[19] Patents Registry
The Hong Kong Special Administrative Region
香港特別行政區
專利註冊處

[11] 1246648 B
EP 3305302 B1

[12] **STANDARD PATENT SPECIFICATION**
標準專利說明書

[21] Application no. 申請編號
18106100.4

[22] Date of filing 提交日期
10.05.2018

[51] Int. Cl.
A61K C12N

[54] COMPOSITIONS AND METHODS FOR MODULATION OF SMN2 SPLICING IN A SUBJECT
用於在受試者中調節 SMN2 剪接的組合物和方法

[30] Priority 優先權
17.06.2009 US 218031 P

[43] Date of publication of application 申請發表日期
14.09.2018

[45] Date of publication of grant of patent 批予專利的發表日期
06.09.2019

EP Application no. & date 歐洲專利申請編號及日期
EP 17203913.3 17.06.2010

EP Publication no. & date 歐洲專利申請發表編號及日期
EP 3305302 11.04.2018

Date of grant in designated patent office 指定專利當局批予專利日期
19.09.2018

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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 305 302 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
19.09.2018 Bulletin 2018/38

(21) Application number: **17203913.3**

(22) Date of filing: **17.06.2010**

(51) Int Cl.:
A61K 31/7088 *(2006.01)* ***C12N 15/113*** *(2010.01)*

(54) **COMPOSITIONS AND METHODS FOR MODULATION OF SMN2 SPLICING IN A SUBJECT**

ZUSAMMENSETZUNGEN UND VERFAHREN ZUR MODULATION DER SMN2-AUFSPALTUNG IM KÖRPER EINES PATIENTEN

COMPOSITIONS ET MÉTHODES POUR MODULER L'ÉPISSAGE DE SMN2 CHEZ UN SUJET

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR

(30) Priority: **17.06.2009 US 218031 P**

(43) Date of publication of application:
11.04.2018 Bulletin 2018/15

(60) Divisional application:
18185820.0

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
10790221.5 / 2 442 816

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(56) References cited:
WO-A2-2007/002390 **WO-A2-2010/091308**
US-A1- 2007 292 408

- **HUA Y ET AL: "Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice", AMERICAN JOURNAL OF HUMAN GENETICS, AMERICAN SOCIETY OF HUMAN GENETICS, CHICAGO, IL, US, vol. 82, no. 4, 1 April 2008 (2008-04-01), pages 834-848, XP002692598, ISSN: 0002-9297, DOI: 10.1016/J.AJHG.2008.01.014 [retrieved on 2008-03-27]**
- **SCHMID ALOICIA ET AL: "Animal models of spinal muscular atrophy", JOURNAL OF CHILD NEUROLOGY, DECKER PERIODICALS, HAMILTON, CA, vol. 22, no. 8, 1 August 2007 (2007-08-01) , pages 1004-1012, XP002550020, ISSN: 0883-0738, DOI: 10.1177/0883073807305667**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- SINGH NIRMAL K ET AL: "Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron", MOLECULAR AND CELLULAR BIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 26, no. 4, 1 February 2006 (2006-02-01), pages 1333-1346, XP002596112, ISSN: 0270-7306
- J. H. WILLIAMS ET AL.: "Oligonucleotide-mediated survival of motor neuron protein expression in CNS improves phenotype in a mouse model of Spinal Muscular Atrophy", JOURNAL OF NEUROSCIENCE, vol. 29, no. 24, 17 June 2009 (2009-06-17) , pages 7633-7638, XP055044377, ISSN: 0270-6474, DOI: 10.1523/JNEUROSCI.0950-09.2009
- LE THANH T. ET AL.: "SMNdelta7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN", HUMAN MOLECULAR GENETICS, OXFORD UNIVERSITY PRESS, SURREY, vol. 14, no. 6, 1 January 2005 (2005-01-01), pages 845-857, XP008073633, ISSN: 0964-6906, DOI: 10.1093/HMG/DDI078
- T. D. BAUGHAN ET AL: "Delivery of bifunctional RNAs that target an intronic repressor and increase SMN levels in an animal model of spinal muscular atrophy", HUMAN MOLECULAR GENETICS, vol. 18, no. 9, 19 February 2009 (2009-02-19), pages 1600-1611, XP055051447, ISSN: 0964-6906, DOI: 10.1093/hmg/ddp076

Description

SEQUENCE LISTING

[0001] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 20100617_CORE0086WOSEQ.txt, created June 17, 2010, which is 5 Kb in size.

BACKGROUND OF THE INVENTION

[0002] Newly synthesized eukaryotic mRNA molecules, known as primary transcripts or pre-mRNA are processed before translation. Processing of the pre-mRNAs includes addition of a 5' methylated cap and an approximately 200-250 base poly(A) tail to the 3' end of the transcript. Processing of mRNA from pre-mRNA also frequently involves splicing of the pre-mRNA, which occurs in the maturation of 90-95% of mammalian mRNAs. Introns (or intervening sequences) are regions of a pre-mRNA (or the DNA encoding it) that are not included in the coding sequence of the mature mRNA. Exons are regions of a primary transcript that remain in the mature mRNA. The exons are spliced together to form the mature mRNA sequence. Splice junctions are also referred to as splice sites with the 5' side of the junction often called the "5' splice site," or "splice donor site" and the 3' side the "3' splice site" or "splice acceptor site." In splicing, the 3' end of an upstream exon is joined to the 5' end of the downstream exon. Thus the unspliced pre-mRNA has an exon/intron junction at the 5' end of an intron and an intron/exon junction at the 3' end of an intron. After the intron is removed, the exons are contiguous at what is sometimes referred to as the exon/exon junction or boundary in the mature mRNA. Cryptic splice sites are those which are less often used but may be used when the usual splice site is blocked or unavailable. Alternative splicing, defined as the splicing together of different combinations of exons, often results in multiple mRNA transcripts from a single gene.

[0003] Up to 50% of human genetic diseases resulting from a point mutation result in aberrant pre-mRNA processing. Such point mutations can either disrupt a current splice site or create a new splice site, resulting in mRNA transcripts comprised of a different combination of exons or with deletions in exons. Point mutations also can result in activation of a cryptic splice site or disrupt regulatory *cis* elements (i.e. splicing enhancers or silencers) (Cartegni et al., Nat. Rev. Genet., 2002, 3, 285-298; Drawczak et al., Hum. Genet., 1992, 90, 41-54). Antisense oligonucleotides have been used to target mutations that lead to aberrant splicing in several genetic diseases in order to redirect splicing to give a desired splice product (Kole, Acta Biochimica Polonica, 1997, 44, 231-238).

[0004] Antisense compounds have also been used to alter the ratio of naturally occurring alternate splice variants such as the long and short forms of Bcl-x pre-mRNA (U.S. Patent 6,172,216; U.S. Patent 6,214,986; Taylor et al., Nat. Biotechnol. 1999, 17, 1097-1100) or to force skipping of specific exons containing premature termination codons (Wilton et al., Neuromuscul. Disord., 1999, 9, 330-338). U.S. Patent 5,627,274 and WO 94/26887 disclose compositions and methods for combating aberrant splicing in a pre-mRNA molecule containing a mutation using antisense oligonucleotides which do not activate RNAse H.

[0005] Proximal spinal muscular atrophy (SMA) is a genetic, neurodegenerative disorder characterized by the loss of spinal motor neurons. SMA is an autosomal recessive disease of early onset and is currently the leading cause of death among infants. The severity of SMA varies among patients and has thus been classified into three types. Type I SMA is the most severe form with onset at birth or within 6 months and typically results in death within 2 years. Children with type I SMA are unable to sit or walk. Type II SMA is the intermediate form and patients are able to sit, but cannot stand or walk. Patients with type III SMA, a chronic form of the disease, typically develop SMA after 18 months of age (Lefebvre et al., Hum. Mol. Genet., 1998, 7, 1531-1536).

[0006] The molecular basis of SMA is caused by the loss of both copies of survival motor neuron gene 1 (SMN1), which may also be known as SMN Telomeric, a protein that is part of a multi-protein complex thought to be involved in snRNP biogenesis and recycling. A nearly identical gene, SMN2, which may also be known as SMN Centromeric, exists in a duplicated region on chromosome 5q13 and modulates disease severity. Expression of the normal SMN1 gene results solely in expression of survival motor neuron (SMN) protein. Although SMN1 and SMN2 have the potential to code for the same protein, SMN2 contains a translationally silent mutation at position +6 of exon 7, which results in inefficient inclusion of exon 7 in SMN2 transcripts. Thus, the predominant form of SMN2 is a truncated version, lacking exon 7, which is unstable and inactive (Cartegni and Krainer, Nat. Genet., 2002, 30, 377-384). Expression of the SMN2 gene results in approximately 10-20% of the SMN protein and 80-90% of the unstable/non-functional SMNdelta7 protein. SMN protein plays a well-established role in assembly of the spliceosome and may also mediate mRNA trafficking in the axon and nerve terminus of neurons.

[0007] Antisense technology is an effective means for modulating the expression of one or more specific gene products, including alternative splice products, and is uniquely useful in a number of therapeutic, diagnostic, and research applications. The principle behind antisense technology is that an antisense compound, which hybridizes to a target nucleic acid, modulates gene expression activities such as transcription, splicing or translation through one of a number of

antisense mechanisms. The sequence specificity of antisense compounds makes them extremely attractive as tools for target validation and gene functionalization, as well as therapeutics to selectively modulate the expression of genes involved in disease.

Certain antisense compounds complementary to SMN2 are known in the art. See for example, WO 2007/002390; Hua et al., American J. of Human Genetics (April 2008) 82, 1-15; Singh et al., RNA Bio. 6:3, 1-10 (2009). Certain antisense compounds and methods disclosed herein possess desirable characteristics compared to such compounds and methods known in the art. Chimeric peptide nucleic acid molecules designed to modulate splicing of SMN2 have been described (WO 02/38738; Cartegni and Krainer, Nat. Struct. Biol., 2003, 10, 120-125). Hua Y et al (2008) The American Journal of Human Genetics 82: 834-848, describes antisense compounds which target human Smn2 pre-mRNA, and their testing for an effect on exon 7 inclusion in human *SMN2* transgenic mice.

SUMMARY OF THE INVENTION

[0008] In one aspect, the present invention provides an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a pre-mRNA encoding human SMN2, for use in treating a human subject having spinal muscular atrophy (SMA), wherein the compound is administered into the cerebrospinal fluid in the intrathecal space of the human subject, wherein the antisense oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence of SEQ ID NO: 1, wherein each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety, wherein each modified sugar moiety is a 2'-methoxyethyl sugar moiety and wherein each internucleoside linkage is a phosphorothioate linkage.

[0009] The invention also provides use of an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a pre-mRNA encoding human SMN2 in the manufacture of a medicament for use in treating a human subject having spinal muscular atrophy (SMA), wherein the medicament is administered into the cerebrospinal fluid in the intrathecal space of the human subject, wherein the antisense oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence of SEQ ID NO: 1, wherein each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety, wherein each modified sugar moiety is a 2'-methoxyethyl sugar moiety and wherein each internucleoside linkage is a phosphorothioate linkage.

[0010] Further aspects of the invention are set out in the present claims.

[0011] In certain instances, the present disclosure provides methods comprising administering to a subject an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 pre-mRNA, wherein the antisense compound is administered into the cerebrospinal fluid. In certain instances, the administration is into the intrathecal space. In certain instances, the administration is into the cerebrospinal fluid in the brain. In certain instances, the administration comprises a bolus injection. In certain instances, the administration comprises infusion with a delivery pump.

[0012] In certain instances, the antisense compound is administered at a dose from 0.01 to 10 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the dose is from 0.01 to 10 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the dose is from 0.01 to 5 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the dose is from 0.05 to 1 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the dose is from 0.01 to 0.5 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the dose is from 0.05 to 0.5 milligrams of antisense compound per kilogram of body weight of the subject.

[0013] In certain instances, the dose is administered daily. In certain instances, the dose is administered weekly. In certain instances, the antisense compound is administered continuously and wherein the dose is the amount administered per day. In certain instances, the method comprises administering at least one induction dose during an induction phase and administering at least one maintenance dose during a maintenance phase. In certain instances, the induction dose is from 0.05 to 5.0 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the maintenance dose is from 0.01 to 1.0 milligrams of antisense compound per kilogram of body weight of the subject. In certain instances, the duration of the induction phase is at least 1 week. In certain instances, the duration of the maintenance phase is at least 1 week. In certain instances, each induction dose and each maintenance dose comprises a single injection. In certain instances, each induction dose and each maintenance dose independently comprise two or more injections. In certain instances, antisense compound is administered at least 2 times over a treatment period of at least 1 week. In certain instances, the treatment period is at least one month. In certain instances, the treatment period is at least 2 months. In certain instances, the treatment period is at least 4 months. In certain instances, the induction dose is administered by one or more bolus injections and the maintenance dose is administered by an infusion pump.

[0014] In certain instances, the method comprises assessing the tolerability and/or effectiveness of the antisense compound. In certain instances, dose amount or frequency of antisense compound is reduced following an indication that administration of the antisense compound is not tolerated. In certain instances, the dose amount or frequency of antisense compound is maintained or reduced following an indication that administration of the antisense compound is

effective. In certain instances, the dose of antisense compound is increased following an indication that administration of the antisense compound is not effective. In certain instances, frequency of administration of antisense compound is reduced following an indication that administration of the antisense compound is effective. In certain instances, frequency of administration of antisense compound is increased following an indication that administration of the antisense compound is not effective.

[0015] In certain instances, the methods comprise co-administration of the antisense compound and at least one other therapy. In certain instances, an antisense compound and at least one other therapy are co-administered at the same time. In certain instances, an antisense compound is administered prior to administration of the at least one other therapy. In certain instances, an antisense compound is administered after administration of the at least one other therapy. In certain instances, the at least one other therapy comprises administration of one or more of valproic acid, riluzole, hydroxyurea, and a butyrate. In certain instances, at least one other therapy comprises administration of trichostatin-A. In certain instances, the at least one other therapy comprises administration of stem cells. In certain instances, at least one other therapy is gene therapy. In certain instances, gene therapy is administered to the CSF and an antisense compound is administered systemically. In certain instances, gene therapy is administered to the CSF and an antisense compound is administered systemically and to the CSF. In certain instances, the disclosure provides treatment regimens where initially, an antisense compound is administered to the CSF and systemically, followed by gene therapy administration to the CSF and systemic administration of antisense compound. In certain such instances, the subject is an infant at the time of initial treatment. In certain such instances, the subject is less than 2 years old. In certain instances, antisense compound is administered to the CNS of a subject until the subject is old enough for gene therapy. In certain such instances, antisense compound is administered systemically throughout.

[0016] In certain instances, the antisense compound is administered at a concentration of about 0.01 mg/ml, about 0.05 mg/ml, about 0.1 mg/ml, about 0.5 mg/ml, about 1 mg/ml, about 5 mg/ml, about 10 mg/ml, about 50 mg/ml, or about 100 mg/ml.

[0017] In certain instances, inclusion of exon 7 of SMN2 mRNA in a motoneuron in the subject is increased. In certain instances, inclusion of exon 7 amino acids in SMN2 polypeptide in a motoneuron in the subject is increased.

[0018] In certain instances, the disclosure provides methods of increasing inclusion of exon 7 of SMN2 mRNA in a motoneuron in a subject comprising administering to the subject an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 and thereby increasing inclusion of exon 7 of SMN2 mRNA in the motoneuron in the subject.

[0019] In certain instances, the disclosure provides methods of increasing inclusion of exon 7 amino acids in SMN2 polypeptide in a motoneuron in a subject comprising administering to the subject an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 and thereby increasing inclusion of exon 7 amino acids in SMN2 polypeptide in the motoneuron in the subject.

[0020] In certain instances, the subject has SMA. In certain instances, the subject has type I SMA. In certain instances, the subject has type II SMA. In certain instances, the subject has type III SMA.

[0021] In certain instances, a first dose is administered in utero. In certain instances, the first dose is administered prior to complete formation of the blood-brain-barrier. In certain instances , a first dose is administered within 1 week of birth of the subject. In certain instances, a first dose is administered within 1 month of birth of the subject. In certain instances, a first dose is administered within 3 months of birth of the subject. In certain instances, a first dose is administered within 6 months of birth of the subject. In certain instances, a first dose is administered when the subject is from 1 to 2 years of age. In certain instances, a first dose is administered when the subject is from 1 to 15 years of age. In certain instances, a first dose is administered when the subject is older than 15 years of age.

[0022] In certain instances, the subject is a mammal. In certain instances, the subject is a human.

[0023] In certain instances, the methods comprise identifying a subject having SMA. In certain instances, the subject is identified by measuring electrical activity of one or more muscles of the subject. In certain instances, the subject is identified by a genetic test to determine whether the subject has a mutation in the subject's SMN1 gene. In certain instances, the subject is identified by muscle biopsy.

[0024] In certain instances, administering the antisense compound results in an increase in the amount of SMN2 mRNA having exon 7 of at least 10%. In certain instances, the increase in the amount of SMN2 mRNA having exon 7 is at least 20%. In certain instances, the increase in the amount of SMN2 mRNA having exon 7 is at least 50%. In certain instances, the amount of SMN2 mRNA having exon 7 is at least 70%.

[0025] In certain instances, administering of the antisense compound results in an increase in the amount of SMN2 polypeptide having exon 7 amino acids of at least 10%. In certain instances, wherein the increase in the amount of SMN2 polypeptide having exon 7 amino acids is at least 20%. In certain instances, the increase in the amount of SMN2 polypeptide having exon 7 amino acids is at least 50%. In certain instances, the increase in the amount of SMN2 polypeptide having exon 7 amino acids is at least 70%.

[0026] In certain instances, the administering of the antisense compound ameliorates at least one symptom of SMA in the subject. In certain instances, the administering of the antisense compound results in improved motor function in

the subject. In certain instances, the administering of the antisense compound results in delayed or reduced loss of motor function in the subject. In certain instances, administering of the antisense compound results in improved respiratory function. In certain instances, the administering of the antisense compound results in improved survival.

[0027] In certain instances, at least one nucleoside of the antisense oligonucleotide comprises a modified sugar moiety. In certain instances, at least one modified sugar moiety comprises a 2'-methoxyethyl sugar moiety. In certain instances, essentially each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety. In certain instances, the nucleosides comprising a modified sugar moiety all comprise the same sugar modification. In certain instances, wherein each modified sugar moiety comprises a 2'-methoxyethyl sugar moiety. In certain instances, each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety. In certain instances, the nucleosides all comprise the same sugar modification. In certain instances, each modified sugar moiety comprises a 2'-methoxyethyl sugar moiety. In certain instances, at least one internucleoside linkage is a phosphorothioate internucleoside linkage. In certain instances, each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0028] In certain instances, the antisense oligonucleotide consists of 10 to 25 linked nucleosides. In certain instances, the antisense oligonucleotide consists of 12 to 22 linked nucleosides. In certain instances, the antisense oligonucleotide consists of 15 to 20 linked nucleosides. In certain instances, the antisense oligonucleotide consists of 18 linked nucleosides.

[0029] In certain instances, the antisense oligonucleotide is at least 90% complementary to the nucleic acid encoding human SMN2. In certain instances, the antisense oligonucleotide is fully complementary to the nucleic acid encoding human SMN2. In certain instances, the oligonucleotide has a nucleobase sequence comprising at least 10 contiguous nucleobases of the nucleobase sequence SEQ ID NO: 1. In certain instances, the oligonucleotide has a nucleobase sequence comprising at least 15 contiguous nucleobases of the nucleobase sequence SEQ ID NO: 1. In certain instances, the oligonucleotide has a nucleobase sequence comprising the nucleobase sequence SEQ ID NO: 1. In certain instances, the oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence SEQ ID NO: 1.

[0030] In certain instances, the antisense compound comprises a conjugate group or terminal group.

[0031] In certain instances, the antisense compound consists of the antisense oligonucleotide.

[0032] In certain instances, the antisense compound is also administered systemically. In certain instances, the systemic administration is by intravenous or intraperitoneal injection. In certain instances, systemic administration and the administration into the central nervous system are performed at the same time. In certain instances, systemic administration and the administration into the central nervous system are performed at different times.

[0033] In certain instances, the disclosure provides systemic administration of antisense compounds, either alone or in combination with delivery into the CSF. In certain instances, pharmaceutical compositions are administered systemically. In certain instances, pharmaceutical compositions are administered subcutaneously. In certain instances, pharmaceutical compositions are administered intravenously. In certain instances, pharmaceutical compositions are administered by intramuscular injection.

[0034] In certain instances, pharmaceutical compositions are administered both directly to the CSF (e.g., IT and/or ICV injection and/or infusion) and systemically.

[0035] In certain instances, the disclosure provides methods of administering to a subject having at least one symptom associated with SMA, at least one dose of an antisense compound comprising an oligonucleotide consisting of 15 to 20 linked nucleosides and having a nucleobase sequence which is 100% complementary to SEQ ID NO. 7 over its entire length, and wherein each nucleoside is a 2'-MOE modified nucleoside; and wherein at least one dose is between 0.1 mg/kg and 5 mg/kg administered to the CSF. In certain such instances, the dose is between 0.5 mg/kg and 2 mg/kg. In certain instances, at least one dose is administered by bolus injection. In certain such instances, the dose is administered by bolus intrathecal injection. In certain instances, at least one second dose is administered. In certain such instances, the second dose is administered at least 2 weeks after the first dose. In certain instances, the second dose is administered at least 4 weeks after the first dose. In certain instances, the second dose is administered at least 8 weeks after the first dose. In certain instances, the second dose is administered at least 12 weeks after the first dose. In certain instances, the second dose is administered at least 16 weeks after the first dose. In certain instances, the second dose is administered at least 20 weeks after the first dose. In certain instances, the subject is under 2 years old at the time of the first dose. In certain instances, the subject is between 2 and 15 years old. In certain instances, the subject is between 15 and 30 years old. In certain instances, the subject is older than 30 years old. In certain instances, at least one symptom associated with SMA is reduced its progression has slowed. In certain instances, the oligonucleotide is ISIS396443.

[0036] In certain instances, the disclosure provides methods of administering to a subject having at least one symptom associated with SMA, at least one dose of an antisense compound comprising an oligonucleotide consisting of 15 to 20 linked nucleosides and having a nucleobase sequence comprising which is 100% complementary to SEQ ID NO. 7 over its entire length, and wherein each nucleoside is a 2'-MOE modified nucleoside; and wherein at least one dose is administered systemically. In certain such instances, at least one dose is administered by bolus injection. In certain such instances, the dose is administered by bolus subcutaneous injection. In certain, the dose administered is between 0.5mg/kg and 50mg/kg. In certain instances, the dose is between 1 mg/kg and 10mg/kg. In certain instances, the dose

is between 1 mg/kg and 5 mg/kg. In certain instances, the dose is between 0.5mg/kg and 1mg/kg. In certain instances, at least one second dose is administered. In certain such instances, the second dose is administered at least 2 weeks after the first dose. In certain instances, the second dose is administered at least 4 weeks after the first dose. In certain instances, the second dose is administered at least 8 weeks after the first dose. In certain instances, the second dose is administered at least 12 weeks after the first dose. In certain instances, the second dose is administered at least 16 weeks after the first dose. In certain instances, the second dose is administered at least 20 weeks after the first dose. In certain instances, the subject is under 2 years old at the time of the first dose. In certain instances, the subject is between 2 and 15 years old. In certain instances, the subject is between 15 and 30 years old. In certain instances, the subject is older than 30 years old. In certain instances, at least one symptom associated with SMA is reduced its progression has slowed. In certain instances, the oligonucleotide is ISIS396443.

