sooooooooo oss	None
3494	TAGATGACCCGCTGGGA	1347	0	eekk-d8- kkeee	sooooooooo oss	None
3495	GGTAGATGACCCGCTGG	1349	0	eekk-d8- kkeee	sooooooooo oss	None
3496	GGAGGTAGATGACCCGC	1352	0	eekk-d8- kkeee	sooooooooo oss	None
3497	CTGGAGGTAGATGACCC	1354	0	eekk-d8- kkeee	sooooooooo oss	None
3498	ATGTGGTACACCTCGTT	1746	0	eekk-d8- kkeee	sooooooooo oss	None
3499	TGCGGATGTGGTACACC	1751	0	eekk-d8- kkeee	sooooooooo oss	None
3500	CCCATGCGGATGTGGTA	1755	0	eekk-d8- kkeee	sooooooooo oss	None
3501	AGCTCTTGCCCTCGTAC	1793	0	eekk-d8- kkeee	sooooooooo oss	None
3502	AGGTGAAGCTCTTGCCC	1799	0	eekk-d8- kkeee	sooooooooo oss	None
3503	GCGTAGGTGAAGCTCTT	1803	0	eekk-d8- kkeee	sooooooooo oss	None
3504	GGCCGCGTAGGTGAAGC	1807	0	eekk-d8- kkeee	sooooooooo oss	None
3505	AAGGCCGCGTAGGTGAA	1809	0	eekk-d8- kkeee	sooooooooo oss	None
3506	GATCCACAGGTCGCCCT	3112	0	eekk-d8- kkeee	sooooooooo oss	None
3507	CGGATCCACAGGTCGCC	3114	0	eekk-d8- kkeee	sooooooooo oss	None
3508	GGCCGTACGTGCGGATC	3125	0	eekk-d8- kkeee	sooooooooo oss	None

3509	GCGGCCGTACGTGCGGA	3127	0	eekk-d8-kkeee	sooooooooooss	None
3510	ATCTCGGCGCTGGAGGA	3162	0	eekk-d8-kkeee	sooooooooooss	None
3511	CCAATGGGGATCTCGGC	3171	0	eekk-d8-kkeee	sooooooooooss	None
3512	GTTGGCTTAGTACCTGTGGT	661	4	d20	oooooooooooo	None
3513	CTTTGGTTGCGAGGTTAGCT	661	10	d20	oooooooooooo	None
3514	GCAGGAGTGGAAGAGATCA	979	4	d20	oooooooooooo	None
3515	AGTAGGCAGAGGTCAAAGGA	979	18	d20	oooooooooooo	None
3516	GTTGGCTTAGTACCTGTGGT	661	4	eeee-d10-eeee	oooooooooooo	None
3517	GGTACATGTCCCCCTGCGAC	1349	4	eeee-d10-eeee	oooooooooooo	None
3518	GGAGCTAGTTGAGCCGGTGG	1352	4	eeee-d10-eeee	oooooooooooo	None
3519	ATGTCGTAGACCACGTAGCC	1746	4	eeee-d10-eeee	oooooooooooo	None
3520	CCAAGCGCATGAGGTTAC	1755	4	eeee-d10-eeee	oooooooooooo	None
3521	GCGTTGGTCAAGGTCTAGCC	1803	4	eeee-d10-eeee	oooooooooooo	None
3522	GGCCCCGTTGGTCAAGGTCT	1807	4	eeee-d10-eeee	oooooooooooo	None
3523	GCGGGCGTTCGTCCGGTCC	3127	4	eeee-d10-eeee	oooooooooooo	None
3524	ATCTGGGCCCTGCAGGTGCA	3162	4	eeee-d10-eeee	oooooooooooo	None
3525	CAAAGGGCATCACGGGGCT	3171	4	eeee-d10-eeee	oooooooooooo	None

Table 6 provides the oligonucleotide ASO sequences, positions in the KCNT1 transcript (NCBI NM_020822.2), chemistries used to modify the ASO, and percent knockdown of KCNT1 in BE(2)-M17 cells after treatment with the indicated ASO. Here, the data corresponding to different ASO sequences are organized according to the KCNT position, as denoted in the first column. In the ASO Gap column, “e” indicates a 2'-O-(2-methoxyethyl) (2'MOE) modified nucleoside, and “k” indicates a locked nucleoside (LNAs). In the ASO Linkages column, “s” indicates a phosphorothiate linkage and “o” indicates a phosphodiester linkage. In the “ASO Cytosines” column, “None” indicates that all cytosines are unmodified, while “Modified” indicates that all cytosines are 5-Methyl-2'-deoxycytosine (5-Methyl-dC).

All ASO specific negative controls (SEQ ID NOs: 3512-3525) generated less KCNT1 knockdown in BE(2)-M17 cells compared to their matched targeted ASO. These data confirm the specificity of the assay and highlight the dependence of knockdown on sequence

homology. There was generally good concordance between the knockdown obtained with the various chemistries. However, some ASO sequences demonstrated substantially different activity depending on the chemistry used (position 1354: SEQ ID NO: 1208 with 21% knockdown versus SEQ ID NO: 3457 with 59% knockdown). In addition, cytosine modified ASOs were consistently more potent for the majority of tested ASOs. Although activity was observed across each of the entire hotspots tested (NM_020822.2 (SEQ ID NO: 3526): 655 to 680, 1340 to 1370, 1740 to 1815, and 3110 to 3171), there were certain ASOs with greater activity which was not predicted by the sequence homology.

Table 6: Percent Knock down of KCNT1 expressed in human (BE(2)-M17) neuronal cells. Sequences are organized according to KCNT1 position.

Position	SEQ ID NO	ASO Gap	ASO Linkages	ASO Cytosines	BE(2)-M17 100nM ASO Mean
374	1	eeee-d10-eeee	ssssssssssssssssss	None	7
	3410	d20	ssssssssssssssssss	None	14
655	625	eeee-d10-eeee	ssssssssssssssssss	None	11
656	626	eeee-d10-eeee	ssssssssssssssssss	None	34
657	627	eeee-d10-eeee	ssssssssssssssssss	None	37
658	628	eeee-d10-eeee	ssssssssssssssssss	None	51
659	629	eeee-d10-eeee	ssssssssssssssssss	None	44
660	630	eeee-d10-eeee	ssssssssssssssssss	None	43
661	4	eeee-d10-eeee	ssssssssssssssssss	None	44
	3411	d20	ssssssssssssssssss	None	39
	3420	eeee-d10-eeee	ssssssssssssssssss	Modified	49
	3452	eeee-d12-eeee	ssssssssssssssssss	None	39
	3472	eeee-d8-eeee	sooossssssssooss	None	38
	3492	eeek-d8-keee	soossssssssooss	None	40
	3512	d20	ssssssssssssssssss	None	6
	3513	d20	ssssssssssssssssss	None	5
	3516	eeee-d10-eeee	ssssssssssssssssss	None	11
662	631	eeee-d10-eeee	ssssssssssssssssss	None	33
663	632	eeee-d10-eeee	ssssssssssssssssss	None	5
664	633	eeee-d10-eeee	ssssssssssssssssss	None	34
665	634	eeee-d10-eeee	ssssssssssssssssss	None	37
666	635	eeee-d10-eeee	ssssssssssssssssss	None	35
666	3421	eeee-d10-eeee	ssssssssssssssssss	Modified	59
666	3453	eeee-d12-eeee	ssssssssssssssssss	None	41

