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(54) Title: LNA OLIGONUCLEOTIDES FOR SPLICE MODULATION OF STMN2

(57) Abstract: The present invention relates to splice modulating LNA oligonucleotides (oligomers) that are complementary to the STMN2 pre-mRNA, for inhibiting the inclusion of a STMN2 cryptic exon into STMN2 transcripts which is associated with neurological diseases. The oligonucleotides of the invention are useful in the treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS).

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LNA OLIGONUCLEOTIDES FOR SPLICE MODULATION OF STMN2

FIELD OF INVENTION

The present invention relates to splice modulating oligonucleotides (oligomers) that are complementary to the STMN2 pre-mRNA, for inhibiting the inclusion of a STMN2 cryptic exon during STMN2 pre-mRNA splicing, which results in STMN2 transcript variants that are associated with neurodegenerative disorders characterized by TDP-43 pathology or mislocalization of TDP-43. The oligonucleotides of the invention are useful in the treatment of neurodegenerative diseases and disorder, such as neurodegenerative diseases and disorders characterized by TDP-43 pathology or mislocalization of TDP-43 from the nucleus, such as amyotrophic lateral sclerosis (ALS).

BACKGROUND

Klim et al., Nature Neuroscience 2019, vol 22 167-179 reports that ALS-implicated protein TDP-43 sustains levels of STMN2, a mediator of motor neuron growth and repair, and in particular certain STMN2 transcripts whose abundances in human motor neurons are sensitive to TDP-43 depletion, and proposes that restoring STMN2 expression warrants examination as a therapeutic strategy for ALS. Melamed et al., Nature Neuroscience 22, 180-190 (2019) reports that premature polyadenylation-mediated loss of stathmin-2 is a hallmark of TDP-43-dependent neurodegeneration.

TDP-43 inclusions are observed in a variety of neurodegenerative disorders (Lagier-Tourenne et al., HMG, 2010). TDP-43 pathology is associated to higher cognitive impairment and medial temporal atrophy in AD (Josephs et al., Acta Neurop, 2014; Mayo Clinic). Aberrant splicing of TDP-43 targets is observed in AD patient brains with TDP-43 aggregates or TDP-43 nuclear exclusion (Sun et al., Acta Neuropath, 2017, Johns Hopkins)

Antisense-mediated splicing modulation is a tool that can be exploited in several ways to provide a potential therapy for rare genetic diseases. This approach has resulted in approved therapeutics for Duchenne muscular dystrophy and spinal muscular atrophy – see Arechavala-Gomez et al., Appl Clin Genet. 2014; 7: 245–252 for a review of various approaches for splice modulation using antisense oligonucleotides.

WO2019/241648 discloses 2'-O-methoxyethyl ASOs for increasing STMN2 expression.

The present inventors have taken an approach by the use of splice switching LNA mixmer compounds targeting a STMN2 cryptic exon present in the intronic region between exon1 and exon 2 of the human STMN2 pre-mRNA. The compounds of the invention may inhibit the inclusion of the cryptic intron and/or enhance the expression of wildtype STMN2 mRNA.

OBJECTIVE OF THE INVENTION

The present invention provides splice modulating oligonucleotides (oligomers) that are complementary to the STMN2 pre-mRNA, for inhibiting the inclusion of a STMN2 cryptic exon and/or enhancing the expression of wildtype STMN2 mRNA. The oligonucleotides of the invention are useful in the treatment of neurodegenerative diseases such as neurodegenerative disorders characterized by TDP-43 pathology or mislocalization of TDP-43 from the nucleus. In some embodiments, the oligonucleotides of the invention are for use in the treatment of amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD).

SUMMARY OF INVENTION

The present invention provides antisense oligonucleotides that are complementary to, and are capable of modulating the expression of a STMN2 nucleic acid, and for their use in medicine.

The present invention relates to the identification of advantageous target site sequences present within a STMN2 nucleic acid, which are suitable for targeting with LNA antisense oligonucleotides.

The invention provides for an LNA antisense oligonucleotide of 14 to 18 contiguous nucleotides in length, and comprises a contiguous nucleotide sequence of at least 14 nucleotides in length which are 100% complementarity to a sequence selected from the group consisting of SEQ ID Nos 144, 91, 92, 93, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, , 108, 109, 110, , 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, , 160, 161, 162, 163, 164, 165, 166, 167, 168, 170, 174, 175, 176, 177, 178, 179, and 180. The LNA antisense oligonucleotide of the invention is capable of modulating the expression of human STMN2 pre-mRNA in a cell which is expressing human STMN2 pre-mRNA, suitably by modulating the splicing of the human STMN2 pre-mRNA and/or by enhancing the expression of human mature STMN2 mRNA which comprises a contiguous sequence between exon 1 and exon 2 (referred to herein as wildtype STMN2 transcript, or WT STMN2 transcript).

Advantageously, the LNA antisense oligonucleotide of the invention is capable of either (i) reducing the level of human STMN2 mature mRNA which comprise an STMN2 intron 1 derived cryptic exon, such as that illustrated within SEQ ID NO 182; or (ii) enhancing the expression level of wildtype STMN2 mature mRNA in a TDP-43 depleted cell which is expressing human STMN2 pre-mRNA (for example SEQ ID NO 183).

Advantageously, the LNA antisense oligonucleotide of the invention is capable of (i) reducing the level of human STMN2 mature mRNA which comprise a intron 1 derived cryptic exon, such as that illustrated within SEQ ID NO 182; and (ii) enhancing the expression level of wildtype

STMN2 mature mRNA; in a TDP-43 depleted cell which is expressing human STMN2 premRNA (for example SEQ ID NO 183).

Suitably, in some embodiments, the reduction in the level of human STMN2 mature mRNA which comprise a intron 1 derived cryptic exon and the enhancement of the expression level of wildtype STMN2 mature mRNA may be achieved concurrently in the TDP-43 depleted cell by the administration of the oligonucleotides of the invention to the TDP-43 depleted cell in an effective amount.

Suitably, the antisense oligonucleotides of the invention are capable of modulating the splicing on the STMN2 pre-mRNA so as to inhibit the inclusion of a region of intronic sequence in STMN2 transcripts, such as a region of intron 1 of the STMN2 pre-mRNA. Inclusion of a region of intron 1 (referred to as cryptic exon, or ce1), results in the inclusion of a region of intron 1 sequence positioned 3' of exon 1 in the resulting mature STMN2 mRNA transcript. By way of example a mRNA which comprises the cryptic exon may comprise the underlined sequence shown in SEQ ID NO 182.

It will be understood that where the reference sequence provided herein is a DNA sequence, and the target is a RNA, the reference sequence it refers to the equivalent genomic DNA sequence or cDNA sequence. For example the equivalent RNA sequence will comprise ribonucleotides, and the RNA sequence will comprise U rather than T bases.

The presence of the cryptic intron within the STMN2 mRNA transcript is associated with TDP-43 pathologies.

The antisense oligonucleotide of the invention may suitably be referred to as a splice modulating oligonucleotide.

Advantageously, the antisense oligonucleotides of the invention modulate the splicing of STMN2 pre-mRNA so that there is a change in ratio of the STMN2 transcripts: with a decrease in the proportion of STMN2 transcripts which comprise the cryptic intron as compared to the STMN2 which comprise an intact exon1:exon2 STMN2 boundary (as illustrated in SEQ ID NO 184 – also referred to herein as the WT STMN2 mRNA).

The present invention relates to the identification of advantageous target site sequences present within a STMN2 pre-mRNA, which are suitable for targeting with antisense oligonucleotides of the invention to modulate the splicing of the STMN2 pre-mRNA, to inhibit the inclusion of the cryptic exon in STMN2 transcripts.

The present invention provides an antisense oligonucleotide targeting a STMN2 nucleic acid for use in the treatment of a disease selected from the group consisting of neurodegenerative diseases as neurodegenerative disorders characterized by TDP-43 pathology or mislocalization

of TDP-43 from the nucleus, such as amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD).

Advantageously, the antisense oligonucleotides of the invention comprises a contiguous nucleotide sequence of at least 14 nucleotides in length, which are complementary to such as fully complementary to SEQ ID NO 182:

SEQ ID NO 182:

TTGGATTTTTAAAATTATATTCATATTGCAG^vGACTCGGCAGAAGACCTTCGAGAGAAA
GGTAGAAAATAAGAATTTGGCTCTCTGTGTGAGCATGTGTGCGTGTGTGCGAGAG
AGAGAGACAGACAGCCTGCCTAAGAAGAAATGAATGTGAATGCGGCTTGTGGCAC
AGTTGACAAGGATGATAAATCAATAATGCAAGCTTACTATCATTATGAATAGCAAT
ACTGAAGAAATTAAACAAAAGATTGCTGTCTCAATATATC

SEQ ID NO 182 is a part of human STMN2 intron 1 which is targeted by the compounds of the invention. The ^v symbol represents an alternative splice acceptor site which is often activated in TDP-43 depleted cells, the bold text represents the part of intron 1 which is spliced out in the mature ce1 STMN2 (aberrant form), whilst the underlined sequence is STMN2 intron 1 sequence which is incorporated as a cryptic exon (ce1). As reported in Melamed et al., Nature Neuroscience 22 (2019), 180 – 190, the presence of the cryptic exon results in the introduction of a premature polyadenylation site (italicized ATTAAA), resulting in a truncated protein and loss of STMN2 function.

Please note in the STMN2 transcript sequence, uracil nucleosides are present instead of the thymine nucleosides illustrated in the SEQ ID NO 182 references sequence). The underlined region represents an exemplary sequence of a cryptic exon, originating from intron 1 of the STMN2 pre-mRNA.

In some embodiments, the antisense oligonucleotides of the invention comprise a contiguous nucleotide sequence of at least 14 nucleotides in length, such as 14 – 18 nucleotides in length, which are complementary to such as fully complementary to nucleotides 32 – 266 of SEQ ID NO 182.

In some embodiments, the antisense oligonucleotides of the invention comprises a contiguous nucleotide sequence of at least 14 nucleotides in length, such as at least 16 nucleotides in length, which are complementary to such as fully complementary to a sequence selected from SEQ ID NO 91 – 180.

In some embodiments, the antisense oligonucleotides of the invention comprises a contiguous nucleotide sequence of at least 14 nucleotides in length, such as at least 16 nucleotides in length present in a sequence selected from SEQ ID NO 1 – 90.

In some embodiments, the antisense oligonucleotides of the invention comprises a contiguous nucleotide sequence of 16 nucleotides in length present in a sequence selected from the group consisting of SEQ ID NO 1 – 90.

In some embodiments, the antisense oligonucleotides of the invention comprises a contiguous nucleotide sequence selected from the group consisting of SEQ ID NO 1 – 90.

In some embodiments, the invention provides a LNA antisense oligonucleotide of 14 to 18 contiguous nucleotides in length, and comprises a contiguous nucleotide sequence of at least 14 nucleotides in length which are 100% complementarity to a sequence selected from the group consisting of SEQ ID Nos 144, 91, 92, 93, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, , 108, 109, 110, ,113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, , 160, 161, 162, 163, 164, 165, 166, 167, 168, 170, 174, 175, 176, 177, 178, 179, and 180, wherein the antisense oligonucleotide is capable of either (i) reducing the level of human STMN2 mature mRNA which comprise a intron 1 derived cryptic exon, such as illustrated in SEQ ID NO 182; or (ii) enhancing the expression level of wildtype STMN2 mature mRNA in a TDP-43 depleted cell which is expressing human STMN2 pre-mRNA. Suitably, the antisense oligonucleotide is capable of (i) reducing the level of human STMN2 mature mRNA which comprise a intron 1 derived cryptic exon, such as illustrated within SEQ ID NO 182; and (ii) enhancing the expression level of wildtype mature STMN2 mRNA, in a TDP-43 depleted cell which is expressing human STMN2 pre-mRNA.

In some embodiments, the LNA antisense oligonucleotide is 14, 15, 16 or 17 contiguous nucleotides in length which together form the contiguous nucleotide sequence which is 100% complementarity to a sequence selected from the group consisting of SEQ ID Nos 144, 91, 92, 93, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, , 108, 109, 110, ,113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, , 160, 161, 162, 163, 164, 165, 166, 167, 168, 170, 174, 175, 176, 177, 178, 179, and 180.

In some embodiments, the LNA antisense oligonucleotide comprises LNA and DNA nucleosides, such as a LNA mixmer oligonucleotide.

It is advantageous that the LNA antisense oligonucleotide of the invention is capable of enhancing the expression of wildtype STMN2 in a TDP-43 depleted cell which is expressing human STMN2 pre-mRNA, when administered to said cell in suitably effective amount. In this regard, the LNA antisense oligonucleotide of the invention should advantageously not deplete the level of WT STMN2 mature mRNA in the cell. Advantageously the LNA antisense oligonucleotide of the

invention is capable of enhancing the expression of WT STMN2 mature mRNA in a TDP-43 depleted cell, which is expressing human STMN2 pre-mRNA. Advantageously, the LNA antisense oligonucleotide is not capable of recruiting RNaseH. Advantageously, LNA antisense oligonucleotide is not capable of recruiting the RNAi machinery.

- 5 In some embodiments, the antisense oligonucleotide comprises of a contiguous nucleotide sequence selected from the group consisting of SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.
- 10 In some embodiments, the LNA antisense oligonucleotide comprises of a contiguous nucleotide sequence selected from the group consisting of SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.
- 15 In some embodiments, the antisense oligonucleotide comprises of a contiguous nucleotide sequence selected from the group consisting of compound ID NO # 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.
- 20 The invention provides for an antisense oligonucleotide selected from the group consisting of compound ID NO # 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.

- 25 The invention provides for an antisense oligonucleotide of formula (5' – 3')

Compound ID NO 54: ${}^m\text{C}_s\text{a}_s\text{c}_s\text{A}_s\text{c}_s\text{A}_s\text{c}_s\text{G}_s\text{c}_s\text{A}_s\text{c}_s\text{A}_s\text{c}_s\text{a}_s\text{T}_s\text{G}$

wherein capital letters are beta-D-oxy LNA nucleotides, lower case letters are 2'deoxyribose nucleosides (DNA nucleoside), ${}^m\text{C}$ are 5-methyl cytosine beta-D-oxy LNA nucleosides, and subscript s is a phosphorothioate internucleoside linkage.

- 30 The invention provides for an antisense oligonucleotide of formula (5' – 3')

Compound ID NO 42: $\text{T}_s\text{t}_s\text{T}_s\text{c}_s\text{t}_s\text{T}_s\text{c}_s\text{t}_s\text{T}_s\text{a}_s\text{g}_s\text{G}_s\text{c}_s\text{a}_s\text{G}_s\text{G}$

wherein capital letters are beta-D-oxy LNA nucleotides, lower case letters are 2'deoxyribose nucleosides, and subscript s is a phosphorothioate internucleoside linkage.

The invention provides for an antisense oligonucleotide of formula (5' – 3')

- 35 Compound ID NO 35: ${}^m\text{C}_s\text{a}_s\text{c}_s\text{A}_s\text{a}_s\text{G}_s\text{c}_s\text{c}_s\text{G}_s\text{c}_s\text{A}_s\text{t}_s\text{T}_s\text{c}_s\text{A}_s{}^m\text{C}$

wherein capital letters are beta-D-oxy LNA nucleotides, lower case letters are 2'deoxyribose nucleosides, ^mC are 5-methyl cytosine beta-D-oxy LNA nucleosides, and subscript s is a phosphorothioate internucleoside linkage.

The invention provides for an antisense oligonucleotide of formula (5' – 3')

5 Compound ID NO 51: T_sC_sT_sC_st_s^mC_st_sC_sG_sC_sA_sC_sA_sC_sA_s^mC

wherein capital letters are beta-D-oxy LNA nucleotides, lower case letters are 2'deoxyribose nucleosides, ^mC are 5-methyl cytosine beta-D-oxy LNA nucleosides, and subscript s is a phosphorothioate internucleoside linkage.

10 The oligonucleotide of the invention may comprise one or more conjugate groups, *i.e.* the oligonucleotide may be an antisense oligonucleotide conjugate.

The invention provides pharmaceutical compositions comprising the antisense oligonucleotide of the invention and a pharmaceutically acceptable diluents, carriers, salts and/or adjuvants.

15 The invention provides pharmaceutical compositions comprising the antisense oligonucleotide of the invention and a pharmaceutically acceptable diluents, carriers, salts or adjuvants.

The invention provides for a pharmaceutically acceptable salt of the antisense oligonucleotide of the invention. In some embodiments, the pharmaceutically acceptable salt is a sodium salt, a potassium salt or an ammonium salt.

20 The invention provides for a pharmaceutical solution of the oligonucleotide of the invention, wherein the pharmaceutical solution comprises the oligonucleotide of the invention and a pharmaceutically acceptable solvent, such as phosphate buffered saline.

The invention provides for the oligonucleotide of the invention in solid powdered form, such as in the form of a lyophilized powder.

25 Typically, the antisense oligonucleotide of the invention comprises a contiguous nucleotide sequence of at least 10 nucleotides in length, such as 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to, such as fully complementary to, the STMN2 target nucleic acid, such as SEQ ID NO 183. In some embodiments, the all of the nucleosides of the antisense oligonucleotide form the contiguous nucleotide sequence.