[0037] In certain instances, the disclosure provides methods of administering to a subject having at least one symptom associated with SMA, at least one dose to the CSF and at least one systemic dose of an antisense compound comprising an oligonucleotide consisting of 15 to 20 linked nucleosides and having a nucleobase sequence which is 100% complementary to SEQ ID NO. 7 over its entire length, and wherein each nucleoside is a 2'-MOE modified nucleoside. In certain such instances, the CSF dose is between 0.1 mg/kg and 5 mg/kg. In certain instances, the systemic dose is between 0.5mg/kg and 50mg/kg. In certain instances, at least one CSF dose is administered by bolus injection. In certain such instances, at least one CSF dose is administered by bolus intrathecal injection. In certain instances, at least one systemic dose is administered by bolus injection. In certain such instances, at least one systemic dose is administered by subcutaneous injection. In certain instances, the CSF dose and the systemic dose are administered at the same time. In certain instances, the CSF dose and the systemic dose are administered at different times. In certain instances, the subject is under 2 years old at the time of the first dose. In certain instances, the subject is between 2 and 15 years old. In certain instances, the subject is between 15 and 30 years old. In certain instances, the subject is older than 30 years old. In certain instances, at least one symptom associated with SMA is reduced its progression has slowed. In certain instances, the oligonucleotide is ISIS396443.

[0038] In certain instances, the disclosure provides methods of administering to a subject having at least one symptom associated with SMA, at least one systemic dose of an antisense compound comprising an oligonucleotide consisting of 15 to 20 linked nucleosides and having a nucleobase sequence which is 100% complementary to SEQ ID NO. 7 over its entire length, and wherein each nucleoside is a 2'-MOE modified nucleoside; and at least one dose of a gene therapy agent. In certain instances, the systemic dose is between 0.5mg/kg and 50mg/kg. In certain instances, at least one systemic dose is administered by bolus injection. In certain such instances, at least one systemic dose is administered by subcutaneous injection. In certain instances, the systemic dose and the gene therapy agent are administered at the same time. In certain instances, the systemic dose and the gene therapy agent are administered at different times. In certain instances, the gene therapy agent is administered to the CSF. In certain such instances, the gene therapy agent is administered by intrathecal injection and/or infusion. In certain such instances, the gene therapy agent is administered by intracerebroventricular injection and/or infusion. In certain instances, the subject is under 2 years old at the time of the first dose. In certain instances, the subject is between 2 and 15 years old. In certain instances, the subject is between 15 and 30 years old. In certain instances, the subject is older than 30 years old. In certain instances, at least one symptom associated with SMA is reduced or its progression has slowed. In certain instances, the oligonucleotide is ISIS396443.

[0039] In certain instances, the disclosure provides methods of selecting a subject having at least one symptom associated with SMA and administering an antisense compound according to any of the methods above. In certain such instances, at least one symptom of SMA is assessed after administration. In certain such instances, at least one symptom of SMA is improved. In certain such instances, at least one symptom of SMA does not progress or progresses more slowly compared to a subject who has not received administration of antisense compound.

[0040] In certain instances, the disclosure provides an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2, for use in any of the above methods. In certain instances, the disclosure provides such a compound for use in treating a disease or condition associated with survival motor neuron 1 (SMN1).

[0041] In certain instances, the disclosure provides use of an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 in the manufacture of a medicament for use in any of the above methods. In certain instances, the medicament is for treating a disease or condition associated with survival motor neuron 1 (SMN1).

BRIEF DESCRIPTION OF THE FIGURES

[0042]

Figure 1 shows results from a duration of action study discussed in Example 4, in which the percent of SMN2 that includes exon 7 (y-axis) was assessed at 0, 2, 4, 6, and 8 weeks after termination of 7 days of treatment (x-axis).

The week "0" sample was taken 1 day after termination of treatment. CON represents saline treated mice. There was no difference in % inclusion among control saline treated mice at different time points from 0 to 6 months.

Figure 2 shows results from a duration of action study discussed in Example 4, in which the percent of SMN2 that includes exon 7 was assessed at 0, 0.5, 1, 2, 5, and 6 months after termination of 7 days of treatment. The month "0" sample was taken 1 day after termination of treatment. CON represents saline treated mice. There was no difference in % inclusion among control saline treated mice at different time points from 0 to 6 months.

Figure 3 shows the results from an experiments discussed in Example 6 measuring the effect of embryonic administration of ISIS396443 on tail-length in Taiwan strain of SMA mice. Figure 3A shows the first such experiment and Figure 3B shows a repeated experiment testing a different concentration of antisense compound, as noted and including data for normal mice for comparison.

Figure 4 shows results from western blots discussed in Example 7. The Y axis is the percent of SMN in the various samples that includes exon 7.

Figures 5 and 6 show results from experiments discussed in Example 7. A number of assessments of SMA mice (Taiwan strain) were performed following treatment with either an antisense compound or with a control oligonucleotide.

Figure 7 shows a survival curve from an experiment discussed in Example 7.

Figure 8 shows results from an assessment of the number of motor neurons in different portions of the spinal cord following treatment with an antisense compound or with a control oligonucleotide, as discussed in Example 7.

Figure 9 shows results from an assessment of full SMN RNA (including exon 7) in animals treated with antisense as discussed in Example 7.

Figure 10 shows a survival curve from an experiment discussed in Example 7 in which animals were either (1) untreated; (2) given a single dose of antisense compound at birth (Day P0); or (3) given a first dose at P0 and a second dose at day 21 (P21).

Figure 11 shows a survival curve from an experiment described in Example 7 comparing animals that received the second dose with animals that received only the first dose.

Figure 12 shows results from an experiment discussed in Example 9 in which antisense compound was administered to monkeys by intrathecal infusion and concentration of the compound was assessed in different tissues 96 hours later.

Figure 13 shows a survival curve for experiments discussed in Example 12, in which different doses of antisense compound were administered to severe SMA mice by subcutaneous injection.

DETAILED DESCRIPTION OF THE INVENTION

[0043] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

[0044] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. Definitions

[0045] Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis,

and chemical analysis. Certain such techniques and procedures may be found for example in "Carbohydrate Modifications in Antisense Research" Edited by Sangvi and Cook, American Chemical Society , Washington D.C., 1994; "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., 18th edition, 1990; and "Antisense Drug Technology, Principles, Strategies, and Applications" Edited by Stanley T. Crooke, CRC Press, Boca Raton, Florida; and Sambrook et al., "Molecular Cloning, A laboratory Manual," 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

[0046] Unless otherwise indicated, the following terms have the following meanings:

"Nucleoside" means a compound comprising a heterocyclic base moiety and a sugar moiety. Nucleosides include, but are not limited to, naturally occurring nucleosides, modified nucleosides, and nucleosides having mimetic bases and/or sugar groups. Nucleosides may be modified with any of a variety of substituents.

[0047] "Sugar moiety" means a natural or modified sugar or sugar surrogate.

[0048] "Natural sugar" means a ribofuranose moiety of DNA (2'-H) or RNA (2'-OH).

[0049] "Modified sugar" means a ribofuranose moiety comprising at least one substituent other than that of a natural sugar.

[0050] "Sugar surrogate" means a structure other than a ribofuranose ring which is capable of substituting for the sugar of a nucleoside. Examples of sugar surrogates include, but are not limited to, open ring systems, 6-membered rings, sugars in which the oxygen is replace with, for example, sulfur or nitrogen. For example, sugar surrogates include, but are not limited to morpholinos and 4'-thio-containing sugars.

[0051] "Nucleobase" means the heterocyclic base portion of a nucleoside. Nucleobases may be naturally occurring or may be modified. In certain instances, a nucleobase may comprise any atom or group of atoms capable of hydrogen bonding to a nucleobase of another nucleic acid.

[0052] "Nucleotide" means a nucleoside comprising a phosphate linking group. As used herein, nucleosides include nucleotides.

[0053] "Modified nucleoside" a nucleoside comprising at least one modification compared to naturally occurring RNA or DNA nucleosides. Such modification may be at the sugar moiety and/or at the nucleobase.

[0054] "Bicyclic nucleoside" or "BNA" means a nucleoside wherein the sugar moiety of the nucleoside comprises a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic sugar moiety.

[0055] "4'-2' bicyclic nucleoside" means a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

[0056] "2'-modified" or "2'-substituted" means a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH.

[0057] "2'-OMe" or "2'-OCH_3" or "2'-O-methyl" each means a nucleoside comprising a sugar comprising an -OCH_3 group at the 2' position of the sugar ring.

[0058] "MOE" or "2'-MOE" or "2'-$OCH_2CH_2OCH_3$" or "2'-O-methoxyethyl" each means a nucleoside comprising a sugar comprising a -OCH_2CH_2OCH3 group at the 2' position of the sugar ring.

[0059] "Oligonucleotide" means a compound comprising a plurality of linked nucleosides. In certain instances, one or more of the plurality of nucleosides is modified. In certain instances, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

[0060] "Oligonucleoside" means an oligonucleotide in which none of the internucleoside linkages contains a phosphorus atom. As used herein, oligonucleotides include oligonucleosides.

[0061] "Modified oligonucleotide" means an oligonucleotide comprising at least one modified nucleoside and/or at least one modified internucleoside linkage.

[0062] "Internucleoside linkage" means a covalent linkage between adjacent nucleosides of an oligonucleotide.

[0063] "Naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage.

[0064] "Modified internucleoside linkage" means any internucleoside linkage other than a naturally occurring internucleoside linkage.

[0065] "Oligomeric compound" means a compound comprising an oligonucleotide. In certain instances, an oligomeric compound consists of an oligonucleotide. In certain instances, an oligomeric compound further comprises one or more conjugate and/or terminal groups.

[0066] "Antisense compound" means an oligomeric compound, at least a portion of which is at least partially complementary to a target nucleic acid to which it hybridizes, wherein such hybridization results at least one antisense activity.

[0067] "Antisense oligonucleotide" means an antisense compound wherein the oligomeric compound consists of an oligonucleotide.

[0068] "Antisense activity" refers to any detectable and/or measurable effect attributable to the hybridization of an antisense compound to its target nucleic acid. In certain instances, such antisense activity is an increase or decrease in an amount of a nucleic acid or protein. In certain instances, such antisense activity is a change in the ratio of splice variants of a nucleic acid or protein. In certain instances, such antisense activity is a phenotypic change in a cell and/or subject.

[0069] "Detecting" or "measuring" of antisense activity may be direct or indirect. For example, in certain instances,

antisense activity is assessed by detecting and/or measuring the amount of target nucleic acid or protein or the relative amounts of splice variants of a target nucleic acid or protein. In certain instances, antisense activity is detected by observing a phenotypic change in a cell or animal. In connection with any activity, response, or effect, the terms "detecting" and "measuring," indicate that a test for detecting or measuring is performed. Such detection and/or measuring may include values of zero. Thus, if a test for detection or measuring results in a finding of no activity (activity of zero), the step of detecting or measuring the activity has nevertheless been performed.

[0070] "Target nucleic acid" refers to any nucleic acid molecule the expression, amount, or activity of which is capable of being modulated by an antisense compound.

[0071] "Target mRNA" means a pre-selected RNA molecule that encodes a protein.

[0072] "Target pre-mRNA" means a pre-selected RNA transcript that has not been fully processed into mRNA. Notably, pre-mRNA includes one or more intron.

[0073] "Target protein" means a protein encoded by a target nucleic acid.

[0074] "Modulation" means to a perturbation of function or activity. In certain instances, modulation means an increase in gene expression. In certain instances, modulation means a decrease in gene expression.

[0075] "Expression" means any functions and steps by which a gene's coded information is converted into structures present and operating in a cell.

[0076] "Nucleobase sequence" means the order of contiguous nucleobases, in a 5' to 3' orientation, independent of any sugar, linkage, and/or nucleobase modification.

[0077] "Contiguous nucleobases" means nucleobases immediately adjacent to each other in a nucleic acid.

[0078] "Nucleobase complementarity" means the ability of two nucleobases to pair non-covalently via hydrogen bonding.

[0079] "Complementary" means that a first nucleic acid is capable of hybridizing to a second nucleic acid under stringent hybridization conditions. For example, an antisense compound is complementary to its target nucleic acid if it is capable of hybridizing to the target nucleic acid under stringent hybridization conditions.

[0080] "Fully complementary" means each nucleobase of a first nucleic acid is capable of pairing with a nucleobase at each corresponding contiguous position in a second nucleic acid.

[0081] "Percent complementarity" of an antisense compound means the percentage of nucleobases of the antisense compound that are complementary to an equal-length portion of a target nucleic acid. Percent complementarity is calculated by dividing the number of nucleobases of the antisense oligonucleotide that are complementary to nucleobases at corresponding contiguous positions in the target nucleic acid by the total length of the antisense compound.

[0082] "Percent identity" means the number of nucleobases in first nucleic acid that are identical to nucleobases at corresponding positions in a second nucleic acid, divided by the total number of nucleobases in the first nucleic acid.

[0083] "Hybridize" means the annealing of complementary nucleic acids that occurs through nucleobase complementarity.

[0084] "Mismatch" means a nucleobase of a first nucleic acid that is not capable of pairing with a nucleobase at a corresponding position of a second nucleic acid.

[0085] "Identical nucleobase sequence" means having the same nucleobase sequence, independent of any chemical modifications to the nucleosides.

[0086] "Different modifications" or "differently modified" refer to nucleosides or internucleoside linkages that have different nucleoside modifications or internucleoside linkages than one another, including absence of modifications. Thus, for example, a MOE nucleoside and an unmodified DNA nucleoside are "differently modified," even though the DNA nucleoside is unmodified. Likewise, DNA and RNA are "differently modified," even though both are naturally-occurring unmodified nucleosides. Nucleosides that are the same but for comprising different nucleobases are not differently modified, unless otherwise indicated. For example, a nucleoside comprising a 2'-OMe modified sugar and an adenine nucleobase and a nucleoside comprising a 2'-OMe modified sugar and a thymine nucleobase are not differently modified.

[0087] "The same modifications" refer to nucleosides and internucleoside linkages (including unmodified nucleosides and internucleoside linkages) that are the same as one another. Thus, for example, two unmodified DNA nucleoside have "the same modification," even though the DNA nucleoside is unmodified.

[0088] "Type of modification" or nucleoside of a "type" means the modification of a nucleoside and includes modified and unmodified nucleosides. Accordingly, unless otherwise indicated, a "nucleoside having a modification of a first type" may be an unmodified nucleoside.

[0089] "Separate regions" of an oligonucleotide means a portion of an oligonucleotide wherein the nucleosides and internucleoside linkages within the region all comprise the same modifications; and the nucleosides and/or the internucleoside linkages of any neighboring portions include at least one different modification.

[0090] "Motif' means a pattern of modified and/or unmodified nucleobases, sugars, and/or internucleoside linkages in an oligonucleotide.

[0091] "Fully modified oligonucleotide" means each nucleobase, each sugar, and/or each internucleoside linkage is

modified.

[0092] "Uniformly modified oligonucleotide" means each nucleobase, each sugar, and/or each internucleoside linkage has the same modification throughout the modified oligonucleotide.

[0093] "Alternating motif' means an oligonucleotide or a portion thereof, having at least four separate regions of modified nucleosides in a pattern $(AB)_nA_m$ where A represents a region of nucleosides having a first type of modification; B represent a region of nucleosides having a different type of modification; n is 2-15; and m is 0 or 1. Thus, in certain instances, alternating motifs include 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more alternating regions. In certain instances, each A region and each B region independently comprises 1-4 nucleosides.

[0094] "Subject" means a human or non-human animal selected for treatment or therapy.

[0095] "Subject in need thereof' means a subject identified as in need of a therapy or treatment. In such instances, a subject has one or more indications of having or developing SMA.

[0096] "Administering" means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

[0097] "Parenteral administration," means administration through injection or infusion. Parenteral administration includes, but is not limited to, subcutaneous administration, intravenous administration, or intramuscular administration.

[0098] "Systemic administration" means administration to an area other than the intended locus of activity. Examples or systemic administration are subcutaneous administration and intravenous administration, and intraperitoneal administration.

[0099] "Subcutaneous administration" means administration just below the skin.

[0100] "Intravenous administration" means administration into a vein.

[0101] "Cerebrospinal fluid" or "CSF" means the fluid filling the space around the brain and spinal cord.

[0102] "Administration into the cerebrospinal fluid" means any administration that delivers a substance directly into the CSF.

[0103] "Intracerebroventricular" or "ICV" mean administration into the ventricular system of the brain.

[0104] "Intrathecal" or "IT" means administration into the CSF under the arachnoid membrane which covers the brain and spinal cord. IT injection is performed through the theca of the spinal cord into the subarachnoid space, where a pharmaceutical agent is injected into the sheath surrounding the spinal cord.

[0105] "Induction phase" means a dosing phase during which administration is initiated and steady state concentrations of active pharmaceutical agent are achieved in a target tissue. For example, an induction phase is a dosing phase during which steady state concentrations of antisense oligonucleotide are achieved in liver.

[0106] "Maintenance phase" means a dosing phase after target tissue steady state concentrations of drug have been achieved.

[0107] "Duration" means the period of time during which an activity or event continues. For example, the duration of an induction phase is the period of time during which induction doses are administered.

[0108] "Maintenance dose" means a dose administered at a single administration during the maintenance phase. As used herein, "induction dose" means a dose administered at a single administration during the induction phase.

[0109] "Co-administration" means administration of two or more pharmaceutical agents to a subject. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents may be administered through the same or different routes of administration. Co-administration encompasses administration in parallel or sequentially.

[0110] "Therapy" means a disease treatment method. In certain instances, therapy includes, but is not limited to surgical therapies, chemical therapies, and physical interventions, such as assisted respiration, feeding tubes, and physical therapy for the purpose of increasing strength.

[0111] "Treatment" means the application of one or more specific procedures used for the cure or amelioration of a disease. In certain instances, the specific procedure is the administration of one or more pharmaceutical agents.

[0112] "Amelioration" means a lessening of severity of at least one indicator of a condition or disease. In certain instances, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures which are known to those skilled in the art.

[0113] "Prevent the onset of' means to prevent the development a condition or disease in a subject who is at risk for developing the disease or condition. In certain instances, a subject at risk for developing the disease or condition receives treatment similar to the treatment received by a subject who already has the disease or condition.

[0114] "Delay the onset of' means to delay the development of a condition or disease in a subject who is at risk for developing the disease or condition.

[0115] "Slow the progression of' means that the severity of at least one symptom associated with a disease or condition worsens less quickly.

[0116] "Exon 7 amino acids" means the portion of an SMN protein that correspond to exon 7 of the SMN RNA. Exon 7 amino acids are present in SMN protein expressed from SMN RNA where exon 7 was not excluded during splicing.

[0117] "SMN protein" means normal full length survival motor neuron protein. SMN may be expressed from either an SMN1 gene or from an SMN2 gene, provided that exon 7 is present in the mature mRNA and the exon 7 amino acids are present in the SMN protein.

[0118] "Dose" means a specified quantity of a pharmaceutical agent provided in a single administration or over a specified amount of time. In certain instances, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain instances, where subcutaneous or intrathecal or ICV administration is desired, the desired dose requires a volume not easily accommodated by a single injection. In such instances, two or more injections may be used to achieve the desired dose. In the setting of continuous infusion, dose may be expressed as the quantity of a pharmaceutical agent delivered per unit of time.

[0119] "Dosage unit" means a form in which a pharmaceutical agent is provided. In certain instances, a dosage unit is a vial containing lyophilized oligonucleotide. In certain instances a dosage unit is a vial containing reconstituted oligonucleotide.

[0120] "Therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to an animal.

[0121] "Pharmaceutical composition" means a mixture of substances suitable for administering to an individual that includes a pharmaceutical agent. For example, a pharmaceutical composition may comprise a modified oligonucleotide and a sterile aqueous solution.

[0122] "Acceptable safety profile" means a pattern of side effects that is within clinically acceptable limits.

[0123] "Side effect" means a physiological response attributable to a treatment other than desired effects.

1. Certain Modified Oligonucleotides

[0124] In certain instances, the present disclosure provides methods and compositions involving antisense oligonucleotides comprising one or more modification compared to oligonucleotides of naturally occurring oligomers, such as DNA or RNA. Such modified antisense oligonucleotides may possess one or more desirable properties. Certain such modifications alter the antisense activity of the antisense oligonucleotide, for example by increasing affinity of the antisense oligonucleotide for its target nucleic acid, increasing its resistance to one or more nucleases, and/or altering the pharmacokinetics or tissue distribution of the oligonucleotide. In certain instances, such modified antisense oligonucleotides comprise one or more modified nucleosides and/or one or more modified nucleoside linkages and/or one or more conjugate groups.

a. Certain Modified Nucleosides

[0125] In certain instances, antisense oligonucleotides comprise one or more modified nucleosides. Such modified nucleosides may include a modified sugar and/or a modified nucleobase. In certain instances, incorporation of such modified nucleosides in an oligonucleotide results in increased affinity for a target nucleic acid and/or increased stability, including but not limited to, increased resistance to nuclease degradation, and or improved toxicity and/or uptake properties of the modified oligonucleotide.

i. Certain Nucleobases

[0126] The naturally occurring base portion of nucleosides are heterocyclic base, typically purines and pyrimidines. In addition to "unmodified" or "natural" nucleobases such as the purine nucleobases adenine (A) and guanine (G), and the pyrimidine nucleobases thymine (T), cytosine (C) and uracil (U), many modified nucleobases or nucleobase mimetics known to those skilled in the art are amenable to incorporation into the compounds described herein. In certain instances, a modified nucleobase is a nucleobase that is fairly similar in structure to the parent nucleobase, such as for example a 7-deaza purine, a 5-methyl cytosine, or a G-clamp. In certain instances, nucleobase mimetic include more complicated structures, such as for example a tricyclic phenoxazine nucleobase mimetic. Methods for preparation of the above noted modified nucleobases are well known to those skilled in the art.

ii. Certain Modified Sugars and Sugar Surrogates

[0127] Antisense oligonucleotides of the present disclosure can optionally contain one or more nucleosides wherein the sugar moiety is modified, compared to a natural sugar. Oligonucleotides comprising such sugar modified nucleosides may have enhanced nuclease stability, increased binding affinity or some other beneficial biological property. Such modifications include without limitation, addition of substituent groups, bridging of non-geminal ring atoms to form a bicyclic nucleic acid (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or $C(R_1)(R)_2$ (R = H, C_1-C_{12} alkyl or a protecting group) and combinations of these such as for example a 2'-F-5'-methyl substituted nucleoside (see PCT

International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

[0128] Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3 and 2'-$O(CH_2)_2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF3, $O(CH_2)_2SCH_3$, $O(CH2)_2$-O-N(Rm)(R_n), and O-CH_2-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

[0129] Examples of bicyclic nucleic acids (BNAs) include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain instances, antisense compounds provided herein include one or more BNA nucleosides wherein the bridge comprises one of the formulas: 4'-β-D-(CH_2)-O-2' (β-D-LNA); 4'-(CH_2)-S-2'; 4'-α-L-(CH_2)-O-2' (α-L-LNA); 4'-$(CH_2)_2$-O-2' (ENA); 4'-C$(CH_3)_2$-O-2' (see PCT/US2008/068922, published as WO 2009/006478); 4'-CH(CH_3)-O-2' and 4'-CH(CH_2OCH_3)-O-2' (see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-CH_2-N(OCH_3)-2' (see PCT/US2008/ 064591, published as WO 2008/150729); 4'-CH_2-O-N(CH_3)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2' (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-C(CH_3)-2' and 4'-CH_2-C(=CH_2)-2' (see PCT/US2008/ 066154, published as WO 2008/154401); and wherein R is, independently, H, C_1-C_{12} alkyl, or a protecting group.