666	3473	eeee-d8-eeee	sooooooooooooo	None	19
666	3493	eeek-d8-kkee	sooooooooooooo	None	52
667	636	eeee-d10-eeee	oooooooooooooooo	None	40
668	637	eeee-d10-eeee	oooooooooooooooo	None	30
669	638	eeee-d10-eeee	oooooooooooooooo	None	17
670	639	eeee-d10-eeee	oooooooooooooooo	None	26
671	640	eeee-d10-eeee	oooooooooooooooo	None	49
672	641	eeee-d10-eeee	oooooooooooooooo	None	38
673	642	eeee-d10-eeee	oooooooooooooooo	None	56
674	643	eeee-d10-eeee	oooooooooooooooo	None	48
675	644	eeee-d10-eeee	oooooooooooooooo	None	21
676	645	eeee-d10-eeee	oooooooooooooooo	None	50
677	646	eeee-d10-eeee	oooooooooooooooo	None	64
678	647	eeee-d10-eeee	oooooooooooooooo	None	39
679	648	eeee-d10-eeee	oooooooooooooooo	None	41
680	649	eeee-d10-eeee	oooooooooooooooo	None	52
765	5	eeee-d10-eeee	oooooooooooooooo	None	36
765	3412	d20	oooooooooooooooo	None	45
837	6	eeee-d10-eeee	oooooooooooooooo	None	31
837	3413	d20	oooooooooooooooo	None	40
979	3414	d20	oooooooooooooooo	None	35
979	3514	d20	oooooooooooooooo	None	14
979	3515	d20	oooooooooooooooo	None	4
1340	1195	eeee-d10-eeee	oooooooooooooooo	None	49
1341	1196	eeee-d10-eeee	oooooooooooooooo	None	51
1342	1197	eeee-d10-eeee	oooooooooooooooo	None	41
1343	1198	eeee-d10-eeee	oooooooooooooooo	None	33
1344	1199	eeee-d10-eeee	oooooooooooooooo	None	38
1345	1200	eeee-d10-eeee	oooooooooooooooo	None	14
1346	1201	eeee-d10-eeee	oooooooooooooooo	None	11
1347	8	eeee-d10-eeee	oooooooooooooooo	None	25
1347	3415	d20	oooooooooooooooo	None	32
1347	3422	eeee-d10-eeee	oooooooooooooooo	Modified	59
1347	3454	eeee-d12-eeee	oooooooooooooooo	None	37
1347	3474	eeee-d8-eeee	sooooooooooooo	None	25
1347	3494	eeek-d8-kkee	sooooooooooooo	None	26
1348	1202	eeee-d10-eeee	oooooooooooooooo	None	24
1349	1203	eeee-d10-eeee	oooooooooooooooo	None	41

1349	3423	eeee-d10-eeee	ssssssssssssssssss	Modified	60
1349	3455	eeee-d12-eeee	ssssssssssssssssss	None	49
1349	3475	eeee-d8-eeee	sooooooooooooo	None	51
1349	3495	eeek-d8-kkee	sooooooooooooo	None	53
1349	3517	eeee-d10-eeee	ssssssssssssssssss	None	33
1350	1204	eeee-d10-eeee	ssssssssssssssssss	None	44
1351	1205	eeee-d10-eeee	ssssssssssssssssss	None	65
1352	1206	eeee-d10-eeee	ssssssssssssssssss	None	56
1352	3424	eeee-d10-eeee	ssssssssssssssssss	Modified	63
1352	3456	eeee-d12-eeee	ssssssssssssssssss	None	43
1352	3476	eeee-d8-eeee	sooooooooooooo	None	18
1352	3496	eeek-d8-kkee	sooooooooooooo	None	50
1352	3518	eeee-d10-eeee	ssssssssssssssssss	None	39
1353	1207	eeee-d10-eeee	ssssssssssssssssss	None	55
1354	1208	eeee-d10-eeee	ssssssssssssssssss	None	21
1354	3425	eeee-d10-eeee	ssssssssssssssssss	Modified	62
1354	3457	eeee-d12-eeee	ssssssssssssssssss	None	59
1354	3477	eeee-d8-eeee	sooooooooooooo	None	34
1354	3497	eeek-d8-kkee	sooooooooooooo	None	37
1355	1209	eeee-d10-eeee	ssssssssssssssssss	None	45
1356	1210	eeee-d10-eeee	ssssssssssssssssss	None	41
1357	1211	eeee-d10-eeee	ssssssssssssssssss	None	52
1358	1212	eeee-d10-eeee	ssssssssssssssssss	None	54
1359	1213	eeee-d10-eeee	ssssssssssssssssss	None	41
1360	1214	eeee-d10-eeee	ssssssssssssssssss	None	41
1361	1215	eeee-d10-eeee	ssssssssssssssssss	None	11
1362	1216	eeee-d10-eeee	ssssssssssssssssss	None	22
1363	1217	eeee-d10-eeee	ssssssssssssssssss	None	32
1364	1218	eeee-d10-eeee	ssssssssssssssssss	None	50
1365	1219	eeee-d10-eeee	ssssssssssssssssss	None	38
1366	1220	eeee-d10-eeee	ssssssssssssssssss	None	34
1367	1221	eeee-d10-eeee	ssssssssssssssssss	None	25
1368	1222	eeee-d10-eeee	ssssssssssssssssss	None	20
1369	1223	eeee-d10-eeee	ssssssssssssssssss	None	-3
1370	1224	eeee-d10-eeee	ssssssssssssssssss	None	62
1629	10	eeee-d10-eeee	ssssssssssssssssss	None	53
1629	3416	d20	ssssssssssssssssss	None	45
1740	1496	eeee-d10-eeee	ssssssssssssssssss	None	45

1741	1497	eeee-d10-eeee	ssssssssssssssssss	None	64
1742	1498	eeee-d10-eeee	ssssssssssssssssss	None	67
1743	1499	eeee-d10-eeee	ssssssssssssssssss	None	44
1744	1500	eeee-d10-eeee	ssssssssssssssssss	None	42
1745	1501	eeee-d10-eeee	ssssssssssssssssss	None	21
1746	1502	eeee-d10-eeee	ssssssssssssssssss	None	32
1746	3426	eeee-d10-eeee	ssssssssssssssssss	Modified	17
1746	3458	eeee-d12-eeee	ssssssssssssssssss	None	44
1746	3478	eeee-d8-eeee	sooooooooooooo	None	41
1746	3498	eeek-d8-keee	sooooooooooooo	None	26
1746	3519	eeee-d10-eeee	ssssssssssssssssss	None	15
1747	1503	eeee-d10-eeee	ssssssssssssssssss	None	30
1748	1504	eeee-d10-eeee	ssssssssssssssssss	None	46
1749	1505	eeee-d10-eeee	ssssssssssssssssss	None	54
1750	1506	eeee-d10-eeee	ssssssssssssssssss	None	46
1751	1507	eeee-d10-eeee	ssssssssssssssssss	None	47
1751	3427	eeee-d10-eeee	ssssssssssssssssss	Modified	39
1751	3459	eeee-d12-eeee	ssssssssssssssssss	None	69
1751	3479	eeee-d8-eeee	sooooooooooooo	None	41
1751	3499	eeek-d8-keee	sooooooooooooo	None	42
1752	28	eeee-d10-eeee	ssssssssssssssssss	None	49
1753	1508	eeee-d10-eeee	ssssssssssssssssss	None	54
1754	1509	eeee-d10-eeee	ssssssssssssssssss	None	43
1755	1510	eeee-d10-eeee	ssssssssssssssssss	None	72
1755	3428	eeee-d10-eeee	ssssssssssssssssss	Modified	79
1755	3460	eeee-d12-eeee	ssssssssssssssssss	None	61
1755	3480	eeee-d8-eeee	sooooooooooooo	None	49
1755	3500	eeek-d8-keee	sooooooooooooo	None	37
1755	3520	eeee-d10-eeee	ssssssssssssssssss	None	-1
1756	1511	eeee-d10-eeee	ssssssssssssssssss	None	44
1757	1512	eeee-d10-eeee	ssssssssssssssssss	None	36
1758	1513	eeee-d10-eeee	ssssssssssssssssss	None	35
1759	1514	eeee-d10-eeee	ssssssssssssssssss	None	44
1760	17	eeee-d10-eeee	ssssssssssssssssss	None	32
1761	1515	eeee-d10-eeee	ssssssssssssssssss	None	11
1762	1516	eeee-d10-eeee	ssssssssssssssssss	None	28
1763	1517	eeee-d10-eeee	ssssssssssssssssss	None	28
1764	1518	eeee-d10-eeee	ssssssssssssssssss	None	26