30 In some embodiments, the antisense oligonucleotide of the invention has a length of up to 30 nucleotides, and comprises a contiguous nucleotide sequence of at least 10 nucleotides in length, such as 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to, such as fully complementary to, the STMN2 target nucleic acid, such as SEQ ID NO 183, or a target site there in such as a target site (target sequence) selected from the group consisting of SEQ ID NO 91 – 180.

In some embodiments, the antisense oligonucleotide of the invention has a length of up to 30 nucleotides, and comprises a contiguous nucleotide sequence of at least 12 nucleotides in length, such as 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to, such as fully complementary to SEQ ID NO 182.

- 5 Advantageously, the antisense oligonucleotide of the invention is capable of modulating the splicing of the human STMN2 pre-mRNA, such as to inhibit or reduce the inclusion of STMN2 intron 1 sequence in the population of STMN2 mature mRNA transcripts (also referred to as transcripts herein).

The invention provides an *in vivo* or *in vitro* method for modulating the splicing of a mammalian
10 STMN2 pre-mRNA in a target cell which is expressing a STMN2 pre-mRNA, by administering an oligonucleotide or composition of the invention in an effective amount to said cell, wherein the administering an oligonucleotide results in a decrease in the level, or decrease in the expression of, a mature STMN2 mRNA which comprises a sequence of RNA nucleotides positioned between the 3' splice site of exon 1 and the 5' splice site of exon 2, such the intron 1
15 sequence shown in SEQ ID NO 182 (the nucleotides in position 32 – 266 of sequence ID NO 182 are intron 1 (cryptic exon, ce1) sequence).

Modulation of STMN2 expression may therefore refer to a change in the ratio of splice variant transcripts of STMN2, such as an increase in the level of the wildtype STMN2 mRNA (such as SEQ ID NO 184), and/ or a decrease in the level of STMN2 mRNA transcript which comprises
20 STMN2 intron 1 sequence (cryptic exon 1, ce1), such the sequence shown in SEQ ID NO 182 (nucleotides 23-266 of SEQ ID NO 182).

In an advantageous embodiment, the oligonucleotide of the invention is a splice modulating oligonucleotide, such as a splice switching oligonucleotide, which targets the STMN2 pre-mRNA, preventing or inhibiting the splicing event which results in a mature mRNA which
25 comprises the cryptic exon, such as ce1. Splice modulating oligonucleotides typically operate via an occupation based mechanism rather than via RNaseH or siRNA degradation.

In a further aspect the invention provides methods for treating or preventing neurodegenerative disease such as amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD)., comprising administering a therapeutically or prophylactically effective amount of the
30 oligonucleotide of the invention to a subject suffering from or susceptible to the disease.

In a further aspect the oligonucleotide or composition of the invention is used for the treatment or prevention of a neurodegenerative disease as neurodegenerative disorders characterized by TDP-43 pathology or mislocalization of TDP-43 from the nucleus, such as the disorders and diseases referred to herein, such as amyotrophic lateral sclerosis (ALS) or frontotemporal lobar
35 degeneration (FTLD).

SEQUENCE LISTING

The sequence listing submitted with this application is hereby incorporated by reference. It will be understood that in reference to target nucleic acids or target sequences or target sites, the sequences disclosed herein refer to DNA sequences, the target nucleic acids in the cell, in vitro or in vivo, are however the RNA versions (for instance in the RNA target sequences uracil (U) is present in place of the thymine (T) shown in the enclosed DNA sequences). SEQ ID NO 184 is the reference human STMN2 mature mRNA (correctly spliced);

SEQ ID NOs 1 – 90 are the nucleobase sequence motif of compounds #1 – 90. SEQ ID NOs 91 – 180 are the nucleobase sequence of the target sites (DNA equivalent of the RNA is provided) which are complementary to compounds #1 – 90 respectively. SEQ ID NO 181 is the TDP-43 targeting LNA gapmer which is used to deplete TDP-43 in the neuronal cells in the examples; SEQ ID NO 183 is the reference GRCh38:8:79610814:79666175:1 genomic sequence of human STMN2 (pre-mRNA sequence); SEQ ID NO 182 is the reference sequence which contains the part of intron 1 which forms the STMN2 cryptic exon in TDP-43 depleted cells; SEQ ID NO 184 is the reference STMN2 mature mRNA, correctly spliced; SEQ ID NO 185 – 188 are selected regions which may be targeted by the compounds of the invention; SEQ ID NO 190 is a reference STMN2 mRNA which comprises a cryptic exon sequence derived from STMN2 intron 1; SEQ ID NO 189 is a reference sequence of a STMN2 cryptic exon; SEQ ID NO 191 is the reference STMN2 amino acid sequence, encoded by SEQ ID NO 184; SEQ ID NO 192 is a reference amino acid sequence encoded by SEQ ID NO 190; SEQ ID NOs 194 – 201 are various probes, primers and PCR product sequences referred to in the examples.

BRIEF DESCRIPTION OF FIGURES

Figure 1. RNA-Seq analysis of *TARDBP* and *STMN2* in human motor neurons (hMNS) treated with or without antisense oligonucleotide targeting *TARDBP* encoding TDP-43. A) *TARDBP* expression is reduced 72x on average upon treatment with SEQ ID NO 181. B) Measurement of the wild type *STMN2* transcript (SEQ ID NO 183) shows a 101x reduction upon treatment with SEQ ID NO 181 and C) shows an increase in the transcript including the cryptic exon (2a) (SEQ ID NO 184) by 138x. The analysis was performed in duplicates untreated cells (black dots) and SEQ ID NO 181 treated cells (light grey dots). Counts corresponds to the number of transcripts based on RNA-Seq analysis using Cufflinks to map reads against human genome (*hg38*).

Figure 2. Genome viewer (CLC genomic Workbench by Qiagen) showing the inclusion of the *STMN2* cryptic exon (2a) and the overall reduction of wild type *STMN2* upon treatment of human motor neurons with antisense oligonucleotide. Upper panel shows *hg38* and the lower panel shows the annotated isoform of *STMN2* pre-mRNAs. The upper two traces show untreated hMNS and the exon coverage in duplicates. The lower two traces show the exon coverage in hMNS treated with SEQ ID NO 181. The blue box marks the included cryptic exon.

Figure 3. Removal of *TDP-43* removes *STMN2* wild type expression and induce inclusion of cryptic exon (exon 2a). Illustration of RNA-Seq analysis showing the coverage at exon boundaries within the *STMN2* gene. (A) Coverage at single nucleotide position (first or last bp in predicted exons) of untreated cells (striped bars), cells treated with SEQ ID NO 181 (filled light grey bars). The analysis was performed in duplicates with SD shown. (B) Graphical illustration of the coverage in the untreated and treated cells with inclusion of the polyadenylation. Positions within *hg38*, *STMN2*: exon 1 (3') chr8:79,311,214, exon 2a (5') chr8:79,616,822, exon 2 (5') chr8:79,636,802, exon 2 (3') chr8:79,636,897, exon 3 (5') chr8:79,641,378, exon 4 (5') chr8:79,654,871, exon 4 (3') chr8:79,655,062, exon 5 (5') chr8:79,664,815.

Figure 4. Illustration showing the position of antisense oligonucleotides (small grey bars) blocking the inclusion of the *STMN2* cryptic exon (2a) (big light grey box) to restore canonical splicing *STMN2* (exon 1 to 2).

Figure 5. Illustration of an example of the aberrant splicing event which results in the cryptic exon inclusion in *STMN2* mRNA in TDP-43 depleted cells. The nucleotide sequence is SEQ ID NO 190 (*STMN2* cryptic exon containing mRNA transcript) and the amino acid sequence is the resulting truncated *STMN2* protein. The shaded bold nucleotide sequence is the *STMN2* exon 1 sequence and the underlined nucleotide sequence is the cryptic exon sequence which originates from intron 1. The ATG start codon, TAG stop codon and polyadenylation signal are highlighted.

DEFINITIONS

Oligonucleotide

The term “oligonucleotide” as used herein is 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 and isolation. 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 is man-made, and is chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides such as 2' sugar modified nucleosides. The oligonucleotide of the invention may comprise one or more modified internucleoside linkages, such as one or more phosphorothioate internucleoside linkages.

Antisense oligonucleotides

The term “antisense oligonucleotide” as used herein is defined as oligonucleotides capable of modulating expression of a target gene by hybridizing to a target nucleic acid, in particular to a

contiguous sequence on a target nucleic acid. Antisense oligonucleotides are not essentially double stranded and are therefore not siRNAs or shRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded. It is understood that single stranded oligonucleotides of the present invention can form hairpins or intermolecular duplex structures (duplex between two molecules of the same oligonucleotide), as long as the degree of intra or inter self-complementarity is less than 50% across of the full length of the oligonucleotide.

In some embodiments, the single stranded antisense oligonucleotide of the invention may not contain RNA nucleosides.

Advantageously, the antisense oligonucleotide of the invention comprises one or more modified nucleosides or nucleotides, such as 2' sugar modified nucleosides. Furthermore, it is advantageous that the nucleosides which are not modified are DNA nucleosides.

An LNA antisense oligonucleotide is an antisense oligonucleotide which comprises at least one LNA nucleoside.

Contiguous Nucleotide Sequence

The term "contiguous nucleotide sequence" refers to the region of the oligonucleotide which is complementary to the target nucleic acid. The term is used interchangeably herein with the term "contiguous nucleobase sequence" and the term "oligonucleotide motif sequence". In some embodiments all the nucleosides of the oligonucleotide constitute the contiguous nucleotide sequence. The contiguous nucleotide sequence is the sequence of nucleotides in the oligonucleotide of the invention which are complementary to, preferably fully complementary to the target nucleic acid or target sequence.

In some embodiments the oligonucleotide comprises the contiguous nucleotide sequence, such as a F-G-F' gapmer region or a splice modulating region (e.g. a mixmer or totalmer), and may optionally comprise further nucleotide(s), for example a nucleotide linker region which may be used to attach a functional group (e.g. a conjugate group) to the contiguous nucleotide sequence. The nucleotide linker region may or may not be complementary to the target nucleic acid. It is understood that the contiguous nucleotide sequence of the oligonucleotide cannot be longer than the oligonucleotide as such and that the oligonucleotide cannot be shorter than the contiguous nucleotide sequence.

Nucleotides and nucleosides

Nucleotides and nucleosides are the building blocks of oligonucleotides and polynucleotides, and for the purposes of the present invention include both naturally occurring and non-naturally occurring nucleotides and nucleosides. In nature, nucleotides, such as DNA and RNA nucleotides comprise a ribose sugar moiety, a nucleobase moiety and one or more phosphate

groups (which is absent in nucleosides). Nucleosides and nucleotides may also interchangeably be referred to as “units” or “monomers”.

Modified nucleoside

The term “modified nucleoside” or “nucleoside modification” as used herein refers to nucleosides modified as compared to the equivalent DNA or RNA nucleoside by the introduction of one or more modifications of the sugar moiety or the (nucleo)base moiety. Advantageously, one or more of the modified nucleosides of the antisense oligonucleotide of the invention comprise a modified sugar moiety. The term modified nucleoside may also be used herein interchangeably with the term “nucleoside analogue” or modified “units” or modified “monomers”. Nucleosides with an unmodified DNA or RNA sugar moiety are termed DNA or RNA nucleosides herein. Nucleosides with modifications in the base region of the DNA or RNA nucleoside are still generally termed DNA or RNA if they allow Watson Crick base pairing. Exemplary modified nucleosides which may be used in the compounds of the invention include LNA, 2'-O-MOE and morpholino nucleoside analogues.

Modified internucleoside linkage

The term “modified internucleoside linkage” is defined as generally understood by the skilled person as linkages other than phosphodiester (PO) linkages, that covalently couples two nucleosides together. The oligonucleotides of the invention may therefore comprise one or more modified internucleoside linkages such as a one or more phosphorothioate internucleoside linkage.

In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate, such as at least 60%, such as at least 70%, such as at least 75%, such as at least 80% or such as at least 90% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate.

Advantageously, all the internucleoside linkages of the contiguous nucleotide sequence of the oligonucleotide are phosphorothioate, or all the internucleoside linkages of the oligonucleotide are phosphorothioate linkages.

It is recognized that, as disclosed in EP 2 742 135, antisense oligonucleotides may comprise other internucleoside linkages (other than phosphodiester and phosphorothioate), for example alkyl phosphonate/methyl phosphonate internucleoside, which according to EP 2 742 135 may for example be tolerated in an otherwise DNA phosphorothioate the gap region.

Nucleobase

The term nucleobase includes 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 modified 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 non-naturally occurring variants. 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.

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 a nucleobase selected from isocytosine, pseudoisocytosine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uracil, 5-bromouracil 5-thiazolo-uracil, 2-thio-uracil, 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 modified nucleobases of equivalent function. For example, in the exemplified oligonucleotides, the nucleobase moieties are selected from A, T, G, C, and 5-methyl cytosine. Optionally, for LNA gapmers, 5-methyl cytosine LNA nucleosides may be used.

Modified oligonucleotide

The term modified oligonucleotide describes an oligonucleotide comprising one or more sugar-modified nucleosides and/or modified internucleoside linkages. The term chimeric oligonucleotide is a term that has been used in the literature to describe oligonucleotides comprising sugar modified nucleosides and DNA nucleosides. The antisense oligonucleotide of the invention is advantageously a chimeric oligonucleotide.

Complementarity

The term "complementarity" describes the capacity for Watson-Crick base-pairing of nucleosides/nucleotides. Watson-Crick base pairs are guanine (G)-cytosine (C) and adenine (A) - thymine (T)/uracil (U). It will be understood that oligonucleotides may comprise nucleosides with modified nucleobases, for example 5-methyl cytosine is often used in place of cytosine, and as such the term complementarity encompasses Watson Crick base-pairing between non-modified and modified nucleobases (see for example 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 “% complementary” as used herein, refers to the proportion of nucleotides (in percent) of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which across the contiguous nucleotide sequence, are complementary to a reference sequence (e.g. a target sequence or sequence motif). The percentage of complementarity is thus calculated by counting the number of aligned nucleobases that are complementary (from Watson Crick base pair) between the two sequences (when aligned with the target sequence 5'-3' and the oligonucleotide sequence from 3'-5'), dividing that number by the total number of nucleotides in the oligonucleotide and multiplying by 100. In such a comparison, a nucleobase/nucleotide which does not align (form a base pair) is termed a mismatch. Insertions and deletions are not allowed in the calculation of % complementarity of a contiguous nucleotide sequence. It will be understood that in determining complementarity, chemical modifications of the nucleobases are disregarded as long as the functional capacity of the nucleobase to form Watson Crick base pairing is retained (e.g. 5'-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

The term “fully complementary”, refers to 100% complementarity.

Identity

The term “Identity” as used herein, refers to the proportion of nucleotides (expressed in percent) of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which across the contiguous nucleotide sequence, are identical to a reference sequence (e.g. a sequence motif). The percentage of identity is thus calculated by counting the number of aligned nucleobases that are identical (a Match) between two sequences (in the contiguous nucleotide sequence of the compound of the invention and in the reference sequence), dividing that number by the total number of nucleotides in the oligonucleotide and multiplying by 100. Therefore, Percentage of Identity = (Matches x 100)/Length of aligned region (e.g. the contiguous nucleotide sequence). Insertions and deletions are not allowed in the calculation the percentage of identity of a contiguous nucleotide sequence. It will be understood that in determining identity, chemical modifications of the nucleobases are disregarded as long as the functional capacity of the nucleobase to form Watson Crick base pairing is retained (e.g. 5-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

Hybridization

The term “hybridizing” or “hybridizes” as used herein is to be understood as two nucleic acid strands (e.g. an oligonucleotide and a target nucleic acid) forming hydrogen bonds between base pairs on opposite strands thereby forming a duplex. The affinity of the binding between two nucleic acid strands is the strength of the hybridization. It is often described in terms of the melting temperature (T_m) defined as the temperature at which half of the oligonucleotides are duplexed with the target nucleic acid. At physiological conditions, T_m is not strictly proportional

to the affinity (Mergny and Lacroix, 2003, *Oligonucleotides* 13:515–537). The standard state Gibbs free energy ΔG° is a more accurate representation of binding affinity and is related to the dissociation constant (K_d) of the reaction by $\Delta G^\circ = -RT \ln(K_d)$, where R is the gas constant and T is the absolute temperature. Therefore, a very low ΔG° of the reaction between an

5 oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. ΔG° is the energy associated with a reaction where aqueous concentrations are 1M, the pH is 7, and the temperature is 37°C. The hybridization of oligonucleotides to a target nucleic acid is a spontaneous reaction and for spontaneous reactions ΔG° is less than zero. ΔG° can be measured experimentally, for example, by use of

10 the isothermal titration calorimetry (ITC) method as described in Hansen et al., 1965, *Chem. Comm.* 36–38 and Holdgate et al., 2005, *Drug Discov Today*. The skilled person will know that commercial equipment is available for ΔG° measurements. ΔG° can also be estimated numerically by using the nearest neighbor model as described by SantaLucia, 1998, *Proc Natl Acad Sci USA*. 95: 1460–1465 using appropriately derived thermodynamic parameters

15 described by Sugimoto et al., 1995, *Biochemistry* 34:11211–11216 and McTigue et al., 2004, *Biochemistry* 43:5388–5405. In order to have the possibility of modulating its intended nucleic acid target by hybridization, oligonucleotides of the present invention hybridize to a target nucleic acid with estimated ΔG° values below -10 kcal for oligonucleotides that are 10-30 nucleotides in length. In some embodiments, the degree or strength of hybridization is

20 measured by the standard state Gibbs free energy ΔG° . The oligonucleotides may hybridize to a target nucleic acid with estimated ΔG° values below the range of -10 kcal, such as below -15 kcal, such as below -20 kcal and such as below -25 kcal for oligonucleotides that are 8-30 nucleotides in length. In some embodiments, the oligonucleotides hybridize to a target nucleic acid with an estimated ΔG° value of -10 to -60 kcal, such as -12 to -40, such as from -15 to -30

25 kcal or -16 to -27 kcal such as -18 to -25 kcal.