[0130] In certain instances, the present disclosure provides modified nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. Certain such modified nucleosides are known. In certain instances, the sugar ring of a nucleoside may be modified at any position. Examples of sugar modifications useful in this disclosure include, but are not limited to compounds comprising a sugar substituent group selected from: OH, F, O-alkyl, S-alkyl, N-alkyl, or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. In certain such instances, such substituents are at the 2' position of the sugar.

[0131] In certain instances, modified nucleosides comprise a substituent at the 2' position of the sugar. In certain instances, such substituents are selected from among: a halide (including, but not limited to F), allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, -OCF_3, O-$(CH_2)_2$-O-CH_3, 2'-$O(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-N(R_m)(R_n), or O-CH2-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

[0132] In certain instances, modified nucleosides suitable for use in the present disclosure are: 2-methoxyethoxy, 2'-O-methyl (2'-O-CH_3), 2'-fluoro (2'-F).

[0133] In certain instances, modified nucleosides having a substituent group at the 2'-position selected from: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'-sugar substituent groups include: C_1 to C_{10} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an oligomeric compound, and other substituents having similar properties.

[0134] In certain instances, modifed nucleosides comprise a 2'-MOE side chain (Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- O-methyl, O-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, P., Helv. Chim. Acta, 1995, 78, 486-504; Altmann et al., Chimia, 1996, 50, 168-176; Altmann et al., Biochem. Soc. Trans., 1996, 24, 630-637; and Altmann et al., Nucleosides Nucleotides, 1997, 16, 917-926).

[0135] In certain instances, 2'-sugar substituent groups are in either the arabino (up) position or ribo (down) position. In certain such instances, a 2'-arabino modification is 2'-F arabino (FANA). Similar modifications can also be made at other positions on the sugar, particularly the 3' position of the sugar on a 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide.

[0136] In certain instances, nucleosides suitable for use in the present disclosure have sugar surrogates such as cyclobutyl in place of the ribofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920.

[0137] In certain instances, the present disclosure provides nucleosides comprising a modification at the 2'-position of the sugar. In certain instances, the disclosure provides nucleosides comprising a modification at the 5'-positin of the sugar. In certain instances, the disclosure provides nucleosides comprising modifications at the 2'-position and the 5'-position of the sugar. In certain instances, modified nucleosides may be useful for incorporation into oligonucleotides. In certain instance, modified nucleosides are incorporated into oligonucleosides at the 5'-end of the oligonucleotide.

b. Certain Internucleoside Linkages

[0138] Antisense oligonucleotides of the present disclosure can optionally contain one or more modified internucleoside linkages. The two main classes of linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus containing linkages include, but are not limited to, phosphodiesters (P=O), phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates (P=S). Representative non-phosphorus containing linking groups include, but are not limited to, methylenemethylimino (-CH2-N(CH3)-O-CH2-), thiodiester (-O-C(O)-S-), thionocarbamate (-O-C(O)(NH)-S-); siloxane (-O-Si(H)2-O-); and N,N'-dimethylhydrazine (-CH2-N(CH3)-N(CH3)-). Oligonucleotides having non-phosphorus linking groups are referred to as oligonucleosides. Modified linkages, compared to natural phosphodiester linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotides. In certain instances, linkages having a chiral atom can be prepared as racemic mixtures, as separate enantiomers. Representative chiral linkages include, but are not limited to, alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known to those skilled in the art.

[0139] The antisense oligonucleotides described herein contain one or more asymmetric centers and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), such as for sugar anomers, or as (D) or (L) such as for amino acids et al. Included in the antisense compounds provided herein are all such possible isomers, as well as their racemic and optically pure forms.

[0140] In certain instances, antisense oligonucleotides have at least one modified internucleoside linkage. In certain instances, antisense oligonucleotides have at least 2 modified internucleoside linkages. In certain instances, antisense oligonucleotides have at least 3 modified internucleoside linkages. In certain instances, antisense oligonucleotides have at least 10 modified internucleoside linkages. In certain instances, each internucleoside linkage of an antisense oligonucleotide is a modified internucleoside linkage. In certain instances, such modified internucleoside linkages are phosphorothioate linkages.

c. Lengths

[0141] In certain instances, the present disclosure provides antisense oligonucleotides of any of a variety of ranges of lengths. In certain instances, the disclosure provides antisense compounds or antisense oligonucleotides comprising or consisting of X-Y linked nucleosides, where X and Y are each independently selected from 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 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, and 50; provided that X<Y. For example, in certain instances, the disclosure provides antisense compounds or antisense oligonucleotides comprising or consisting of: 8-9, 8-10, 8-11, 8-12, 8-13, 8-14, 8-15, 8-16, 8-17, 8-18, 8-19, 8-20, 8-21, 8-22, 8-23, 8-24, 8-25, 8-26, 8-27, 8-28, 8-29, 8-30, 9-10, 9-11, 9-12, 9-13, 9-14, 9-15, 9-16, 9-17, 9-18, 9-19, 9-20, 9-21, 9-22, 9-23, 9-24, 9-25, 9-26, 9-27, 9-28, 9-29, 9-30, 10-11, 10-12, 10-13, 10-14, 10-15, 10-16, 10-17, 10-18, 10-19, 10-20, 10-21, 10-22, 10-23,10-24,10-25,10-26,10-27,10-28,10-29,10-30, 11-12, 11-13, 11-14, 11-15, 11-16, 11-17, 11-18, 11-19, 11-20, 11-21, 11-22, 11-23, 11-24, 11-25, 11-26, 11-27, 11-28, 11-29, 11-30, 12-13, 12-14, 12-15, 12-16, 12-17, 12-18, 12-19, 12-20, 12-21, 12-22, 12-23, 12-24, 12-25, 12-26, 12-27, 12-28, 12-29, 12-30, 13-14, 13-15, 13-16, 13-17, 13-18, 13-19, 13-20, 13-21, 13-22, 13-23, 13-24, 13-25, 13-26, 13-27, 13-28, 13-29, 13-30, 14-15, 14-16, 14-17, 14-18, 14-19, 14-20, 14-21, 14-22, 14-23, 14-24, 14-25, 14-26, 14-27, 14-28, 14-29, 14-30, 15-16, 15-17, 15-18, 15-19, 15-20, 15-21, 15-22, 15-23, 15-24, 15-25, 15-26, 15-27, 15-28, 15-29, 15-30, 16-17, 16-18, 16-19, 16-20, 16-21, 16-22, 16-23, 16-24, 16-25, 16-26, 16-27, 16-28, 16-29, 16-30, 17-18, 17-19, 17-20, 17-21, 17-22, 17-23, 17-24, 17-25, 17-26, 17-27, 17-28, 17-29, 17-30, 18-19, 18-20, 18-21, 18-22, 18-23, 18-24, 18-25, 18-26, 18-27, 18-28, 18-29, 18-30, 19-20, 19-21, 19-22, 19-23, 19-24, 19-25, 19-26, 19-29, 19-28, 19-29, 19-30, 20-21, 20-22, 20-23, 20-24, 20-25, 20-26, 20-27, 20-28, 20-29, 20-30, 21-22, 21-23, 21-24, 21-25, 21-26, 21-27, 21-28, 21-29, 21-30, 22-23, 22-24, 22-25, 22-26, 22-27, 22-28, 22-29, 22-30, 23-24, 23-25, 23-26, 23-27, 23-28, 23-29, 23-30, 24-25, 24-26, 24-27, 24-28, 24-29, 24-30, 25-26, 25-27, 25-28, 25-29, 25-30, 26-27, 26-28, 26-29, 26-30, 27-28, 27-29, 27-30, 28-29, 28-30, or 29-30 linked nucleosides.

[0142] In certain instances, antisense compounds or antisense oligonucleotides of the present disclosure are 15 nucleosides in length. In certain instances, antisense compounds or antisense oligonucleotides of the present disclosure are 16 nucleosides in length. In certain instances, antisense compounds or antisense oligonucleotides of the present disclosure are 17 nucleosides in length. In certain instances, antisense compounds or antisense oligonucleotides of the present disclosure are 18 nucleosides in length. In certain instances, antisense compounds or antisense oligonucleotides of the present disclosure are 19 nucleosides in length. In certain instances, antisense compounds or antisense oligonucleotides of the present disclosure are 20 nucleosides in length.

d. Certain Oligonucleotide Motifs

[0143] In certain instances, antisense oligonucleotides have chemically modified subunits arranged in specific orientations along their length. In certain instances, antisense oligonucleotides of the disclosure are fully modified. In certain instances, antisense oligonucleotides of the disclosure are uniformly modified. In certain instances, antisense oligonucleotides of the disclosure are uniformly modified and each nucleoside comprises a 2'-MOE sugar moiety. In certain instances, antisense oligonucleotides of the disclosure are uniformly modified and each nucleoside comprises a 2'-OMe sugar moiety. In certain instances, antisense oligonucleotides of the disclosure are uniformly modified and each nucleoside comprises a morpholino sugar moiety.

[0144] In certain instances, oligonucleotides of the disclosure comprise an alternating motif. In certain such instances, the alternating modification types are selected from among 2'-MOE, 2'-F, a bicyclic sugar-modifed nucleoside, and DNA (unmodified 2'-deoxy). In certain such instances, each alternating region comprises a single nucleoside.

[0145] In certain instances, oligonucleotides of the disclosure comprise one or more block of nucleosides of a first type and one or more block of nucleosides of a second type.

[0146] In certan instances, one or more alternating regions in an alternating motif include more than a single nucleoside of a type. For example, oligomeric compounds of the present disclosure may include one or more regions of any of the following nucleoside motifs:

$Nu_1\ Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_1$;
$Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_2\ Nu_2$;
$Nu_1\ Nu_1\ Nu_2\ Nu_1\ Nu_1\ Nu_2$;
$Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_1\ Nu_2\ Nu_2$;
$Nu_1\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_1$;
$Nu_1\ Nu_1\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_2$;
$Nu_1\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_1$;
$Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_1$;
$Nu_2\ Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_2\ Nu_1Nu_2\ Nu_1\ Nu_1$; or
$Nu_1\ Nu_2Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_1\ Nu_2\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_2\ Nu_1\ Nu_1$;

wherein Nu_1 is a nucleoside of a first type and Nu_2 is a nucleoside of a second type. In certain instances, one of Nu_1 and Nu_2 is a 2'-MOE nucleoside and the other of Nu_1 and Nu_2 is a selected from: a 2'-OMe modified nucleoside, BNA, and an unmodifed DNA or RNA nucleoside.

2. Oligomeric Compounds

[0147] In certain instances, the present disclosure provides oligomeric compounds. In certain instances, oligomeric compounds are comprised only of an oligonucleotide. In certain instances , an oligomeric compound comprises an oligonucleotide and one or more conjugate and/or terminal group. Such conjugate and/or terminal groups may be added to oligonucleotides having any of the chemical motifs discussed above. Thus, for example, an oligomeric compound comprising an oligonucleotide having region of alternating nucleosides may comprise a terminal group.

a. Certain Conjugate Groups

[0148] In certain instances, oligonucleotides of the present disclosure are modified by attachment of one or more conjugate groups. In general, conjugate groups modify one or more properties of the attached oligomeric compound including but not limited to, pharmacodynamics, pharmacokinetics, stability, binding, absorption, cellular distribution, cellular uptake, charge and clearance. Conjugate groups are routinely used in the chemical arts and are linked directly or via an optional conjugate linking moiety or conjugate linking group to a parent compound such as an oligomeric compound, such as an oligonucleotide. Conjugate groups includes without limitation, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins and dyes. Certain conjugate groups have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., do-decan-diol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate

(Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

[0149] In certain instances, a conjugate group comprises an active drug substance, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fen-bufen, ketoprofen, (*S*)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic. Oligonucleotide-drug conjugates and their preparation are described in U.S. Patent Application 09/334,130, published as US 6,656,730.

[0150] Representative U.S. patents that teach the preparation of oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

[0151] Conjugate groups may be attached to either or both ends of an oligonucleotide (terminal conjugate groups) and/or at any internal position.

b. Terminal Groups

[0152] In certain instances, oligomeric compounds comprise terminal groups at one or both ends. In certain instances , a terminal group may comprise any of the conjugate groups discussed above. In certain instances, terminal groups may comprise additional nucleosides and/or inverted abasic nucleosides. In certain instances, a terminal group is a stabilizing group.

[0153] In certain instances, oligomeric compounds comprise one or more terminal stabilizing group that enhances properties such as for example nuclease stability. Included in stabilizing groups are cap structures. The terms "cap structure" or "terminal cap moiety," as used herein, refer to chemical modifications, which can be attached to one or both of the termini of an oligomeric compound. Certain such terminal modifications protect the oligomeric compounds having terminal nucleic acid moieties from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both termini. (for more details see Wincott et al., International PCT publication No. WO 97/26270; Beaucage and Tyer, 1993, Tetrahedron 49, 1925; U.S. Patent Application Publication No. US 2005/0020525; and WO 03/004602.

[0154] In certain instances, one or more additional nucleosides is added to one or both terminal ends of an oligonucleotide of an oligomeric compound. Such additional terminal nucleosides are referred to herein as terminal-group nucleosides. In a double-stranded compound, such terminal-group nucleosides are terminal (3' and/or 5') overhangs. In the setting of double-stranded antisense compounds, such terminal-group nucleosides may or may not be complementary to a target nucleic acid. In certain instances, the terminal group is a non-nucleoside terminal group. Such non-terminal groups may be any terminal group other than a nucleoside.

c. Oligomeric Compound Motifs

[0155] In certain instances, oligomeric compounds of the present disclosure comprise a motif: T_1-$(Nu_1)_{n1}$-$(Nu_2)_{n2}$-$(Nu_1)_{n3}$-$(Nu_2)_{n4}$-$(Nu_1)_{n5}$-T_2, wherein:

Nu_1, is a nucleoside of a first type;
Nu_2, is a nucleoside of a second type;
each of n1 and n5 is, independently from 0 to 3;
the sum of n2 plus n4 is between 10 and 25;
n3 is from 0 and 5; and
each $T_1$and T_2 is, independently, H, a hydroxyl protecting group, an optionally linked conjugate group or a capping group.

[0156] In certain such instances, the sum of n2 and n4 is 13 or 14; n1 is 2; n3 is 2 or 3; and n5 is 2. In certain such instances, oligomeric compounds of the present disclosure comprise a motif selected from Table A.

Table A				
n1	n2	n3	n4	n5
2	16	0	0	2
2	2	3	11	2
2	5	3	8	2
2	8	3	5	2
2	11	3	2	2
2	9	3	4	2
2	10	3	3	2
2	3	3	10	2
2	4	3	9	2
2	6	3	7	2
2	7	3	6	2
2	8	6	2	2
2	2	2	12	2
2	3	2	11	2
2	4	2	10	2
2	5	2	9	2
2	6	2	8	2
2	7	2	7	2
2	8	2	6	2
2	9	2	5	2
2	10	2	4	2
2	11	2	3	2
2	12	2	2	2

[0157] Table A is intended to illustrate, but not to limit the present disclosure. The oligomeric compounds depicted in Table A each comprise 20 nucleosides. Oligomeric compounds comprising more or fewer nucleosides can easily by designed by selecting different numbers of nucleosides for one or more of n1-n5. In certain instances, Nu_1 and Nu_2 are each selected from among: 2'-MOE, 2'-OMe, DNA, and a bicyclic nucleoside.

3. Antisense

[0158] In certain instances, oligomeric compounds of the present disclosure are antisense compounds. Accordingly, in such instances, oligomeric compounds hybridize with a target nucleic acid, resulting in an antisense activity.

a. Hybridization

[0159] In certain instances, the disclosure provides antisense compounds that specifically hybridize to a target nucleic acid when there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

[0160] Thus, "stringent hybridization conditions" or "stringent conditions" means conditions under which an antisense compounds hybridize to a target sequence, but to a minimal number of other sequences. Stringent conditions are

sequence-dependent and will be different in different circumstances, and "stringent conditions" under which antisense oligonucleotides hybridize to a target sequence are determined by the nature and composition of the antisense oligonucleotides and the assays in which they are being investigated.

[0161] It is understood in the art that incorporation of nucleotide affinity modifications may allow for a greater number of mismatches compared to an unmodified compound. Similarly, certain nucleobase sequences may be more tolerant to mismatches than other nucleobase sequences. One of ordinary skill in the art is capable of determining an appropriate number of mismatches between oligonucleotides, or between an antisense oligonucleotide and a target nucleic acid, such as by determining melting temperature (Tm). Tm or ΔTm can be calculated by techniques that are familiar to one of ordinary skill in the art. For example, techniques described in Freier et al. (Nucleic Acids Research, 1997, 25, 22: 4429-4443) allow one of ordinary skill in the art to evaluate nucleotide modifications for their ability to increase the melting temperature of an RNA:DNA duplex.

b. pre-mRNA Processing

[0162] In certain instances, antisense compounds provided herein are complementary to a pre-mRNA. In certain instances, such antisense compounds alter splicing of the pre-mRNA. In certain such instances , the ratio of one variant of a mature mRNA corresponding to a target pre-mRNA to another variant of that mature mRNA is altered. In certain such instances, the ratio of one variant of a protein expressed from the target pre-mRNA to another variant of the protein is altered. Certain oligomeric compounds and nucleobase sequences that may be used to alter splicing of a pre-mRNA may be found for example in US 6,210,892; US 5,627,274; US 5,665,593; 5,916,808; US 5,976,879; US2006/0172962; US2007/002390; US2005/0074801; US2007/0105807; US2005/0054836; WO 2007/090073; WO2007/047913, Hua et al., PLoS Biol 5(4):e73; Vickers et al., J. Immunol. 2006 Mar 15; 176(6):3652-61; and Hua et al., American J. of Human Genetics (April 2008) 82, 1-15. In certain instances antisense sequences that alter splicing are modified according to motifs of the present disclosure.

[0163] Antisense is an effective means for modulating the expression of one or more specific gene products and is uniquely useful in a number of therapeutic, diagnostic, and research applications. Provided herein are antisense compounds useful for modulating gene expression via antisense mechanisms of action, including antisense mechanisms based on target occupancy. In one aspect, the antisense compounds provided herein modulate splicing of a target gene. Such modulation includes promoting or inhibiting exon inclusion. Further provided herein are antisense compounds targeted to *cis* splicing regulatory elements present in pre-mRNA molecules, including exonic splicing enhancers, exonic splicing silencers, intronic splicing enhancers and intronic splicing silencers. Disruption of *cis* splicing regulatory elements is thought to alter splice site selection, which may lead to an alteration in the composition of splice products.

[0164] Processing of eukaryotic pre-mRNAs is a complex process that requires a multitude of signals and protein factors to achieve appropriate mRNA splicing. Exon definition by the spliceosome requires more than the canonical splicing signals which define intron-exon boundaries. One such additional signal is provided by *cis*-acting regulatory enhancer and silencer sequences. Exonic splicing enhancers (ESE), exonic splicing silencers (ESS), intronic splicing enhancers (ISE) and intron splicing silencers (ISS) have been identified which either repress or enhance usage of splice donor sites or splice acceptor sites, depending on their site and mode of action (Yeo et al. 2004, Proc. Natl. Acad. Sci. U.S.A. 101(44):15700-15705). Binding of specific proteins (*trans* factors) to these regulatory sequences directs the splicing process, either promoting or inhibiting usage of particular splice sites and thus modulating the ratio of splicing products (Scamborova et al. 2004, Mol. Cell. Biol. 24(5):1855-1869; Hovhannisyan and Carstens, 2005, Mol. Cell. Biol. 25(1):250-263; Minovitsky et al. 2005, Nucleic Acids Res. 33(2):714-724).

4. Pharmaceutical Compositions

[0165] In certain instances, the present disclosure provides pharmaceutical compositions comprising one or more antisense compound. In certain instances, such pharmaceutical composition comprises a sterile saline solution and one or more antisense compound. In certain instances, such pharmaceutical composition consists of a sterile saline solution and one or more antisense compound.

[0166] In certain instances, antisense compounds may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[0167] In certain instances antisense compounds, can be utilized in pharmaceutical compositions by combining such oligomeric compounds with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in certain instances, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound and a pharmaceutically acceptable diluent. In certain instances, the pharmaceutically

acceptable diluent is PBS.

[0168] Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters. In certain instances, pharmaceutical compositions comprising antisense compounds comprise one or more oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

[0169] A prodrug can include the incorporation of additional nucleosides at one or both ends of an oligomeric compound which are cleaved by endogenous nucleases within the body, to form the active antisense oligomeric compound.

[0170] Lipid-based vectors have been used in nucleic acid therapies in a variety of methods. For example, in one method, the nucleic acid is introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In another method, DNA complexes with mono- or poly-cationic lipids are formed without the presence of a neutral lipid.

[0171] Certain preparations are described in Akinc et al., Nature Biotechnology 26, 561 - 569 (01 May 2008).

5. Administration to a Subject

[0172] In certain instances, pharmaceutical compositions comprising one or more antisense compound are administered to a subject. In certain instances, such pharmaceutical compositions are administered by injection. In certain instances, such pharmaceutical compositions are administered by infusion.

[0173] In certain instances, pharmaceutical compositions are administered by injection or infusion into the CSF. In certain such instances, pharmaceutical compositions are administered by direct injection or infusion into the spine. In certain instances, pharmaceutical compositions are administered by injection or infusion into the brain. In certain instances, pharmaceutical compositions are administered by intrathecal injection or infusion rather than into the spinal cord tissue itself. Without being limited as to theory, in certain instances, the antisense compound released into the surrounding CSF and may penetrate into the spinal cord parenchyma. An additional advantage of intrathecal delivery is that the intrathecal route mimics lumbar puncture administration (i.e., spinal tap) already in routine use in humans.

[0174] In certain instances, pharmaceutical compositions are administered by intracerebroventricular (ICV) injection or infusion. Intracerebroventricular, or intraventricular, delivery of a pharmaceutical composition comprising one or more antisense compounds may be performed in any one or more of the brain's ventricles, which are filled with cerebrospinal fluid (CSF). CSF is a clear fluid that fills the ventricles, is present in the subarachnoid space, and surrounds the brain and spinal cord. CSF is produced by the choroid plexuses and via the weeping or transmission of tissue fluid by the brain into the ventricles. The choroid plexus is a structure lining the floor of the lateral ventricle and the roof of the third and fourth ventricles. Certain studies have indicated that these structures are capable of producing 400-600 ccs of fluid per day consistent with an amount to fill the central nervous system spaces four times in a day. In adult humans, the volume of this fluid has been calculated to be from 125 to 150 ml (4-5 oz). The CSF is in continuous formation, circulation and absorption. Certain studies have indicated that approximately 430 to 450 ml (nearly 2 cups) of CSF may be produced every day. Certain calculations estimate that production equals approximately 0.35 ml per minute in adults and 0.15 per minute in infant humans. The choroid plexuses of the lateral ventricles produce the majority of CSF. It flows through the foramina of Monro into the third ventricle where it is added to by production from the third ventricle and continues down through the aqueduct of Sylvius to the fourth ventricle. The fourth ventricle adds more CSF; the fluid then travels into the subarachnoid space through the foramina of Magendie and Luschka. It then circulates throughout the base of the brain, down around the spinal cord and upward over the cerebral hemispheres. The CSF empties into the blood via the arachnoid villi and intracranial vascular sinuses.

[0175] In certain instances, such pharmaceutical compositions are administered systemically. In certain e instances, pharmaceutical compositions are administered subcutaneously. In certain instances pharmaceutical compositions are administered intravenously. In certain instances, pharmaceutical compositions are administered by intramuscular injection.

[0176] In certain instances, pharmaceutical compositions are administered both directly to the CSF (e.g., IT and/or ICV injection and/or infusion) and systemically.