1765	1519	eeee-d10-eeee	ssssssssssssssssss	None	35
1766	1520	eeee-d10-eeee	ssssssssssssssssss	None	46
1767	1521	eeee-d10-eeee	ssssssssssssssssss	None	31
1768	1522	eeee-d10-eeee	ssssssssssssssssss	None	42
1769	1523	eeee-d10-eeee	ssssssssssssssssss	None	21
1770	1524	eeee-d10-eeee	ssssssssssssssssss	None	12
1771	1525	eeee-d10-eeee	ssssssssssssssssss	None	-4
1772	1526	eeee-d10-eeee	ssssssssssssssssss	None	31
1773	1527	eeee-d10-eeee	ssssssssssssssssss	None	25
1774	1528	eeee-d10-eeee	ssssssssssssssssss	None	36
1775	62	eeee-d10-eeee	ssssssssssssssssss	None	49
1776	1529	eeee-d10-eeee	ssssssssssssssssss	None	36
1777	1530	eeee-d10-eeee	ssssssssssssssssss	None	52
1778	1531	eeee-d10-eeee	ssssssssssssssssss	None	54
1779	1532	eeee-d10-eeee	ssssssssssssssssss	None	22
1780	1533	eeee-d10-eeee	ssssssssssssssssss	None	63
1781	1534	eeee-d10-eeee	ssssssssssssssssss	None	63
1782	1535	eeee-d10-eeee	ssssssssssssssssss	None	34
1783	1536	eeee-d10-eeee	ssssssssssssssssss	None	17
1784	1537	eeee-d10-eeee	ssssssssssssssssss	None	24
1785	1538	eeee-d10-eeee	ssssssssssssssssss	None	40
1786	1539	eeee-d10-eeee	ssssssssssssssssss	None	53
1787	1540	eeee-d10-eeee	ssssssssssssssssss	None	46
1788	1541	eeee-d10-eeee	ssssssssssssssssss	None	46
1789	1542	eeee-d10-eeee	ssssssssssssssssss	None	71
1790	1543	eeee-d10-eeee	ssssssssssssssssss	None	41
1791	1544	eeee-d10-eeee	ssssssssssssssssss	None	34
1792	1545	eeee-d10-eeee	ssssssssssssssssss	None	38
1793	1546	eeee-d10-eeee	ssssssssssssssssss	None	52
1793	3429	eeee-d10-eeee	ssssssssssssssssss	Modified	59
1793	3461	eeee-d12-eeee	ssssssssssssssssss	None	25
1793	3481	eeee-d8-eeee	sooossssssooss	None	52
1793	3501	eekk-d8-kkee	soossssssooss	None	42
1794	1547	eeee-d10-eeee	ssssssssssssssssss	None	58
1795	29	eeee-d10-eeee	ssssssssssssssssss	None	51
1796	1548	eeee-d10-eeee	ssssssssssssssssss	None	40
1797	1549	eeee-d10-eeee	ssssssssssssssssss	None	16
1798	1550	eeee-d10-eeee	ssssssssssssssssss	None	33

1799	1551	eeee-d10-eeee	ssssssssssssssssss	None	63
1799	3430	eeee-d10-eeee	ssssssssssssssssss	Modified	61
1799	3462	eeee-d12-eeee	ssssssssssssssssss	None	39
1799	3482	eeee-d8-eeee	sooooooooooooo	None	21
1799	3502	eeek-d8-keee	sooooooooooooo	None	52
1800	1552	eeee-d10-eeee	ssssssssssssssssss	None	22
1801	1553	eeee-d10-eeee	ssssssssssssssssss	None	16
1802	1554	eeee-d10-eeee	ssssssssssssssssss	None	33
1803	1555	eeee-d10-eeee	ssssssssssssssssss	None	60
1803	3431	eeee-d10-eeee	ssssssssssssssssss	Modified	60
1803	3463	eeee-d12-eeee	ssssssssssssssssss	None	59
1803	3483	eeee-d8-eeee	sooooooooooooo	None	52
1803	3503	eeek-d8-keee	sooooooooooooo	None	49
1803	3521	eeee-d10-eeee	ssssssssssssssssss	None	25
1804	1556	eeee-d10-eeee	ssssssssssssssssss	None	67
1805	1557	eeee-d10-eeee	ssssssssssssssssss	None	63
1806	1558	eeee-d10-eeee	ssssssssssssssssss	None	39
1807	1559	eeee-d10-eeee	ssssssssssssssssss	None	24
1807	3432	eeee-d10-eeee	ssssssssssssssssss	Modified	63
1807	3464	eeee-d12-eeee	ssssssssssssssssss	None	59
1807	3484	eeee-d8-eeee	sooooooooooooo	None	13
1807	3504	eeek-d8-keee	sooooooooooooo	None	41
1807	3522	eeee-d10-eeee	ssssssssssssssssss	None	1
1808	1560	eeee-d10-eeee	ssssssssssssssssss	None	24
1809	1561	eeee-d10-eeee	ssssssssssssssssss	None	9
1809	3433	eeee-d10-eeee	ssssssssssssssssss	Modified	64
1809	3465	eeee-d12-eeee	ssssssssssssssssss	None	61
1809	3485	eeee-d8-eeee	sooooooooooooo	None	-17
1809	3505	eeek-d8-keee	sooooooooooooo	None	39
1810	1562	eeee-d10-eeee	ssssssssssssssssss	None	38
1811	1563	eeee-d10-eeee	ssssssssssssssssss	None	44
1812	1564	eeee-d10-eeee	ssssssssssssssssss	None	-10
1813	1565	eeee-d10-eeee	ssssssssssssssssss	None	27
1814	1566	eeee-d10-eeee	ssssssssssssssssss	None	15
1815	1567	eeee-d10-eeee	ssssssssssssssssss	None	44
2879	12	eeee-d10-eeee	ssssssssssssssssss	None	40
2879	3417	d20	ssssssssssssssssss	None	23
3008	13	eeee-d10-eeee	ssssssssssssssssss	None	24

3008	3418	d20	ssssssssssssssssss	None	6
3110	2591	eeee-d10-eeee	ssssssssssssssssss	None	21
3111	2592	eeee-d10-eeee	ssssssssssssssssss	None	20
3112	2593	eeee-d10-eeee	ssssssssssssssssss	None	7
3112	3434	eeee-d10-eeee	ssssssssssssssssss	Modified	54
3112	3466	eeee-d12-eeee	ssssssssssssssssss	None	58
3112	3486	eeee-d8-eeee	sooooooooooooo	None	44
3112	3506	eeek-d8-keee	sooooooooooooo	None	51
3113	2594	eeee-d10-eeee	ssssssssssssssssss	None	46
3114	2595	eeee-d10-eeee	ssssssssssssssssss	None	29
3114	3435	eeee-d10-eeee	ssssssssssssssssss	Modified	70
3114	3467	eeee-d12-eeee	ssssssssssssssssss	None	41
3114	3487	eeee-d8-eeee	sooooooooooooo	None	45
3114	3507	eeek-d8-keee	sooooooooooooo	None	29
3115	2596	eeee-d10-eeee	ssssssssssssssssss	None	44
3116	2597	eeee-d10-eeee	ssssssssssssssssss	None	30
3117	2598	eeee-d10-eeee	ssssssssssssssssss	None	30
3118	2599	eeee-d10-eeee	ssssssssssssssssss	None	48
3119	2600	eeee-d10-eeee	ssssssssssssssssss	None	54
3120	2601	eeee-d10-eeee	ssssssssssssssssss	None	31
3121	2602	eeee-d10-eeee	ssssssssssssssssss	None	42
3122	2603	eeee-d10-eeee	ssssssssssssssssss	None	57
3123	2604	eeee-d10-eeee	ssssssssssssssssss	None	62
3124	2605	eeee-d10-eeee	ssssssssssssssssss	None	56
3125	2606	eeee-d10-eeee	ssssssssssssssssss	None	-1
3125	3436	eeee-d10-eeee	ssssssssssssssssss	Modified	61
3125	3468	eeee-d12-eeee	ssssssssssssssssss	None	55
3125	3488	eeee-d8-eeee	sooooooooooooo	None	57
3125	3508	eeek-d8-keee	sooooooooooooo	None	60
3126	3395	eeee-d10-eeee	ssssssssssssssssss	None	46
3127	3396	eeee-d10-eeee	ssssssssssssssssss	None	55
3127	3437	eeee-d10-eeee	ssssssssssssssssss	Modified	77
3127	3469	eeee-d12-eeee	ssssssssssssssssss	None	58
3127	3489	eeee-d8-eeee	sooooooooooooo	None	54
3127	3509	eeek-d8-keee	sooooooooooooo	None	42
3127	3523	eeee-d10-eeee	ssssssssssssssssss	None	15
3128	3397	eeee-d10-eeee	ssssssssssssssssss	None	65
3129	3398	eeee-d10-eeee	ssssssssssssssssss	None	50