The Target or Target Nucleic Acid

The term “target” as used herein is used to refer to the STMN2 nucleic acids, and in particular the STMN2 pre-mRNA. The STMN2 target nucleic acid encodes the STMN2 protein, alternatively known as stathmin 2, or SCG10, SCGN10, and is disclosed as Gene: STMN2

30 ENSG00000104435 (ensemble.org), encoded on human Chromosome 8: 79,610,814-79,666,175 forward strand (GRCh38:CM000670.2), as illustrated in SEQ ID NO 183.

In some embodiments, the target nucleic acid comprises one or more of the following SNPs or polymorphisms (chromosome location & SNP substitution or deletion or insertion as compared to SEQ ID NO 183) – these polymorphisms are naturally occurring variants within the target

35 sequence region SEQ ID NO 182:

rs116751431	28:79616775	C/T	-	SNP
rs747165292	8:79616777	T/C	-	SNP

	rs906464557	8:79616784	C/T	-	SNP
	rs1169222402	8:79616787-79616788			TT/- deletion
	rs1453733842	8:79616791	T/C	-	SNP
	rs1244853275	8:79616801	A/T	-	SNP
5	rs993993236	8:79616810	T/C	-	SNP
	rs1025914136	8:79616813	A/G	-	SNP
	rs1249430782	8:79616817	T/G	-	SNP
	rs888190199	8:79616826	C/T	-	SNP
	rs879805065	8:79616827	G/A/T	-	SNP
10	rs879805065	8:79616827	G/A/T	-	SNP
	rs1313826159	8:79616830	A/C	-	SNP
	rs1344938894	8:79616836	C/T	-	SNP
	rs1230800262	8:79616837	C/G	-	SNP
	rs1034864135	8:79616840	C/T	-	SNP
15	rs567291555	8:79616841	G/A		SNP
	rs1351732399	8:79616845	G/C	-	SNP
	rs1332005676	8:79616850	G/A	-	SNP
	rs1328489488	8:79616853	G/A	-	SNP
	rs187783264	8:79616861	G/A		SNP
20	rs1372573028	8:79616871	C/G	-	SNP
	rs1261206094	8:79616887	G/A	-	SNP
	rs150697010	8:79616890	C/T	-	SNP
	rs552819092	8:79616891	G/A		SNP
	rs976424506	8:79616893	G/A/T	-	SNP
25	rs976424506	8:79616893	G/A/T	-	SNP
	rs191086701	8:79616898	C/T	-	SNP
	rs1362998638	8: between 79616898 & 79616899			-/GA insertion
	rs141543143	8:79616899-79616900			GA/- deletion
	rs781392795	8:79616899	G/A	-	SNP
30	rs1209558043	8:79616901	G/A	-	SNP
	rs571388018	8:79616905	G/A	SNP	
	rs1030547261	8:79616907	G/A/C	-	SNP
	rs1030547261	8:79616907	G/A/C	-	SNP
	rs955146963	8:79616908-79616911			AGAC/- - deletion
35	rs1288125593	8:79616915	C/G	-	SNP
	rs984549688	8:79616935	G/A	-	SNP
	rs866086409	8:79616939	G/T	-	SNP
	rs534735285	8:79616946	C/T		SNP
	rs1040072763	8:79616947	G/A	-	SNP
40	rs1160083850	8:79616956	C/T	-	SNP
	rs1358143504	8:79616960	G/A	-	SNP
	rs936750850	8:79616963	G/T	-	SNP
	rs1357480507	8:79616968-79616992			GGATGATAAATCAATAAT.../deletion
	rs1336159365	8:79616972	G/-	-	deletion
45	rs1414968044	8:79616978	T/C	-	SNP
	rs1398863395	8:79616981-79616982			AT/- deletion
	rs1384069685	8:79616982	T/C	-	SNP
	rs989563219	8:79616986	G/A	-	SNP
	rs1473422200	8:79616991	C/T	-	SNP
50	rs916572979	8:79616992	T/G	-	SNP
	rs1303163410	8:79616995	C/T	-	SNP
	rs902069445	8:79616997	A/G	-	SNP
	rs377037510	8:79617001	T/A/G		SNP
	rs377037510	8:79617001	T/A/G		SNP
55	rs1443515501	8:79617002	T/A	-	SNP
	rs1268003825	8:79617009	T/C	-	SNP

rs10511432108:79617011 G/A - SNP
rs13316334288:79617028-79617029 AA/- deletion
rs893294869 8:79617038 A/G - SNP
rs12279468068:79617040 T/A - SNP
5 rs13771573258:79617046 C/G - SNP
rs929912224 8:79617050 A/G - SNP
rs756125650 8:79617051 T/C - SNP
rs13361229188:79617053 T/G - SNP

- 10 In some embodiments the target nucleic acid is SEQ ID NO 183, or a naturally occurring variant thereof. Naturally occurring variants of STMN2 pre-mRNA, include STMN2 pre-mRNAs which comprise one or more of the above polymorphisms (taking into account RNA has U rather than T nucleosides).

STMN2 pre-mRNA Exons and Introns

- 15 The following table provides the exons and introns present in the reference human STMN2 pre-mRNA illustrated by reference to SEQ ID NO 183 (the STMN2 genomic sequence). Note pre-RNA sequence will comprise U rather than T nucleosides.

Exemplary exonic regions in the human STMN2 premRNA (SEQ ID NO 183)			Exemplary intronic regions in the human STMN2 premRNA (SEQ ID NO 183)		
ID	start	end	ID	start	end
Exon 1	339	401	Intron1	402	25988
Exon 2	25989	26084	Intron 2	26085	30564
Exon 3	30565	30737	Intron 3	30738	44057
Exon 4	44058	44249	Intron 4	44250	52766
Exon 5	52767	52848	Intron 5	52849	54001
Exon 6	54002	54194			

- 20 The exons illustrated in the above table are the exons present in the wildtype STMN2 mature mRNA, and the introns illustrated are in control cells effectively spliced from the STMN2 pre-mRNA, resulting in wildtype mature mRNA (for example as illustrated in SEQ ID NO 184).

SEQ ID NO 182 represents a preferred target site region of the STMN2 pre-mRNA (Target Sequence), and comprises part of region intron 1, which forms into the cryptic exon, or 5' end of the cryptic exon. Note the STMN2 pre-mRNA target nucleic acid in a cell, may comprise one or more polymorphisms, such as those described herein.

- 25 The targeting of the STMN2 pre-mRNA with the compounds of the invention inhibits or prevents the inclusion of intron 1 sequence, for example cryptic exon, ce1 (underlined sequence in SEQ ID NO 182) into STMN2 mRNA transcripts.

Target Sequence

The term “target sequence” as used herein refers to a sequence of nucleotides present in the target nucleic acid which comprises the nucleobase sequence which is complementary to the oligonucleotide of the invention. In some embodiments, the target sequence consists of a region on the target nucleic acid with a nucleobase sequence that is complementary to the contiguous nucleotide sequence of the oligonucleotide of the invention. This region of the target nucleic acid may interchangeably be referred to as the target nucleotide sequence, target sequence or target region. In some embodiments the target sequence is longer than the complementary sequence of a single oligonucleotide, and may, for example represent a preferred region of the target nucleic acid which may be targeted by several oligonucleotides of the invention.

In some embodiments, the antisense oligonucleotide of the invention, or the contiguous nucleotide sequence thereof, is complementary to, such as fully complementary a target sequence selected from the group consisting of SEQ ID NO 91 – 180.

Target Cell

The term a “target cell” as used herein refers to a cell which is expressing the target nucleic acid. In some embodiments the target cell may be *in vivo* or *in vitro*. In some embodiments the target cell is a mammalian cell such as a rodent cell, such as a mouse cell or a rat cell, or a primate cell such as a monkey cell or a human cell. In some embodiments the target cell is a transgenic animal cell which is expressing the human STMN2 target nucleic acid.

For *in vitro* evaluation, for example, primary human motor neurons or human pluripotent stem cell-derived motor neurons cells may be used.

Typically, the target cell expresses the STMN2 pre-mRNA. For experimental evaluation a target cell may be used which expresses a nucleic acid which comprises a target sequence. In a particularly advantageous embodiment the target cell expresses a target nucleic acid which comprises a cryptic exon, such as ce1, such as that illustrated within SEQ ID NO 182.

The contiguous sequence of nucleobases of the oligonucleotide of the invention is typically complementary to the STMN2 target nucleic acid, such as SEQ ID NO 183 as measured across the length of the oligonucleotide, optionally with the exception of a mismatches, and optionally excluding nucleotide based linker regions which may link the oligonucleotide to an optional functional group such as a conjugate, or other non-complementary terminal nucleotides (e.g. region D' or D'').

Advantageously, the contiguous sequence of nucleobases of the oligonucleotide of the invention is complementary to a target sequence selected from the group consisting of SEQ ID NO 91 – 180.

Naturally occurring variant

The term “naturally occurring variant” refers to variants of STMN2 gene or transcripts which originate from the same genetic loci as the target nucleic acid, but may differ for example, by virtue of degeneracy of the genetic code causing a multiplicity of codons encoding the same amino acid, or due to alternative splicing of pre-mRNA, or the presence of polymorphisms, such as single nucleotide polymorphisms (SNPs), and allelic variants – see the exemplary list provided herein. Based on the presence of the sufficient complementary sequence to the oligonucleotide, the oligonucleotide of the invention may therefore target the target nucleic acid and naturally occurring variants thereof.

In some embodiments, the naturally occurring variants have at least 95% such as at least 98% or at least 99% homology to a mammalian STMN2 target nucleic acid, such as a target nucleic acid selected from the group consisting of SEQ ID NO 183. In some embodiments, the naturally occurring variants have at least 99% homology to the human STMN2 target nucleic acid of SEQ ID NO: 183.

Splice Modulation

Splice modulation can be used to correct cryptic splicing, modulate alternative splicing, restore the open reading frame, and induce protein knockdown. In the context of the present invention, a preferred modulation is the correction of cryptic splicing, such as the reduction of the splicing event which results in the synthesis of a mature mRNA which comprises STMN2 intron 1 derived sequence positioned downstream of exon 1, or positioned between exon1 and exon 2 of a STMN2 transcript.

Splice modulation may be assayed by RNA sequencing (RNAseq), which allows for a quantitative assessment of the different splice products of a pre-mRNA. In some embodiments of the invention, the antisense oligonucleotides modulate the splicing of the STMN2 pre-mRNA so as to reduce the level of mature mRNA (transcript) which comprises a RNA sequence positioned between the exon 1 and exon 2, such as nucleosides derived from STMN2 intron 1, for example as illustrated in the RNA sequence shown in SEQ ID NO 182.

The mature STMN2 mRNA transcripts (transcript variant) comprising the cryptic exon may for example be characterized by the presence of a sequence within the transcript, selected from the group consisting of: 182, 185, 186 and 187 (Note in the RNA transcripts U replaces T):

SEQ ID NO 185:

CCCCATCACTCTCTCTTAATTGGATTTTTTAAAATTATATTCATATTGCAGGACTCGGCAGAAGACCTTCG
AGAGAAAGGTAGAAAATAAGAATTTGGCTCT

SEQ ID NO 186: TTATATTCATATTGCAGGACTCGGCAGAAGACCTTCG

SEQ ID NO 187: TCATATTGCAGGACTCGGCAGAA

SEQ ID NO 188: ATATTGCAGGACTCGGCAGAA

The underlined region is intron 1 derived sequence, the non-underlined is exon 1 derived sequence. The transcripts comprising the cryptic exon may therefore comprise a sequence selected from the group consisting of SEQ ID NO 185, 186, 187 and 188. Note in the mRNA transcript U residues will be present in place of T residues.

In some embodiments, the mRNA transcript variant which comprises the cryptic exon comprises the sequence shown in SEQ ID NO 189 (Note in the RNA transcripts U replaces T), or at least the 600 3' most nucleosides thereof, i.e. the first 600 nucleotides of SEQ ID NO 189, counting from in the 3' to 5' direction:

SEQ ID NO 189:

AGCTCCTAGGAAGCTTCAGGGCTTAAAGCTCCACTCTACTTGGACTGTACTATCAGGCCCCCAAAATGGGGGGAGCC
GACAGGGAAGGACTGATTTCCATTTCAAAGTGCATTCTGGTACTTTGTACTCCAGCACCATTTGGCCGATCAATATTT
AATGCTTGGAGATTCTGACTCTGCGGGAGTCATGTCAGGGGACCTTGGGAGCCAATCTGCTTGAGCTTCTGAGTGAT
AATTATTCATGGGCTCCTGCCTCTTGCTCTTTCTCTAGCACGGTCCCCTCTGTCAGACTCAGTGCCTTATTCAGTCT
TCTCTCTCGCTCTCTCCGCTGCTGTAGCCGGACCCCTTTGCCTTCGCCACTGCTCAGCGTCTGCACATCCCTACAATG
GCTAAACAGCAATGGGACTCGGCAGAGACACCTTCGAGAGAAAGGTAGAAAATAAGAATTTGGCTCTCTGTGTGAGC
ATGTGTGCGTGTGTGCGAGAGAGAGACAGACAGCCTGCCTAAGAAGAAATGAATGTGAATGCGGCTTGTGGCACA
GTTGACAAGGATGATAAATCAATAATGCAAGCTTACTATCATTATGAATAGCAATACTGAAGAAATTAAACAAAA
GATTGCTGTCTCAATATATC

High affinity modified nucleosides

A high affinity modified nucleoside is a modified nucleotide which, when incorporated into the oligonucleotide enhances the affinity of the oligonucleotide for its complementary target, for example as measured by the melting temperature (T_m). A high affinity modified nucleoside of the present invention preferably result in an increase in melting temperature between +0.5 to +12°C, more preferably between +1.5 to +10°C and most preferably between +3 to +8°C per modified nucleoside. Numerous high affinity modified nucleosides are known in the art and include for example, many 2' substituted nucleosides as well as locked nucleic acids (LNA) (see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213).

Sugar modifications

The oligomer of the invention may comprise one or more nucleosides which have a modified sugar moiety, i.e. a modification of the sugar moiety when compared to the ribose sugar moiety found in DNA and RNA.

Numerous nucleosides with modification of the ribose sugar moiety have been made, primarily with the aim of improving certain properties of oligonucleotides, such as affinity and/or nuclease resistance.

Such modifications include those where the ribose ring structure is modified, e.g. by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradicle bridge

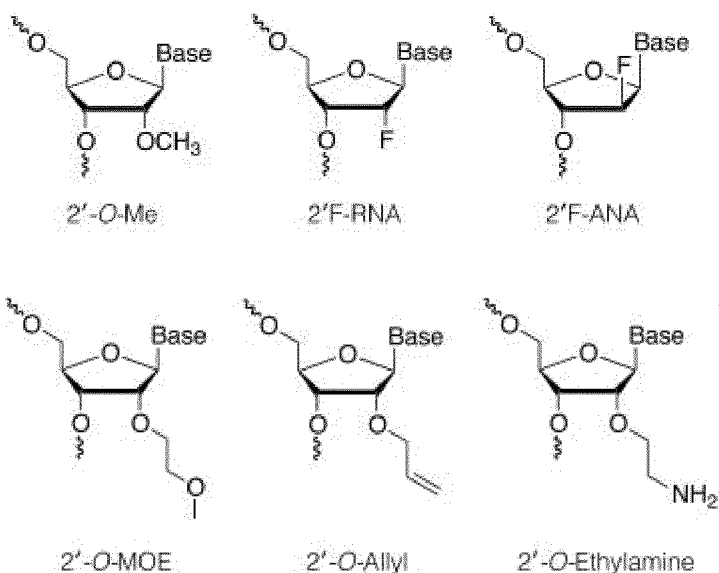
between the C2 and C4 carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g. UNA). Other sugar modified nucleosides include, for example, bicyclohexose nucleic acids (WO2011/017521) or tricyclic nucleic acids (WO2013/154798). Modified nucleosides also include nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

Sugar modifications also include modifications made via altering the substituent groups on the ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in DNA and RNA nucleosides. Substituents may, for example be introduced at the 2', 3', 4' or 5' positions.

10 **2' sugar modified nucleosides**

A 2' sugar modified nucleoside is a nucleoside which has a substituent other than H or -OH at the 2' position (2' substituted nucleoside) or comprises a 2' linked biradicle capable of forming a bridge between the 2' carbon and a second carbon in the ribose ring, such as LNA (2' - 4' biradicle bridged) nucleosides.

15 Indeed, much focus has been spent on developing 2' sugar substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial properties when incorporated into oligonucleotides. For example, the 2' modified sugar may provide enhanced binding affinity and/or increased nuclease resistance to the oligonucleotide. Examples of 2' substituted modified nucleosides are 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, and 2'-F-ANA nucleoside. For further
20 examples, please see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213, and Deleavey and Damha, Chemistry and Biology 2012, 19, 937. Below are illustrations of some 2' substituted modified nucleosides.