[0177] In certain instances, an antisense compound administered systemically enters neurons. In certain instances, systemically administered antisense compounds may penetrate the blood-brain barrier, particularly in young subjects where the blood-brain barrier is not fully formed (e.g., in subjects in utero and/or in newborn subjects). In certain instances, some amount of systemically administered antisense compound may be taken up by nerve cells, even in subjects in which the blood-brain barrier is fully formed. For example, antisense compounds may enter a neuron at or near the neuromuscular junction (retrograde uptake). In certain instances, such retrograde uptake results in antisense activity inside the neuron, including, but not limited to, a motor neuron, and provides a therapeutic benefit by antisense activity

inside the neuron.

[0178] In certain instances, systemic administration provides therapeutic benefit by antisense activity occurring in cells and/or tissues other than neurons. While evidence suggests that functional SMN inside neurons is required for normal neuron function, the consequence of reduced functional SMN in other cells and tissues is not well characterized. In certain instances, antisense activity in non-neuronal cells results in restoration of SMN function in those non-neuronal cells, which in turn results in therapeutic benefit.

[0179] In certain instances, improved SMN function in non-neuronal cells provides improved neuronal cell function, whether or not SMN function inside neurons is improved. For example, in certain instances, systemic administration of pharmaceutical compositions of the present disclosure results in antisense activity in muscle cells. Such antisense activity in muscle cells may provide a benefit to the motor-neurons associated with that muscle cell or to neurons generally. In such instances, the muscle cell having restored SMN function may provide a factor that improves neuronal viability and/or function. In certain instances, such antisense activity is independent of benefit from antisense activity occurring from antisense compounds inside neurons. In certain instances, systemic administration of pharmaceutical compositions of the present disclosure results in antisense activity in other non-neuronal cells, including cells not in immediate association with neurons. Such antisense activity in non-neuronal cells may improve function of neurons. For example, antisense activity in a non-neuronal cell (e.g., liver cell) may result in that cell producing a factor that improves function of neurons. Note: since the term "antisense activity" includes direct and indirect activities, a benefit to neuronal function is an "antisense activity" even if no antisense compound enters the neuron.

[0180] In certain instances, systemic administration of a pharmaceutical composition results in therapeutic benefit independent of direct or indirect antisense activities in neurons. Typically, in the setting of SMA, neuronal function is diminished, resulting in significant symptoms. Additional symptoms may result from diminished SMN activity in other cells. Certain such symptoms may be masked by the relative severity of symptoms from diminished neuronal function. In certain instances, systemic administration results in restored or improved SMN function in non-neuronal cells. In certain such instances, such restored or improved SMN function in non-neuronal cells has therapeutic benefit. For example, in certain instances, subjects having SMA have reduced growth. Such reduced growth may not result from diminished function in neuronal cells. Indeed, reduced growth may be related to impaired function of cells in another organ, such as the pituitary gland, and/or may be the result of SMN deficiencies throughout the cells of the body. In such instances, systemic administration may result in improved SMN activity in pituitary cells and/or other cells, resulting in improved growth. In certain instances, administration to the CSF restores sufficient neuronal function to allow a subject to live longer, however one or more symptoms previously unknown because subjects typically died before such symptoms appeared emerges, because the subject lives longer. Certain such emergent symptoms may be lethal. In certain instances, emergent symptoms are treated by systemic administration. Regardless of mechanism, in certain instances, a variety of symptoms of SMA, including, but not limited to symptoms previously masked by more severe symptoms associated with impaired neuronal function, may be treated by systemic administration.

[0181] In certain instances, systemic administration of pharmaceutical compositions of the present disclosure result in increased SMN activity in muscle cells. In certain instances, such improved SMN activity in muscle cells provides therapeutic benefit. Improved SMN activity in muscle alone has been reported to be insufficient to provide therapeutic benefit (e.g., Gravrilina, et al., Hum Mol Genet 2008 17(8):1063-1075). In certain instances, the present disclosure provides methods that result improve SMN function in muscle and do provide therapeutic benefit. In certain instances, therapeutic benefit may be attributable to improved SMN function in other cells (alone or in combination with muscle cells). In certain instances, improved SMN function in muscle alone may provide benefit.

[0182] In certain instances, systemic administration results in improved survival.

6. Spinal Muscular Atrophy (SMA)

[0183] SMA is a genetic disorder characterized by degeneration of spinal motor neurons. SMA is caused by the homozygous loss of both functional copies of the SMN1 gene. However, the SMN2 gene has the potential to code for the same protein as SMN1 and thus overcome the genetic defect of SMA patients. SMN2 contains a translationally silent mutation (C→T) at position +6 of exon 7, which results in inefficient inclusion of exon 7 in SMN2 transcripts. Therefore, the predominant form of SMN2, one which lacks exon 7, is unstable and inactive. Thus, therapeutic compounds capable of modulating SMN2 splicing such that the percentage of SMN2 transcripts containing exon 7 is increased, would be useful for the treatment of SMA.

[0184] In certain instances, the present disclosure provides antisense compounds complementary to a pre-mRNA encoding SMN2. In certain such instances, the antisense compound alters splicing of SMN2. Certain sequences and regions useful for altering splicing of SMN2 may be found in PCT/US06/024469, published as WO 2007/002390. In certain instances, oligomeric compounds having any motif described herein have a nucleobase sequence complementary to intron 7 of SMN2. Certain such nucleobase sequences are exemplified in the non-limiting table below.

Sequence	Length	SEQ ID
TGCTGGCAGACTTAC	15	3
CATAATGCTGGCAGA	15	4
TCATAATGCTGGCAG	15	5
TTCATAATGCTGGCA	15	6
TTTCATAATGCTGGC	15	2
ATTCACTTTCATAATGCTGG	20	7
TCACTTTCATAATGCTGG	18	1
CTTTCATAATGCTGG	15	8
TCATAATGCTGG	12	9
ACTTTCATAATGCTG	15	10
TTCATAATGCTG	12	11
CACTTTCATAATGCT	15	12
TTTCATAATGCT	12	13
TCACTTTCATAATGC	15	14
CTTTCATAATGC	12	15
TTCACTTTCATAATG	15	16
ACTTTCATAATG	12	17
ATTCACTTTCATAAT	15	18
CACTTTCATAAT	12	19
GATTCACTTTCATAA	15	20
TCACTTTCATAA	12	21
TTCACTTTCATA	12	22
ATTCACTTTCAT	12	23
AGTAAGATTCACTTT	15	24

[0185] Antisense compounds of the present disclosure can be used to modulate the expression of SMN2 in a subject, such as a human. In certain instances, the subject has spinal muscular atrophy. In certain such subjects, the SMN1 gene is absent or otherwise fails to produce sufficient amounts of functional SMN protein. In certain instances, the antisense compounds of the present disclosure effectively modulate splicing of SMN2, resulting in an increase in exon 7 inclusion in SMN2 mRNA and ultimately in SMN2 protein that includes the amino acids corresponding to exon 7. Such alternate SMN2 protein resembles wild-type SMN protein. Antisense compounds of the present disclosure that effectively modulate expression of SMN2 mRNA or protein products of expression are considered active antisense compounds.

[0186] Modulation of expression of SMN2 can be measured in a bodily fluid, which may or may not contain cells; tissue; or organ of the animal. Methods of obtaining samples for analysis, such as body fluids (e.g., sputum, serum, CSF), tissues (e.g., biopsy), or organs, and methods of preparation of the samples to allow for analysis are well known to those skilled in the art. Methods for analysis of RNA and protein levels are discussed above and are well known to those skilled in the art. The effects of treatment can be assessed by measuring biomarkers associated with the target gene expression in the aforementioned fluids, tissues or organs, collected from an animal contacted with one or more compounds of the disclosure, by routine clinical methods known in the art.

[0187] Methods whereby bodily fluids, organs or tissues are contacted with an effective amount of one or more of the antisense compounds or compositions of the disclosure are also contemplated. Bodily fluids, organs or tissues can be contacted with one or more of the compounds of the disclosure resulting in modulation of SMN2 expression in the cells of bodily fluids, organs or tissues. An effective amount can be determined by monitoring the modulatory effect of the antisense compound or compounds or compositions on target nucleic acids or their products by methods routine to the

skilled artisan.

[0188] The disclosure also provides an antisense compound as described herein, for use in any of the methods as described herein. For example, the disclosure provides an antisense compound comprising an antisense oligonucleotide complementary to a nucleic acid encoding human SMN2, for use in treating a disease or condition associated with survival motor neuron protein (SMN), such as spinal muscular atrophy (SMA). As a further example, the disclosure provides an antisense compound comprising an antisense oligonucleotide complementary to a nucleic acid encoding human SMN2, for use in treating a disease or condition associated with survival motor neuron protein (SMN) by administering the antisense compound directly into the central nervous system (CNS) or CSF.

[0189] The disclosure also provides the use of an antisense compound as described herein in the manufacture of a medicament for use in any of the methods as described herein. For example, the disclosure provides the use of an antisense compound comprising an antisense oligonucleotide complementary to a nucleic acid encoding human SMN2 in the manufacture of a medicament for treating a disease or condition associated with survival motor neuron protein (SMN), such as spinal muscular atrophy (SMA). As a further example, the disclosure provides the use of an antisense compound comprising an antisense oligonucleotide complementary to a nucleic acid encoding human SMN2 in the manufacture of a medicament for treating a disease or condition associated with survival motor neuron protein (SMN) by administration of the medicament directly into the central nervous system (CNS) or CSF.

[0190] In certain instances, oligomeric compounds having any motif described herein have a nucleobase sequence complementary to exon 7 of SMN2.

[0191] In certain instances, oligomeric compounds having any motif described herein have a nucleobase sequence complementary to intron 6 of SMN2.

[0192] In certain instances, an antisense compound comprises an antisense oligonucleotide having a nucleobase sequence comprising at least 10 nucleobases of the sequence: TCACTTTCATAATGCTGG (SEQ ID NO: 1). In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 11 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 12 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 13 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 14 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 15 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 16 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising at least 17 nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence comprising the nucleobases of such sequence. In certain instances, an antisense oligonucleotide has a nucleobase sequence consisting of the nucleobases of such sequence. In certain instances, an antisense oligonucleotide consists of 10-18 linked nucleosides and has a nucleobase sequence 100% identical to an equal-length portion of the sequence: TCACTTTCATAATGCTGG (SEQ ID NO: 1).

7. Certain Subjects

[0193] In certain instances, a subject has one or more indicator of SMA. In certain instances, the subject has reduced electrical activity of one or more muscles. In certain instances, the subject has a mutant SMN1 gene. In certain instances, the subject's SMN1 gene is absent or incapable of producing functional SMN protein. In certain instances, the subject is diagnosed by a genetic test. In certain instances , the subject is identified by muscle biopsy. In certain instances, a subject is unable to sit upright. In certain instances, a subject is unable to stand or walk. In certain instances, a subject requires assistance to breathe and/or eat. In certaininstances , a subject is identified by electrophysiological measurement of muscle and/or muscle biopsy.

[0194] In certain instances, the subject has SMA type I. In certain instances, the subject has SMA type II. In certain instances, the subject has SMA type III. In certain instances, the subject is diagnosed as having SMA in utero. In certain instances, the subject is diagnosed as having SMA within one week after birth. In certain instances, the subject is diagnosed as having SMA within one month of birth. In certain instances, the subject is diagnosed as having SMA by 3 months of age. In certain instances, the subject is diagnosed as having SMA by 6 months of age. In certain instances, the subject is diagnosed as having SMA by 1 year of age. In certain instances, the subject is diagnosed as having SMA between 1 and 2 years of age. In certain instances, the subject is diagnosed as having SMA between 1 and 15 years of age. In certain instances, the subject is diagnosed as having SMA when the subject is older than 15 years of age.

[0195] In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered in utero. In certain such instances, the first dose is administered before complete development of the blood-brain-barrier. In certain instances, the first dose is administered to the subject in utero systemically. In certain instances, the first dose is administered in utero after formation of the blood-brain-barrier. In certain instances, the first dose is administered to the CSF.

[0196] In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is

administered when the subject is less than one week old. In certain instances s, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is less than one month old. In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is less than 3 months old. In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is less than 6 months old. In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is less than one year old. In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is less than 2 years old. In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is less than 15 years old. In certain instances, the first dose of a pharmaceutical composition according to the present disclosure is administered when the subject is older than 15 years old.

8. Certain Doses

[0197] In certain instances, the present disclosure provides dose amounts and frequencies. In certain instances, pharmaceutical compositions are administered as a bolus injection. In certain such instances, the dose of the bolus injection is from 0.01 to 25 milligrams of antisense compound per kilogram body weight of the subject. In certain such instances, the dose of the bolus injection is from 0.01 to 10 milligrams of antisense compound per kilogram body weight of the subject. In certain instances, the dose is from 0.05 to 5 milligrams of antisense compound per kilogram body weight of the subject. In certain instances, the dose is from 0.1 to 2 milligrams of antisense compound per kilogram body weight of the subject. In certain instances, the dose is from 0.5 to 1 milligrams of antisense compound per kilogram body weight of the subject. In certain instances, such doses are administered twice monthly. In certain instances, such doses are administered every month. In certain instances, such doses are administered every 2 months. In certain instances, such doses are administered every 6 months. In certain instances such doses are administered by bolus injection into the CSF. In certain instances such doses are administered by intrathecal bolus injection. In certain instances , such doses are administered by bolus systemic injection (e.g., subcutaneous, intramuscular, or intravenous injection). In certain instances, subjects receive bolus injections into the CSF and bolus systemic injections. In such instances, the doses of the CSF bolus and the systemic bolus may be the same or different from one another. In certain instances, the CSF and systemic doses are administered at different frequencies. In certain instances, the disclosure provides a dosing regimen comprising at least one bolus intrathecal injection and at least one bolus subcutaneous injection.

[0198] In certain instances, pharmaceutical compositions are administered by continuous infusion. Such continuous infusion may be accomplished by an infusion pump that delivers pharmaceutical compositions to the CSF. In certain instances, such infusion pump delivers pharmaceutical composition IT or ICV. In certain such instances, the dose administered is between 0.05 and 25 milligrams of antisense compound per kilogram body weight of the subject per day. In certain instances, the dose administered is from 0.1 to 10 milligrams of antisense compound per kilogram body weight of the subject per day. In certain instances, the dose administered is from 0.5 to 10 milligrams of antisense compound per kilogram body weight of the subject per day. In certain instances, the dose administered is from 0.5 to 5 milligrams of antisense compound per kilogram body weight of the subject per day. In certain instances, the dose administered is from 1 to 5 milligrams of antisense compound per kilogram body weight of the subject per day. In certain instances, the disclosure provides a dosing regimen comprising infusion into the CNS and at least one bolus systemic injection. In certain instances, the disclosure provides a dosing regimen comprising infusion into the CNS and at least one bolus subcutaneous injection. In certain instances, the dose, whether by bolus or infusion, is adjusted to achieve or maintain a concentration of antisense compound from 0.1 to 100 microgram per gram of CNS tissue. In certain instances, the dose, whether by bolus or infusion, is adjusted to achieve or maintain a concentration of antisense compound from 1 to 10 microgram per gram of CNS tissue. In certain instances, the dose, whether by bolus or infusion, is adjusted to achieve or maintain a concentration of antisense compound from 0.1 to 1 microgram per gram of CNS tissue.

[0199] In certain instances, dosing a subject is divided into an induction phase and a maintenance phase. In certain such instances, the dose administered during the induction phase is greater than the dose administered during the maintenance phase. In certain instances, the dose administered during the induction phase is less than the dose administered during the maintenance phase. In certain instances, the induction phase is achieved by bolus injection and the maintenance phase is achieved by continuous infusion.

[0200] In certain instances, the disclosure provides systemic administration of antisense compounds, either alone or in combination with delivery into the CSF. In certain instances, the dose for systemic administration is from 0.1 mg/kg to 200 mg/kg. In certain instances, the dose for systemic administration is from 0.1 mg/kg to 100 mg/kg. In certain instances, the dose for systemic administration is from 0.5 mg/kg to 100 mg/kg. In certain instances, the dose for systemic administration is from 1 mg/kg to 100 mg/kg. In certain instances , the dose for systemic administration is from 1 mg/kg to 50 mg/kg. In certain instances, the dose for systemic administration is from 1 mg/kg to 25 mg/kg. In certain instances , the dose for systemic administration is from 0.1 mg/kg to 25 mg/kg. In certain instances, the dose for systemic admin-

istration is from 0.1 mg/kg to 10 mg/kg. In certain instances , the dose for systemic administration is from 1 mg/kg to 10 mg/kg. In certain instances, the dose for systemic administration is from 1 mg/kg to 5 mg/kg. In certain instances comprising both systemic and CSF delivery, the doses for those two routes are independently determined.

a. Calculation of Appropriate Human Doses

[0201] In certain instances, the subject is a human. In certain instances, a human dose is calculated or estimated from data from animal experiments, such as those described herein. In certain instances, a human dose is calculated or estimated from data from monkey and/or mouse experiments, such as those described herein. In certain instances, a human dose is calculated or estimated from data from mouse experiments, such as those described herein. In certain instances, appropriate human doses can be calculated using pharmacokinetic data from mouse along with knowledge of brain weight and/or cerebrospinal fluid (CSF) turnover rates. For example, the mouse brain weight is approximately 0.4 g, which is approximately 2% of its body weight. In humans, the average brain weight is 1.5 kg which is approximately 2.5% of body weight. In certain instances, administration into the CSF results in elimination of a portion of the compound through uptake in brain tissue and subsequent metabolism. By using the ratio of human to mouse brain weight as a scaling factor an estimate of the elimination and clearance through the brain tissue can be calculated. Additionally, the CSF turnover rate can be used to estimate elimination of compound from the CSF to blood. Mouse CSF turnover rate is approximately 10-12 times per day (0.04 mL produced at 0.325 μl/min). Human CSF turnover rate is approximately 4 times per day (100-160 mL produced at 350 - 400 μl/min). Clearance, and therefore dosing requirements, can be based on brain weight elimination scaling, and/or the CSF turnover scaling. The extrapolated human CSF clearance can be used to estimate equivalent doses in humans that approximate doses in mice. In this way, human doses can be estimated that account for differences in tissue metabolism based on brain weight and CSF turnover rates. Such methods of calculation and estimate are known to those skilled in the art.

[0202] By way of non-limiting example, in certain instances, an equivalent human dose can be estimated from a desired mouse dose by multiplying the mg/kg mouse dose by a factor from about 0.25 to about 1.25 depending on the determined clearance and elimination of a particular compound. Thus, for example, in certain instances, a human dose equivalent of a 0.01 mg dose for a 20 g mouse will range from about 8.75 mg to about 43.75 mg total dose for a 70 kg human. Likewise, in certain instances, a human dose equivalent of a 0.01 mg dose for a 4 g newborn mouse will range from about 1.9 mg to about 9.4 mg total dose for a 3 kg newborn human. These example doses are merely to illustrate how one of skill may determine an appropriate human dose and are not intended to limit the present disclosure.

[0203] In certain instances, a human dose for systemic delivery (whether administered alone or in combination with CSF delivery) is calculated or estimated from data from animal experiments, such as those described herein. Typically, an appropriate human dose (in mg/kg) for systemic dose is between 0.1 and 10 times an effective dose in animals. Thus, solely for example, a subcutaneous dose of 50μg in a 2g newborn mouse is a dose of 25mg/kg. The corresponding dose for a human is predicted to be between 2.5mg/kg and 250mg/kg. For a 3 kilogram infant, the corresponding dose is between 7.5 mg and 750 mg. For a 25 kg child, the corresponding dose is from 62.5 mg to 6250 mg.

9. Treatment Regimens

[0204] In certain instances, the above dose amounts, dose frequencies, routes of administration, induction and maintenance phases, and timing of first dose are combined to provide dosing regimens for subjects having SMA. Such dosing regimens may be selected and adjusted to provide amelioration of one or more symptom of SMA and/or to reduce or avoid toxicity or side effects attributable to the administration of the pharmaceutical composition. In certain instances, subjects are in utero or newborn. In such instances, administration of pharmaceutical compositions, particularly by continuous infusion, presents particular challenges. Accordingly, in certain instances, the present disclosure provides for administration of pharmaceutical compositions by bolus administration while the subject is in utero or very young, followed by continuous infusion via an implanted infusion pump when the subject is older and placement of such pump is more practical. Further, in certain instances, as a subject grows, the absolute dose is increased to achieve the same or similar dose:body-weight ratio. The following table is intended to exemplify treatment regimens and is not intended to limit the possible combinations of treatments which will be easily accomplished by one of skill in the art.

Dosing period	First	Second	Third	Fourth	Fifth
Regimen 1					
Subject Age	In utero, prior to formation of blood-brain-barrier	In utero, after formation of blood-brain-barrier	> 1 week	6 months	1.5 years

(continued)

Dosing period	First	Second	Third	Fourth	Fifth
Regimen 1					
Dose Amount	50 μg	50 μg	100 μg	10 μg/day	50 μg/day
Frequency	Single admin	Single admin	Monthly	Continuous	Continuous
Route of Administration	Systemic injection	IT injection	IT injections	IT infusion	IT infusion
Duration	N/A	N/A	6 months	1 year	Ongoing
Regimen 2					
Subject Age	In utero, after formation of blood-brain-barrier	> 1 week	6 months	1.5 years	N/A
Dose Amount	50 μg	100 μg	5 mg/day	10 mg/day	N/A
Frequency	Single admin	Monthly	Continuous	Continuous	N/A
Route of Administration	ICV injection	ICV injection	ICV infusion	ICV infusion	N/A
Duration	N/A	6 months	1 year	Ongoing	N/A
Regimen 3					
Subject Age	> 1 week	6 months	1.5 years	2.5 years*	
Dose Amount	100 μg	500 μg/day	20 mg/day	20 mg/day	100 mg
Frequency	2xMonthly	Continuous	Continuous	Continuous	2xMonthly
Route of Administration	ICV injection	ICV infusion	ICV infusion	ICV infusion	IP
Duration	6 months	1 year	1 year	Ongoing	Ongoing
* Note: the 4th dosing period in regimen 3 exemplifies continuous CSF infusion combined with periodic systemic administration. These treatment regimens are intended to exemplify and not to limit the present disclosure.					

[0205] In certain instances, the dosing regimen comprises a systemic administration, either alone or in combination with administration into the CSF (for example regimen 3, above). The table, below further exemplifies such regimens.

Systemic administration			CSF administration		
Dose	Route	Frequency	Dose	Route	Frequency
1-5 mg/kg	subcutaneous	weekly	5-10 mg/kg	bolus IT	monthly
1-5 mg/kg	subcutaneous	monthly	1-5 mg/kg	bolus ICV	2 months
10-50 mg/kg	subcutaneous	monthly	0.5-1 mg/kg	bolus IT	6 months
0.5-25 mg/kg	subcutaneous	monthly	10 mg/kg/day	IT infusion	continuous for 7 days every 6 months
0.1-10 mg/kg	subcutaneous	monthly	none		
none			0.5-1 mg/kg	bolus IT	6 months

These treatment regimens are intended to exemplify and not to limit the present disclosure. One of skill in the art will be able to select an appropriate combination of the doses and deliveries in view of the present disclosure and based on a variety of factors, such as the severity of the condition and the overall health and age of the subject.

10. Co-administration

[0206] In certain instances, pharmaceutical compositions of the present disclosure are co-administered with at least one other pharmaceutical composition for treating SMA and/or for treating one or more symptom associated with SMA. In certain instances, such other pharmaceutical composition is selected from trichostatin-A, valproic acid, riluzole, hydroxyurea, and a butyrate or butyrate derivative. In certain instances, pharmaceutical compositions of the present disclosure are co-administered with trichostatin A. In certain instances, pharmaceutical compositions of the present disclosure are co-administered with a derivative of quinazoline, for example as described in Thurmond, et al., J. Med Chem. 2008, 51, 449-469. In certain instances, a pharmaceutical composition of the present disclosure and at least one other pharmaceutical composition are co-administered at the same time. In certain instances, a pharmaceutical composition of the present disclosure and at least one other pharmaceutical composition are co-administered at different times.