3130	3399	eeee-d10-eeee	ssssssssssssssssss	None	52
3131	3400	eeee-d10-eeee	ssssssssssssssssss	None	39
3132	3401	eeee-d10-eeee	ssssssssssssssssss	None	34
3133	3440	eeee-d10-eeee	ssssssssssssssssss	None	56
3134	3441	eeee-d10-eeee	ssssssssssssssssss	None	16
3135	3442	eeee-d10-eeee	ssssssssssssssssss	None	35
3136	3443	eeee-d10-eeee	ssssssssssssssssss	None	62
3137	3444	eeee-d10-eeee	ssssssssssssssssss	None	59
3138	3445	eeee-d10-eeee	ssssssssssssssssss	None	56
3139	3446	eeee-d10-eeee	ssssssssssssssssss	None	52
3140	3447	eeee-d10-eeee	ssssssssssssssssss	None	55
3141	2607	eeee-d10-eeee	ssssssssssssssssss	None	33
3142	2608	eeee-d10-eeee	ssssssssssssssssss	None	36
3143	2609	eeee-d10-eeee	ssssssssssssssssss	None	27
3144	2610	eeee-d10-eeee	ssssssssssssssssss	None	19
3145	2611	eeee-d10-eeee	ssssssssssssssssss	None	32
3146	2612	eeee-d10-eeee	ssssssssssssssssss	None	43
3147	2613	eeee-d10-eeee	ssssssssssssssssss	None	24
3148	2614	eeee-d10-eeee	ssssssssssssssssss	None	39
3149	2615	eeee-d10-eeee	ssssssssssssssssss	None	29
3150	2616	eeee-d10-eeee	ssssssssssssssssss	None	38
3151	2617	eeee-d10-eeee	ssssssssssssssssss	None	59
3152	2618	eeee-d10-eeee	ssssssssssssssssss	None	39
3153	2619	eeee-d10-eeee	ssssssssssssssssss	None	58
3154	2620	eeee-d10-eeee	ssssssssssssssssss	None	41
3155	2621	eeee-d10-eeee	ssssssssssssssssss	None	32
3156	2622	eeee-d10-eeee	ssssssssssssssssss	None	63
3157	2623	eeee-d10-eeee	ssssssssssssssssss	None	63
3158	2624	eeee-d10-eeee	ssssssssssssssssss	None	57
3159	2625	eeee-d10-eeee	ssssssssssssssssss	None	59
3160	2626	eeee-d10-eeee	ssssssssssssssssss	None	65
3161	2627	eeee-d10-eeee	ssssssssssssssssss	None	45
3162	2628	eeee-d10-eeee	ssssssssssssssssss	None	37
3162	3438	eeee-d10-eeee	ssssssssssssssssss	Modified	22
3162	3470	eeee-d12-eeee	ssssssssssssssssss	None	48
3162	3490	eeee-d8-eeee	soooossssssooss	None	29
3162	3510	eeek-d8-kkeee	soossssssssooss	None	35
3162	3524	eeee-d10-eeee	ssssssssssssssssss	None	7

3163	2629	eeee-d10-eeee	ssssssssssssssssss	None	60
3164	2630	eeee-d10-eeee	ssssssssssssssssss	None	52
3165	2631	eeee-d10-eeee	ssssssssssssssssss	None	52
3166	3448	eeee-d10-eeee	ssssssssssssssssss	None	31
3167	3449	eeee-d10-eeee	ssssssssssssssssss	None	49
3168	15	eeee-d10-eeee	ssssssssssssssssss	None	33
3168	3419	d20	ssssssssssssssssss	None	22
3169	3450	eeee-d10-eeee	ssssssssssssssssss	None	26
3170	3451	eeee-d10-eeee	ssssssssssssssssss	None	34
3171	3402	eeee-d10-eeee	ssssssssssssssssss	None	54
3171	3439	eeee-d10-eeee	ssssssssssssssssss	Modified	69
3171	3471	eeee-d12-eeee	ssssssssssssssssss	None	50
3171	3491	eeee-d8-eeee	sooooooooooooo	None	41
3171	3511	eeek-d8-kkeee	sooooooooooooo	None	32
3171	3525	eeee-d10-eeee	ssssssssssssssssss	None	16

Table 7 below shows the percent knockdown of KCNT1 in BE(2)-M17 cells after treatment with the ASO with the sequence of a corresponding SEQ ID NO.

Table 7: Percent Knock down of KCNT1 expressed in human BE(2)-M17 cells organized according to SEQ ID NO.

	BE(2)-M17		BE(2)-M17		BE(2)-M17		BE(2)-M17
	100nM ASO		100nM ASO		100nM ASO		100nM ASO
SED ID NO:	Mean	SED ID NO:	Mean	SED ID NO:	Mean	SED ID NO:	Mean
1	7	1505	54	2605	56	3452	39
4	44	1506	46	2606	-1	3453	41
5	36	1507	47	2607	33	3454	37
6	31	1508	54	2608	36	3455	49
8	25	1509	43	2609	27	3456	43
10	53	1510	72	2610	19	3457	59
12	40	1511	44	2611	32	3458	44
13	24	1512	36	2612	43	3459	69
15	33	1513	35	2613	24	3460	61
17	32	1514	44	2614	39	3461	25
28	49	1515	11	2615	29	3462	39
29	51	1516	28	2616	38	3463	59
62	49	1517	28	2617	59	3464	59
625	11	1518	26	2618	39	3465	61
626	34	1519	35	2619	58	3466	58

	BE(2)-M17		BE(2)-M17		BE(2)-M17		BE(2)-M17
	100nM ASO		100nM ASO		100nM ASO		100nM ASO
SED ID NO:	Mean	SED ID NO:	Mean	SED ID NO:	Mean	SED ID NO:	Mean
627	37	1520	46	2620	41	3467	41
628	51	1521	31	2621	32	3468	55
629	44	1522	42	2622	63	3469	58
630	43	1523	21	2623	63	3470	48
631	33	1524	12	2624	57	3471	50
632	5	1525	-4	2625	59	3472	38
633	34	1526	31	2626	65	3473	19
634	37	1527	25	2627	45	3474	25
635	35	1528	36	2628	37	3475	51
636	40	1529	36	2629	60	3476	18
637	30	1530	52	2630	52	3477	34
638	17	1531	54	2631	52	3478	41
639	26	1532	22	3395	46	3479	41
640	49	1533	63	3396	55	3480	49
641	38	1534	63	3397	65	3481	52
642	56	1535	34	3398	50	3482	21
643	48	1536	17	3399	52	3483	52
644	21	1537	24	3400	39	3484	13
645	50	1538	40	3401	34	3485	-17
646	64	1539	53	3402	54	3486	44
647	39	1540	46	3410	14	3487	45
648	41	1541	46	3411	39	3488	57
649	52	1542	71	3412	45	3489	54
1195	49	1543	41	3413	40	3490	29
1196	51	1544	34	3414	35	3491	41
1197	41	1545	38	3415	32	3492	40
1198	33	1546	52	3416	45	3493	52
1199	38	1547	58	3417	23	3494	26
1200	14	1548	40	3418	6	3495	53
1201	11	1549	16	3419	22	3496	50
1202	24	1550	33	3420	49	3497	37
1203	41	1551	63	3421	59	3498	26
1204	44	1552	22	3422	59	3499	42
1205	65	1553	16	3423	60	3500	37
1206	56	1554	33	3424	63	3501	42
1207	55	1555	60	3425	62	3502	52
1208	21	1556	67	3426	17	3503	49
1209	45	1557	63	3427	39	3504	41
1210	41	1558	39	3428	79	3505	39
1211	52	1559	24	3429	59	3506	51

	BE(2)-M17		BE(2)-M17		BE(2)-M17		BE(2)-M17
	100nM ASO		100nM ASO		100nM ASO		100nM ASO
SED ID NO:	Mean	SED ID NO:	Mean	SED ID NO:	Mean	SED ID NO:	Mean
1212	54	1560	24	3430	61	3507	29
1213	41	1561	9	3431	60	3508	60
1214	41	1562	38	3432	63	3509	42
1215	11	1563	44	3433	64	3510	35
1216	22	1564	-10	3434	54	3511	32
1217	32	1565	27	3435	70	3512	6
1218	50	1566	15	3436	61	3513	5
1219	38	1567	44	3437	77	3514	14
1220	34	2591	21	3438	22	3515	4
1221	25	2592	20	3439	69	3516	11
1222	20	2593	7	3440	56	3517	33
1223	-3	2594	46	3441	16	3518	39
1224	62	2595	29	3442	35	3519	15
1496	45	2596	44	3443	62	3520	-1
1497	64	2597	30	3444	59	3521	25
1498	67	2598	30	3445	56	3522	1
1499	44	2599	48	3446	52	3523	15
1500	42	2600	54	3447	55	3524	7
1501	21	2601	31	3448	31	3525	16
1502	32	2602	42	3449	49		
1503	30	2603	57	3450	26		
1504	46	2604	62	3451	34		

Example 4. Evaluation of Select Antisense Oligonucleotides

For select ASOs, the degree of KCNT1 mRNA knock-down was determined using a taqman quantitative polymerase chain reaction (qPCR assay). Human (SH-Sy5Y) neuronal cell lines were transfected with between 500nM and 10,000 nM ASO using the Amaxa nucleofection (protocol CA137). After 48 hour incubation at 37 °C, cDNA was prepared from each well using the Cell-to-Ct Kit (ThermoFisher Scientific). The expression level of KCNT1 was determined using taqman qPCR assays for either KCNT1 (human Hs.PT.58.19442766) or the housekeeping gene HPRT1 (human Hs.PT.58v.45621572). All taqman assays were predesigned by Integrated DNA Technologies. KCNT1 and HPRT1 detection were multiplexed in a single well. The fold change in KCNT1 was calculated using the $\Delta\Delta C_p$ method whereby the expression of KCNT1 is first normalized to HPRT1 ($2^{-(Cp_KCNT1 - Cp_HPRT1)}$) in the same well (multiplexed reaction) followed by a secondary

normalization to the vehicle, non-transfected control ($2^{-(Cp_ASO-Cp_vehicle)}$). The assay was performed in biological duplicates and technical triplicates.