In relation to the present invention 2' substituted sugar modified nucleosides does not include 2' bridged nucleosides like LNA.

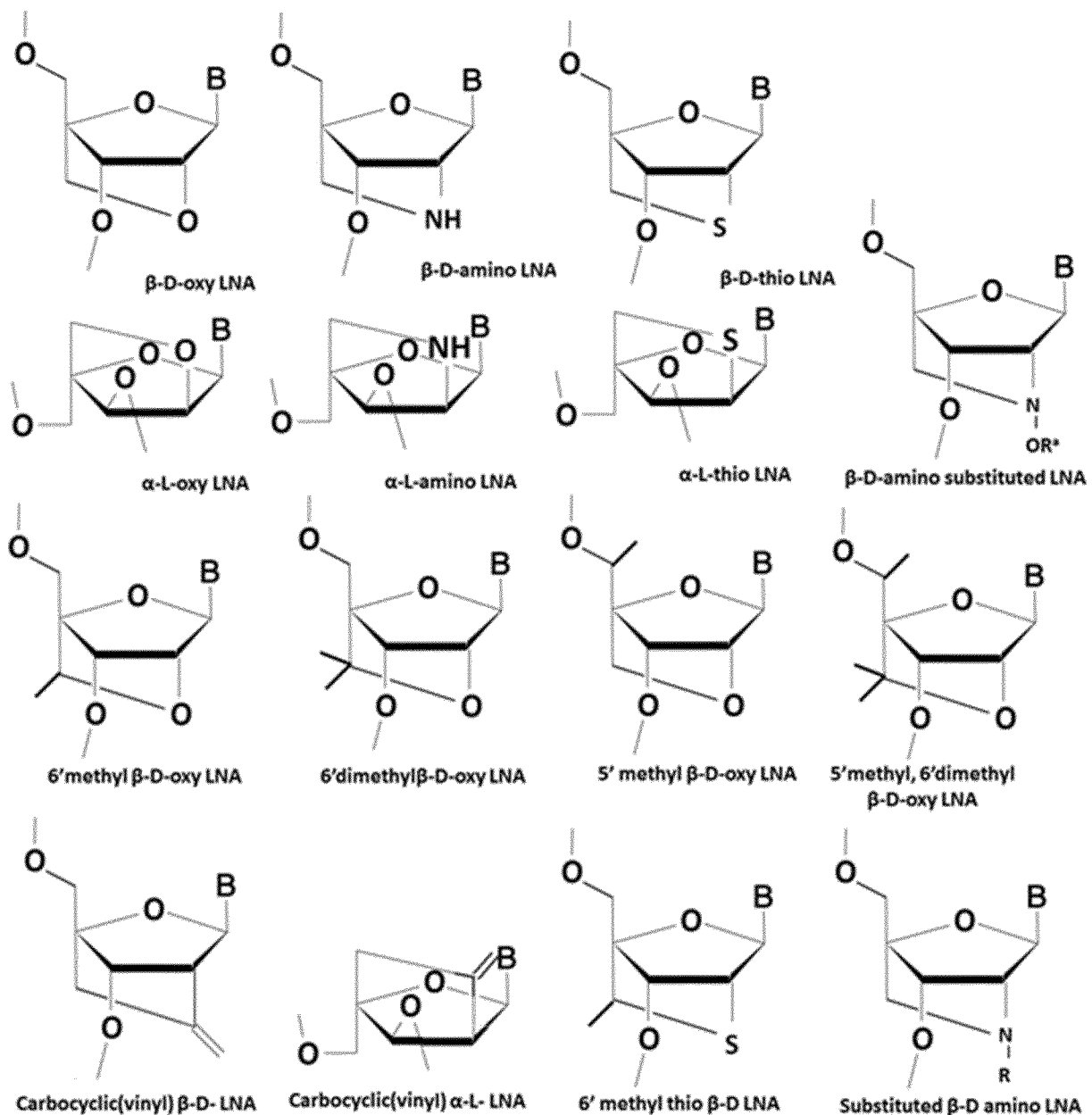
Locked Nucleic Acid Nucleosides (LNA nucleoside)

5 A "LNA nucleoside" is a 2'- modified nucleoside which comprises a biradical linking the C2' and C4' of the ribose sugar ring of said nucleoside (also referred to as a "2'- 4' bridge"), which restricts or locks the conformation of the ribose ring. These nucleosides are also termed bridged nucleic acid or bicyclic nucleic acid (BNA) in the literature. The locking of the conformation of the ribose is associated with an enhanced affinity of hybridization (duplex stabilization) when the LNA is incorporated into an oligonucleotide for a complementary RNA or
10 DNA molecule. This can be routinely determined by measuring the melting temperature of the oligonucleotide/complement duplex.

Non limiting, exemplary LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352 , WO 2004/046160, WO 00/047599, WO 2007/134181, WO 2010/077578, WO 2010/036698, WO 2007/090071, WO 2009/006478, WO 2011/156202, WO 2008/154401, WO
15 2009/067647, WO 2008/150729, Morita et al., Bioorganic & Med.Chem. Lett. 12, 73-76, Seth et al. J. Org. Chem. 2010, Vol 75(5) pp. 1569-81, and Mitsuoka et al., Nucleic Acids Research 2009, 37(4), 1225-1238, and Wan and Seth, J. Medical Chemistry 2016, 59, 9645-9667.

Further non limiting, exemplary LNA nucleosides are disclosed in Scheme 1.

Scheme 1:



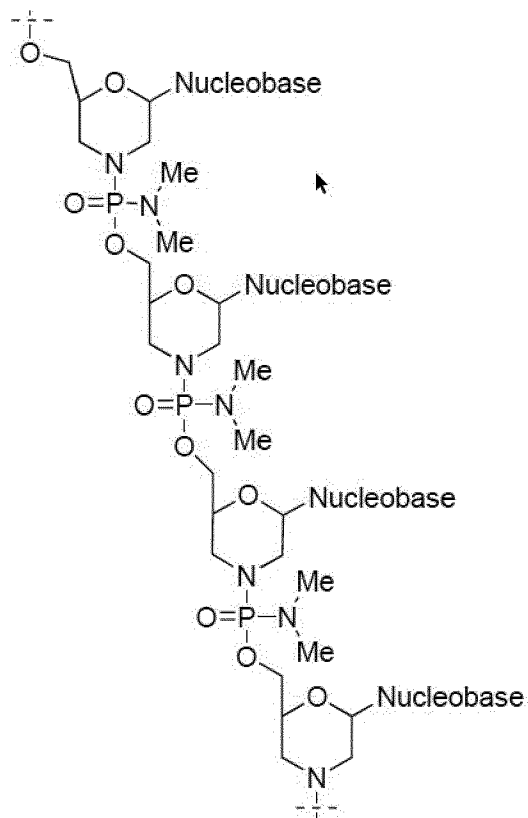
Particular LNA nucleosides are beta-D-oxy-LNA, 6'-methyl-beta-D-oxy LNA such as (S)-6'-methyl-beta-D-oxy-LNA (ScET) and ENA.

A particularly advantageous LNA is beta-D-oxy-LNA.

5 Morpholino Oligonucleotides

In some embodiments, the oligonucleotide of the invention comprises or consists of morpholino nucleosides (*i.e.* is a Morpholino oligomer and as a phosphorodiamidate Morpholino oligomer (PMO)). Splice modulating morpholino oligonucleotides have been approved for clinical use – see for example eteplirsen, a 30nt morpholino oligonucleotide targeting a frame shift mutation in DMD, used to treat Duchenne muscular dystrophy. Morpholino oligonucleotides have nucleobases attached to six membered morpholine rings rather ribose, such as

methylenemorpholine rings linked through phosphorodiamidate groups, for example as illustrated by the following illustration of 4 consecutive morpholino nucleotides:



In some embodiments, morpholino oligonucleotides of the invention may be, for example 20 – 40 morpholino nucleotides in length, such as morpholino 25 – 35 nucleotides in length.

RNase H Activity and Recruitment

The RNase H activity of an antisense oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule. WO01/23613 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. 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 a 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). For use in determining RNase H activity, recombinant RNase H1 is available from Lubio Science GmbH, Lucerne, Switzerland.

Mixmers and Totalmers

For splice modulation it is often advantageous to use antisense oligonucleotides which do not recruit RNAaseH. As RNaseH activity requires a contiguous sequence of DNA nucleotides,

RNaseH activity of antisense oligonucleotide may be achieved by designing antisense oligonucleotides which do not comprise a region of more than 3 or more than 4 contiguous DNA nucleosides. This may be achieved by using antisense oligonucleotides or contiguous nucleoside regions thereof with a mixmer design, which comprise sugar modified nucleosides, such as 2' sugar modified nucleosides, and short regions of DNA nucleosides, such as 1, 2 or 3 DNA nucleosides. Mixmers are exemplified herein by every second design, wherein the nucleosides alternative between 1 LNA and 1 DNA nucleoside, e.g. LDLDLDLDLDLL, with 5' and 3' terminal LNA nucleosides, and every third design, such as LDDLDDLDDLDDL, where every third nucleoside is a LNA nucleoside.

A totalmer is an antisense oligonucleotide or a contiguous nucleotide sequence thereof which does not comprise DNA or RNA nucleosides, and may for example comprise only 2'-O-MOE nucleosides, such as a fully MOE phosphorothioate, e.g. MMMMMMMMMMMMMMMMMMMM, where M = 2'-O-MOE, which are reported to be effective splice modulators for therapeutic use. Alternatively, a mixmer may comprise a mixture of modified nucleosides, such as MLMLMLMLMLMLMLMLML, wherein L = LNA and M = 2'-O-MOE nucleosides.

Advantageously, the internucleoside nucleosides in mixmers and totalmers may be phosphorothioate, or a majority of nucleoside linkages in mixmers may be phosphorothioate. Mixmers and totalmers may comprise other internucleoside linkages, such as phosphodiester or phosphorodithioate, by way of example.

Region D' or D'' in an oligonucleotide

The oligonucleotide of the invention may in some embodiments comprise or consist of the contiguous nucleotide sequence of the oligonucleotide which is complementary to the target nucleic acid or mixmer or totalmer region, and further 5' and/or 3' nucleosides. The further 5' and/or 3' nucleosides may or may not be fully complementary to the target nucleic acid. Such further 5' and/or 3' nucleosides may be referred to as region D' and D'' herein.

The addition of region D' or D'' may be used for the purpose of joining the contiguous nucleotide sequence to a conjugate moiety or another functional group. When used for joining the contiguous nucleotide sequence with a conjugate moiety it can serve as a biocleavable linker. Alternatively, it may be used to provide exonuclease protection or for ease of synthesis or manufacture.

Region D' and D'' can be attached to the 5' end of region F or the 3' end of region F', respectively to generate designs of the following formulas D'-CNS, CNS -D'' or D'- CNS -D''. Wherein CNS is the contiguous nucleotide sequence of the antisense oligonucleotide, such as a mixmer or totalmer region.

Region D' or D'' may independently comprise or consist of 1, 2, 3, 4 or 5 additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. The nucleotide adjacent to the F or F' region is not a sugar-modified nucleotide, such as a DNA or RNA or base modified versions of these. The D' or D' region may serve as a nuclease susceptible

5 biocleavable linker (see definition of linkers). In some embodiments the additional 5' and/or 3' end nucleotides are linked with phosphodiester linkages, and are DNA or RNA. Nucleotide based biocleavable linkers suitable for use as region D' or D'' are disclosed in WO2014/076195, which include by way of example a phosphodiester linked DNA dinucleotide. The use of biocleavable linkers in poly-oligonucleotide constructs is disclosed in WO2015/113922, where
10 they are used to link multiple antisense constructs within a single oligonucleotide.

In one embodiment the oligonucleotide of the invention comprises a region D' and/or D'' in addition to the contiguous nucleotide sequence which constitutes the a mixmer or a totalmer.

In some embodiments the internucleoside linkage positioned between region D' and region F is a phosphodiester linkage. In some embodiments the internucleoside linkage positioned
15 between region F' and region D'' is a phosphodiester linkage.

Conjugate

The term conjugate as used herein refers to an oligonucleotide which is covalently linked to a non-nucleotide moiety (conjugate moiety or region C or third region). The conjugate moiety may be covalently linked to the antisense oligonucleotide, optionally via a linker group, such as
20 region D' or D''.

Oligonucleotide conjugates and their synthesis has also been reported in comprehensive reviews by Manoharan in Antisense Drug Technology, Principles, Strategies, and Applications, S.T. Crooke, ed., Ch. 16, Marcel Dekker, Inc., 2001 and Manoharan, Antisense and Nucleic Acid Drug Development, 2002, 12, 103.

25 In some embodiments, the non-nucleotide moiety (conjugate moiety) is selected from the group consisting of carbohydrates (e.g. GalNAc), cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins (e.g. bacterial toxins), vitamins, viral proteins (e.g. capsids) or combinations thereof.

Linkers

30 A linkage or linker 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 (Region C), to a first region, e.g. an oligonucleotide or contiguous nucleotide sequence
35 complementary to the target nucleic acid (region A).

In some embodiments of the invention the conjugate or oligonucleotide conjugate of the invention may optionally, comprise a linker region (second region or region B and/or region Y) which is positioned between the oligonucleotide or contiguous nucleotide sequence complementary to the target nucleic acid (region A or first region) and the conjugate moiety (region C or third region).

Region B refers to biocleavable linkers comprising or consisting of a physiologically labile bond that is cleavable under conditions normally encountered or analogous to those encountered within a mammalian body. Conditions under which physiologically labile linkers undergo chemical transformation (e.g., cleavage) include chemical conditions such as pH, temperature, oxidative or reductive conditions or agents, and salt concentration found in or analogous to those encountered in mammalian cells. Mammalian intracellular conditions also include the presence of enzymatic activity normally present in a mammalian cell such as from proteolytic enzymes or hydrolytic enzymes or nucleases. In one embodiment the biocleavable linker is susceptible to S1 nuclease cleavage. In some embodiments the nuclease susceptible linker comprises between 1 and 5 nucleosides, such as DNA nucleoside(s) comprising at least two consecutive phosphodiester linkages,. Phosphodiester containing biocleavable linkers are described in more detail in WO 2014/076195.

Region Y refers to linkers that are not necessarily biocleavable but primarily serve to covalently connect a conjugate moiety (region C or third region), to an oligonucleotide (region A or first region). The region Y linkers may comprise a chain structure or an oligomer of repeating units such as ethylene glycol, amino acid units or amino alkyl groups. The oligonucleotide conjugates of the present invention can be constructed of the following regional elements A-C, A-B-C, A-B-Y-C, A-Y-B-C or A-Y-C. In some embodiments the linker (region Y) is an amino alkyl, such as a C2 – C36 amino alkyl group, including, for example C6 to C12 amino alkyl groups. In some embodiments the linker (region Y) is a C6 amino alkyl group.

Treatment

The term 'treatment' as used herein refers to both treatment of an existing disease (e.g. a disease or disorder as herein referred to), or prevention of a disease, *i.e.* prophylaxis. It will therefore be recognized that treatment as referred to herein may, in some embodiments, be prophylactic.

The compounds of the invention may be used in medicine, such as for the use in the treatment of a disease or disorders associated with TDP-43 aggregation and/or mislocalization from the nucleus, such as a disease or disorder selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD), Alzheimer's Disease, Hippocampal sclerosis dementia, Down syndrome, Parkinson disease, Huntington's disease,

polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and Chronic Traumatic Encephalopathy.

DETAILED DESCRIPTION OF THE INVENTION

The Oligonucleotides of the Invention

5 The antisense oligonucleotides of the invention are capable of modulating the splicing on the STMN2 pre-mRNA so as to inhibit or prevent the inclusion of a region of intronic sequence in STMN2 mRNA transcripts, such as a region of intron 1 sequence of the STMN2 pre-mRNA. Inclusion of the region of intron 1 (referred to as a cryptic exon), results in the inclusion of a region of intron 1 sequence positioned adjacent to 3' of exon 1 in the resulting STMN2 transcript
10 which is associated with TDP-43 pathologies.

The invention provides for an antisense oligonucleotide, wherein the antisense oligonucleotide is 10 to 40 nucleotides in length, and comprises a contiguous nucleotide sequence of at least 10 nucleotides in length with at least 90% complementarity, such as 100% complementarity to SEQ ID NO 182, or a pharmaceutically acceptable salt thereof.

15 In some embodiments, the antisense oligonucleotide is for or is capable of modulation the splicing of human STMN2 pre-mRNA in a cell which is expressing STMN2 pre-mRNA

In some embodiments, the antisense oligonucleotide is capable of reducing or preventing the splicing events of STMN2 pre-mRNA which result STMN2 transcript variant(s) (mature mRNA) which comprise a cryptic exon, such as a region of STMN2 intron 1 positioned downstream of
20 STMN2 exon 1 in a cell which is expressing said STMN2 transcript variant(s).

In some embodiments, the oligonucleotide has a length of 10-25 nucleotides.

In some embodiments, the contiguous nucleotide sequence has 100% complementarity to SEQ ID NO 182.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence thereof,
25 is complementary to, such as fully complementary to a sequence selected from the group consisting of SEQ ID NO 91 – 180.