[0207] In certain instances, pharmaceutical compositions of the present disclosure are co-administered with a gene therapy agent. In certain such instances, the gene therapy agent is administered to the CSF and the pharmaceutical composition of the present disclosure is administered systemically. In certain such instances, the gene therapy agent is administered to the CSF and the pharmaceutical composition of the present disclosure is administered to the CSF and systemically. In certain instances, a pharmaceutical composition of the present disclosure and a gene therapy agent are co-administered at the same time. In certain instances a pharmaceutical composition of the present disclosure and a gene therapy agent are co-administered at different times. Certain gene therapy approaches to SMA treatment have been reported (e.g.,Coady et al., PLoS ONE 2008 3(10): e3468; Passini et al., J Clin Invest 2010 Apr 1, 120(4): 1253-64).

[0208] In certain instances, pharmaceutical compositions of the present disclosure are co-administered with at least one other therapy for SMA. In certain instances, such other therapy for SMA is surgery. In certain instances, such other therapy is physical therapy, including, but not limited to exercises designed to strengthen muscles necessary for breathing, such as cough therapy. In certain instances, other therapy is a physical intervention, such as a feeding tube or device for assisted breathing.

[0209] In certain instances, pharmaceutical compositions of the present disclosure are co-administered with one or more other pharmaceutical compositions that reduce an undesired side-effect of the pharmaceutical compositions of the present disclosure.

11. Phenotypic Effects

[0210] In certain instances, administration of at least one pharmaceutical composition of the present disclosure results in a phenotypic change in the subject. In certain instances, such phenotypic changes include, but are not limited to: increased absolute amount of SMN mRNA that includes exon 7; increase in the ratio SMN mRNA that includes exon 7 to SMN mRNA lacking exon 7; increased absolute amount of SMN protein that includes exon 7; increase in the ratio SMN protein that includes exon 7 to SMN protein lacking exon 7; improved muscle strength, improved electrical activity in at least one muscle; improved respiration; weight gain; and survival. In certain instances, at least one phenotypic change is detected in a motoneuron of the subject. In certain instances, administration of at least one pharmaceutical composition of the present disclosure results in a subject being able to sit-up, to stand, and/or to walk. In certain instances, administration of at least one pharmaceutical composition of the present disclosure results in a subject being able to eat, drink, and/or breathe without assistance. In certain instances, efficacy of treatment is assessed by electrophysiological assessment of muscle. In certain instances, administration of a pharmaceutical composition of the present disclosure improves at least one symptom of SMA and has little or no inflammatory effect. In certain such instance, absence of inflammatory effect is determined by the absence of significant increase in Aifl levels upon treatment.

[0211] In certain instances, administration of at least one pharmaceutical composition of the present disclosure delays the onset of at least one symptom of SMA. In certain instances, administration of at least one pharmaceutical composition of the present disclosure slows the progression of at least one symptom of SMA. In certain instances, administration of at least one pharmaceutical composition of the present disclosure reduces the severity of at least one symptom of SMA.

[0212] In certain instances, administration of at least one pharmaceutical composition of the present disclosure results in an undesired side-effect. In certain instances, a treatment regimen is identified that results in desired amelioration of symptoms while avoiding undesired side-effects.

12. Dosage units

[0213] In certain instances pharmaceutical compositions of the present disclosure are prepared as dosage units for administration. Certain such dosage units are at concentrations selected from 0.01 mg to 100mg. In certain such instances, a pharmaceutical composition of the present disclosure comprises a dose of antisense compound selected from 0.01 mg, 0.1 mg, 0.5 mg, 1 mg, 5 mg, 10 mg, 20 mg, 25 mg, 50 mg, 75 mg, 100 mg, 150 mg, and 200 mg. In certain instances, a pharmaceutical composition is comprises a dose of oligonucleotide selected from 0.1 mg, 0.5 mg, 1 mg, 5

mg, 10 mg, 25 mg, and 50 mg.

13. Kits

[0214] In certain instances, the present disclosure provides kits comprising at least one pharmaceutical composition. In certain instances, such kits further comprise a means of delivery, for example a syringe or infusion pump.

Nonlimiting disclosure

[0215] While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain instances, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Although the sequence listing accompanying this filing identifies each sequence as either "RNA" or "DNA" as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

[0216] Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligomeric compound having the nucleobase sequence "ATCGATCG" encompasses any oligomeric compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG" and oligomeric compounds having other modified bases, such as "AT^{me}CGAUCG," wherein ^{me}C indicates a cytosine base comprising a methyl group at the 5-position.

Example 1 - Antisense compounds targeting SMN2

[0217] The following oligonucleotides were synthesized using standard techniques previously reported.

Reference #	Sequence	Length	Chemistry	SEQ ID
ISIS396443	TCACTTTCATAATGCTGG	18	Full 2'-MOE; full PS	1
ISIS396449	TTTCATAATGCTGGC	15	Full 2'-MOE; full PS	2
PS = phosphorothioate internucleoside linkages				

Example 2 - **Smn-/- SMN Transgenic Mice**

[0218] Therapeutic effectiveness and safety using the antisense compounds as described above can be tested in an appropriate animal model. For example, animal models which appear most similar to human disease include animal species which either spontaneously develop a high incidence of the particular disease or those that have been induced to do so.

[0219] In particular, animal models for SMA are known. As explained above, the molecular basis of SMA, an autosomal recessive neuromuscular disorder, is the homozygous loss of the survival motor neuron gene 1 (SMN1). A nearly identical copy of the SMN1 gene, called SMN2 is found in humans and modulates the disease severity. In contrast to humans, mice have a single gene (Smn) that is equivalent to SMN1. Homozygous loss of this gene is lethal to embryos and results in massive cell death, which indicates that the Smn gene product is necessary for cellular survival and function. The introduction of 2 copies of SMN2 into mice lacking SMN rescues the embryonic lethality, resulting in mice with the SMA phenotype (Monani et al., Hum. Mol. Genet. (2000) 9:333-339. A high copy number of SMN2 rescues the mice because sufficient SMN protein is produced in motor neurons. See, also, Hsieh-Li, et al., Nat. Genet. (2000) 24:66-70, reporting the production of transgenic mouse lines that expressed human SMN2. In particular, transgenic mice harboring SMN2 in the Smn-/- background showed pathological changes in the spinal cord and skeletal muscles similar to those of SMA patients. The severity of the pathological changes in these mice correlated with the amount of SMN protein that contained the region encoded by exon 7. Heterozygous mice lacking one copy of Smn are designated Smn -/+ and are a model for the less severe form of SMA, type III.

[0220] The severity of the SMA phenotype is a function of the number of copies of human SMN2 in the mice. The

"Taiwan" strain has 4 copies of human SMN2, resulting in mice that have moderate to severe SMA phenotype, similar to Type I or Type II.

[0221] Delta-7 mice ($Smn^{-/-}$, $hSMN2^{+/+}$, $SMN\Delta7^{+/+}$) also lack mouse Smn and express human SMN2. Delta 7 mice have a more severe phenotype and die shortly after birth, typically about 15-20 days after birth.

Example 3 - **Systemic Administration of Antisense compounds *in vivo* in Smn -/- SMN2 (Taiwan Strain)**

[0222] Taiwan mice were treated by intraperitoneal injection with saline or with 35 mg/kg of ISIS396443 or a mismatched antisense oligonucleotide control once each day for 5 days and were sacrificed 2 days later on day 7. Liver and kidney were collected and RNA was isolated using standard techniques. SMN2 with and without exon 7 was visualized by RT-PCR. Administration with ISIS396443 resulted in substantial increase in exon 7 inclusion in the SMN2 from kidney and liver compared to saline and mismatch control treated animals.

Example 4 -Intracerebroventricular (ICV) Administration of Antisense compounds *in vivo* in Smn -/- SMN2 (Taiwan Strain)

[0223] Taiwan mice were injected ICV either with saline or with 150 μg of ISIS396443 each day for 7 days. The mice were sacrificed on day 8 and RNA from brain and spinal cord was extracted. RT-PCR analysis showed substantial increase in exon 7 inclusion in the SMN2 in brain and spinal cord samples obtained from animals treated with ISIS396443. These results indicate that ICV treatment with antisense oligonucleotide targeting SMN can rescue the SMA condition because exclusion of exon 7 is associated with the SMA phenotype.

Dose-Response

[0224] Taiwan mice were injected ICV either with saline or with 10, 50, 100, or 150 μg of ISIS396443 each day for 7 days (5 mice in each treatment group) and were sacrificed on day 8. RNA was isolated and analyzed by RT-PCR. The 10 μg treatment group showed moderate exon 7 inclusion. The 50 μg, 100 μg, and 150 μg groups all showed substantial exon 7 inclusion.

Duration of Response

[0225] To determine the duration of effect, 24 mice were injected ICV with 50 μg of ISIS396443 each day for 7 days. Four mice were sacrificed at the time of the final dose (time 0) and four mice were sacrificed at each of: 1 week, 2 weeks, 4 weeks and 8 weeks after the final dose. All treated mice showed substantial exon 7 inclusion by RT-PCR with the effect at week 8 showing no difference with the other groups, as shown in Figure 1:
These results indicate that ICV administration of ISIS396443 at 50 μg per day for 7 days is effective for at least 8 weeks following treatment.

[0226] The experiment was repeated to test longer time points. Type III mice were treated by ICV infusion of ISIS396443 at 50μg/day for 7 days. Mice were sacrificed 0, 0.5, 1, 2, 4, and 6 months after the end of the 7 day infusion period. RNA was collected from the spinal cords and analyzed by northern blot. As shown in the graph below, the effect of ISIS396443 infusion persisted for 6 months after infusion, as shown in Figure 2. This long duration of effect has several possible explanations. It may reflect stability of ISIS396443, stability of the corrected SMN protein and/or that the dose was high enough that even after loss of compound to metabolism, the remaining dose continued to provide benefit. Thus, these data may support administration of lower doses as well as infrequent doses.

Example 5 - Administration of Antisense compounds by Continuous Intracerebroventricular (ICV) Infusion

[0227] Using a micro-osmotic pump (Azlet Osmotic Pumps, Cupertino, CA, USA), ISIS396443 was delivered into cerebrospinal fluid (CSF) through the right lateral ventricle in adult type-III Smn+/- or Smn-/- SMA mice with a human *SMN2* transgene (Taiwan strain). Dose-response studies revealed that intracerebroventricular (ICV) infusion of the ISIS396443 increased *SMN2* exon 7 inclusion in spinal cord to ~90%, compared to ~10% in saline-treated mice. Western blotting and immunohistochemical analysis demonstrated a robust increase of the human transgenic SMN protein levels in spinal-cord motor neurons. These results indicate that CNS infusion of antisense oligonucleotide ISIS396443 can rescue the SMA condition because exclusion of exon 7 is associated with the SMA phenotype.

Example 6 - Embryonic Administration

[0228] A single ICV injection of either 20 μg or 10 μg of ISIS396443 was administered to embryonic Taiwan mice at

day 15 of gestation (E15). Animals were sacrificed at day 7 after birth (P7). RNA was isolated from the lumbar spinal cord and analyzed by RT-PCR. The single embryonic administration of ISIS 396443 resulted in substantial exon 7 inclusion. These results indicate that treatment with antisense oligonucleotide ISIS396443 in utero can rescue the SMA condition because exclusion of exon 7 is associated with the SMA phenotype.

[0229] The above experiment was repeated and the animals were sacrificed at 11 weeks. Untreated Taiwan mice develop necrotic tails, which shorten over time. The single embryonic injection of 20 μg of ISIS396443 significantly delayed the onset of tail degradation, as shown in Figure 3A. These results indicate that embryonic treatment with antisense oligonucleotide targeting SMN delays onset of SMA.

[0230] These results were confirmed in another study using the same conditions, except the doses tested were 20 μg and 10 μg of ISIS396443 and the study included normal mice for comparison. Results from that experiment are shown in Figure 3B.

Example 7 - In vivo administration in the delta-7 mouse model

[0231] Heterozygote ($SMN^{+/-}$, $hSMN2^{+/+}$, $SMN\Delta7^{+/+}$) breeding pairs were mated and, on the day of birth (P0), newborn pups were treated with ISIS396443 (18-mer, SEQ ID NO. 1), ISIS396449 (15-mer, SEQ ID NO. 2), ISIS387954 (20-mer, SEQ ID NO.7) or a scrambled control ASO (ISIS439273; 18-mer). Mice were injected bilaterally into the cerebral lateral ventricles for a total dose of 8 μg (4 μg in each lateral ventricle). All the injections were performed with a finely drawn glass micropipette needle as described (Passini et al, J. Virol. (2001) 75:12382-12392). Following the injections, the pups were toe-clipped and genotyped (Le et al., Hum. Mol. Genet. (2005) 14:845-857) to identify SMA ($SMN^{-/-}$, $hSMN2^{+/+}$, $SMN\Delta7^{+/+}$), heterozygote, and wild type ($SMN^{+/+}$, $hSMN2^{+/+}$, $SMN\Delta7^{+/+}$) mice. All the litters were culled to 7 pups to control for litter size on survival. Some of the litters were not injected in order to generate untreated control groups.

[0232] Widespread distribution of the 18-mer was detected in the spinal cord at 14 days post-injection in SMA mice, including the thoracic, lumbar, and cervical regions of the spinal cord. Furthermore, co-localization studies with ChAT confirmed the vast majority of the cells targeted by ISIS396443 in the spinal cord were motor neurons. No signal was detected in control, untreated mice.

[0233] Western blot analysis at 14 days showed the amount of SMN in the brain and spinal cord were at 40-60% wild type levels, compared to 10% in untreated SMA controls. No signal above background was detected in control mice treated with a scrambled version of the ASO. Results of the western blots are provided in Figure 4.

[0234] SMA mice treated with SMA ASOs also exhibited a significant increase in weight, ambulatory function (righting reflex and grip strength), and coordination (hindlimb splay) regardless of the length of the ASO as compared to untreated SMA mice or SMA mice treated with a scrambled ASO. No significant increase in body weight, ambulatory function (righting reflex and grip strength), or coordination was observed in SMA mice treated with a scrambled ASO as compared to untreated SMA mice. Results are provided in Figures 5 and 6.

[0235] Importantly, SMA mice treated with ASOs regardless of length of ASO produced a significant increase in median survival as shown in Figure 7. Survival was from birth was 31.5 (15-mer), 27.0 (18-mer), and 28.0 (20-mer) days, compared to 16.0 days in untreated SMA controls. In contrast, SMA mice treated with an 18-mer scrambled control did not improve survival. These results demonstrate that treatment with antisense oligonucleotide targeting SMN treatment increases lifespan in SMA affected subjects.

[0236] The SMA ASOs also increased motor neuron cell counts in the spinal cord as shown in Figure 8.

[0237] SMN RNA was measured by RT-PCR. Animals treated with SMA ASOs had increased SMN RNA levels compared to untreated SMA mice. Results from mice treated with the 20-mer ASO compared to untreated SMA mice are shown in Figure 9.

[0238] To determine whether survival could be further increased by administration of a second dose, the above experiment was repeated with an additional dose of 20 μg at day 21. Results are shown in Figure 10. The graph above shows the effect of the first dose of 8 μg at day 0. At day P21, half of the treated mice were given a second treatment.

[0239] The effect of the second treatment compared to mice that received only the first treatment is shown in Figure 11. This result indicates that a second ICV treatment with antisense oligonucleotide further increases survival.

Example 8 - Activity in SMA type III mice

[0240] Two antisense compounds and one control compound were tested in a mouse model of SMA. The compounds are described in table below.

Compounds Tested in Taiwan Strain SMA Mice

ISIS#	Sequence	Description	SEQ ID
396443	TCACTTTCATAATGCTGG	Uniform 2'-MOE, full PS; 18-mer; complementary to intron 7 of human SMN2	1
449220	ATTCACTTTCATAATGCTGG	Uniform 2-OMe; full PS; 20-mer; complementary to intron 7 of human SMN2	3
439272	TTAGTTTAATCACGCTCG	Uniform 2'-MOE; full PS; 18-mer; control sequence	4

[0241] Taiwan strain of SMA type III mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). These mice lack mouse SMN and are homozygous for human SMN2 (mSMN -/-; hSMN2 +/+). These mice have been described in Hsieh-Li HM, et al., Nature Genet. 24, 66-70 2000.

[0242] Mice were treated with 3, 10, 30, or 100 μg of ISIS396443 or ISIS449220 per day or with 30 or 100 μg of control compound ISIS439272 per day in phosphate buffered saline (PBS). Control mice were treated with PBS alone (dose of 0). All treatments were administered by intracerebroventricular (ICV) infusion using an Azlet 1007D osmotic pump. There were five animals for each dose, however, two of the mice from the highest dose of ISIS449220 died prior to completion of the study. Animals were sacrificed on day 9 (two days after final dose) and brain and lumbar sections of the spinal cords were collected from each animal. Real time PCR was performed on each sample to determine the amount of human SMN2 message including exon 7 ((+)exon 7) and the amount of human SMN2 message lacking exon 7((-)exon 7). Real time PCR was also performed to determine the expression levels of allograft inflammatory factor (AIF1) and glyceraldehyde 3-phosphate dehyrogenase (GADPH).

[0243] Expression levels for (+)exon 7 and (-)exon 7were normalized to GADPH levels. Those normalized expression levels were then divided by the GADPH-normalized levels from the PBS treated control mice. The resulting fold-control values are reported in Table 17, below. Data represent mean fold of control for all five mice in each group, except the highest dose of ISIS449220, which represent the 3 surviving mice.

[0244] Administration of ISIS396443 resulted in a striking increase in inclusion of exon 7. At 10 μg/day, ISIS396443 resulted in nearly twice as much (1.8 fold) exon 7 retained SMN2 message in brain, and in lumbar spinal cord it was more than twice as much compared to untreated control.

Ability of Antisense Compounds to Alter Splicing in SMA Mice

Compound	Dose (μg/day)	Brain		Lumbar Cord	
		(+)exon 7	(-)exon 7	(+)exon 7	(-)exon 7
396443 (2'-MOE)	0	1.0	1.0	1.0	1.0
	3	1.3	1.0	1.4	1.0
	10	1.8	0.7	2.1	0.6
	30	2.4	0.6	3.4	0.3
	100	3.0	0.3	3.8	0.1
449220 (2'-OMe)	0	1.0	1.0	1.0	1.0
	3	0.9	1.1	1.0	1.1
	10	1.0	1.1	1.0	1.2
	30	1.0	1.2	1.1	1.2
	100*	1.0	1.0	1.2	1.1
439272 Control	0	1.0	1.0	1.0	1.0
	30	1.0	1.1	0.9	1.1
	100	1.0	1.0	1.0	1.0
* data from only 3 mice for this dose					

[0245] Expression of allograft inflammatory factor (AIF1) was tested as a measure of inflammation. After normalization of all samples to (GADPH), the ratio of AIF1 for each treatment group was divided by the value for the PBS control.

ISIS396443 resulted in no increase in AIF1, even at the highest dose. ISIS449220 resulted in increased AIF1 in both brain and lumbar spinal cord. Data in Table 18 represent mean fold of control for all five mice in each group, except the highest dose of ISIS449220, which represent the 3 surviving mice.

Toxicity of antisense compounds in SMA Mice

Compound	Dose (μg/day)	AIF-1/GAPDH	
		Brain	Lumbar
396443 (2'-MOE)	0	1.0	1.0
	3	10	1.0
	10	1.1	1.2
	30	1.0	1.0
	100	0.9	1.0
449220 (2'-OMe)	0	1.0	1.0
	3	1.0	1.0
	10	1.0	1.8
	30	1.2	2.9
	100*	1.8	3.3
439272 Control	0	0.9	0.9
	30	0.9	1.0
	100	0.9	1.2
* data from only 3 mice for this dose			

Example 9 - Administration to Monkeys

[0246] Cynomolgus monkeys were used to assess distribution of ISIS395443 at different doses and routes of administration. ISIS396443 was administered to 2 monkeys. One monkey received a dose of 3 mg by ICV infusion and the other monkey received a dose of 3 mg by IT infusion. Both infusions were delivered over a 24 hour period. The monkeys were sacrificed and tissues were harvested 96 hours after the end of the infusion period. The concentration of ISIS396443 was measured in samples from Cervical, Thoracic, and Lumbar sections of the spinal cord. Results are summarized in the table below.

Animal #	Dose	Route	Tissue	Concentration of ISIS396443 (μg/g)
1	3 mg over 24 hours	ICV infusion	Cervical	21.5
			Thoracic	9.4
			Lumbar	23.9
2	3 mg over 24 hours	IT infusion	Cervical	12.5
			Thoracic	22.6
			Lumbar	42.6

Since cynomolgus monkeys are approximately 3 kg, this dose is about 1 mg/kg.

[0247] To further assess distribution of ISIS39644, twenty-six monkeys were divided into six groups as provided in the table below.

Group	Dose	Route	Concentration of compound (mg/ml)	Duration of infusion	Day sacrificed	Number of monkeys
1	0	ICV	0	14 days	Day 19	2M/2F

(continued)

Group	Dose	Route	Concentration of compound (mg/ml)	Duration of infusion	Day sacrificed	Number of monkeys
2	3 mg	ICV	0.09	14 days	Day 19	2M/2F
3	3 mg	IT	1.25	1 day	Day 6	3M/2F
4	3 mg	IT	0.42	3 days	Day 8	2M/2F
5	3 mg	IT	0.18	7 days	Day 12	3M/2F
6	3 mg	IT	0.09	14 days	Day 19	2M/2F

Infusion rate for all groups was 100 μL/hour. All monkeys received a total of 3 mg of ISIS39644 in saline, except for group 1, which received saline only. Monkeys were sacrificed and tissues were harvested 5 days after the end of infusion.

[0248] Concentrations of ISIS39644 in tissue samples from the monkeys were evaluated using standard techniques. Summaries of the results are provided in the graphs in Figure 12. Samples were also evaluated by histology. The histology did not show any adverse effect of treatment and confirmed presence of ISIS396443. There was no evidence of Purkinje cell loss.

[0249] Rapid infusion appeared to have more ISIS396443 than slower infusion. These results suggest that faster infusion rates or bolus injection may be preferred in certain instances. Since bolus administration has certain practical advantages over infusion, in certain instances, it is the preferred method of administration into the CSF. In certain instances, the preferred method of administration into the CSF is by bolus IT injection.

Example 10 - Generation of a mouse model of severe SMA and ICV treatment

[0250] Mice having a severe SMA phenotype (sSMA mice) were generated. Homozygote sSMA mice carry 2 copies of human SMN2 and no mouse SMN. The average lifespan is about 10 days. In addition, the SMA mice are smaller and have shorter tails. Heterozygotes carry mouse SMN and develop normally.

[0251] To study the effect of antisense compounds in these sSMA mice, 20μg of ISIS396443 was injected ICV at day P1. Treatment resulted in an increase in average survival from 9.9 days (saline treated control) to 16.7 days. RT-PCR analysis showed increased full-length SMN RNA in tissues from the treated mice.

Example 11 - Systemic administration of ISIS 396443

[0252] sSMA mice and healthy heterozygote control mice were divided into groups to study the effect of ISIS396443 by bolus ICV injection and/or bolus subcutaneous injection (SC) as follows:

Group 1 - ICV+SC
One ICV injection of 20 μg at P1 or P2 (day 1 or 2 after birth); and two subcutaneous injections of 50 μg/g delivered between P0 and P3.

Group 2 - SC+SC
Two SC injections of 50 μg/g delivered between P0 and P3; and one subcutaneous injection of 50 μg/g delivered between P5 and P6; and subcutaneous injection of 50 μg/g delivered between P9 and P10.