FIG. 1 is a plot demonstrating percentage knockdown of hKCNT1 in response to different antisense oligonucleotide treatments (specifically, antisense oligonucleotides corresponding to SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 751, SEQ ID NO: 759, SEQ ID NO: 1206, and SEQ ID NO: 1546). The numerical percentage knockdown values are shown below in Table 8. The positive control is SEQ ID NO: 7. The negative control is an ASO with identical chemistry but with a sequence not found in the human genome.

Table 8: Percent knockdown data expressed as average percent knock-down.

	% Knockdown							
	SEQ ID NO							
ASO μ M	4	5	751	759	1206	1546	Pos Control	Neg Control
0.5	36.55	13.42	7.09	12.03	18.95	25.50	-27.31	-98.79
1	61.60	36.27	24.74	25.59	26.96	42.66	7.02	-98.58
2	85.97	55.76	42.84	43.51	46.64	69.39	27.66	-98.69
5	94.33	79.72	62.23	73.50	70.44	84.36	74.18	-98.69
10	98.61	84.89	76.00	87.23	84.34	94.51	88.02	-98.81

All ASO's tested exhibited a dose dependent knock down of the KCNT1 expression. ASOs as shown in SEQ ID NO. 4 and 1546 exhibited the most potent knockdown of the KCNT1 gene, with greater than 80% gene expression reduction when treated with 5 μ M of the ASO oligonucleotide. The IC₅₀ of each ASO is shown in Table 9.

Table 9. IC₅₀ of select ASOs in SH-SY5Y neuronal cells

SEQ ID NO.	SH-SY5Y Cells IC ₅₀ (uM)
4	0.716
5	1.733
7	3.218
751	3.041
759	2.342
1206	2.255
1546	1.177

OTHER EMBODIMENTS

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference in their entirety to the same extent as if each individual

publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

5 While the invention has been described in connection with specific embodiments thereof, it will be understood that invention is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the
10 invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claimed.

CLAIMS

WHAT IS CLAIMED IS:

1. A compound comprising an oligonucleotide comprising a nucleobase sequence at least 90% complementary to at least 10 contiguous nucleobases of a transcript comprising a sequence at least 90% identity to SEQ ID NO: 3526, or a contiguous 15 to 50 nucleobase portion of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.
2. An oligonucleotide comprising a nucleobase sequence at least 90% complementary to at least 10 contiguous nucleobases of a transcript comprising a sequence at least 90% identity to SEQ ID NO: 3526, or a contiguous 15 to 50 nucleobase portion of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.
3. The compound comprising an oligonucleotide of claim 1, or the oligonucleotide of claim 2, wherein the oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares 90% identity with an equal length portion of any one of SEQ ID NOs: 1-3525.
4. The compound comprising an oligonucleotide of claims 1 or 3, or the oligonucleotide of claims 2 or 3, wherein the oligonucleotide comprises at least a contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 1-3525.
5. The compound comprising an oligonucleotide of claims 1 or 3 or the oligonucleotide of claims 2 or 3, wherein the oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares 90% identity with an equal length portion of any one of SEQ ID NOs: 1-116.
6. The compound comprising an oligonucleotide of claims 1 or 3 or the oligonucleotide of claims 2 or 3, wherein the oligonucleotide comprises at least a contiguous 10 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.
7. The compound comprising an oligonucleotide of any one of claims 1 or 3-6 or the oligonucleotide of any one of claims 2-6, wherein the oligonucleotide comprises at least a

contiguous 10 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595.

8. The compound comprising an oligonucleotide of any one of claims 1 or 3-7 or the oligonucleotide of any one of claims 2-7, wherein the oligonucleotide comprises at least a
5 contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525.

9. The compound comprising an oligonucleotide of any one of claims 1 or 3-8 or the oligonucleotide of any one of claims 2-8, wherein the oligonucleotide comprises at least a
10 contiguous 11, 12, 13, 14, 15, 16, or 17 nucleobase sequence that shares at least 90% identity with an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595.

10. A compound comprising an oligonucleotide comprising at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID 1, 4, 5,
15 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1388, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

11. An oligonucleotide comprising at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17,
20 28, 29, 62, 625-649, 1195-1224, 1496-1567, 2591-2631, or 3395-3525, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

12. The compound comprising an oligonucleotide of claim 10, or the oligonucleotide of claim 11, wherein the oligonucleotide comprises at least 10 contiguous nucleobases that share 90% identity to an equal length portion of any one of SEQ ID NOs: 4, 1046, 1071, 1388,
25 1551, 1546, or 2595.

13. The compound comprising an oligonucleotide of claim 10, or the oligonucleotide of claim 11, wherein the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleobases of any one of SEQ ID NOs: 1, 4, 5, 6, 8, 10, 12, 13, 15, 17, 28, 29, 62, 625-649, 1046, 1071, 1195-1224, 1338, 1496-1567, 2591-2631, or 3395-3525.

14. The compound comprising an oligonucleotide of claim 13, or the oligonucleotide of claim 13, wherein the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19

contiguous nucleobases of any one of SEQ ID NOs: 4, 1046, 1071, 1388, 1551, 1546, or 2595.

15. A compound comprising an oligonucleotide comprising at least 10 contiguous nucleobases which is at least 90% complementary to an equal length portion of nucleobases within a 10 nucleobase range of any one of positions 374, 661, 655-680, 765, 837, 1347, 1340-1370, 1629, 1760, 1752, 1795, 1775, 1740-1815, 2879, 3008, 3168, or 3110-3171 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

16. An oligonucleotide comprising at least 10 contiguous nucleobases which is at least 90% complementary to an equal length portion of nucleobases within a 10 nucleobase range of positions 374, 661, 655-680, 765, 837, 1347, 1340-1370, 1629, 1760, 1752, 1795, 1775, 1740-1815, 2879, 3008, 3168, or 3110-3171 of SEQ ID NO: 3526, wherein at least one nucleoside linkage of the nucleobase sequence is a modified internucleoside linkage.

17. The compound comprising an oligonucleotide of claim 15, or the oligonucleotide of claim 16, wherein the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 655-680, 1340-137, 1740-1815, or 3110-3175 of SEQ ID NO: 3526.

18. The compound comprising an oligonucleotide of claim 17, or the oligonucleotide of claim 17, wherein the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 655-665, 660-670, 665-675, or 670-680 of SEQ ID NO: 3526.

19. The compound comprising an oligonucleotide of claim 17, or the oligonucleotide of claim 17, wherein the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 1340-1350, 1345-1355, 1350-1360, 1355-1365, or 1360-1370 of SEQ ID NO: 3526.

20. The compound comprising an oligonucleotide of claim 17, or the oligonucleotide of claim 17, wherein the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 1740-1750, 1745-1755, 1750-1760, 1755-1765, 1760-1770, 1765-1775, 1770-1780, 1775-1785, 1780-1790, 1785-1795, 1790-1800, 1795-1805, 1800-1810, or 1805-1815 of SEQ ID NO: 3526.