In some embodiments, antisense oligonucleotide or contiguous nucleotide sequence thereof, comprises a sequence selected from the group consisting of SEQ ID NO 1 - 90, or at least 10 contiguous nucleotides thereof.

30 In some embodiments, oligonucleotide is or comprises an antisense oligonucleotide mixmer or totalmer.

In some embodiments, antisense oligonucleotide or contiguous nucleotide sequence thereof is 12 – 20 nucleotides in length.

In some embodiments, antisense oligonucleotide comprises an oligonucleotide provided herein,
35 such as a compound selected from the group consisting of compound ID NO #1 - #90

The invention provides for a conjugate comprising the antisense oligonucleotide according to any one of the invention, and at least one conjugate moiety covalently attached to said oligonucleotide.

The invention provides for a pharmaceutically acceptable salt of the antisense oligonucleotide, or the conjugate according to the invention.

The invention provides for a pharmaceutical composition comprising the antisense oligonucleotide or the conjugate or salt of the invention.

The invention provides for an *in vivo* or *in vitro* method for modulating the splicing of a STMN2 pre-mRNA in a target cell which is expressing STMN2 pre-mRNA, said method comprising administering an antisense oligonucleotide or the conjugate or salt or the pharmaceutical composition of the invention in an effective amount to said cell.

In some embodiments, the administration of the antisense oligonucleotide results in reduced expression of, or prevents the expression of a STMN2 mRNA transcript variant which comprises a cryptic exon, or which comprises SEQ ID NO 188 (such as comprises any one of SEQ ID NO 182, 185, 186, or 187)

In some embodiments, the administration of the antisense oligonucleotide results in increased expression of WT STMN2 mRNA.

In some embodiments, the administration of the antisense oligonucleotide the cell is either a human cell or a mammalian cell which is expressing a human STMN2 sequence, such as a human STMN2 pre-mRNA sequence. In some embodiments the cell is a neuronal cell.

The invention provides for a method for treating or preventing neurological disease comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide or the conjugate or the salt or pharmaceutical composition of the invention to a subject suffering from or susceptible to the neurological disease (a subject in need of treatment for said disease).

The invention provides for an antisense oligonucleotide or the conjugate or the salt or pharmaceutical composition of the invention for use as a medicament.

The invention provides for the antisense oligonucleotide or the conjugate or the salt or pharmaceutical composition of the invention for use in medicine.

The invention provides for the antisense oligonucleotide or the conjugate or the salt or pharmaceutical composition of the invention for use in the treatment of an neurological disease.

The invention provides for the use of the antisense oligonucleotide or the conjugate or the salt or pharmaceutical composition of the invention, in the preparation of a medicament for treatment or prevention of a neurological disease.

In some embodiments the neurological disease is a disease or disorders associated with TDP-43 aggregation and/or mislocalization from the nucleus.

In some embodiments the neurological disease is a disease or disorder selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD), Alzheimer's Disease, Hippocampal sclerosis dementia, Down syndrome, Parkinson disease, Huntington's disease, polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and
5 Chronic Traumatic Encephalopathy.

An aspect of the present invention relates to an antisense oligonucleotide which comprises a contiguous nucleotide sequence of 10 to 40 nucleotides in length with at least 90% complementarity, such as fully complementarity SEQ ID NO 183, such as the region of SEQ ID NO 183 provided herein as SEQ ID NO 182, and a target sequence selected from the group
10 consisting of SEQ ID NO 91 – 180.

An aspect of the present invention relates to an antisense oligonucleotide which comprises a contiguous nucleotide sequence of 12 to 25 nucleotides in length with at least 90% complementarity, such as fully complementarity SEQ ID NO 183, such as the region of SEQ ID NO 183 provided herein as SEQ ID NO 182, and a target sequence selected from the group
15 consisting of SEQ ID NO 91 – 180.

In some embodiments, the oligonucleotide comprises a contiguous sequence of 10 to 30 nucleotides in length, which is at least 90% complementary, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or 100% complementary with a region of the
20 target nucleic acid or a target sequence.

It is advantageous if the oligonucleotide of the invention, or contiguous nucleotide sequence thereof is fully complementary (100% complementary) to a region of the target nucleic acid, or in some embodiments may comprise one or two mismatches between the oligonucleotide and the target nucleic acid.

25 In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementary, such as fully (or 100%) complementary, to a region target nucleic acid region present in SEQ ID NO: 183, such as SEQ ID NO 182, or a sequence selected from SEQ ID NO 91-180.

In some embodiments, the antisense oligonucleotide of the invention or the contiguous
30 nucleotide sequence thereof comprises or consists of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides in length.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting of sequences SEQ ID NO 1 – 90 or at least 10 contiguous nucleotides thereof. It will be understood that the sequence shown in
35 SEQ ID NO 1 – 90 may include modified nucleobases which function as the shown nucleobase in base pairing, for example 5-methyl cytosine may be used in place of methyl cytosine.

Furthermore it will be understood that with reference to T residues, U residues may be used, for example when utilizing 2'-O-methoxyethyl nucleosides.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 1 to 90.

It is understood that the contiguous nucleobase sequences (motif sequence) can be modified to for example increase nuclease resistance and/or binding affinity to the target nucleic acid.

The pattern in which the modified nucleosides (such as high affinity modified nucleosides) are incorporated into the oligonucleotide sequence is generally termed oligonucleotide design.

The oligonucleotides of the invention are designed with modified nucleosides and DNA nucleosides. Advantageously, high affinity modified nucleosides are used.

In an embodiment, the oligonucleotide comprises at least 1 modified nucleoside, such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 modified nucleosides. In an embodiment the oligonucleotide comprises from 1 to 10 modified nucleosides, such as from 2 to 9 modified nucleosides, such as from 3 to 8 modified nucleosides, such as from 4 to 7 modified nucleosides, such as 6 or 7 modified nucleosides. Suitable modifications are described in the "Definitions" section under "modified nucleoside", "high affinity modified nucleosides", "sugar modifications", "2' sugar modifications" and Locked nucleic acids (LNA)".

In some embodiments, the antisense oligonucleotide is or comprises an antisense oligonucleotide mixmer or totalmer.

In some embodiments, the antisense oligonucleotide is a morpholino oligonucleotide.

In some embodiments, the antisense oligonucleotide is a 2'-O-MOE oligonucleotide, *i.e.* comprises one or more 2'-O-MOE nucleosides.

In some embodiments, the antisense oligonucleotide is an LNA oligonucleotide, *i.e.* comprises one or more LNA nucleosides.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence thereof is 10 – 25 or 10 - 20 nucleotides in length.

In an embodiment, the oligonucleotide comprises one or more sugar modified nucleosides, such as 2' sugar modified nucleosides. Preferably the oligonucleotide of the invention comprise one or more 2' sugar modified 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 LNA nucleosides. It is advantageous if one or more of the modified nucleoside(s) is a locked nucleic acid (LNA).

In a further embodiment the oligonucleotide comprises at least one modified internucleoside linkage. Suitable internucleoside modifications are described in the “Definitions” section under “Modified internucleoside linkage”. It is advantageous if at least 75%, such as all, 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.

Compounds

Compound Table

SEQ ID NO	Nucleobase Motif	Compound ID	Compound	SEQ ID NO	Target site sequence (note DNA sequence shown – actual RNA target will have U rather than T residues)
1	GATATATTGAGACAGC	#1	GatAtAttGagAcaGC	91	GCTGTCTCAATATATC
2	ATATTGAGACAGCAAT	#2	AtAttGaGaCaGcaAT	92	ATTGCTGTCTCAATAT
3	TTGAGACAGCAATCTT	#3	TtGagAcAgcAaTcTT	93	AAGATTGCTGTCTCAA
4	AGACAGCAATCTTTTG	#4	AgAcAgcAaTctTtTG	94	CAAAGATTGCTGTCT
5	CAGCAATCTTTTGTTT	#5	CagCaaTctTtGtTT	95	AAACAAAAGATTGCTG
6	CAATCTTTTGTTTAA	#6	CAaTctTtGtTtTAA	96	TTAAAACAAAAGATTG
7	TCTTTTGTTTAAATTT	#7	TcTtTtGtTtTtAaTTT	97	AAATTAACAAAAGA
8	TTTGTTTAAATTTCTT	#8	TtTgTtTtAaTTTcTT	98	AAGAAATTAACAAA
9	GTTTAAATTTCTTCAG	#9	GtTtAaTTTcTcAG	99	CTGAAGAAATTAAC
10	TTAATTTCTTCAGTAT	#10	TtAaTTTcTcAGtAT	100	ATACTGAAGAAATTA
11	ATTTCTTCAGTATTGC	#11	AttTcTcAGtAttGC	101	GCAATACTGAAGAAAT
12	TCTTCAGTATTGCTAT	#12	TctTcAGtAttGctAT	102	ATAGCAATACTGAAGA
13	TCAGTATTGCTATTCA	#13	TcAGtAttGctAttCA	103	TGAATAGCAATACTGA
14	GTATTGCTATTTCATAA	#14	GtaTtGcTAtTcAtAA	104	TTATGAATAGCAATAC
15	TTGCTATTCATAAATG	#15	TtGctAtTCAtAaaTG	105	CATTTATGAATAGCAA
16	CTATTCATAAATGATA	#16	CtaTtCaTAaAtgATA	106	TATCATTTATGAATAG
17	TTCATAAATGATAGTA	#17	TtCaTaAaTGaTagTA	107	TACTATCATTTATGAA
18	ATAAATGATAGTAAGC	#18	AtAaaTgaTaGtaAGC	108	GCTTACTATCATTTAT
19	AATGATAGTAAGCTTG	#19	AAtGatAgTaaGctTG	109	CAAGCTTACTATCATT
20	GATAGTAAGCTTGCAT	#20	GatAgTaaGctGcAT	110	ATGCAAGCTTACTATC
21	AGTAAGCTTGCATTAT	#21	AgTaaGctGcAttAT	111	ATAATGCAAGCTTACT
22	AAGCTTGCATTATTGA	#22	AagCttGcAttAttGA	112	TCAATAATGCAAGCTT
23	CTTGCATTATTGATTT	#23	CttGcAttATtGatTT	113	AAATCAATAATGCAAG
24	GCATTATTGATTTATC	#24	GcAttATtGatTtATC	114	GATAAATCAATAATGC
25	TTATTGATTTATCATC	#25	TTaTTGaTtAtCaTC	115	GATGATAAATCAATAA
26	TTGATTTATCATCCTT	#26	TtGatTtAtCcTtCTT	116	AAGGATGATAAATCAA
27	ATTTATCATCCTTGTC	#27	AtTtAtCcTtGtTC	117	GACAAGGATGATAAAT
28	TATCATCCTTGTC AAC	#28	TaTcAtCcTtGtAAC	118	GTTGACAAGGATGATA
29	CATCCTTGTC AACTGT	#29	CaTccTtGtCAActGT	119	ACAGTTGACAAGGATG

30	CCTTGTCAACTGTGCC	#30	CcTtGtcAAcTgtGcC	120	GGCACAGTTGACAAGG
31	TGTCAACTGTGCCACA	#31	TgtCaActGtGccACA	121	TGTGGCACAGTTGACA
32	CAACTGTGCCACAAGC	#32	CaActGtGccAcAaGC	122	GCTTGTGGCACAGTTG
33	CTGTGCCACAAGCCGC	#33	CtGtGccAcAaGccGC	123	GCGGCTTGTGGCACAG
34	TGCCACAAGCCGCATT	#34	TgcCacAaGccGcaTT	124	AATGCGGCTTGTGGCA
35	CACAAGCCGCATTAC	#35	CacAaGccGcAtTcAC	125	GTGAATGCGGCTTGTG
36	AAGCCGCATTACATT	#36	AaGccGcAtTcAcaTT	126	AATGTGAATGCGGCTT
37	CCGCATTACATTCAT	#37	CcGcAtTcAcAtTcAT	127	ATGAATGTGAATGCGG
38	CATTCACATTCATTC	#38	CatTcaCatTcAttTC	128	GAAATGAATGTGAATG
39	TCACATTCATTTCTTC	#39	TcAcAtTcAttTctTC	129	GAAGAAATGAATGTGA
40	CATTCATTTCTTCTTA	#40	CatTcAttTctTctTA	130	TAAGAAGAAATGAATG
41	TCATTTCTTCTTAGGC	#41	TcAttTctTctTagGC	131	GCCTAAGAAGAAATGA
42	TTTCTTCTTAGGCAGG	#42	TtTctTctTagGcaGG	132	CCTGCCTAAGAAGAAA
43	CTTCTTAGGCAGGCTG	#43	CtTcTtAggCagGcTG	133	CAGCCTGCCTAAGAAG
44	CTTAGGCAGGCTGTCT	#44	CttAggCagGctGtCT	134	AGACAGCCTGCCTAAG
45	AGGCAGGCTGTCTGTC	#45	AggCagGctGtCtgTC	135	GACAGACAGCCTGCCT
46	CAGGCTGTCTGTCTCT	#46	CagGctGtCtgTctCT	136	AGAGACAGACAGCCTG
47	GCTGTCTGTCTCTCTC	#47	GctGtCtGtcTcTcTC	137	GAGAGAGACAGACAGC
48	GTCTGTCTCTCTCTCT	#48	GtCtGtcTcTcTctCT	138	AGAGAGAGAGACAGAC
49	TGTCTCTCTCTCTCGC	#49	TgtCtcTcTctCtcGC	139	GCGAGAGAGAGAGACA
50	CTCTCTCTCTCGCACA	#50	CtcTcTctCtcGcaCA	140	TGTGCGAGAGAGAGAG
51	TCTCTCTCGCACACAC	#51	TcTctCtcGcAcAcAC	141	GTGTGTGCGAGAGAGA
52	CTCTCGCACACAEGCA	#52	CtcTcGcAcAcAegCA	142	TGCGTGTGTGCGAGAG
53	TCGCACACACGCACAC	#53	TcGcAcAcAcGcAcAC	143	GTGTGCGTGTGTGCGA
54	CACACACGCACACATG	#54	CacAcAcGcAcAcaTG	144	CATGTGTGCGTGTGTG
55	ACACGCACACATGCTC	#55	AcAcGcAcAcAtGcTC	145	GAGCATGTGTGCGTGT
56	CGCACACATGCTCACA	#56	CgcAcAcAtGcTcaCA	146	TGTGAGCATGTGTGCG
57	ACACATGCTCACACAG	#57	AcAcAtGctCacAcAG	147	CTGTGTGAGCATGTGT
58	CATGCTCACACAGAGA	#58	CatGctCacAcAgaGA	148	TCTCTGTGTGAGCATG
59	GCTCACACAGAGAGCC	#59	GctCacAcAgaGagCC	149	GGCTCTCTGTGTGAGC
60	CACACAGAGAGCCAAA	#60	CacAcAgaGagCcaAA	150	TTTGGCTCTCTGTGTG
61	ACAGAGAGCCAAATTC	#61	AcAgaGaGccAaAtTC	151	GAATTTGGCTCTCTGT
62	GAGAGCCAAATTCTTA	#62	GagAgcCaaAtTctTA	152	TAAGAATTTGGCTCTC
63	AGCCAAATTCTTATTT	#63	AgcCaaAtTctTatTT	153	AAATAAGAATTTGGCT
64	CAAATTCTTATTTTCT	#64	CaaAtTcTTaTtTtCT	154	AGAAAATAAGAATTTG
65	ATTCTTATTTTCTACC	#65	AtTcTtAttTtcTaCC	155	GGTAGAAAATAAGAAT
66	CTTATTTTCTACCTTT	#66	CttAtTtTctAcCtTT	156	AAAGGTAGAAAATAAG
67	ATTTTCTACCTTTCTC	#67	AtTtTctAccTtTcTC	157	GAGAAAGGTAGAAAAT
68	TTCTACCTTTCTCTCG	#68	TTctAccTtTcTctCG	158	CGAGAGAAAGGTAGAA
69	TACCTTTCTCTCGAAG	#69	TacCttTctCtcGaAG	159	CTTCGAGAGAAAGGTA
70	CTTTCTCTCGAAGGTC	#70	CttTctCtcGaAggTC	160	GACCTTCGAGAGAAAG
71	TCTCTCGAAGGTCTTC	#71	TctCtcGaAggTctTC	161	GAAGACCTTCGAGAGA
72	CTCGAAGGTCTTCTGC	#72	CtcGaAggTctTctGC	162	GCAGAAGACCTTCGAG

73	GAAGGTCTTCTGCCGA	#73	GaAggTctTctGccGA	163	TCGGCAGAAGACCTTC
74	GGTCTTCTGCCGAGTC	#74	GgTctTctGccGagTC	164	GAATCGGCAGAAGACC
75	CTTCTGCCGAGTCCTG	#75	CtTctGccGagTccTG	165	CAGGACTCGGCAGAAG
76	CTGCCGAGTCCTGCAA	#76	CtGccGagTccTgcAA	166	TTGCAGGACTCGGCAG
77	CCGAGTCCTGCAATAT	#77	CcGagTccTgcAatAT	167	ATATTGCAGGACTCGG
78	AGTCCTGCAATATGAA	#78	AgtCctGcAatAtgAA	168	TTCATATTGCAGGACT
79	CCTGCAATATGAATAT	#79	CctGcaAtaTgAatAT	169	ATATTCATATTGCAGG
80	GCAATATGAATATAAT	#80	GCaaTaTgaAtAtAAT	170	ATTATATTCATATTGC
81	ATATGAATATAATTTT	#81	ATAtGAatATAaTtTT	171	AAAATTATATTCATAT
82	TGAATATAATTTTAAA	#82	TGAaTATaAtTTTaAA	172	TTTAAAATTATATTCA
83	ATATAATTTTAAAAAT	#83	ATAtaATTtTAAaAAT	173	ATTTTTAAAATTATAT
84	TAATTTTAAAAATCCA	#84	TAAaTtTAAaAAtCCA	174	TGGATTTTAAAATTA
85	TTTTAAAAATCCAATT	#85	TTTtAAaaAtCCaATT	175	AATTGGATTTTAAAA
86	TAAAAATCCAATTAAG	#86	TAAaaATcCaATTaA G	176	CTTAATTGGATTTTAA
87	AAATCCAATTAAGAGA	#87	AAaTccAatTaAGaGA	177	TCTCTTAATTGGATTT
88	TCCAATTAAGAGAGAG	#88	TccAaTtaAgaGagAG	178	CTCTCTCTTAATTGGA
89	AATTAAGAGAGAGTGA	#89	AAiTaaGagAgaGtGA	179	TCACTCTCTCTTAATT
90	TAAGAGAGAGTGATGG	#90	TaaGaGagAgtGatGG	180	CCATCACTCTCTCTTA

The nucleobase motif refers to the sequence of nucleobases in the oligonucleotide. In the compounds, a capital letter is a beta-D-oxy LNA nucleoside, a lower case letter is a DNA nucleoside, and all internucleoside linkages in the compounds are phosphorothioate internucleoside linkages. LNA cytosines are 5-methyl cytosine.