Group 3 - SC
Two SC injections of 50μg/g delivered between P0 and P3.

Group 4 - SMA saline control
One ICV injection of saline at P1 or P2; and two subcutaneous injections of saline delivered between P0 and P3.

Group 5 - Heterozygous control
One ICV injection of 20 μg at P1 or P2; and two subcutaneous injections of 50 μg/g delivered between P0 and P3 of heterozygous mice.

Each group included from 14 to 22 mice. Survival (in days) for individual mice in each group is provided in the table, below. Many mice in this study remain alive at the time that this patent application is being prepared. Thus, a value

proceeded by a ">" indicates that the mouse has lived that number of days and is still alive.

Mouse	Group 1 ICV+SC	Group 2 SC+SC	Group 3 SC	Group 4 Saline	Group 5 Het
1	> 141	> 130	> 103	8	> 146
2	> 141	127	94	8	> 146
3	22	> 114	61	8	> 146
4	> 140	73	> 103	8	> 146
5	117	27	> 103	8	> 145
6	> 124	27	> 103	8	> 145
7	> 111	18	34	8	> 145
8	> 111	> 102	26	8	> 145
9	> 111	>98	31	8	> 145
10	> 111	> 98	69	9	> 144
11	29	> 102	69	9	> 144
12	> 110	> 102	67	9	> 144
13	> 110	> 102	> 91	9	> 144
14	> 110	> 102	> 90	9	> 143
15	> 110	ND	> 90	9	> 143
16	> 108	ND	> 90	9	> 143
17	> 108	ND	> 90	10	> 129
18	> 109	ND	86	10	> 129
19	18	ND	> 75	10	> 129
20	ND	ND	69	10	ND
21	ND	ND	18	11	ND
22	ND	ND	> 71	12	ND
23	ND	ND	ND	12	ND
24	ND	ND	ND	13	ND
25	ND	ND	ND	13	ND
26	ND	ND	ND	14	ND

Example 12 - Dose-Response of SC administration

[0253] Survival of sSMA mice receiving different doses of subcutaneous ISIS396443 was assessed by the following dosing groups.

Group 1-SC400 (dose ranges from 80 mg/kg to 180 mg/kg)
Two SC injections totaling 400 μg per mouse delivered between P0 to P3, first dose was 150 μg at P0 or P1 (volume of 3 μl) and second was 250 μg delivered P2 or P3 (volume of 5 μl).

Group 2-SC200 (dose ranges from 40 mg/kg to 90 mg/kg)
Two SC injections totaling 200 μg per mouse delivered between P0 and P3, first dose was 75 μg at P0-P1 (volume of 1.5 μl) and second was 125 μg delivered P2or P3 (volume of 2.5 μl).

Group 3-SC100 (dose ranges from 20 mg/kg to 45 mg/kg)
Two SC injections totaling 100 μg per mouse delivered between P0 and P3, first dose was 40 μg at P0 or P1 (volume

of 2 μl) and second was 60 μg delivered P2 or P3 (volume of 3μl).

Group 4-SMA saline (negative controls)
Two SC injections of saline between P0 and P3, first was at P0 or P1 (volume of 5 μl) and second delivered P2 or P3 (volume of 5 μl).

Group 5 - Heterozygous control (positive controls)
Mice without any treatment.

Each group included from 14 to 26 mice. Survival (in days) for individual mice in each group is provided in the table, below. Many mice in this study remain alive at the time that this patent application is being prepared. Thus, a value proceeded by a ">" indicates that the mouse has lived that number of days and is still alive.

Mouse	Group 1 SC400	Group 2 SC200	Group 3 SC100	Group 4 Saline	Group 5 Het
1	> 82	>93	11	8	> 87
2	> 82	>91	11	8	> 87
3	> 82	>91	11	9	> 87
4	> 82	> 91	11	9	> 87
5	> 82	14	11	9	> 87
6	> 82	25	12	9	> 87
7	> 82	92	18	9	> 86
8	> 82	>93	19	9	> 86
9	> 82	>93	22	9	> 86
10	> 82	> 90	69	9	> 86
11	> 82	> 90	> 77	9	> 86
12	> 80	> 91	> 77	10	> 86
13	> 80	> 91	> 77	10	> 86
14	25	> 90	> 77	10	> 86
15	ND	> 90	> 75	10	> 85
16	ND	> 90	> 74	11	> 85
17	ND	86	> 74	11	> 85
18	ND	> 90	> 74	12	ND
19	ND	> 52	> 74	12	ND
20	ND	ND	> 74	13	ND
21	ND	ND	> 74	13	ND
22	ND	ND	>71	13	ND
23	ND	ND	>49	13	ND
24	ND	ND	>49	14	ND
25	ND	ND	>49	15	ND
26	ND	ND	23	ND	ND

Example 13 - ICV Infusion vs. ICV Bolus

[0254] Administration by intracerebroventricular bolus injection (ICV bolus) was compared to administration by continuous intracerebroventricular infusion (ICV infusion). The SMA type III transgenic mice were dosed with ISIS387954.

ICV infusion mice were given a total dose of 0 (PBS control), 87.5 μg, 175 μg, 350 μg, or 700 μg infused over 7 days and were sacrificed 2 days later. ICV bolus mice were given the same total doses, 0 (PBS control), 87.5 μg, 175 μg, 350 μg, or 700 μg, in a single ICV injection and were sacrificed 9 days later. There were 5 mice in each group. RNA was collected from the lumbar spinal cord and was analyzed by real time PCR. Intron 7 inclusion was normalized to the saline-treated controls. Results are summarized in the table below.

Group	Dose	Fold increase in intron 7 inclusion relative to PBS
1	PBS (control)	1.0
2	87.5 μg by ICV infusion over 7 days	2.1
3	175 μg by ICV infusion over 7 days	2.4
4	350 μg by ICV infusion over 7 days	3.2
5	700 μg by ICV infusion over 7 days	3.6
6	PBS (control)	1.0
7	87.5 μg by ICV bolus	3.1
8	175 μg by ICV bolus	3.7
9	350 μg by ICV bolus	3.8
10	700 μg by ICV bolus	3.8

In this experiment, the same dose when delivered by ICV bolus injection resulted in greater activity than when delivered by ICV infusion over 7 days.

[0255] Real time PCR was also performed to determine the expression levels of allograft inflammatory factor (AIF1) to assess inflammation. None of the samples from treated mice showed a significant difference from control mice.

Example 14 - Dose-Response by ICV Bolus

[0256] Administration by intracerebroventricular bolus was tested at additional doses. The transgenic mice were administered 0, 10.9 μg, 21.9 μg, 43.4 μg, 87.5 μg, or 175 μg of ISIS387954 by single bolus ICV injection and were sacrificed 9 days later as described in Example 13. Samples were collected from brain and from lumbar spinal cord. RNA was prepared and analyzed by RT-PCR for change in intron 7 inclusion and for change in AIF1. None of the samples showed a change in AIF1 compared to control. Results from intron 7 inclusion are summarized in the table below. The ED50 is at around 22μg.

Group	Dose	Fold increase in intron 7 inclusion relative to PBS	
		Brain	Lumbar spinal cord
1	PBS (control)	1.0	1.0
2	10.9 μg by ICV bolus	2.4	2.2
3	21.9 μg by ICV bolus	2.8	2.7
4	43.4 μg by ICV bolus	3.2	3.4
5	87.5 μg by ICV bolus	3.5	3.4
6	175 μg by ICV bolus	4.4	3.7

The disclosure further includes the subject matter of the claims of WO 2010/148249 from which this application is derived, the content of which is reproduced below as numbered paragraphs.

1. A method comprising administering to a subject an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 pre-mRNA, wherein the antisense compound is administered into the cerebrospinal fluid.

2. The method of paragraph 1, wherein the administration is into the intrathecal space.

3. The method of paragraph 1, wherein the administration is into the cerebrospinal fluid in the brain.

4. The method of any of paragraphs 1-3, wherein the administration comprises a bolus injection.

5. The method of any of paragraphs 1-3, wherein the administration comprises infusion with a delivery pump.

6. The method of any of paragraphs 1-5, wherein the antisense compound is administered at a dose from 0.01 to 10 milligrams of antisense compound per kilogram of body weight of the subject.

7. The method of paragraph 6, wherein the dose is from 0.01 to 10 milligrams of antisense compound per kilogram of body weight of the subject.

8. The method of paragraph 6, wherein the dose is from 0.01 to 5 milligrams of antisense compound per kilogram of body weight of the subject.

9. The method of paragraph 6, wherein the dose is from 0.05 to 1 milligrams of antisense compound per kilogram of body weight of the subject.

10. The method of paragraph 6, wherein the dose is from 0.01 to 0.5 milligrams of antisense compound per kilogram of body weight of the subject.

11. The method of paragraph 6, wherein the dose is from 0.05 to 0.5 milligrams of antisense compound per kilogram of body weight of the subject.

12. The method of any of paragraphs 6-11, wherein the dose is administered daily.

13. The method of any of paragraphs 6-11, wherein the dose is administered weekly.

14. The method of any of paragraphs 6-11, wherein the antisense compound is administered continuously and wherein the dose is the amount administered per day.

15. The method of any of paragraphs 1-13 comprising administering at least one induction dose during an induction phase and administering at least one maintenance dose during a maintenance phase.

16. The method of paragraph 15, wherein the induction dose is from 0.05 to 5.0 milligrams of antisense compound per kilogram of body weight of the subject.

17. The method of paragraph 15 or 16, wherein the maintenance dose is from 0.01 to 1.0 milligrams of antisense compound per kilogram of body weight of the subject.

18. The method of any of paragraphs 15-17, wherein the duration of the induction phase is at least 1 week.

19. The method of any of paragraphs 15-18, wherein the duration of the maintenance phase is at least 1 week.

20. The method of any of paragraphs 15-19, wherein each induction dose and each maintenance dose comprises a single injection.

21. The method of any of paragraphs 15-19, wherein each induction dose and each maintenance dose independently comprise two or more injections.

22. The method of any of paragraphs 1-21, wherein the antisense compound is administered at least 2 times over a treatment period of at least 1 week.

23. The method of paragraph 22, wherein the treatment period is at least one month.

24. The method of paragraph 22, wherein the treatment period is at least 2 months.

25. The method of paragraph 22, wherein the treatment period is at least 4 months.

26. The method of any of paragraphs 15-25, wherein the induction dose is administered by one or more bolus injections and the maintenance dose is administered by an infusion pump.

27. The method of any of paragraphs 1-26 comprising assessing the tolerability and/or effectiveness of the antisense compound.

28. The method of any of paragraphs 1-27, wherein the dose amount or frequency of antisense compound is reduced following an indication that administration of the antisense compound is not tolerated.

29. The method of any of paragraphs 1-28, wherein the dose amount or frequency of antisense compound is maintained or reduced following an indication that administration of the antisense compound is effective.

30. The method of any of paragraphs 1-29, wherein the dose of antisense compound is increased following an indication that administration of the antisense compound is not effective.

31. The method of any of paragraphs 1-30, wherein frequency of administration of antisense compound is reduced following an indication that administration of the antisense compound is effective.

32. The method of any of paragraphs 1-31, wherein frequency of administration of antisense compound is increased following an indication that administration of the antisense compound is not effective.

33. The method of any of the above paragraphs, comprising co-administration of the antisense compound and at least one other therapy.

34. The method of paragraph 33, wherein antisense compound and at least one other therapy are co-administered at the same time.

35. The method of paragraph 34, wherein the antisense compound is administered prior to administration of the at least one other therapy.

36. The method of paragraph 34, wherein the antisense compound is administered after administration of the at least one other therapy.

37. The method of any of paragraphs 33-36 wherein the at least one other therapy comprises administration of one or more of valproic acid, riluzole, hydroxyurea, and a butyrate.

38. The method of any of paragraphs 33-37 wherein the at least one other therapy comprises administration of trichostatin-A.

39. The method of any of paragraphs 33-38 wherein the at least one other therapy comprises administration of stem cells.

40. The method of any of paragraphs 33-39 wherein the at least one other therapy is gene therapy.

41. The method any of the above paragraphs, wherein the antisense compound is administered at a concentration of about 0.01 mg/ml, about 0.05 mg/ml, about 0.1 mg/ml, about 0.5 mg/ml, about 1 mg/ml, about 5 mg/ml, about 10 mg/ml, about 50 mg/ml, or about 100 mg/ml.

42. The method of any of paragraphs 1-41, wherein inclusion of exon 7 of SMN2 mRNA in a motoneuron in the subject is increased.

43. The method of any of paragraphs 1-42, wherein inclusion of exon 7 amino acids in SMN2 polypeptide in a motoneuron in the subject is increased.

44. A method of increasing inclusion of exon 7 of SMN2 mRNA in a motoneuron in a subject comprising administering to the subject an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a

nucleic acid encoding human SMN2 and thereby increasing inclusion of exon 7 of SMN2 mRNA in the motoneuron in the subject.

45. A method of increasing inclusion of exon 7 amino acids in SMN2 polypeptide in a motoneuron in a subject comprising administering to the subject an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 and thereby increasing inclusion of exon 7 amino acids in SMN2 polypeptide in the motoneuron in the subject.

46. The method of any of paragraphs 1-45, wherein the subject has SMA.

47. The method of any of paragraphs 1-45, wherein the subject has type I SMA

48. The method of any of paragraphs 1-45, wherein the subject has type II SMA.

49. The method of any of paragraphs 1-45, wherein the subject has type III SMA.

50. The method of any of paragraphs 1-49, wherein a first dose is administered in utero.

51. The method of paragraph 50, wherein the first dose is administered prior to complete formation of the blood-brain-barrier.

52. The method of any of paragraphs 1-49, wherein a first dose is administered within 1 week of birth of the subject.

53. The method of any of paragraphs 1-49, wherein a first dose is administered within 1 month of birth of the subject.

54. The method of any of paragraphs 1-49, wherein a first dose is administered within 3 months of birth of the subject.

55. The method of any of paragraphs 1-49, wherein a first dose is administered within 6 months of birth of the subject.

56. The method of any of paragraphs 1-49, wherein a first dose is administered when the subject is from 1 to 2 years of age.

57. The method of any of paragraphs 1-49, wherein a first dose is administered when the subject is from 1 to 15 years of age.

58. The method of any of paragraphs 1-49, wherein a first dose is administered when the subject is older than 15 years of age.

59. The method of any of paragraphs 1-58, wherein the subject is a mammal.

60. The method of paragraph 59, wherein the subject is a human.

61. The method of any of paragraphs 1-60, comprising identifying a subject having SMA.

62. The method of paragraph 61, wherein the subject is identified by measuring electrical activity of one or more muscles of the subject.

63. The method of paragraph 61, wherein the subject is identified by a genetic test to determine whether the subject has a mutation in the subject's SMN1 gene.

64. The method of paragraph 61, wherein the subject is identified by muscle biopsy.

65. The method paragraph 42, wherein the administering of the antisense compound results in an increase in the amount of SMN2 mRNA having exon 7 of at least 10%.

66. The method of paragraph 65, wherein the increase in the amount of SMN2 mRNA having exon 7 is at least 20%.

67. The method of paragraph 65, wherein the increase in the amount of SMN2 mRNA having exon 7 is at least 50%.

68. The method of paragraph 65, wherein the increase in the amount of SMN2 mRNA having exon 7 is at least 70%.

69. The method of paragraph 43, wherein the administering of the antisense compound results in an increase in the amount of SMN2 polypeptide having exon 7 amino acids of at least 10%.

70. The method of paragraph 69, wherein the increase in the amount of SMN2 polypeptide having exon 7 amino acids is at least 20%.

71. The method of paragraph 69, wherein the increase in the amount of SMN2 polypeptide having exon 7 amino acids is at least 50%.

72. The method of paragraph 69, wherein the increase in the amount of SMN2 polypeptide having exon 7 amino acids is at least 70%.

73. The method of any of paragraphs 1-72, wherein the administering of the antisense compound ameliorates at least one symptom of SMA in the subject.

74. The method of paragraph 73, wherein the administering of the antisense compound results in improved motor function in the subject.

75. The method of paragraph 73, wherein the administering of the antisense compound results in delayed or reduced loss of motor function in the subject.

76. The method of paragraph 73, wherein the administering of the antisense compound results in improved respiratory function.

77. The method of paragraph 73, wherein the administering of the antisense compound results in improved survival.

78. The method of any of paragraphs 1-77, wherein at least one nucleoside of the antisense oligonucleotide comprises a modified sugar moiety.

79. The method of paragraph 78, wherein the at least one modified sugar moiety comprises a 2'-methoxyethyl sugar moiety.

80. The method of any of paragraphs 1-79, wherein essentially each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety.

81. The method of paragraph 80, wherein the nucleosides comprising a modified sugar moiety all comprise the same sugar modification.

82. The method of paragraph 81, wherein each modified sugar moiety comprises a 2'-methoxyethyl sugar moiety.

83. The method of any of paragraphs 1-79, wherein each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety.

84. The method of paragraph 83, wherein the nucleosides all comprise the same sugar modification.

85. The method of paragraph 84, wherein each modified sugar moiety comprises a 2'-methoxyethyl sugar moiety.

86. The method of any of paragraphs 1-85, wherein at least one internucleoside linkage is a phosphorothioate internucleoside linkage.

87. The method of paragraph 86, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

88. The method of any of paragraphs 1-87, wherein the antisense oligonucleotide consists of 10 to 25 linked nucleosides.

89. The method of any of paragraphs 1-87, wherein the antisense oligonucleotide consists of 12 to 22 linked nucl-

eosides.

90. The method of any of paragraphs 1-87, wherein the antisense oligonucleotide consists of 15 to 20 linked nucleosides.

91. The method of any of paragraphs 1-87, wherein the antisense oligonucleotide consists of 18 linked nucleosides.

92. The method of any of paragraphs 1-91, wherein the antisense oligonucleotide is at least 90% complementary to the nucleic acid encoding human SMN2.

93. The method of paragraph 92, wherein the antisense oligonucleotide is fully complementary to the nucleic acid encoding human SMN2.

94. The method of any of paragraphs 1-93, wherein the oligonucleotide has a nucleobase sequence comprising at least 10 contiguous nucleobases of the nucleobase sequence SEQ ID NO: 1.

95. The method of paragraph 94, wherein the oligonucleotide has a nucleobase sequence comprising at least 15 contiguous nucleobases of the nucleobase sequence SEQ ID NO: 1.

96. The method of paragraph 94, wherein the oligonucleotide has a nucleobase sequence comprising the nucleobase sequence SEQ ID NO: 1.

97. The method of paragraph 94, wherein the oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence SEQ ID NO: 1.

98. The method of any of paragraphs 1-97, wherein the antisense compound comprises a conjugate group or terminal group.

99. The method of any of paragraphs 1-97, wherein the antisense compound consists of the antisense oligonucleotide.

100. The method of any of paragraphs 1-99, wherein the antisense compound is also administered systemically.

101. The method of paragraph 100, wherein the systemic administration is by intravenous or intraperitoneal injection.

102. The method of paragraph 100 or 101, wherein the systemic administration and the administration into the central nervous system are performed at the same time.

103. The method of paragraph 100 or 101, wherein the systemic administration and the administration into the central nervous system are performed at different times.

104. An antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2, for use in a method according to any of paragraphs 1-103.

105. The antisense compound according to paragraph 104, for use in treating a disease or condition associated with survival motor neuron 1 (SMN1).

106. Use of an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a nucleic acid encoding human SMN2 in the manufacture of a medicament for use in a method according to any preceding paragraph.

107. The use according to paragraph 106, wherein the medicament is for treating a disease or condition associated with survival motor neuron 1 (SMN1).

SEQUENCE LISTING

[0257]

<110> Isis Pharmaceuticals, Inc.
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Katherine W. Klinger

<120> COMPOSITIONS AND METHODS FOR MODULATION OF SMN2 SPLICING

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Claims

1. An antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a pre-mRNA encoding human SMN2, for use in treating a human subject having spinal muscular atrophy (SMA), wherein the compound is administered into the cerebrospinal fluid in the intrathecal space of the human subject, wherein the antisense oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence of SEQ ID NO: 1, wherein each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety, wherein each modified sugar moiety is a 2'-methoxyethyl sugar moiety and wherein each internucleoside linkage is a phosphorothioate linkage.

2. Use of an antisense compound comprising an antisense oligonucleotide complementary to intron 7 of a pre-mRNA encoding human SMN2 in the manufacture of a medicament for use in treating a human subject having spinal muscular atrophy (SMA), wherein the medicament is administered into the cerebrospinal fluid in the intrathecal space of the human subject, wherein the antisense oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence of SEQ ID NO: 1, wherein each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety, wherein each modified sugar moiety is a 2'-methoxyethyl sugar moiety and wherein each internucleoside linkage is a phosphorothioate linkage.

3. The antisense compound for use according to claim 1, or the use of an antisense compound according to claim 2 wherein the administration comprises a bolus injection.

4. The antisense compound for use according to claim 1 or 3 or the use of an antisense compound according to claim 2 or 3, wherein the antisense compound is administered at a dose from 0.01 to 10 milligrams of antisense compound per kilogram of body weight of the human subject.

5. The antisense compound for use according to claim 4, or the use of an antisense compound according to claim 4, wherein the dose is:

   (a) from 0.01 to 5 milligrams of antisense compound per kilogram of body weight of the human subject;
   (b) from 0.05 to 1 milligrams of antisense compound per kilogram of body weight of the human subject;
   (c) from 0.01 to 0.5 milligrams of antisense compound per kilogram of body weight of the human subject; or
   (d) from 0.05 to 0.5 milligrams of antisense compound per kilogram of body weight of the human subject.

6. The antisense compound for use according to any of claims 1 or 3-5 or the use of an antisense compound according to any of claims 2-5, for use in treating a human subject having (i) type I SMA; (ii) type II SMA; or (iii) type III SMA.

7. The antisense compound for use according to any of claims 1 or 3-6 or the use of an antisense compound according to any of claims 2-6, wherein the administering of the antisense compound results in an increase in the amount of SMN2 mRNA having exon 7 of at least 10%.

8. The antisense compound for use according to any of claims 1 or 3-7 or the use of an antisense compound according to any of claims 2-7, wherein the human subject has one or more indicator of SMA.

9. The antisense compound for use according to any of claims 1 or 3-8 or the use of an antisense compound according to any of claims 2-8, wherein the human subject has at least one symptom associated with SMA.

10. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according to any of claims 2-9, wherein the antisense compound is administered both systemically and to the central nervous system and wherein the systemic administration and the administration into the central nervous system are performed at different times.

11. The antisense compound for use or the use of an antisense compound according to claim 10, wherein the systemic administration is by subcutaneous administration, intravenous or intraperitoneal injection.

12. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according to of any of claims 2-9, wherein a first dose of the antisense compound is administered within one week of birth of the human subject.

13. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according

to of any of claims 2-9, wherein a first dose of the antisense compound is administered within one month of birth of the human subject.

14. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according to of any of claims 2-9, wherein a first dose of the antisense compound is administered within three months of birth of the human subject.

15. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according to of any of claims 2-9, wherein a first dose of the antisense compound is administered within six months of birth of the human subject.

16. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according to of any of claims 2-9, wherein a first dose of the antisense compound is administered when the human subject is from 1 to 2 years of age.

17. The antisense compound for use according to any of claims 1 or 3-9 or the use of an antisense compound according to of any of claims 2-9, wherein a first dose of the antisense compound is administered when the human subject is from 1 to 15 years of age.