21. The compound comprising an oligonucleotide of claim 17, or the oligonucleotide of claim 17, wherein the oligonucleotide comprises at least 10 contiguous nucleobases that are complementary to an equal length portion of nucleobases within any one of positions 3110-3120, 3115-3125, 3120-3130, 3125-3135, 3130-3140, 3135-3145, 3140-3150, 3145-3155, 5 3150-3160, 3155-3165, 3160-3170, 3165-3175, 3170-3180 of SEQ ID NO: 3526.
22. The compound comprising an oligonucleotide of claim 15, or the oligonucleotide of claim 16, wherein the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleobases complementary to an equal length portion of nucleobases within any one of positions 374, 661, 765, 837, 1347, 1629, 2879, 3008, 3168, 1760, 1752, 1795, 1775, 10 655-680, 1340-1370, 1740-1815, or 3110-3171 of SEQ ID NO: 3526.
23. The compound comprising an oligonucleotide of claim 15, or the oligonucleotide of claim 16, wherein the oligonucleotide comprises at least 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleobases complementary to an equal length portion of nucleobases within any one of 655-680, 1340-137, 1740-1815, or 3110-3175 of SEQ ID NO: 3526.
- 15 24. The compound comprising an oligonucleotide of any one of claims 1 or 3-23, or the oligonucleotide of any one of claims 2-23, wherein the oligonucleotide is between 12 and 40 nucleobases in length.
25. The compound of any one of claims 1, 10, or 15, wherein the oligonucleotide comprises:
- 20 a. a gap segment comprising one or more of linked deoxyribonucleosides, 2'-Fluoro Arabino Nucleic Acids (FANA), and Fluoro Cyclohexenyl nucleic acid (F-CeNA);
- b. a 5' wing segment comprising linked nucleosides; and
- c. a 3' wing segment comprising linked nucleosides;
- 25 d. wherein the gap segment comprises a region of at least 8 contiguous nucleobases having at least 80% identity to an equal length portion of any one of SEQ ID NOs: 1-3525 positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment each comprises at least two linked nucleosides; and wherein at least one nucleoside
- 30 of each wing segment comprises a modified sugar.
26. The oligonucleotide of any one of claims 2, 11, or 16 comprising;

- a. a gap segment comprising one or more of linked deoxyribonucleosides, 2'-Fluoro Arabino Nucleic Acids (FANA), and Fluoro Cyclohexenyl nucleic acid (F-CeNA);
 - b. a 5' wing segment comprising linked nucleosides; and
 - 5 c. a 3' wing segment comprising linked nucleosides;
 - d. wherein the gap segment comprises a region of at least 8 contiguous nucleobases having at least 80% identity to an equal length portion of any one of SEQ ID NOs: 1-3525 positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment each
 - 10 comprises at least two linked nucleosides; and wherein at least one nucleoside of each wing segment comprises a modified sugar.
27. The compound comprising an oligonucleotide of claim 25, or the oligonucleotide of claim 26, wherein the oligonucleotide comprises at least 13, 14, 15, 16, 17, 18, 19, or 20 linked nucleosides.
- 15 28. The compound comprising an oligonucleotide of any one of claims 1 or 3-27, or the oligonucleotide of any one of claims 2-27, wherein at least one nucleoside linkage of the nucleobase sequence is selected from the group consisting of a phosphodiester linkage, a phosphorothioate linkage, a 2'-alkoxy linkage, an alkyl phosphate linkage, alkyl phosphonate linkage, a phosphorodithioate linkage, a phosphotriester linkage, an alkylphosphonate
 - 20 linkage, a methylphosphonate linkage, a dimethylphosphonate linkage, an aminoalkylphosphotriester linkage, an alkylene phosphonate linkage, a phosphinate linkage, a phosphoramidate linkage, a phosphorodiamidate linkage, an aminoalkylphosphoramidate linkage, a thiophosphoramidate linkage, a thionoalkylphosphonate linkage, a thionoalkylphosphotriester linkage, a thiophosphate linkage, a selenophosphate linkage, and a
 - 25 boranophosphate linkage.
29. The compound comprising an oligonucleotide of claim 25 or 27, or the oligonucleotide of claim 26 or 27, wherein the at least two linked nucleosides of the 5' wing segment are linked through a phosphodiester internucleoside linkage and wherein the at least two linked nucleosides of the 3' wing segment are linked through a phosphodiester
 - 30 internucleoside linkage, and wherein at least one of the internucleoside linkages of the gap segment is a modified internucleoside linkage.

30. The compound comprising an oligonucleotide of any one of claims 25 or 27-29 or the oligonucleotide of any one of claims 26-29, wherein at least two, three, or four internucleoside linkages of the nucleobase sequence are phosphodiester internucleoside linkages.
- 5 31. The compound comprising an oligonucleotide of any one of claims 25 or 27-30, or the oligonucleotide of any one of claims 26-30, wherein at least one, two, three, or four internucleoside linkages between nucleoside bases of the gap segment are phosphodiester internucleoside linkages.
32. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-31,
10 or the oligonucleotide of any one of claims 2-24 or 26-31, wherein at least two internucleoside linkages of the nucleobase sequence is a modified internucleoside linkage.
33. The compound comprising an oligonucleotide of claim 32, or the oligonucleotide of claim 32, wherein the modified internucleoside linkage of the nucleobase sequence is a phosphorothioate linkage.
- 15 34. The compound comprising an oligonucleotide of claim 32 or 33, or the oligonucleotide of claim 32 or 33, wherein all internucleoside linkages of the nucleobase sequence are phosphorothioate linkages.
35. The compound comprising an oligonucleotide of any one of claims 25, 27-28, or 32-34, or the oligonucleotide of any one of claims 26-28 or 32-34, wherein the at least two
20 linked nucleosides of the 5' wing segment are linked through a modified internucleoside linkage.
36. The compound comprising an oligonucleotide of any one of claims 25, 27-28, or 32-34, or the oligonucleotide of any one of claims 26-28 or 32-34, wherein the at least two linked nucleosides of the 3' wing segment are linked through a modified internucleoside
25 linkage.
37. The compound comprising an oligonucleotide of any one of claims 25, 27-28, or 32-36, or the oligonucleotide of any one of claims 26-28 or 32-36, wherein the at least two linked nucleosides of the 5' wing segment are linked through a phosphorothioate internucleoside linkage and wherein the at least two linked nucleosides of the 3' wing
30 segment are linked through a phosphorothioate internucleoside linkage, and wherein at least one of the internucleoside linkages of the gap segment is a modified internucleoside linkage.

38. The compound comprising an oligonucleotide of any one of claims 25, 27-28, or 32-37, or the oligonucleotide of any one of claims 26-28 or 32-37, wherein at least two, three, or four internucleoside linkages of the nucleobase sequence are phosphorothioate internucleoside linkages.

39. The compound comprising an oligonucleotide of any one of claims 25, 27-28, or 32-38, or the oligonucleotide of any one of claims 26-28 or 32-38, wherein at least one, two, three, or four internucleoside linkages between nucleoside bases of the gap segment are phosphorothioate internucleoside linkages.

40. The compound comprising an oligonucleotide of any one of claims 25, 27-28, or 32-39, or the oligonucleotide of any one of claims 26-28 or 32-39, wherein the phosphorothioate internucleoside linkage is in a *Rp* configuration, a *Sp* configuration, or in any combination of *Rp* and *Sp* configuration.

41. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-40, or the oligonucleotide of any one of claims 2-24 or 26-40, wherein the oligonucleotide comprises at least one modified nucleobase.

42. The compound comprising an oligonucleotide of claim 41, or the oligonucleotide of claim 41, wherein the at least one modified nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine.

43. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-42, or the oligonucleotide of any one of claims 2-24 or 26-42, wherein the oligonucleotide comprises at least one modified sugar moiety.

44. The compound comprising an oligonucleotide of claim 43, or the oligonucleotide of claim 43, wherein the at least one modified sugar is a bicyclic sugar.

45. The compound comprising an oligonucleotide of claim 44, or the oligonucleotide of claim 44, wherein the bicyclic sugar comprises a 4'-CH(R)—O-2' bridge wherein R is, independently, H, C₁-C₁₂ alkyl, or a protecting group.