5 **Pharmaceutically acceptable salts**

In a further aspect, the invention provides a pharmaceutically acceptable salt of the antisense oligonucleotide or a conjugate thereof, such as a pharmaceutically acceptable sodium salt or potassium salt.

Method of manufacture

- 10 In a further aspect, the invention provides methods for manufacturing the oligonucleotides of the invention comprising reacting nucleotide units and thereby forming covalently linked contiguous nucleotide units comprised in the oligonucleotide. Preferably, the method uses phosphoramidite chemistry (see for example Caruthers et al, 1987, Methods in Enzymology vol. 154, pages 287-313). In a further embodiment the method further comprises reacting the contiguous nucleotide
- 15 sequence with a conjugating moiety (ligand) to covalently attach the conjugate moiety to the oligonucleotide. In a further aspect, a method is provided for manufacturing the composition of the invention, comprising mixing the oligonucleotide or conjugated oligonucleotide of the invention with a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

Pharmaceutical Composition

In a further aspect, the invention provides pharmaceutical compositions comprising any of the aforementioned oligonucleotides and/or oligonucleotide conjugates or salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS) and pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. In some embodiments the pharmaceutically acceptable diluent is sterile phosphate buffered saline. In some embodiments, the oligonucleotide is used in the pharmaceutically acceptable diluent at a concentration of 50 - 300µM solution.

Applications

The oligonucleotides of the invention may be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

In research, such oligonucleotides may be used to specifically modulate the synthesis of STMN2 protein in cells (e.g. *in vitro* cell cultures) and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention. Typically the target modulation is achieved by degrading or inhibiting the mRNA producing the protein, thereby prevent protein formation or by degrading or inhibiting a modulator of the gene or mRNA producing the protein.

If employing the oligonucleotide of the invention in research or diagnostics the target nucleic acid may be a cDNA or a synthetic nucleic acid derived from DNA or RNA.

The present invention provides an *in vivo* or *in vitro* method for modulating STMN2 expression, such as splice modulation, in a target cell which is expressing STMN2, said method comprising administering an oligonucleotide of the invention in an effective amount to said cell.

In some embodiments, the target cell, is a mammalian cell in particular a human cell. The target cell may be an *in vitro* cell culture or an *in vivo* cell forming part of a tissue in a mammal. In preferred embodiments the target cell is present in a neuronal cell, such as a human primary neuronal cell or pluripotent stem cell derived-neuronal cell (e.g. *in vitro* use), or a neuronal cell *in vivo*, such as a cerebellum cell, a cortex cell, a brain stem cell, or a spinal cord cell.

For therapeutics, the oligonucleotides may be administered to an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the splicing of STMN2 pre-mRNA.

The invention provides methods for treating or preventing a disease, comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide, an oligonucleotide conjugate or a pharmaceutical composition of the invention to a subject suffering from or susceptible to the disease.

The invention also relates to an oligonucleotide, a composition or a conjugate as defined herein for use as a medicament.

The oligonucleotide, oligonucleotide conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

- 5 The invention also provides for the use of the oligonucleotide or oligonucleotide conjugate of the invention as described for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of as a disorder as referred to herein.

The compounds of the invention and the salts, conjugates, compositions thereof, may be used in medicine, for example for the treatment or prevention of diseases and disorders associated
10 with TDP-43 aggregation and/or mislocalization from the nucleus.

The compounds of the invention and the salts, conjugates, compositions thereof, may be used for the use in the treatment of a disease or disorders selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD), Alzheimer's Disease, Hippocampal sclerosis dementia, Down syndrome, Parkinson disease, Huntington's
15 disease, polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and Chronic Traumatic Encephalopathy.

The invention further relates to use of an oligonucleotide, oligonucleotide conjugate or a pharmaceutical composition as defined herein for the manufacture of a medicament for the treatment of a neurological disorder as neurodegenerative disorders characterized by TDP-43
20 pathology or mislocalization of TDP-43 from the nucleus, such as ALS.

The invention further relates to use of an oligonucleotide, oligonucleotide conjugate or a pharmaceutical composition as defined herein for the manufacture of a medicament for the treatment of a disease or disorders selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD), Alzheimer's Disease,
25 Hippocampal sclerosis dementia, Down syndrome, Parkinson disease, Huntington's disease, polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and Chronic Traumatic Encephalopathy

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of a neurological disorder as
30 neurodegenerative disorders characterized by TDP-43 pathology or mislocalization of TDP-43 from the nucleus, such as ALS.

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of a disease or disorders selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar
35 Degeneration (FTLD), Alzheimer's Disease, Hippocampal sclerosis dementia, Down syndrome,

Parkinson disease, Huntington's disease, polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and Chronic Traumatic Encephalopathy.

Administration

The oligonucleotides or pharmaceutical compositions of the present invention may, for example
 5 be administered for example via intracerebral, intracerebroventricular or intrathecal administration.

In a preferred embodiment the oligonucleotide or pharmaceutical compositions of the present invention are administered by a parenteral route including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intrathecal or intracranial,
 10 e.g. intracerebral or intraventricular, intravitreal administration. In one embodiment the active oligonucleotide or oligonucleotide conjugate is administered intravenously. In another embodiment the active oligonucleotide or oligonucleotide conjugate is administered subcutaneously.

CERTAIN EMBODIMENTS THE INVENTION

- 15 1. An antisense oligonucleotide, wherein the antisense oligonucleotide is 10 to 40 nucleotides in length, and comprises a contiguous nucleotide sequence of at least 10 nucleotides in length with at least 90% complementarity, such as 100% complementarity to SEQ ID NO 182.
- 20 2. The antisense oligonucleotide or salt according to embodiment 1, for modulation the splicing of human STMN2 pre-mRNA in a cell which is expressing STMN2 pre-mRNA
3. The antisense oligonucleotide or salt according to embodiment 1 or 2, wherein the antisense oligonucleotide is capable of reducing or preventing the splicing events of STMN2 pre-mRNA which result STMN2 transcript variant(s) (mature mRNA) which comprise a cryptic exon, such as a region of STMN2 intron 1 positioned downstream of STMN2 exon 1
 25 in a cell which is expressing said STMN2 transcript variant(s).
4. The antisense oligonucleotide of any one of embodiments 1 - 3, wherein the oligonucleotide has a length of 10-25 nucleotides.
5. The antisense oligonucleotide of any one of embodiments 1 - 4, wherein the contiguous nucleotide sequence has 100% complementarity to SEQ ID NO 182.
- 30 6. The antisense oligonucleotide of any one of embodiments  - 5, wherein the antisense oligonucleotide or contiguous nucleotide sequence thereof, is complementary to, such as fully complementary to a sequence selected from the group consisting of SEQ ID NO 91 – 180.
7. The antisense oligonucleotide of any one of embodiments  - 6, wherein the antisense oligonucleotide or contiguous nucleotide sequence thereof, comprises a sequence selected
 35 from the group consisting of SEQ ID NO 1 - 90, or at least 10 contiguous nucleotides thereof.

8. The antisense oligonucleotide according to any one of embodiments 1 – 7, wherein the oligonucleotide is or comprises an antisense oligonucleotide mixmer or totalmer.
9. The antisense oligonucleotide of any one of embodiments 8 – 8, wherein the antisense oligonucleotide or contiguous nucleotide sequence thereof is 12 – 20 nucleotides in length.
- 5 10. The antisense oligonucleotide according to any one of embodiments 1 – 9, wherein the antisense oligonucleotide comprises an oligonucleotide provided herein, such as a compound selected from the group consisting of compound ID NO #1 - #90
11. A conjugate comprising the antisense oligonucleotide according to any one of embodiments 8 – 10, and at least one conjugate moiety covalently attached to said oligonucleotide.
- 10 12. A pharmaceutically acceptable salt of the antisense oligonucleotide according to any one of embodiments 1 – 10, or the conjugate according to embodiment 12.
13. A pharmaceutical composition comprising the antisense oligonucleotide of embodiment 8-10 or the conjugate of embodiment 11 or salt of embodiment 12, and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.
- 15 14. An *in vivo* or *in vitro* method for modulating the splicing of a STMN2 pre-mRNA in a target cell which is expressing STMN2 pre-mRNA, said method comprising administering an antisense oligonucleotide of any one of embodiments 8-10 or the conjugate according to embodiment 11, or salt or the pharmaceutical composition according to embodiment 12 or 13 in an effective amount to said cell.
- 20 15. The method according to embodiment 20, wherein the administration of the antisense oligonucleotide results in reduced expression of, or prevents the expression of a STMN2 mRNA transcript variant which comprises a cryptic exon, or which comprises SEQ ID NO 188 (such as comprises any one of SEQ ID NO 182, 185, 186, or 187)
16. The method according to embodiments 14 or 15, wherein the antisense oligonucleotide results in increased expression of WT STMN2 mRNA.
- 25 17. The method according to any one of embodiments 14– 16, wherein the cell is either a human cell or a mammalian cell which is expressing a human STMN2 sequence, such as a human STMN2 pre-mRNA sequence.
18. A method for treating or preventing neurological disease comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide of any one of embodiments 8 – 10 or the conjugate according to embodiment 11 or the salt or pharmaceutical composition of embodiment 12 or 13 to a subject suffering from or susceptible to the neurological disease.
- 30 19. The antisense oligonucleotide of any one of embodiments 1 - 10 or the conjugate according to embodiment 11 or the salt or pharmaceutical composition of embodiment 12 or 13 for use as a medicament.
- 35

20. The antisense oligonucleotide of any one of embodiments 9 - 10 or the conjugate according to embodiment 11 or the salt or pharmaceutical composition of embodiment 12 or 13 for use in the treatment of an neurological disease.
21. Use of the antisense oligonucleotide of embodiment 9 - 10 or the conjugate according to embodiment 11 or the salt or pharmaceutical composition of embodiment 12 or 13, for the preparation of a medicament for treatment or prevention of a neurological disease.
22. The method, use or antisense oligonucleotide, or conjugate, salt, or composition thereof, according to any one of embodiments 18 – 21, wherein the neurological disease is a disease or disorders associated with TDP-43 aggregation and/or mislocalization from the nucleus.
23. The method, use or antisense oligonucleotide, or conjugate, salt, or composition thereof, according to any one of embodiments 18 – 22, wherein the neurological disease is a disease or disorder selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD), Alzheimer's Disease, Hippocampal sclerosis dementia, Down syndrome, Parkinson disease, Huntington's disease, polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and Chronic Traumatic Encephalopathy.

EXAMPLES

Example 1 – Human pluripotent stem cell-derived neuronal culture, oligonucleotide treatment and RNA isolation

Material and methods

Cell culture material:

96 well, black/clear, Tissue Culture Treated plate - Flat bottom with Lid, Falcon 353219

Laminin 521 Biolamina

DPBS (1X) CaCl₂+/MgCl₂+, Gibco 14040-091

DAPT Sigma Aldrich D5942

iCell Motor Neurons Kit, 01279 Cat. R1049 (Fujifilm Cellular Dynamics)

RNA isolation material:

Deoxyribonuclease I, bovine pancreas, Purified Lyophilized powder, J61061 Alfa Aesar

Buffer RDD kit, 1011132 QIAGEN

PureLink® Pro 96 total RNA Purification Kit, Thermo Fisher Cat No.: 12173-011A

Deepwell plate 96/500ul Eppendorf. VWR Cat No.: 737-2504

Cell seeding, maintenance and LNA oligonucleotides treatment:

iCell Glutaneurons with a cell density of 50,000 cells per well were seeded on Laminin coated 96 well plates according to manufacturer instructions (Cellular Dynamics International, X1005 iCell GlutaNeurons User's Guide). After 2 days of restoration, TDP-43 knock down was initiated by addition of 5 uM of compound A. After 7 days, 10 uM of compound B or C was added to the media containing compound A, and the cells incubated for additional 3 days before harvesting cells for RNA isolation.

TDP-43 LNA oligonucleotide - compound A:

SEQ ID NO 181 = TCcacactgaacaAACC (Upper case letters are beta-D-oxy LNA, lower case letters are DNA, LNA Cs are 5-methyl cytosine, all internucleoside linkages are phosphorothioate).

Medium was removed and 100 ul/well (96mwp) Lysis Buffer (PureLink® Pro 96 Thermo Fisher Kit) was added. RNA purification was performed as per the manufacturer's instructions, including DNaseI treatment (RNA Purification PureLink® Pro 96 Thermo Fisher Kit)

Example 2 – RNA sequencing analysis

Material and methods

Sequencing of total RNA isolated from 96 well plates using PureLink 96 well system including Dnase treatment. Next Generation Sequencing library preparation was performed from 100 ng of total RNA using the KAPA RNA HyperPrep Kit with RiboErase (HMR) (Roche, cat. no. KK8561). Sequencing was carried out on the MiniSeq system from Illumina to obtain approximately 4½ million paired-end reads (2x150 bp) per sample.

Samples:

Human motor neurons (hMNS) treated with and without TARDBP targeting (TDP-43 mRNA) antisense oligonucleotide (SEQ ID NO 181):

Data analysis was carried out using Partek Flow (mapping using *hg38* and Cufflinks).

The results are shown in figures 1 – 4

Figure 1 shows the RNA-Seq analysis of *TARDBP* and *STMN2* in human motor neurons

(hMNS) treated with or without antisense oligonucleotide targeting *TARDBP* encoding TDP-43.

A) *TARDBP* expression is reduced 72x on average upon treatment with SEQ ID NO 181. B)

Measurement of the wild type *STMN2* transcript (SEQ ID NO 183) shows a 101x reduction upon treatment with SEQ ID NO 181 and C) shows an increase in the transcript including the cryptic exon (2a) (SEQ ID NO 184) by 138x. The analysis was performed in duplicates untreated cells

(black dots) and SEQ ID NO 181 treated cells (light grey dots). Counts corresponds to the number of transcripts based on RNA-Seq analysis using Cufflinks to map reads against human genome (*hg38*).

Figure 2 shows a genome viewer (CLC genomic Workbench by Qiagen) showing the inclusion of the *STMN2* cryptic exon (2a) and the overall reduction of wild type *STMN2* upon treatment of human motor neurons with antisense oligonucleotide. Upper panel shows *hg38* and the lower panel shows the annotated isoform of *STMN2* pre-mRNAs. The upper two traces show untreated hMNS and the exon coverage in duplicates. The lower two traces show the exon coverage in hMNS treated with SEQ ID NO 181. The blue box marks the included cryptic exon.

Figure 3 illustrates that the removal of *TDP-43* removes *STMN2* wild type expression and induce inclusion of cryptic exon (exon 2a). Illustration of RNA-Seq analysis showing the coverage at exon boundaries within the *STMN2* gene. (A) Coverage at single nucleotide position (first or last bp in predicted exons) of untreated cells (striped bars), cells treated with SEQ ID NO 181 (filled light grey bars). The analysis was performed in duplicates with SD shown. (B) Graphical illustration of the coverage in the untreated and treated cells with inclusion of the polyadenylation. Positions within *hg38*, *STMN2*: exon 1 (3') chr8:79,311,214, exon 2a (5') chr8:79,616,822, exon 2 (5') chr8:79,636,802, exon 2 (3') chr8:79,636,897, exon 3 (5') chr8:79,641,378, exon 4 (5') chr8:79,654,871, exon 4 (3') chr8:79,655,062, exon 5 (5') chr8:79,664,815.

Figure 4 shows the position of antisense oligonucleotides (small grey bars) blocking the inclusion of the *STMN2* cryptic exon (2a) (big light grey box) to restore canonical splicing *STMN2* (exon 1 to 2).