Patentansprüche

1. Antisense-Verbindung, umfassend ein Antisense-Oligonukleotid, das komplementär zu Intron 7 einer prä-mRNA ist, die menschliches SMN2 codiert, zur Verwendung bei der Behandlung eines menschlichen Patienten mit spinaler Muskelatrophie (SMA), wobei die Verbindung in die Cerebrospinalflüssigkeit im Intrathekalraum des menschlichen Patienten verabreicht wird, wobei das Antisense-Oligonukleotid eine Nukleobasensequenz aufweist, die aus der Nukleobasensequenz der SEQ ID NO: 1 besteht, wobei jedes Nukleosid des Antisense-Oligonukleotids einen modifizierten Zuckerrest umfasst, wobei jeder modifizierte Zuckerrest ein 2'-Methoxyethylzuckerrest ist und wobei jede Internukleosidbindung eine Phosphorothioatbindung ist.

2. Verwendung einer Antisense-Verbindung, die ein Antisense-Oligonukleotid umfasst, das komplementär zu Intron 7 einer prä-mRNA ist, die menschliches SMN2 codiert, bei der Herstellung eines Medikaments zur Verwendung bei der Behandlung eines menschlichen Patienten mit spinaler Muskelatrophie (SMA), wobei das Medikament in die Cerebrospinalflüssigkeit im Intrathekalraum des menschlichen Patienten verabreicht wird, wobei das Antisense-Oligonukleotid eine Nukleobasensequenz aufweist, die aus der Nukleobasensequenz der SEQ ID NO: 1 besteht, wobei jedes Nukleosid des Antisense-Oligonukleotids einen modifizierten Zuckerrest umfasst, wobei jeder modifizierte Zuckerrest ein 2'-Methoxyethylzuckerrest ist und wobei jede Internukleosidbindung eine Phosphorothioatbindung ist.

3. Antisense-Verbindung zur Verwendung nach Anspruch 1 oder Verwendung einer Antisense-Verbindung nach Anspruch 2, wobei die Verabreichung eine Bolusinjektion umfasst.

4. Antisense-Verbindung zur Verwendung nach Anspruch 1 oder 3 oder Verwendung einer Antisense-Verbindung nach Anspruch 2 oder 3, wobei die Antisense-Verbindung in einer Dosis von 0,01 bis 10 Milligramm der Antisense-Verbindung pro Kilogramm Körpergewicht des menschlichen Patienten verabreicht wird.

5. Antisense-Verbindung zur Verwendung nach Anspruch 4 oder Verwendung einer Antisense-Verbindung nach Anspruch 4, wobei die Dosis Folgendem entspricht:

(a) 0,01 bis 5 Milligramm der Antisense-Verbindung pro Kilogramm Körpergewicht des menschlichen Patienten;
(b) 0,05 bis 1 Milligramm der Antisense-Verbindung pro Kilogramm Körpergewicht des menschlichen Patienten;
(c) 0,01 bis 0,5 Milligramm der Antisense-Verbindung pro Kilogramm Körpergewicht des menschlichen Patienten;
(d) 0,05 bis 0,5 Milligramm der Antisense-Verbindung pro Kilogramm Körpergewicht des menschlichen Patienten.

6. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-5 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-5 zur Verwendung bei der Behandlung eines menschlichen Patienten mit

(i) SMA Typ 1; (ii) SMA Typ II; oder (iii) SMA Typ III.

7. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-6 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-6, wobei das Verabreichen der Antisense-Verbindung zu einem Anstieg der Menge an SMN2-mRNA, die Exon 7 zu mindestens 10 % aufweist, führt.

8. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-7 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-7, wobei der menschliche Patient einen oder mehrere SMA-Indikatoren aufweist.

9. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 und 3-8 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-8, wobei der menschliche Patient mindestens ein mit SMA einhergehendes Symptom aufweist.

10. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei die Antisense-Verbindung sowohl systemisch als auch in das zentrale Nervensystem verabreicht wird und wobei die systemische Verabreichung und die Verabreichung in das zentrale Nervensystem zu unterschiedlichen Zeitpunkten durchgeführt werden.

11. Antisense-Verbindung zur Verwendung oder Verwendung einer Antisense-Verbindung nach Anspruch 10, wobei die systemische Verabreichung durch subkutane Verabreichung, intravenöse oder intraperitoneale Injektion erfolgt.

12. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei eine erste Dosis der Antisense-Verbindung innerhalb einer Woche nach der Geburt des menschlichen Patienten verabreicht wird.

13. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei eine erste Dosis der Antisense-Verbindung innerhalb eines Monats nach der Geburt des menschlichen Patienten verabreicht wird.

14. Antisense-Verbindung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei eine erste Dosis der Antisense-Verbindung innerhalb von drei Monaten nach der Geburt des menschlichen Patienten verabreicht wird.

15. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei eine erste Dosis der Antisense-Verbindung innerhalb von sechs Monaten nach der Geburt des menschlichen Patienten verabreicht wird.

16. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei eine erste Dosis der Antisense-Verbindung verabreicht wird, wenn der menschliche Patient 1 bis 2 Jahre alt ist.

17. Antisense-Verbindung zur Verwendung nach einem der Ansprüche 1 oder 3-9 oder Verwendung einer Antisense-Verbindung nach einem der Ansprüche 2-9, wobei eine erste Dosis der Antisense-Verbindung verabreicht wird, wenn der menschliche Patient 1 bis 15 Jahre alt ist.

Revendications

1. Composé antisens comprenant un oligonucléotide antisens complémentaire de l'intron 7 d'un pré-ARNm codant pour le SMN2 humain, pour une utilisation dans le traitement d'un sujet humain atteint d'amyotrophie spinale (AMS), dans lequel le composé est administré dans le liquide céphalo-rachidien dans l'espace intrathécal du sujet humain, dans lequel l'oligonucléotide antisens a une séquence de nucléobases constituée de la séquence de nucléobases de SEQ ID NO : 1, dans lequel chaque nucléoside de l'oligonucléotide antisens comprend une fraction de sucre modifiée, dans lequel chaque fraction de sucre modifiée est une fraction de sucre avec un groupe 2'-méthoxyéthyle et dans lequel chaque liaison internucléosidique est une liaison phosphorothioate.

2. Utilisation d'un composé antisens comprenant un oligonucléotide antisens complémentaire de l'intron 7 d'un pré-

ARNm codant pour le SMN2 humain dans la fabrication d'un médicament pour une utilisation dans le traitement d'un sujet humain atteint d'amyotrophie spinale (AMS), dans laquelle le médicament est administré dans le liquide céphalo-rachidien dans l'espace intrathécal du sujet humain, dans laquelle l'oligonucléotide antisens a une séquence de nucléobases constituée de la séquence de nucléobases de SEQ ID NO : 1, dans laquelle chaque nucléoside de l'oligonucléotide antisens comprend une fraction de sucre modifiée, dans laquelle chaque fraction de sucre modifiée est une fraction de sucre avec un groupe 2'-méthoxyéthyle et dans laquelle chaque liaison internucléosidique est une liaison phosphorothioate.

3. Composé antisens pour une utilisation selon la revendication 1, ou utilisation d'un composé antisens selon la revendication 2, dans lequel l'administration comprend une injection de bolus.

4. Composé antisens pour une utilisation selon la revendication 1 ou 3 ou utilisation d'un composé antisens selon la revendication 2 ou 3, dans lequel le composé antisens est administré à une dose de 0,01 à 10 milligrammes de composé antisens par kg de poids corporel du sujet humain.

5. Composé antisens pour une utilisation selon la revendication 4, ou utilisation d'un composé antisens selon la revendication 4, dans lequel la dose est :

(a) de 0,01 à 5 milligrammes de composé antisens par kg de poids corporel du sujet humain ;
(b) de 0,05 à 1 milligramme de composé antisens par kg de poids corporel du sujet humain ;
(c) de 0,01 à 0,5 milligramme de composé antisens par kg de poids corporel du sujet humain ; ou
(d) de 0,05 à 0,5 milligramme de composé antisens par kg de poids corporel du sujet humain.

6. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 5 ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 5, pour une utilisation dans le traitement d'un sujet humain atteint de (i) AMS de type I ; (ii) AMS de type II ; ou (iii) AMS de type III.

7. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 6, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 6, dans lequel l'administration du composé antisens produit une augmentation de la quantité d'ARNm de SMN2 comportant l'exon 7 d'au moins 10 %.

8. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 7, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 7, dans lequel le sujet humain présente un ou plusieurs indicateurs d'AMS.

9. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 8, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 8, dans lequel le sujet humain présente au moins un symptôme associé à l'AMS.

10. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel le composé antisens est administré à la fois par voie systémique et au système nerveux central et dans lequel l'administration systémique et l'administration dans le système nerveux central sont effectuées à des temps différents.

11. Composé antisens pour une utilisation ou utilisation d'un composé antisens selon la revendication 10, dans lequel l'administration systémique est réalisée par une administration sous-cutanée, une injection intraveineuse ou intrapéritonéale.

12. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel une première dose du composé antisens est administrée dans la semaine qui suit la naissance du sujet humain.

13. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel une première dose du composé antisens est administrée dans le mois qui suit la naissance du sujet humain.

14. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel une première dose du composé

antisens est administrée dans les trois mois qui suivent la naissance du sujet humain.

15. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel une première dose du composé antisens est administrée dans les six mois qui suivent la naissance du sujet humain.

16. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel une première dose du composé antisens est administrée lorsque le sujet humain est âgé de 1 à 2 ans.

17. Composé antisens pour une utilisation selon l'une quelconque des revendications 1 ou 3 à 9, ou utilisation d'un composé antisens selon l'une quelconque des revendications 2 à 9, dans lequel une première dose du composé antisens est administrée lorsque le sujet humain est âgé de 1 à 15 ans.

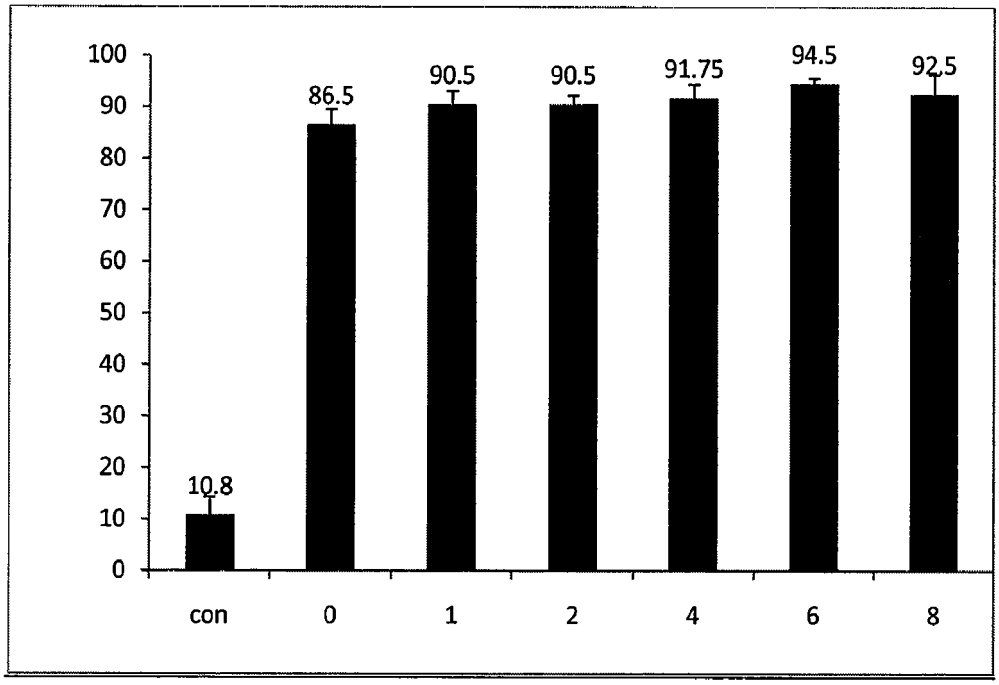

100
90
80
70
60
50
40
30
20
10
0
10.8
86.5
90.5
90.5
91.75
94.5
92.5
con
0
1
2
4
6
8

Figure 1

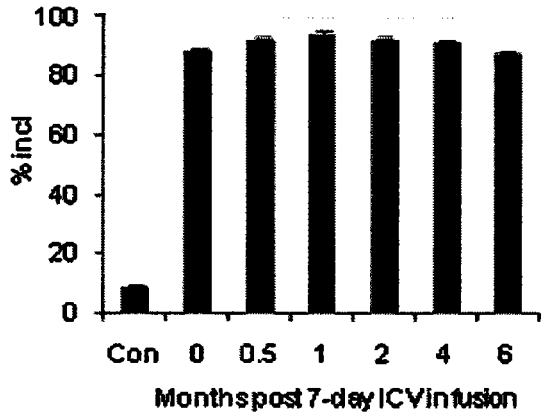
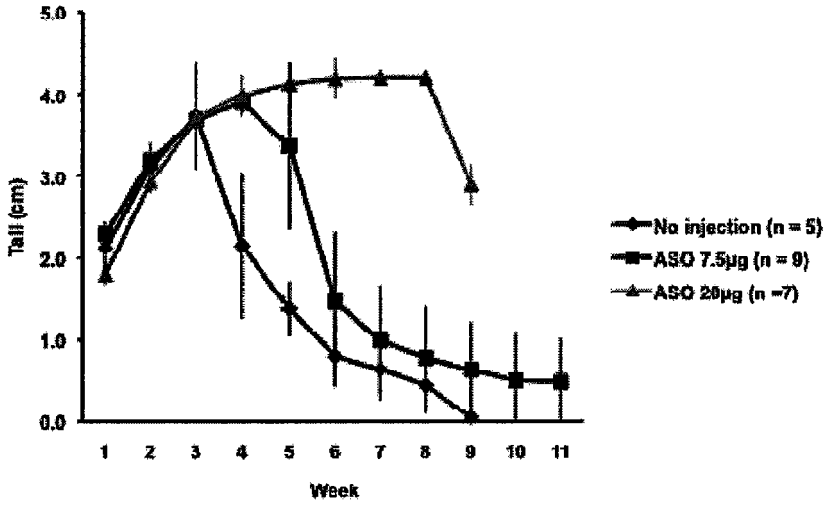

100
80
60
40
20
0
% incl
Con
0
0.5
1
2
4
6
Months post 7-day ICV infusion

Figure 2

A


5.0
4.0
3.0
2.0
1.0
0.0
Tail (cm)
1 2 3 4 5 6 7 8 9 10 11
Week
No injection (n = 5)
ASO 7.5μg (n = 9)
ASO 20μg (n =7)

B

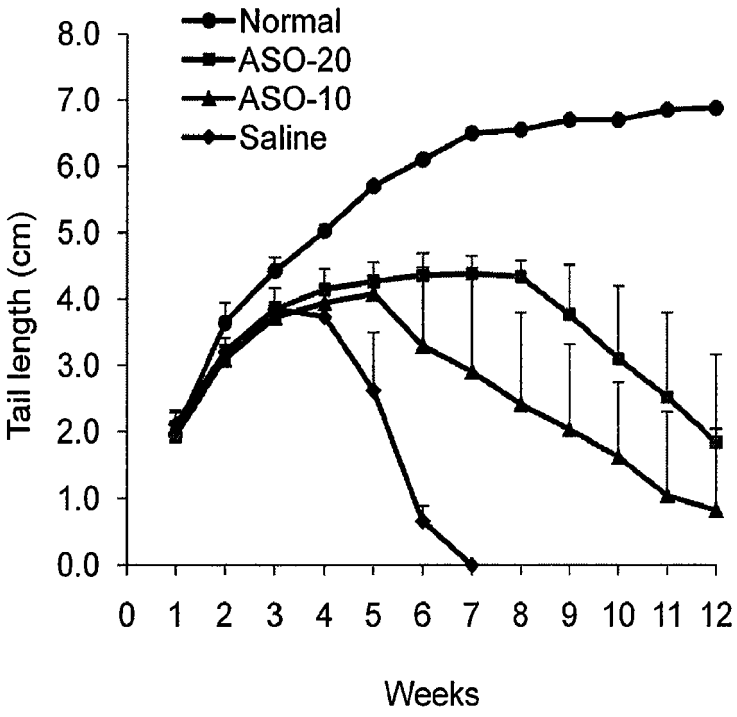

Normal
ASO-20
ASO-10
Saline
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Tail length (cm)
0 1 2 3 4 5 6 7 8 9 10 11 12
Weeks

Figure 3

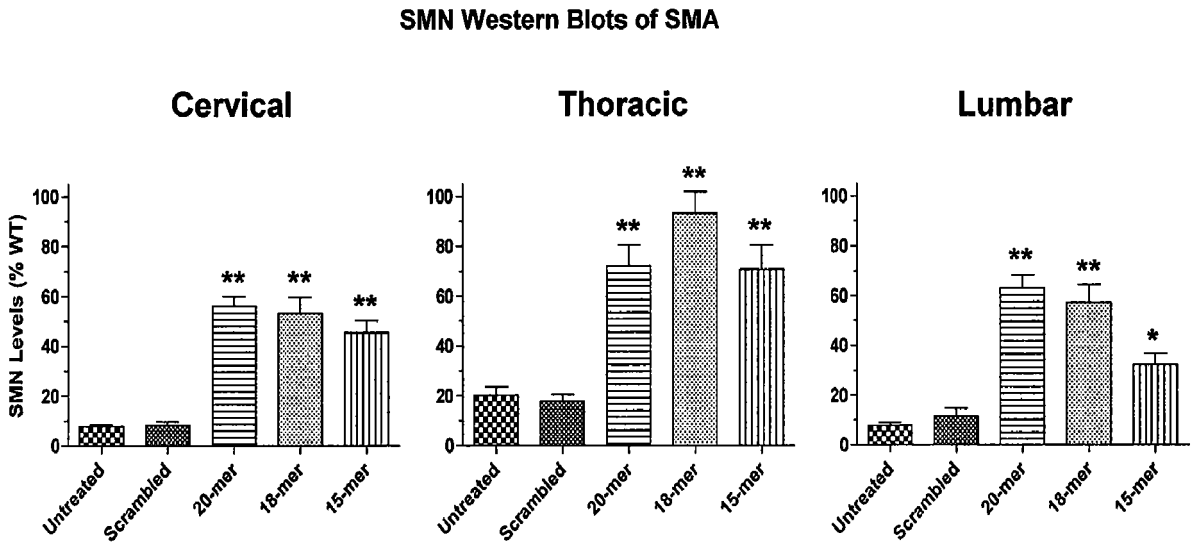

SMN Western Blots of SMA
Cervical
Thoracic
Lumbar
SMN Levels (% WT)
100
80
60
40
20
0
**
*
Untreated
Scrambled
20-mer
18-mer
15-mer
4 µg dose, 16 days post-

Figure 4

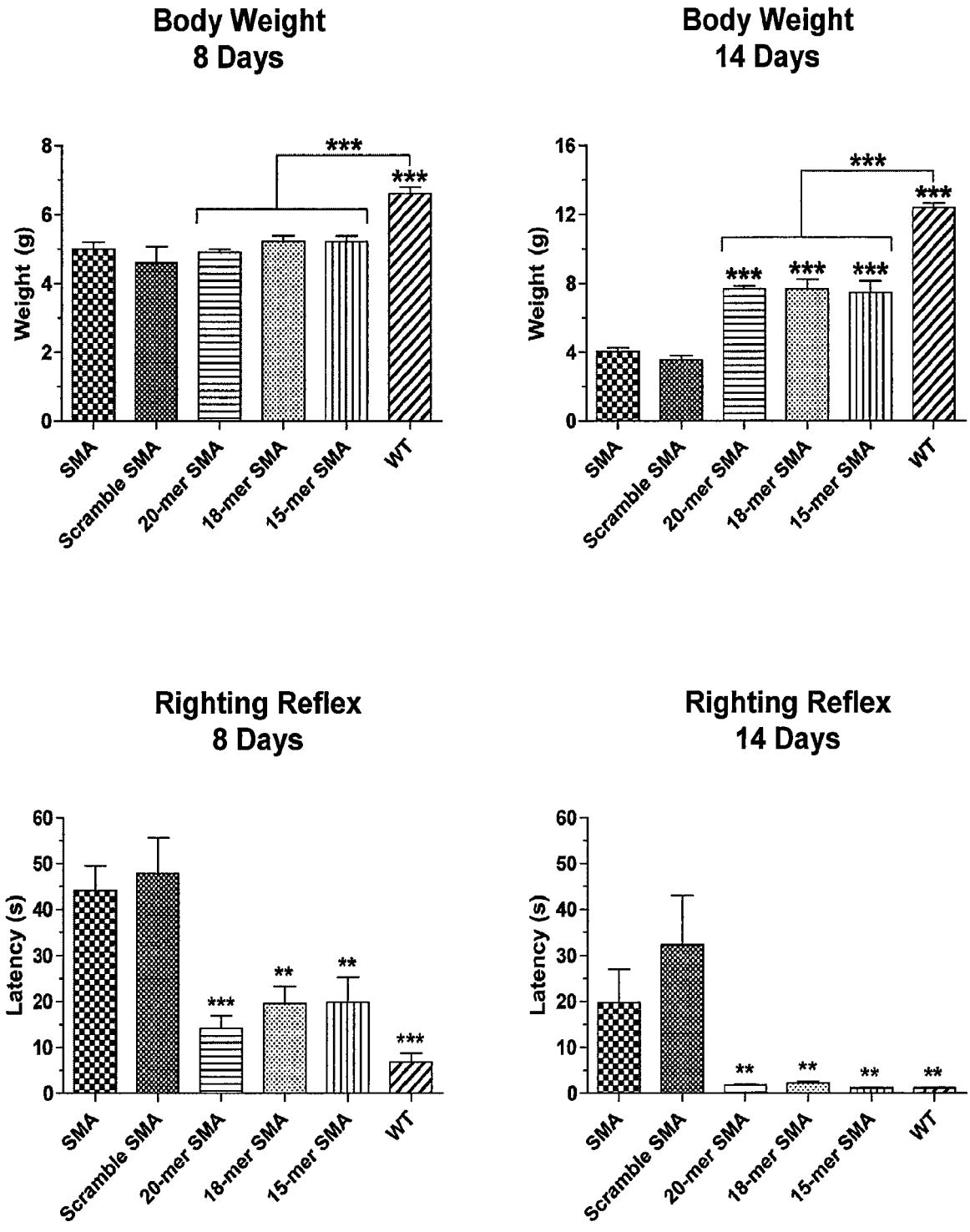

Body Weight
8 Days
Weight (g)
0
2
4
6
8
***
***
SMA
Scramble SMA
20-mer SMA
18-mer SMA
15-mer SMA
WT
Body Weight
14 Days
Weight (g)
0
4
8
12
16
***
***
***
***
***
SMA
Scramble SMA
20-mer SMA
18-mer SMA
15-mer SMA
WT
Righting Reflex
8 Days
Latency (s)
0
10
20
30
40
50
60
***
**
**
***
SMA
Scramble SMA
20-mer SMA
18-mer SMA
15-mer SMA
WT
Righting Reflex
14 Days
Latency (s)
0
10
20
30
40
50
60
**
**
**
**
SMA
Scramble SMA
20-mer SMA
18-mer SMA
15-mer SMA
WT