46. The compound comprising an oligonucleotide of claim 45, or the oligonucleotide of claim 45, wherein R is methyl.

47. The compound comprising an oligonucleotide of claim 45, or the oligonucleotide of claim 45, wherein R is H.

48. The compound comprising an oligonucleotide of claim 43, or the oligonucleotide of claim 43, wherein the modified sugar moiety is one of a 2'-OMe modified sugar moiety, bicyclic sugar moiety, 2'-O-methoxyethyl (MOE), 2'-deoxy-2'-fluoro nucleoside, 2'-fluoro- β -D-arabinonucleoside, locked nucleic acid (LNA), constrained ethyl 2'-4'-bridged nucleic acid (cEt), S-cEt, tcDNA, hexitol nucleic acids (HNA), and tricyclic analog (*e.g.*, tcDNA).
49. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-48, or the oligonucleotide of any one of claims 2-24 or 26-48, wherein the oligonucleotide comprises one or more 2'-O-methoxyethyl nucleosides that are linked through phosphorothioate internucleoside linkages.
50. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-49, or the oligonucleotide of any one of claims 2-24 or 26-49, wherein the oligonucleotide comprises three contiguous nucleoside bases that are linked through phosphorothioate internucleoside linkages at the 5' end and three contiguous nucleoside bases that are linked through phosphorothioate internucleoside linkages at the 3' end.
51. The compound comprising an oligonucleotide of claim 50, or the oligonucleotide of claim 50, wherein the oligonucleotide comprises five contiguous nucleoside bases that are linked through phosphorothioate internucleoside linkages.
52. The compound comprising an oligonucleotide of claim 50 or 51, or the oligonucleotide of claim 50 or 51, wherein the each of the five contiguous nucleoside bases are 2'-O-methoxyethyl nucleosides.
53. The compound comprising an oligonucleotide of any one of claims 43-52, or the oligonucleotide of any one of claims 43-52, wherein each of the nucleoside bases of the oligonucleotide are 2'-O-methoxyethyl nucleosides.
54. The compound comprising an oligonucleotide of any one of claims 43-52, or the oligonucleotide of any one of claims 43-52, wherein the gap segment comprises one or more 2'-O-methoxyethyl nucleosides.
55. The compound comprising an oligonucleotide of any one of claims 43-54, or the oligonucleotide of any one of claims 43-54, wherein the gap segment comprises phosphorothioate internucleoside linkages, wherein the 5' wing segment comprises two contiguous nucleoside bases that are linked through phosphodiester internucleoside linkages, and wherein the 3' wing segment comprises two contiguous nucleoside bases that are linked through phosphodiester internucleoside linkages.

56. The compound comprising an oligonucleotide of any one of claims 43-54, or the oligonucleotide of any one of claims 43-54, wherein five contiguous nucleoside bases in the gap segment are linked through phosphorothioate internucleoside linkages, wherein the 5' wing segment comprises at least one phosphorothioate internucleoside linkage, and wherein the 3' wing segment comprises at least one phosphorothioate internucleoside linkage.

57. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-56, or the oligonucleotide of any one of claims 2-24 or 26-57, comprising one or more chiral centers and/or double bonds.

58. The compound comprising an oligonucleotide of claim 57, or the oligonucleotide of claim 57, wherein the oligonucleotide exist as stereoisomers selected from geometric isomers, enantiomers, and diastereomers.

59. The compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27, or the oligonucleotide of any one of claims 2-24 or 26-27, wherein the oligonucleotide comprises sugar modifications in any of the following patterns: eeeee-d10-eeee, d20, eeeee-d12-eeee, eeeee-d8-eeee, and eekk-d8-kkee, wherein e = 2'-O-methoxyethyl nucleoside; d = a 2'-deoxynucleoside; k = a locked nucleic acid (LNA), constrained methoxyethyl (cMOE) nucleoside, constrained ethyl (cET) nucleoside, or peptide nucleic acid (PNA).

60. The compound comprising an oligonucleotide of any one of claims 1, 3-25, 27, or 59, or the oligonucleotide of any one of claims 2-24, 26, 27, or 59, wherein the oligonucleotide comprises internucleoside linkages in any of the following patterns: sssssssssssssssss; ssssssssssssssssooss; and soossssssssooss; wherein s= a phosphorothioate linkage, and o= a phosphodiester linkage.

61. The compound comprising an oligonucleotide of any one of claims 1, 3-25, 27, 59, or 60, or the oligonucleotide of any one of claims 2-24, 26, 27, 59, or 60, wherein the oligonucleotide comprises sugar modification and internucleoside linkage combinations, respectively, in any of the following patterns:

- a) d20 and sssssssssssssssss;
- b) eeeee-d10-eeee and sssssssssssssssss;
- c) eeeee-d12-eeee and sssssssssssssssss;
- d) eeeee-d8-eeee and soossssssssooss; and
- e) eekk-d8-kkee and soossssssssooss.

62. The compound comprising an oligonucleotide of any one of claims 1, 3-25, 27, or 59-61, or the oligonucleotide of any one of claims 2-24, 26, 27, or 59-61, wherein the oligonucleotide comprises a modified cytosine.
63. The compound comprising an oligonucleotide of claim 62, or the oligonucleotide of claim 62, wherein the modified cytosine is 5-methyl-dC.
64. The compound comprising an oligonucleotide of claim 63, or the oligonucleotide of claim 63, wherein the oligonucleotide comprises sugar modification and internucleoside linkage combinations eeeee-d10-eeee and sssssssssssssssss, and the cytosine are modified as 5-methyl-dC.
65. The compound comprising an oligonucleotide of claim 60 or 61, or the oligonucleotide of claims 60 or 61, wherein the oligonucleotide comprises sugar modification and internucleoside linkage combinations, respectively, in any of the following patterns:
- a) d20 and sssssssssssssssss;
 - b) eeeee-d12-eeee and sssssssssssssssss;
 - c) eeeee-d8-eeee and soossssssssooss; and
 - d) eekk-d8-kkeee and soossssssssooss; and the any cytosine in the oligonucleotide is an unmodified cytosine.
66. A compound or oligonucleotide according to the compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-56, or the oligonucleotide of any one of claims 2-24 or 26-65, complementary to a nucleobase sequence of a target region of a target nucleic acid sequence, wherein the nucleobase sequence of the target region of the target nucleic acid differs from the nucleobase sequence of at least one non-target nucleic acid sequence by 1-3 differentiating nucleobases, and wherein the non-target nucleic acid comprises a sequence of SEQ ID NO: 3526.
67. The compound or oligonucleotide according to claim 66, wherein the 1-3 differentiating nucleobases comprises a single-nucleotide polymorphism (SNP).
68. The compound or oligonucleotide of claim 67, wherein the SNP present in the target region is a SNP compared to an equal length portion of SEQ ID NO: 3526.
69. The compound or oligonucleotide according to claim 66, wherein the single nucleotide polymorphism is selected from the group consisting of: rs397515403,

rs397515402, rs587777264, rs397515404, rs866242631, rs886043455, rs397515407, and rs397515406.

70. The compound or oligonucleotide according to claim 66, wherein the single nucleotide polymorphism is selected from the group consisting of: a C to a G at position 1112 of the sequence shown in SEQ ID NO: 3526, a C to a T at position 2845 of the sequence shown in SEQ ID NO: 3526, and a G to a T at position 885 of the sequence shown in SEQ ID NO: 3526.

71. A pharmaceutical composition comprising the compound or oligonucleotide of any one of the above claims and a pharmaceutically acceptable carrier or excipient.

72. The pharmaceutical composition of claim 71, wherein the pharmaceutical composition is suitable for topical, intrathecal, intracisternal, parenteral, oral, pulmonary, intratracheal, intranasal, transdermal, rectal, buccal, sublingual, vaginal, or intraduodenal administration.

73. A composition comprising the compound or oligonucleotide of any one of the above claims and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome.

74. A method of reducing a level and/or activity of KCNT1 in a cell of a subject having a KCNT1 related disorder, the method comprising contacting the cell with the compound of any one of claims 1, 3-25, or 27-70, the oligonucleotide of any one of claims 2-24, or 26-70, or the pharmaceutical composition of claims 71 or 72 in an amount and for a duration sufficient to reduce the level and/or activity of KCNT1 in the cell.

75. The method of claim 74, wherein the cell is a cell of the central nervous system.

76. A method of treating a neurological disease in a subject in need thereof, the method comprising administering to the patient an inhibitor of a transcript, wherein the transcript shares at least 90% identity with SEQ ID NO: 3526.

77. The method of claim 76, wherein the inhibitor is the compound comprising an oligonucleotide of any one of claims 1, 3-25, or 27-70, or the oligonucleotide of any one of claims 2-24, or 26-70 or the pharmaceutical composition of claims 71 or 72.

78. A method of treating, preventing, or delaying the progression of a KCNT1 related disorder in a subject in need thereof, the method comprising administering to the subject the compound of any one of claims 1, 3-25, or 70, the oligonucleotide of any one of claims 2-24,

or 26-70 or the pharmaceutical composition of claims 71 or 72 in an amount and for a duration sufficient to treat, prevent, or delay the progression of the KCNT1 related disorder.