Example 3 – ddPCR analysis of *STMN2* transcripts

Total RNA from the extraction method described in example 1 was used. cDNA was produced from 1 ug total RNA using the iScript ADV cDNA kit for RT-qPCR (Bio-Rad cat no. 1725038) following the procedure recommended by the manufacturer. Droplets were generated on a Bio-Rad automated droplet generator using ddPCR Supermix for probes without dUTP (Bio-Rad cat no. 186-3024). The droplets generated were run through a PCR reaction on a Bio-Rad C1000 Touch Thermal cycler. The PCR program was: 95 °C for 1 min, followed by 40 cycles of 94 °C for 0.3 min plus 60 °C for 1 minute. Lastly, a step of 98 °C for 10 minutes was performed before hold at 4 °C. The droplets were read on a Bio-Rad QX Droplet Reader.

The method was run as a triplex reaction including the following probes and primers:

1. *STMN2*_cryptic_exon (FAM labelled probe)

TATTCATATTGCAGGACTCGGCAGAAGACC (SEQ ID NO 194)

Forward primer

CATCACTCTCTCTTAATTGG (SEQ ID NO 195)

Reverse primer

CGCACACATGCTCACACAG (SEQ ID NO 196)

PCR product

5 CATCACTCTCTCTTAATTGGATTTTAAAATTATATTCATATTGCAGGACTCGGCAGAAGACC
TTCGAGAGAAAGGTAGAAAATAAGAATTTGGCTCTCTGTGTGAGCATGTGTGCG (SEQ ID
NO 197)

2. STMN2_wild-type_exon1_2 (HEX labelled probe)

10 AACAGCAATGGCCTACAAGGAAAA (SEQ ID NO 198)

Forward primer

GCTCTCTCCGCTGCTGTAG (SEQ ID NO 199)

Reverse primer

AGGTTCCGGGTAAAAGCAAG (SEQ ID NO 200)

15 PCR product

GCTCTCTCCGCTGCTGTAGCCGGACCCTTTGCCTTCGCCACTGCTCAGCGTCTGCACATC
CCTACAATGGCTAAAACAGCAATGGCCTACAAGGAAAAAATGAAGGAGCTGTCCATGCTGT
CACTGATCTGCTCTTGCTTTTACCCGGAACCT (SEQ ID NO 201)

3. HPRT1, Hs.PT.58v.45621572 (IDT pre-designed assay w. HEX/FAM labelled probe)

20 In addition, TARDBP expression was evaluated in a single-plex ddPCR reaction using the
following probes and primers: TARDBP, Hs.PT.58.26912658 (IDT pre-designed assay w. HEX
labelled probe). The results generated with these assays are shown in table 1.

Table 1: Effects of compounds SEQ ID 1-90 on levels of STMN2 transcripts with cryptic exon
25 (SEQ ID) inclusion and of wild-type STMN2 (SEQ ID) after treatment according to the method
described in Example 1. All compounds were tested at 10 uM concentration. Data were
generated by ddPCR according to the method described in Example 3. The wild-type
expression is shown as a relative level to untreated. The cryptic exon (2a) inclusion is shown as
the relative level of cryptic exon signal to total STMN2 signal: (cryptic / (cryptic + wild-type)) x
30 100.

SEQ ID NO	Compound	Nucleobase Motif	Comp ID	% Cryptic exon included	% Wild-type STMN2, relative to untreated
	Untreated cells			0,13	100,0
181	TCcacactgaacaAACC			41,5	54,7
1	GatAtAttGagAcaGC	GATATATTGAGACAGC	#1	8,2	95,5
2	AtAttGaGaCaGcaAT	ATATTGAGACAGCAAT	#2	19,4	86,6
3	TtGagAcAgcAaTcTT	TTGAGACAGCAATCTT	#3	12,9	94,2
4	AgAcAgcAaTctTtTG	AGACAGCAATCTTTTG	#4	15,6	77,6
5	CagCaaTctTtGtTT	CAGCAATCTTTGTTT	#5	31,1	62,1
6	CAaTctTtGtTtTAA	CAATCTTTGTTTTAA	#6	33,6	62,5
7	TcTTtTgTtTtAaTTT	TCTTTGTTTTAATTT	#7	32,7	55,4
8	TtTgTtTtAaTTTcTT	TTTGTTTTAATTTCTT	#8	19,5	48,0
9	GtTtAAtTTcTcAG	GTTTTAATTTCTTCAG	#9	18,4	73,3
10	TtAaTTtcTTcaGtAT	TTAATTTCTTCAGTAT	#10	29,4	64,6
11	AttTctTcAgtAttGC	ATTTCTTCAGTATTGC	#11	21,1	72,2
12	TctTcAgtAttGctAT	TCTTCAGTATTGCTAT	#12	33,7	57,3
13	TcAgtAttGctAttCA	TCAGTATTGCTATTCA	#13	29,6	63,4
14	GtaTtGcTAtTcAtAA	GTATTGCTATTCATAA	#14	38,2	56,1
15	TtGctAtTCaTaaTG	TTGCTATTCATAAATG	#15	39,5	52,0
16	CtaTtCaTaaAtgATA	CTATTCATAAATGATA	#16	31,6	60,1
17	TtCaTaAaTgaTagTA	TTCATAAATGATAGTA	#17	38,4	51,8
18	AtAaaTgaTaGtaAGC	ATAAATGATAGTAAGC	#18	28,8	68,0
19	AAtGatAgTaaGctTG	AATGATAGTAAGCTTG	#19	24,5	66,2
20	GatAgtAagCttGcAT	GATAGTAAGCTTGCAAT	#20	27,7	70,6
21	AgtAagCttGcAttAT	AGTAAGCTTGCAATTAT	#21	34,4	54,6
22	AagCttGcAttAttGA	AAGCTTGCAATTATTGA	#22	39,0	51,2
23	CttGcAttAtTtGatTT	CTTGCAATTATTGATTT	#23	39,4	63,9
24	GcAttAtTgAtTtATC	GCATTATTGATTTATC	#24	29,9	75,7
25	TTaTTgaTttAtCaTC	TTATTGATTTATCATC	#25	36,6	56,8
26	TtGatTtAtcAtCcTT	TTGATTTATCATCCTT	#26	30,2	59,3
27	AtTtAtcAtCcTtgTC	ATTTATCATCCTTGTC	#27	15,4	90,5
28	TaTcAtcCttGtcAAC	TATCATCCTTGTC AAC	#28	14,4	95,8
29	CaTccTtGtcAAcGT	CATCCTTGTC AACTGT	#29	9,9	100,2
30	CcTtGtcAAcGtgCC	CCTTGTC AACTGTGCC	#30	7,8	Not determined
31	TgtCaActGtGccACA	TGTCAACTGTGCCACA	#31	26,7	66,8
32	CaActGtGccAcAaGC	CAACTGTGCCACAAGC	#32	13,8	92,3
33	CtGtGccAcAaGccGC	CTGTGCCACAAGCCGC	#33	15,5	90,4
34	TgcCacAaGccGcaTT	TGCCACAAGCCGCATT	#34	14,7	104,1
35	CacAaGccGcAtTcAC	CACAAGCCGCATTAC	#35	3,9	135,6
36	AaGccGcAtTcAcaTT	AAGCCGCATTACATT	#36	12,8	102,6
37	CcGcAtTcAcAtTcAT	CCGCATTACATTACAT	#37	7,5	130,8
38	CatTcaCatTcAttTC	CATTACATTACATTC	#38	13,4	90,5
39	TcAcAtTcAttTctTC	TCACATTACATTCTTC	#39	8,0	116,2

40	CatTcAttTctTctTA	CATTCATTTCTTCTTA	#40	25,1	78,0
41	TcAttTctTctTagGC	TCATTTCTTCTTAGGC	#41	14,5	105,6
42	TtTctTctTagGcaGG	TTTCTTCTTAGGCAGG	#42	2,3	110,1
43	CtTcTtAggCagGcTG	CTTCTTAGGCAGGCTG	#43	27,9	57,0
44	CttAggCagGctGtCT	CTTAGGCAGGCTGTCT	#44	29,8	73,0
45	AggCagGctGtCtgTC	AGGCAGGCTGTCTGTC	#45	23,4	124,4
46	CagGctGtCtgTctCT	CAGGCTGTCTGTCTCT	#46	25,5	63,7
47	GctGtCtGtcTcTcTC	GCTGTCTGTCTCTCTC	#47	15,6	92,4
48	GtCtGtcTcTcTctCT	GTCTGTCTCTCTCTCT	#48	15,8	83,3
49	TgtCtcTcTctCtcGC	TGTCTCTCTCTCTCGC	#49	15,1	92,4
50	CtcTcTctCtcGcaCA	CTCTCTCTCTCGCACA	#50	10,8	114,8
51	TcTctCtcGcAcAcAC	TCTCTCTCGCACACAC	#51	5,2	149,7
52	CtcTcGcAcAcAegCA	CTCTCGCACACAEGCA	#52	14,7	86,6
53	TcGcAcAcAcGcAcAC	TCGCACACACGCACAC	#53	21,1	89,6
54	CacAcAcGcAcAcaTG	CACACACGCACACATG	#54	3,2	171,4
55	AcAcGcAcAcAtGcTC	ACACGCACACATGCTC	#55	26,3	70,1
56	CgcAcAcAtGcTcaCA	CGCACACATGCTCACA	#56	16,1	102,4
57	AcAcAtGctCacAcAG	ACACATGCTCACACAG	#57	22,9	83,4
58	CatGctCacAcAgaGA	CATGCTCACACAGAGA	#58	36,3	57,4
59	GctCacAcAgaGagCC	GCTCACACAGAGAGCC	#59	42,6	55,2
60	CacAcAgaGagCcaAA	CACACAGAGAGCCAAA	#60	21,7	80,2
61	AcAgaGaGccAaAtTC	ACAGAGAGCCAAATTC	#61	18,7	91,3
62	GagAgcCaaAtTctTA	GAGAGCCAAATTCTTA	#62	34,1	67,5
63	AgcCaaAtTctTatTT	AGCCAAATTCTTATTT	#63	25,4	71,3
64	CaaAtTctTtTaTtTCT	CAAATTCTTATTTTCT	#64	25,5	87,2
65	AtTctTatTtTcTaCC	ATTCTTATTTTCTACC	#65	36,8	57,4
66	CttAtTtTctAcCtTT	CTTATTTTCTACCTTT	#66	31,1	67,5
67	AtTtTctAccTtTcTC	ATTTTCTACCTTTCTC	#67	23,1	96,3
68	TTctAccTtTcTctCG	TTCTACCTTTCTCTCG	#68	9,0	131,6
69	TacCttTctCtcGaAG	TACCTTTCTCTCGAAG	#69	35,4	49,4
70	CttTctCtcGaAggTC	CTTTCTCTCGAAGGTC	#70	29,4	63,9
71	TctCtcGaAggTctTC	TCTCTCGAAGGTCTTC	#71	31,9	64,5
72	CtcGaAggTctTctGC	CTCGAAGGTCTTCTGC	#72	29,2	89,5
73	GaAggTctTctGccGA	GAAGGTCTTCTGCCGA	#73	14,0	Not determined
74	GgTctTctGccGagTC	GGTCTTCTGCCGAGTC	#74	10,5	102,8
75	CtTctGccGagTccTG	CTTCTGCCGAGTCCTG	#75	11,2	119,4
76	CtGccGagTccTgcAA	CTGCCGAGTCCTGCAA	#76	10,8	113,3
77	CcGagTccTgcAatAT	CCGAGTCCTGCAATAT	#77	7,3	136,9
78	AgtCctGcAatAtgAA	AGTCCTGCAATATGAA	#78	38,9	58,9
79	CctGcaAtaTgAatAT	CCTGCAATATGAATAT	#79	45,0	52,3
80	GCaaTaTGaAtAtaAAT	GCAATATGAATATAAT	#80	39,1	74,9
81	ATAtGAatATAaTtTT	ATATGAATATAATTTT	#81	24,5	49,4
82	TGAaTATaAtTTTaAA	TGAATATAATTTTAAA	#82	42,1	52,0

83	ATAtaATTtTAAaAAT	ATATAATTTTAAAAAT	#83	44,9	45,4
84	TAaTtTtAAAAAtCCA	TAATTTTAAAAATCCA	#84	27,4	74,0
85	TTTtAAaaAtCCaATT	TTTAAAAATCCAATT	#85	8,2	95,5
86	TAAaaATcCaATTaAG	TAAAAATCCAATTAAG	#86	19,4	86,6
87	AAaTccAatTaAGaGA	AAATCCAATTAAGAGA	#87	12,9	94,2
88	TccAaTtaAgaGagAG	TCCAATTAAGAGAGAG	#88	15,6	77,6
89	AAtTaaGagAgaGtGA	AATTAAGAGAGAGTGA	#89	31,1	62,1
90	TaaGaGagAgtGatGG	TAAGAGAGAGTGATGG	#90	33,6	62,5

In the compound column, LNA antisense compounds (5' – 3'): Capital letters are beta-D-oxy LNA nucleotides, lower case letters are 2'deoxyribose nucleosides, all LNA C are 5-methyl cytosine beta-D-oxy LNA nucleosides, and all internucleoside linkages are phosphorothioate internucleoside linkages.

CLAIMS

1. An LNA antisense oligonucleotide of 14 to 18 contiguous nucleotides in length, and comprises a contiguous nucleotide sequence of at least 14 nucleotides in length which are 100% complementary to a sequence selected from the group consisting of SEQ ID
5 Nos 144, 91, 92, 93, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, , 108, 109, 110, , 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, , 160, 161, 162, 163, 164, 165, 166, 167, 168, 170, 174, 175, 176, 177, 178, 179, and 180, wherein the antisense
10 oligonucleotide is capable of either (i) reducing the level of human STMN2 mature mRNA which comprise a intron 1 derived cryptic exon, such as illustrated in SEQ ID NO 182; or (ii) enhancing the expression level of wildtype STMN2 mature mRNA in a TDP-43 depleted cell which is expressing human STMN2 pre-mRNA.
2. The LNA antisense oligonucleotide according to claim 1, wherein the antisense
15 oligonucleotide is capable of (i) reducing the level of human STMN2 mature mRNA which comprise a intron 1 derived cryptic exon, such as illustrated in SEQ ID NO 182; and (ii) enhancing the expression level of wildtype STMN2 mature mRNA in a TDP-43 depleted cell which is expressing human STMN2 pre-mRNA.
3. The LNA antisense oligonucleotide according to claim 1 or 2, wherein the LNA
20 antisense oligonucleotide is 14, 15, 16 or 17 contiguous nucleotides in length which together form the contiguous nucleotide sequence which is 100% complementary to a sequence selected from the group consisting of SEQ ID Nos 144, 91, 92, 93, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, , 108, 109, 110, , 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135,
25 136, 137, 138, 139, 140, 141, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, , 160, 161, 162, 163, 164, 165, 166, 167, 168, 170, 174, 175, 176, 177, 178, 179, and 180.
4. The antisense oligonucleotide according to any one of claims 1 – 3, wherein the oligonucleotide comprises LNA and DNA nucleosides, such as a LNA mixmer
30 oligonucleotide.
5. The antisense oligonucleotide according to any one of claims 1 – 3, wherein the oligonucleotide is not capable of recruiting RNaseH.
6. The antisense oligonucleotide according to any one of claims 1 – 5, wherein the
35 antisense oligonucleotide comprises of a contiguous nucleotide sequence selected from the group consisting of SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44,

45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.

7. The antisense oligonucleotide according to any one of claims 1 – 6, wherein the antisense oligonucleotide comprises of a contiguous nucleotide sequence selected from the group consisting of SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.
8. The antisense oligonucleotide according to any one of claims 1 – 6, wherein the antisense oligonucleotide comprises of a contiguous nucleotide sequence selected from the group consisting of compound ID NO # 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.
9. An antisense oligonucleotide selected from the group consisting of compound ID NO # 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 84, 85, 86, 87, 88, 89, and 90.
10. An antisense oligonucleotide of formula (5' – 3')
 Compound ID NO 54: ${}^m\text{C}_s\text{a}_s\text{c}_s\text{A}_s\text{c}_s\text{A}_s\text{c}_s\text{G}_s\text{c}_s\text{A}_s\text{c}_s\text{A}_s\text{c}_s\text{a}_s\text{T}_s\text{G}$
 Compound ID NO 42: $\text{T}_s\text{t}_s\text{T}_s\text{c}_s\text{t}_s\text{T}_s\text{c}_s\text{t}_s\text{T}_s\text{a}_s\text{g}_s\text{G}_s\text{c}_s\text{a}_s\text{G}_s\text{G}$
 Compound ID NO 35: ${}^m\text{C}_s\text{a}_s\text{c}_s\text{A}_s\text{a}_s\text{G}_s\text{c}_s\text{c}_s\text{G}_s\text{c}_s\text{A}_s\text{t}_s\text{T}_s\text{c}_s\text{A}_s{}^m\text{C}$
 Compound ID NO 51: $\text{T}_s\text{c}_s\text{T}_s\text{c}_s\text{t}_s{}^m\text{C}_s\text{t}_s\text{c}_s\text{G}_s\text{c}_s\text{A}_s\text{c}_s\text{A}_s\text{c}_s\text{A}_s{}^m\text{C}$
 Wherein capital letters are beta-D-oxy LNA nucleotides, lower case letters are 2'-deoxyribose nucleosides, ${}^m\text{C}$ are 5-methyl cytosine beta-D-oxy LNA nucleosides, and subscript s is a phosphorothioate internucleoside linkage.
11. A conjugate comprising the antisense oligonucleotide according to any one of claims 1 – 10, and at least one conjugate moiety covalently attached to said oligonucleotide.
12. A pharmaceutically acceptable salt of the antisense oligonucleotide according to any one of claims 1 – 10, or the conjugate according to claim 11.
13. A pharmaceutical composition comprising the antisense oligonucleotide of claim 1 – 10 or the conjugate of claim 11 or salt of claim 12, and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.
14. An *in vivo* or *in vitro* method for modulating the splicing of a STMN2 pre-mRNA in a target cell which is expressing STMN2 pre-mRNA, said method comprising administering an antisense oligonucleotide of any one of claims 1 – 10 or the conjugate according to claim

11, or salt or the pharmaceutical composition according to claim 12 or 13 in an effective amount to said cell.