79. The method of any one of claims 74-78, wherein the KCNT1 related disorder is selected from the group consisting of epilepsy of infancy with migrating focal seizures, autosomal dominant nocturnal frontal lobe epilepsy, West syndrome, infantile spasms, epileptic encephalopathy, focal epilepsy, Ohtahara syndrome, developmental epileptic encephalopathy, and Lennox Gastaut syndrome.

80. The method of any one of claims 74-79, wherein the subject has a gain-of-function mutation in KCNT1.

81. The method of claim 80, wherein the gain-of-function mutation is selected from the group consisting of V271F, L274I, G288S, F346L, R398Q, R428Q, R474H, F502V, M516V, K629N, I760M, Y796H, E893K, M896I, M896K, P924L, R928C, F932I, A934T, A966T, H257D, R262Q, Q270E, V340M, C377S, P409S, L437F, R474C, A477T, R565H, K629E, G652V, I760F, Q906H, R933G, A934T, R950Q, R961H, R1106Q, K1154Q, R474Q, Y1903C, H469L, M896R, K946E, and R950L.

82. The method of claim 80 or 81, wherein the gain-of-function mutation is G288S, R398Q, R428Q, R928C, or A934T.

83. The method of any one of claims 74-82, wherein the method reduces one or more symptoms of the KCNT1 related disorder.

84. The method of claim 83, wherein the one or more symptoms of the KCNT1 related disorder is selected from the group consisting of prolonged seizures, frequent seizures, behavioral and developmental delays, movement and balance issues, orthopedic conditions, delayed language and speech issues, growth and nutrition issues, sleeping difficulties, chronic infection, sensory integration disorder, disruption of the autonomic nervous system, and sweating.

85. The method of any claims 74-84, wherein the oligonucleotide or pharmaceutical composition is administered topically, parenterally, intrathecally, intracisternally, orally, rectally, buccally, sublingually, vaginally, pulmonarily, intratracheally, intranasally, transdermally, or intraduodenally.

86. The method of any one of claims 74-85, wherein the patient is a human.

87. A compound comprising a modified oligonucleotide of 18-22 linked nucleosides in length and having at least 85% sequence complementarity to an equal length portion of *H. sapiens* KCNT1 and *M. musculus* KCNT1 transcript.
88. A compound comprising a modified oligonucleotide of 18-22 linked nucleosides in length and having at least 85% sequence complementarity to an equal length portion of *H. sapiens* KCNT1 and *M. fascicularis* KCNT1 transcript.
89. A compound comprising a modified oligonucleotide of 18-22 linked nucleosides in length and having at least 85% sequence complementarity to an equal length portion of *H. sapiens* KCNT1, *M. musculus* KCNT1, and/or *M. fascicularis* KCNT1 transcript.
90. The compound of any one of claims 87-89, wherein the oligonucleotide comprises a GC content from 40% to 70%.
91. The compound of any one of claims 87-90, wherein the oligonucleotide comprises no more than 2 mismatches to *H. sapiens* KCNT1 transcript.
92. The compound of any one of claims 87-91, wherein the oligonucleotide comprises at least 3 mismatches to any non KCNT1 transcript.
93. The compound of any one of claims 87-92, wherein the oligonucleotide lacks a GGGG tetrad.
94. The method of any one of claims 74-86, wherein the oligonucleotide is not any one of SEQ ID NOs: 3512-3525.
95. The pharmaceutical composition of claim 71 or 72, wherein the oligonucleotide is not any one of SEQ ID NOs: 3512-3525.

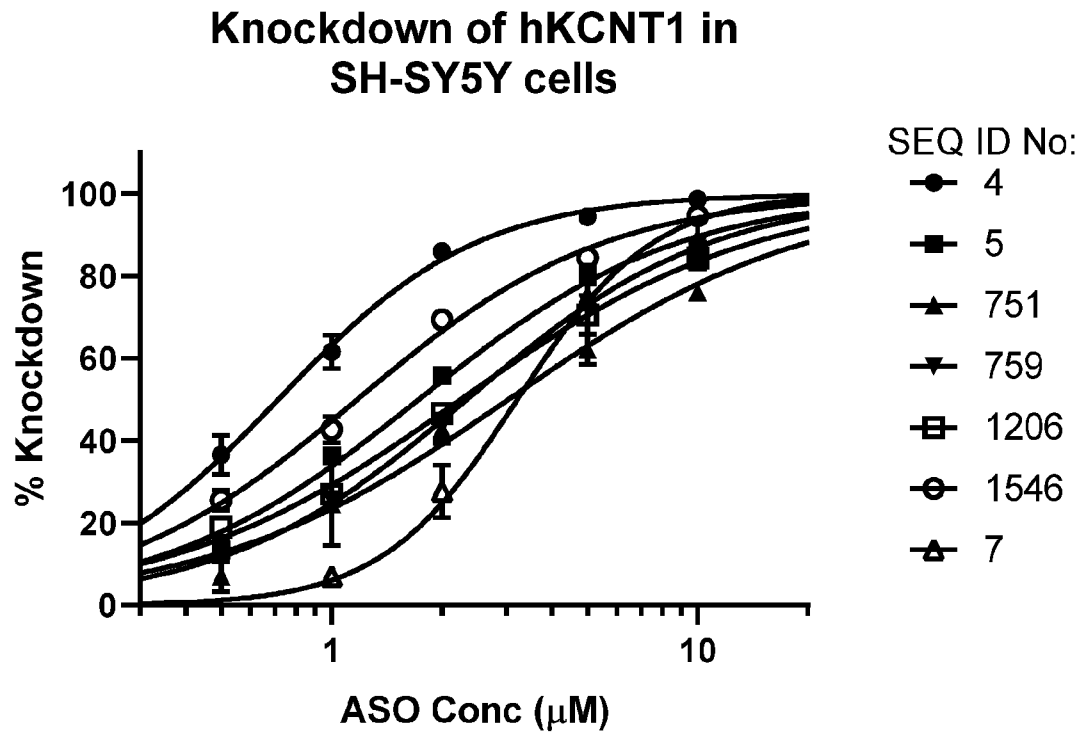


FIG. 1

Knockdown of hKCNT1 in SH-SY5Y cells

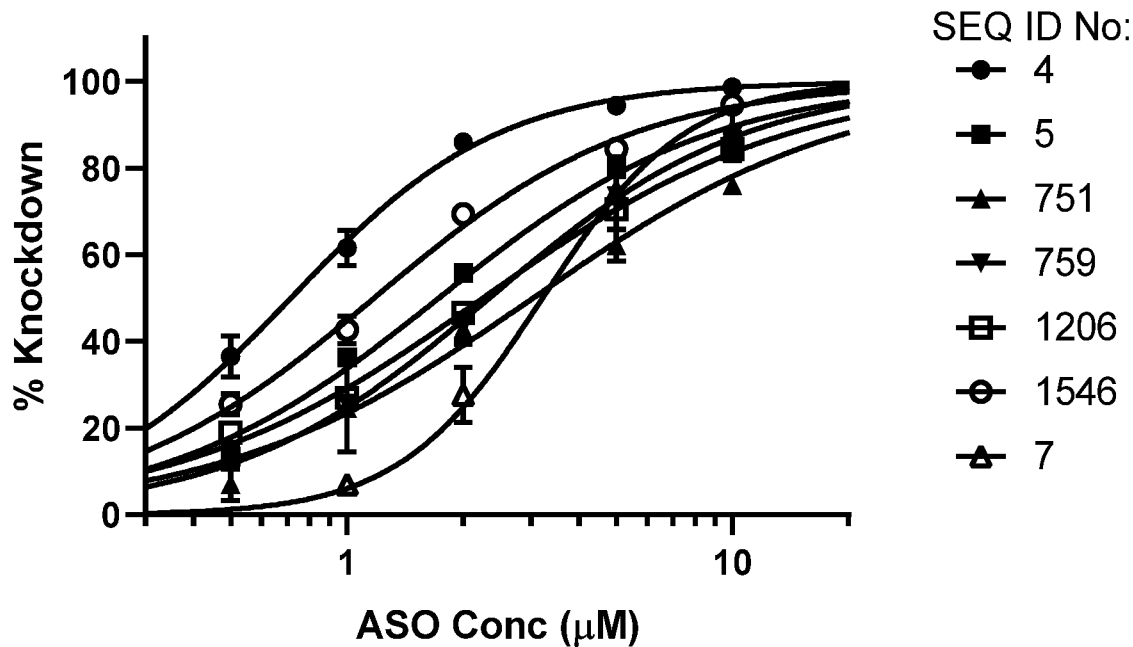


FIG. 1