15. The method according to claim 20, wherein the administration of the antisense oligonucleotide results in reduced expression of, or prevents the expression of a STMN2 mRNA transcript variant which comprises a cryptic exon, or which comprises SEQ ID NO 182.

16. The method according to claims 14 or 15, wherein the antisense oligonucleotide results in increased expression of WT STMN2 mRNA.

17. The method according to any one of claims 14– 16, wherein the cell is either a human cell or a mammalian cell which is expressing a human STMN2 sequence, such as a human STMN2 pre-mRNA sequence.

18. A method for treating or preventing neurological disease comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide of any one of claims 9 - 10 or the conjugate according to claim 11 or the salt or pharmaceutical composition of claim 12 or 13 to a subject suffering from or susceptible to the neurological disease.

19. The antisense oligonucleotide of any one of claims 1 - 10 or the conjugate according to claim 11 or the salt or pharmaceutical composition of claim 12 or 13 for use as a medicament.

20. The antisense oligonucleotide of any one of claims 9 - 10 or the conjugate according to claim 11 or the salt or pharmaceutical composition of claim 12 or 13 for use in the treatment of an neurological disease.

21. Use of the antisense oligonucleotide of claim 9 - 10 or the conjugate according to claim 11 or the salt or pharmaceutical composition of claim 12 or 13, for the preparation of a medicament for treatment or prevention of a neurological disease.

22. The method, use or antisense oligonucleotide, or conjugate, salt, or composition thereof, according to any one of claims 18 – 21, wherein the neurological disease is a disease or disorders associated with TDP-43 aggregation and/or mislocalization from the nucleus.

23. The method, use or antisense oligonucleotide, or conjugate, salt, or composition thereof, according to any one of claims 18 – 22, wherein the neurological disease is a disease or disorder selected from the group consisting of: Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD), Alzheimer's Disease, Hippocampal sclerosis dementia, Down syndrome, Parkinson disease, Huntington's disease, polyglutamine diseases, such as spinocerebellar ataxia 3, Myopathies and Chronic Traumatic Encephalopathy.

FIGURES

Figure 1

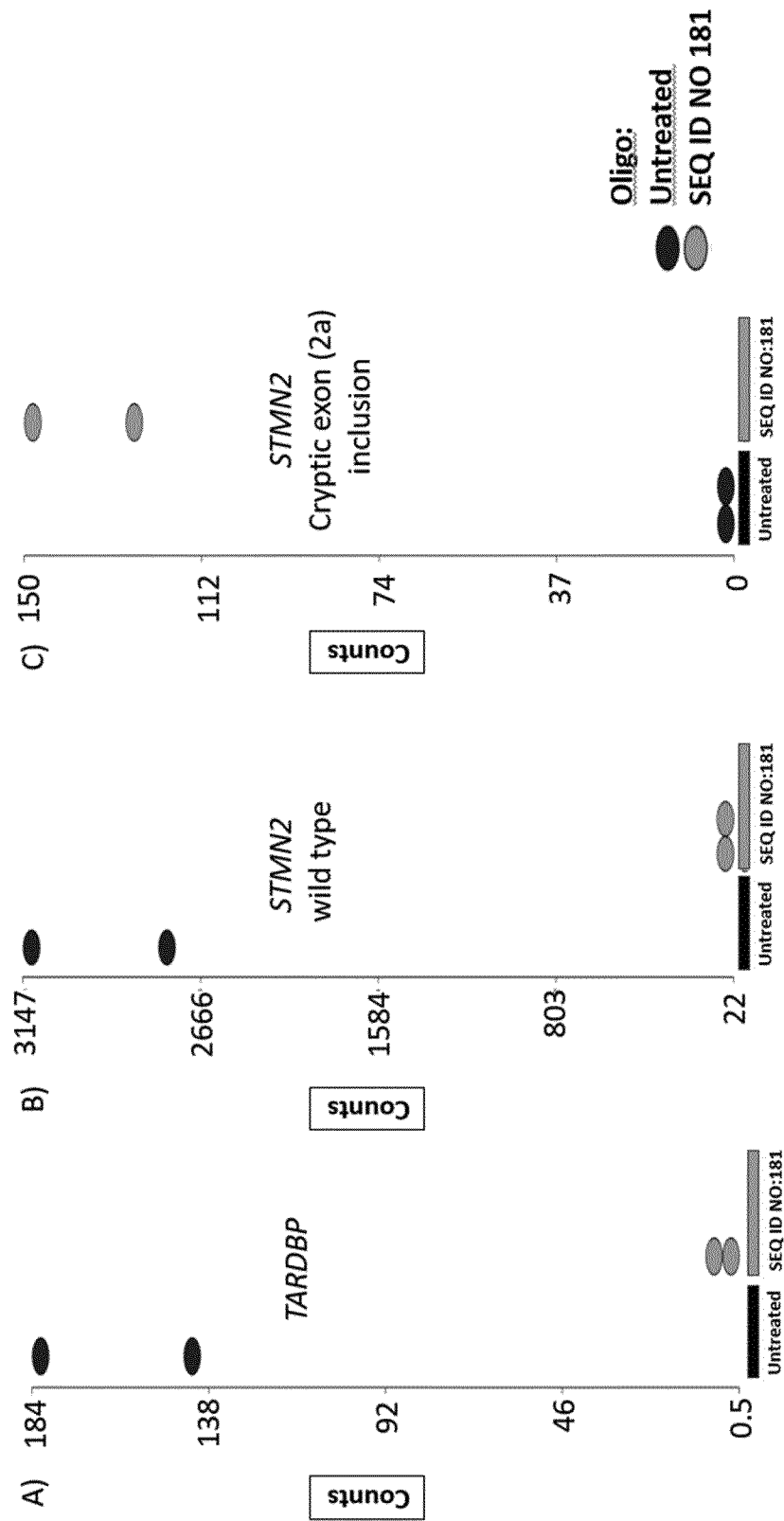


Figure 2

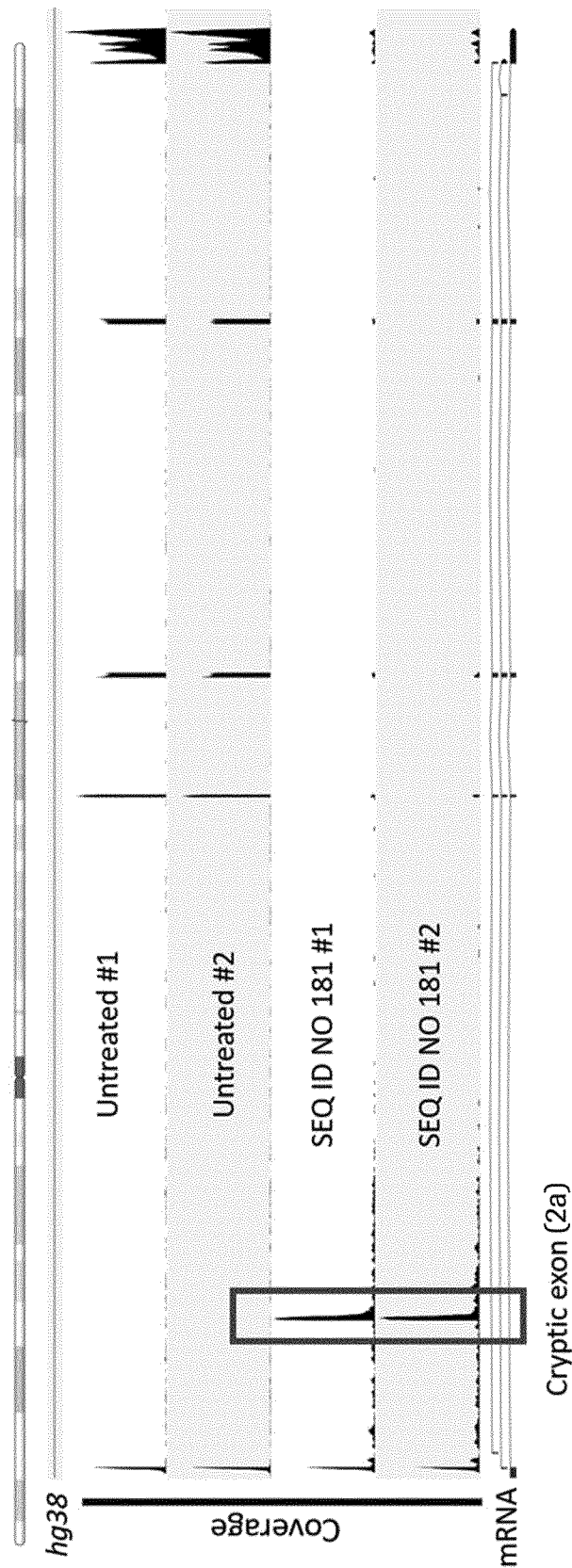


Figure 3

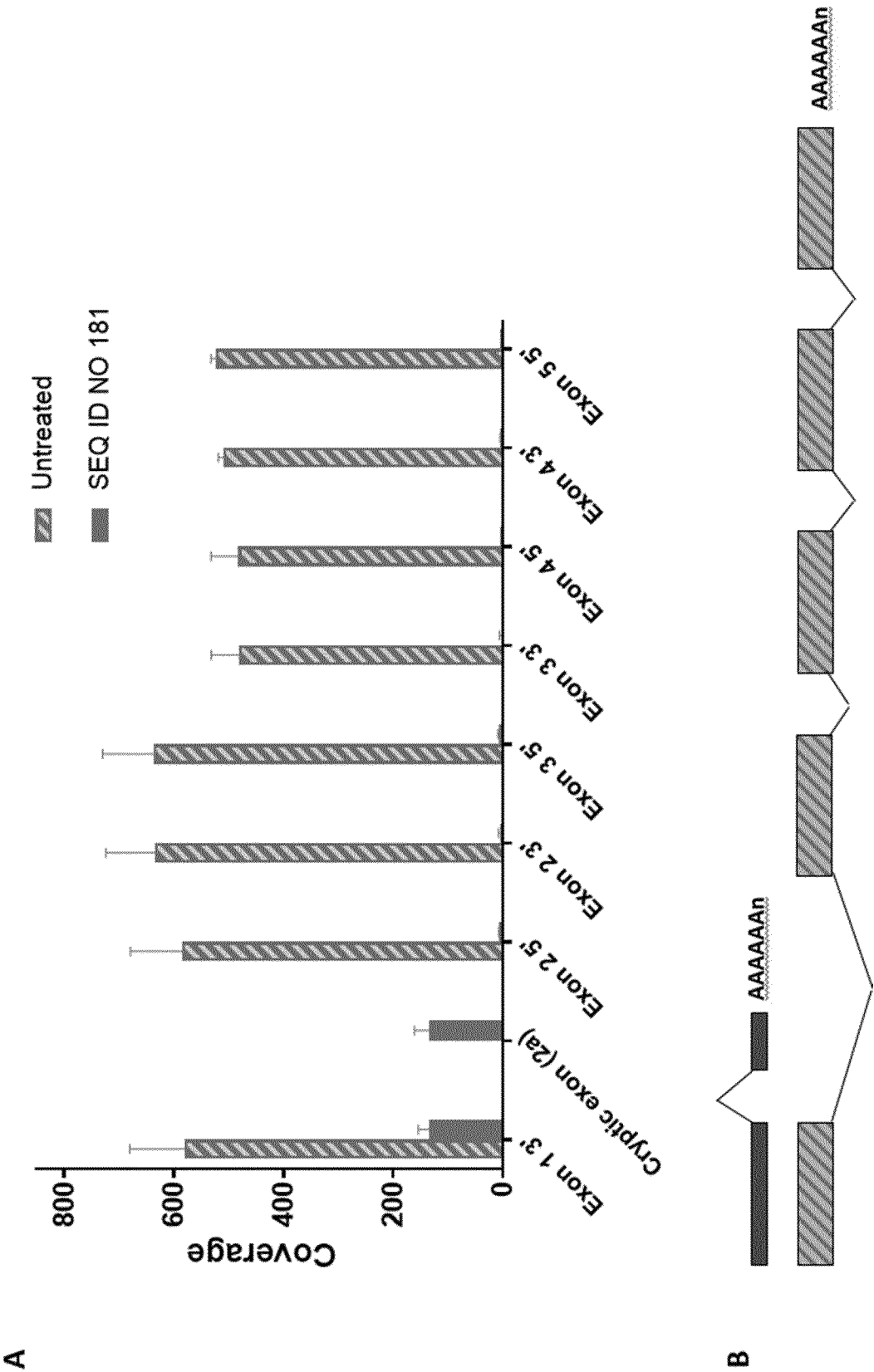


Figure 4

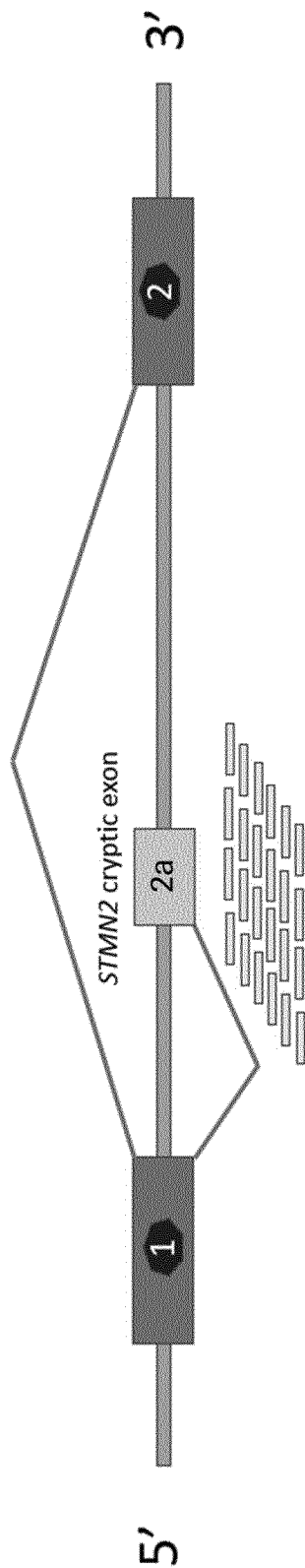


FIGURE 5

AGTCTTCTCTCTCGCTCTCTCCGCTGCTGTAGCCGGACCCCTTTGCCTTCGCCACTGCTCA
GCGTCTGCACATCCCTACAATGGCTAAAACAGCAATGGGACTCGGCAGAAGACCTTCGAG
M A K T A M G L G R R P S R
AGAAAGGT**AG**AAAAATAAGAATTTGGCTCTCTGTGTGAGCATGTGTGCGTGTGTGCGAGAG
E R -
AGAGAGACAGACAGCCTGCCTAAGAAGAAATGAATGTGAATGCGGCTTGTGGCACAGTTG
ACAAGGATGATAAATCAATAATGCAAGCTTACTATCATTTATGAATAGCAATACTGAAGA
AATTAAAACAAAAGATTGCTGTCTCAATATATC

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/070426

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/7088
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, COMPENDEX, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/241648 A1 (IONIS PHARMACEUTICALS INC [US]) 19 December 2019 (2019-12-19) pages 2, 8 - pages 15,21; sequences 21-87, 98-164, 176-242, 254-320 page 36 - page 48; tables 1-4 -----	1-23
A	PETER H. HAGEDORN ET AL: "Locked nucleic acid: modality, diversity, and drug discovery", DRUG DISCOVERY TODAY, vol. 23, no. 1, 1 January 2018 (2018-01-01), pages 101-114, XP055461215, AMSTERDAM, NL ISSN: 1359-6446, DOI: 10.1016/j.drudis.2017.09.018 ----- -/-	1-23



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

2 November 2021

Date of mailing of the international search report

10/11/2021

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/070426

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2020/247419 A2 (QURALIS CORP [US]) 10 December 2020 (2020-12-10) the whole document -----	1-23
X,P	WO 2020/150290 A2 (HARVARD COLLEGE [US]) 23 July 2020 (2020-07-23) the whole document -----	1-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/070426

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2021/070426

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019241648 A1	19-12-2019	AU 2019287635 A1	17-12-2020
		CA 3103429 A1	19-12-2019
		EP 3807411 A1	21-04-2021
		JP 2021526823 A	11-10-2021
		US 2021252039 A1	19-08-2021
		WO 2019241648 A1	19-12-2019

WO 2020247419 A2	10-12-2020	NONE	

WO 2020150290 A2	23-07-2020	CA 3126918 A1	23-07-2020
		WO 2020150290 A2	23-07-2020