Figure 5

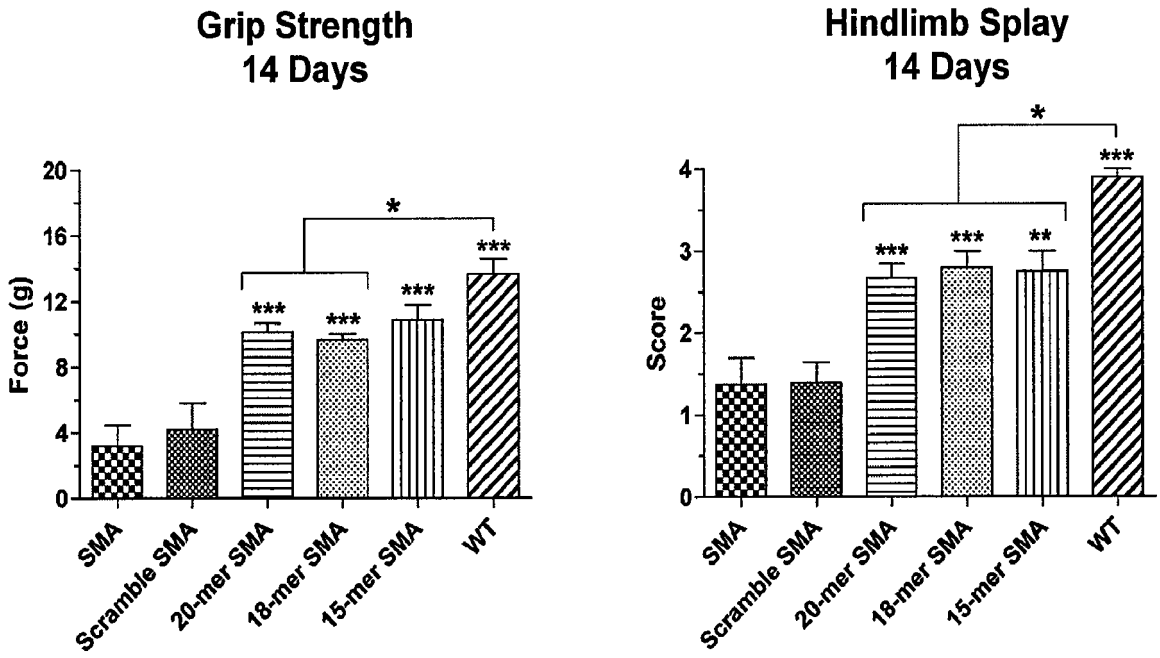

Grip Strength
14 Days
Force (g)
20
16
12
8
4
0
*
***
***
***
***
SMA
Scramble SMA
20-mer SMA
18-mer SMA
15-mer SMA
WT
Hindlimb Splay
14 Days
Score
4
3
2
1
0
*
***
***
***
**
***
SMA
Scramble SMA
20-mer SMA
18-mer SMA
15-mer SMA
WT

Figure 6

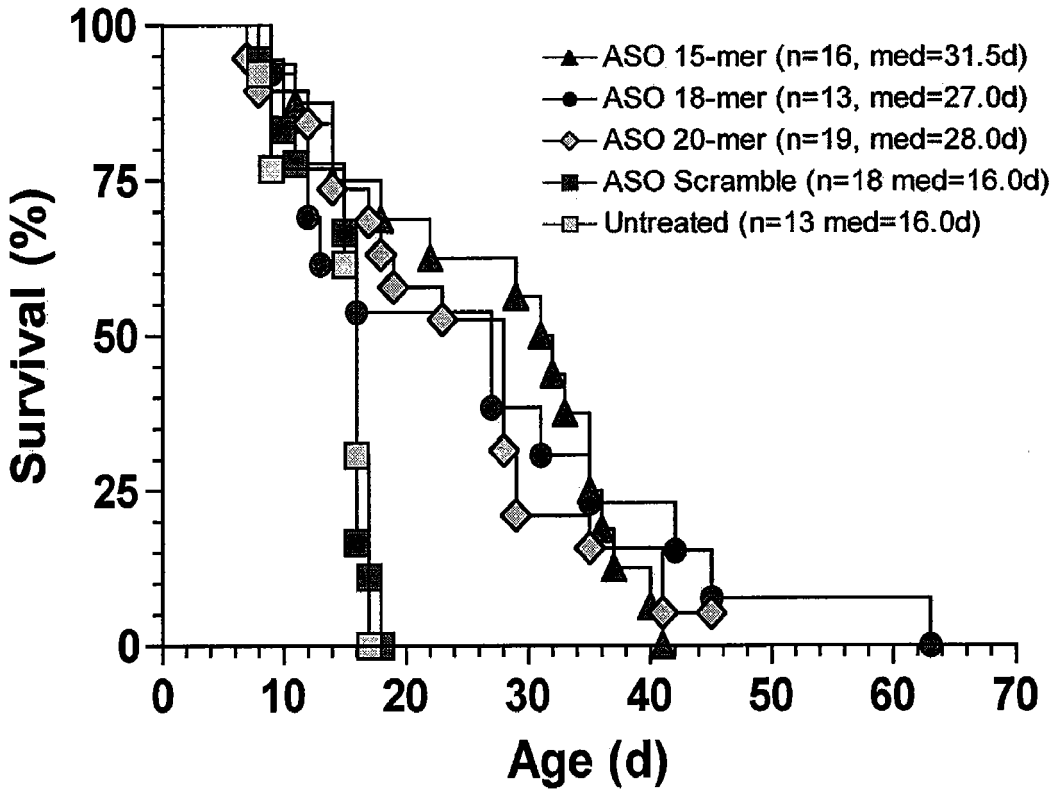
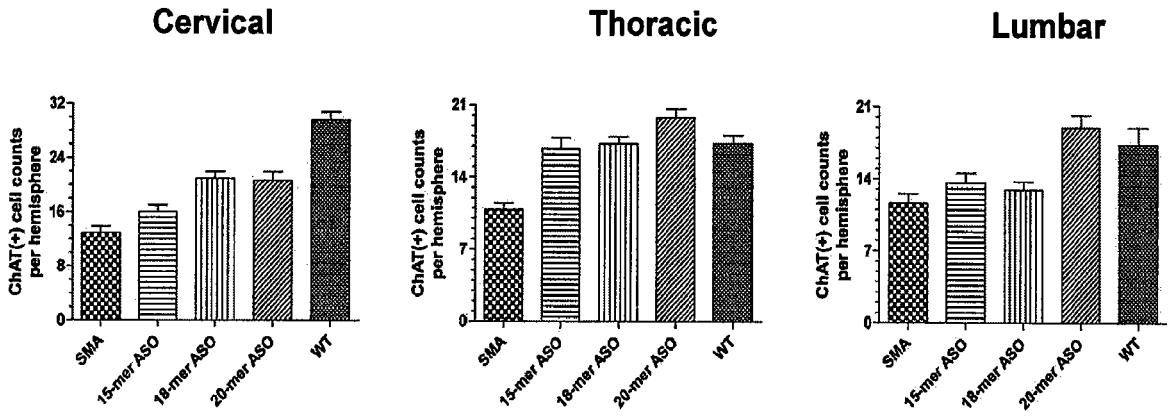

100
75
50
25
0
Survival (%)
0
10
20
30
40
50
60
70
Age (d)
ASO 15-mer (n=16, med=31.5d)
ASO 18-mer (n=13, med=27.0d)
ASO 20-mer (n=19, med=28.0d)
ASO Scramble (n=18 med=16.0d)
Untreated (n=13 med=16.0d)

Figure 7

ASO Treatment Increases Motor Neuron Cell Counts in the Spinal Cord



4 μg dose, 16 days post-injection

Figure 8

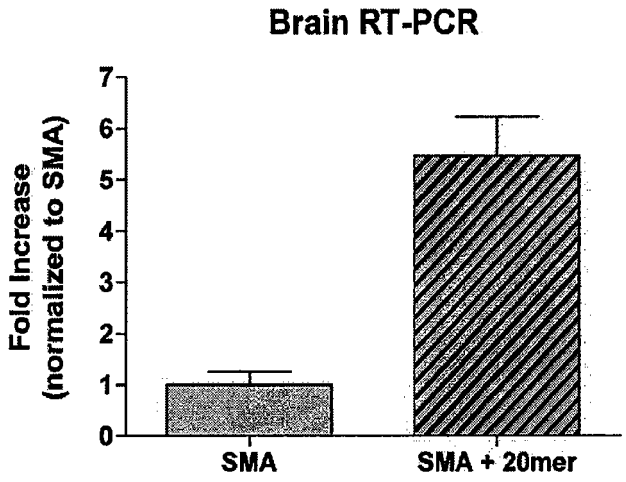

Brain RT-PCR
Fold Increase (normalized to SMA)
7
6
5
4
3
2
1
0
SMA
SMA + 20mer

Figure 9

Injections of 396443 at P0 (8 ug) and P21 (20 ug) vs. P0 alone (8 ug)

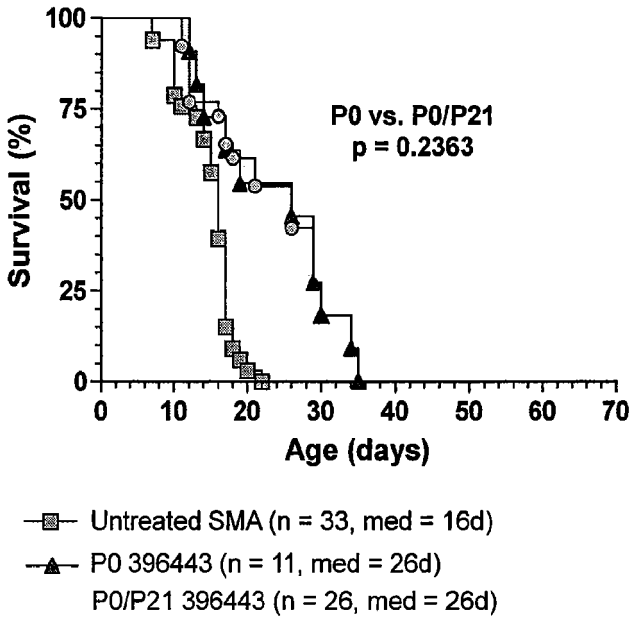


Figure 10

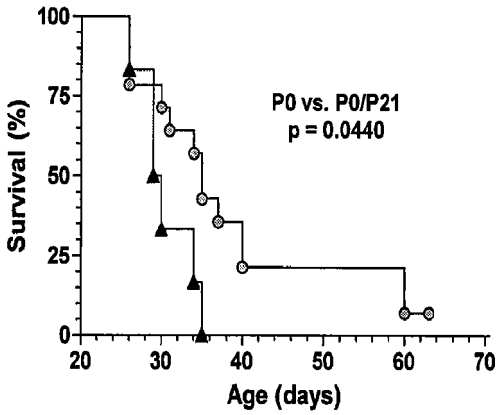

100
75
50
25
0
Survival (%)
20
30
40
50
60
70
Age (days)
P0 vs. P0/P21
p = 0.0440
P0 396443 (n = 6, med = 29.5d)
P0/P21 396443 (n = 14, med = 35d)

Figure 11

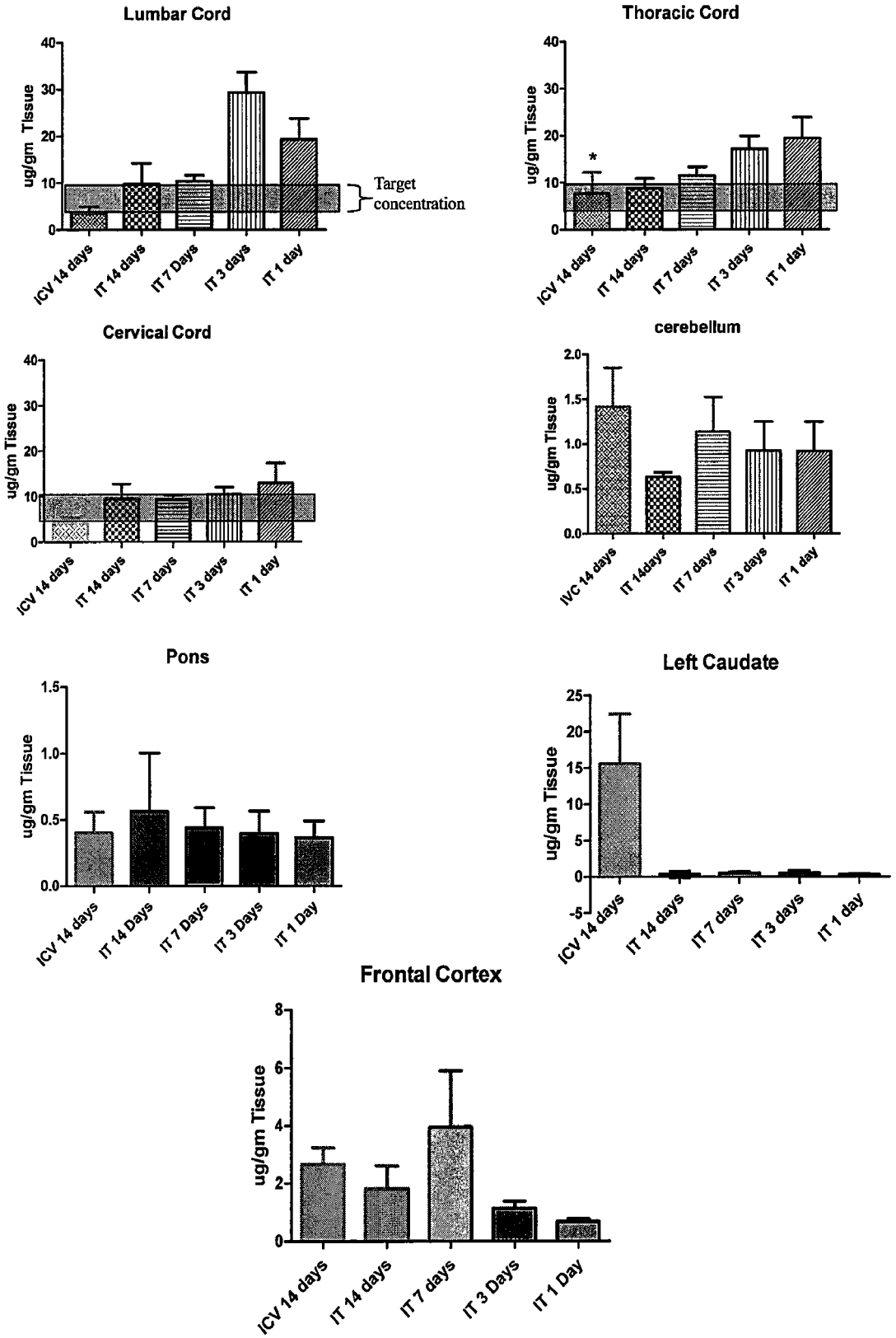

Lumbar Cord
ug/gm Tissue
Target concentration
ICV 14 days
IT 14 days
IT 7 Days
IT 3 days
IT 1 day
Thoracic Cord
ug/gm Tissue
*
ICV 14 days
IT 14 days
IT 7 days
IT 3 days
IT 1 day
Cervical Cord
ug/gm Tissue
ICV 14 days
IT 14 days
IT 7 days
IT 3 days
IT 1 day
cerebellum
ug/gm Tissue
IVC 14 days
IT 14days
IT 7 days
IT 3 days
IT 1 day
Pons
ug/gm Tissue
ICV 14 days
IT 14 Days
IT 7 Days
IT 3 Days
IT 1 Day
Left Caudate
ug/gm Tissue
ICV 14 days
IT 14 days
IT 7 days
IT 3 days
IT 1 day
Frontal Cortex
ug/gm Tissue
ICV 14 days
IT 14 days
IT 7 days
IT 3 Days
IT 1 Day

Figure 12

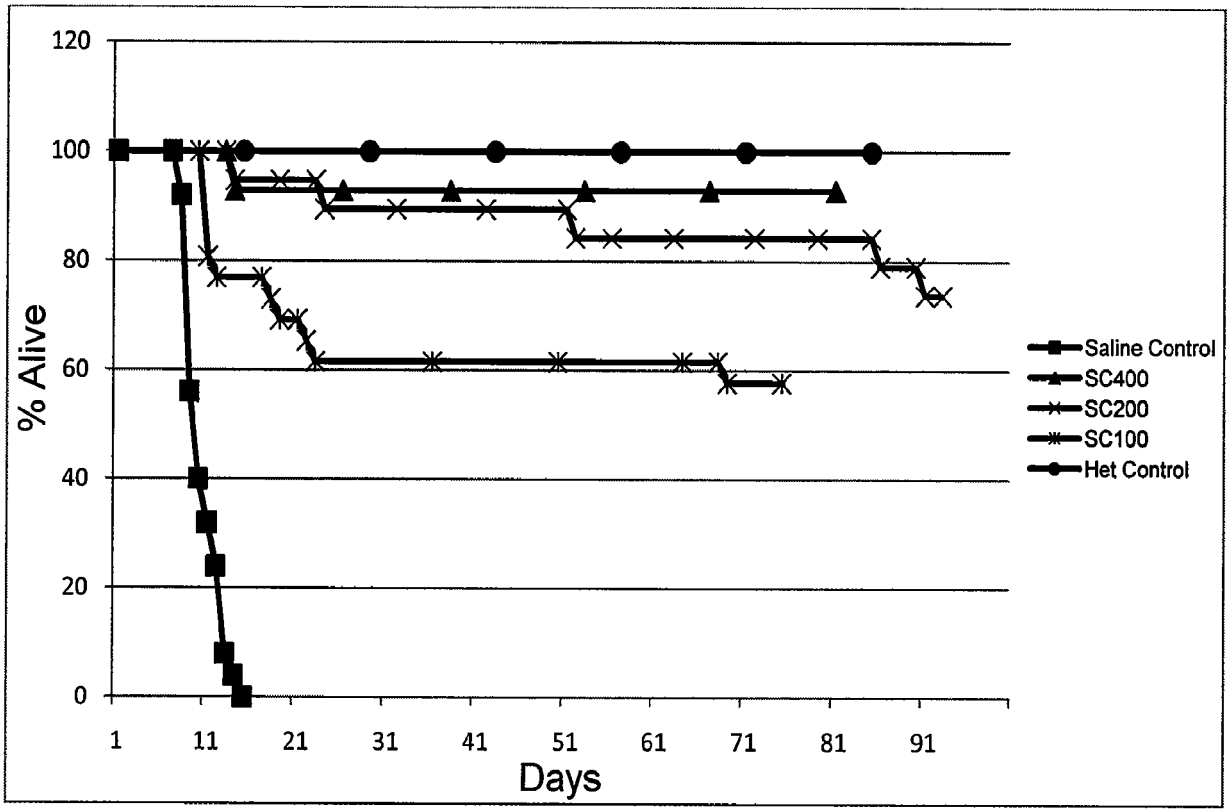

120
100
80
60
40
20
0
% Alive
1
11
21
31
41
51
61
71
81
91
Days
Saline Control
SC400
SC200
SC100
Het Control

Figure 13

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 6172216 B **[0004]**
- US 6214986 B **[0004]**
- US 5627274 A **[0004] [0162]**
- WO 9426887 A **[0004]**
- WO 2007002390 A **[0007] [0184]**
- WO 0238738 A **[0007]**
- WO 2008101157 A **[0127]**
- US 20050130923 A **[0127]**
- WO 2007134181 A **[0127]**
- US 2008068922 W **[0129]**
- WO 2009006478 A **[0129]**
- US 7399845 B **[0129]**
- US 2008064591 W **[0129]**
- WO 2008150729 A **[0129]**
- US 20040171570 A **[0129]**
- US 7427672 B **[0129]**
- US 2008066154 W **[0129]**
- WO 2008154401 A **[0129]**
- US 4981957 A **[0136]**
- US 5118800 A **[0136]**
- US 5319080 A **[0136]**
- US 5359044 A **[0136]**
- US 5393878 A **[0136]**
- US 5446137 A **[0136]**
- US 5466786 A **[0136]**
- US 5514785 A **[0136] [0150]**
- US 5519134 A **[0136]**
- US 5567811 A **[0136]**
- US 5576427 A **[0136]**
- US 5591722 A **[0136]**
- US 5597909 A **[0136]**
- US 5610300 A **[0136]**
- US 5627053 A **[0136]**
- US 5639873 A **[0136]**
- US 5646265 A **[0136]**
- US 5658873 A **[0136]**
- US 5670633 A **[0136]**
- US 5792747 A **[0136]**
- US 5700920 A **[0136]**
- US 334130 A **[0149]**
- US 6656730 B **[0149]**
- US 4828979 A **[0150]**
- US 4948882 A **[0150]**
- US 5218105 A **[0150]**
- US 5525465 A **[0150]**
- US 5541313 A **[0150]**
- US 5545730 A **[0150]**
- US 5552538 A **[0150]**
- US 5578717 A **[0150]**
- US 5580731 A **[0150]**
- US 5591584 A **[0150]**
- US 5109124 A **[0150]**
- US 5118802 A **[0150]**
- US 5138045 A **[0150]**
- US 5414077 A **[0150]**
- US 5486603 A **[0150]**
- US 5512439 A **[0150]**
- US 5578718 A **[0150]**
- US 5608046 A **[0150]**
- US 4587044 A **[0150]**
- US 4605735 A **[0150]**
- US 4667025 A **[0150]**
- US 4762779 A **[0150]**
- US 4789737 A **[0150]**
- US 4824941 A **[0150]**
- US 4835263 A **[0150]**
- US 4876335 A **[0150]**
- US 4904582 A **[0150]**
- US 4958013 A **[0150]**
- US 5082830 A **[0150]**
- US 5112963 A **[0150]**
- US 5214136 A **[0150]**
- US 5245022 A **[0150]**
- US 5254469 A **[0150]**
- US 5258506 A **[0150]**
- US 5262536 A **[0150]**
- US 5272250 A **[0150]**
- US 5292873 A **[0150]**
- US 5317098 A **[0150]**
- US 5371241 A **[0150]**
- US 5391723 A **[0150]**
- US 5416203 A **[0150]**
- US 5451463 A **[0150]**
- US 5510475 A **[0150]**
- US 5512667 A **[0150]**
- US 5565552 A **[0150]**
- US 5567810 A **[0150]**
- US 5574142 A **[0150]**
- US 5585481 A **[0150]**
- US 5587371 A **[0150]**
- US 5595726 A **[0150]**
- US 5597696 A **[0150]**
- US 5599923 A **[0150]**
- US 5599928 A **[0150]**
- US 5688941 A **[0150]**
- WO 9726270 A, Wincott **[0153]**
- US 20050020525 A **[0153]**
- WO 03004602 A **[0153]**

- US 6210892 B **[0162]**
- US 5665593 A **[0162]**
- US 5916808 A **[0162]**
- US 5976879 A **[0162]**
- US 20060172962 A **[0162]**
- US 2007002390 A **[0162]**
- US 20050074801 A **[0162]**
- US 20070105807 A **[0162]**
- US 20050054836 A **[0162]**
- WO 2007090073 A **[0162]**
- WO 2007047913 A **[0162]**
- US 06024469 W **[0184]**
- WO 2010148249 A **[0256]**
- WO 61218031 A **[0257]**

Non-patent literature cited in the description

- **CARTEGNI et al.** *Nat. Rev. Genet.,* 2002, vol. 3, 285-298 **[0003]**
- **DRAWCZAK et al.** *Hum. Genet.,* 1992, vol. 90, 41-54 **[0003]**
- **KOLE.** *Acta Biochimica Polonica,* 1997, vol. 44, 231-238 **[0003]**
- **TAYLOR et al.** *Nat. Biotechnol.,* 1999, vol. 17, 1097-1100 **[0004]**
- **WILTON et al.** *Neuromuscul. Disord.,* 1999, vol. 9, 330-338 **[0004]**
- **LEFEBVRE et al.** *Hum. Mol. Genet.,* 1998, vol. 7, 1531-1536 **[0005]**
- **CARTEGNI ; KRAINER.** *Nat. Genet.,* 2002, vol. 30, 377-384 **[0006]**
- **HUA et al.** *American J. of Human Genetics,* April 2008, vol. 82, 1-15 **[0007] [0162]**
- **SINGH et al.** *RNA Bio.,* 2009, vol. 6 (3), 1-10 **[0007]**
- **CARTEGNI ; KRAINER.** *Nat. Struct. Biol.,* 2003, vol. 10, 120-125 **[0007]**
- **HUA Y et al.** *The American Journal of Human Genetics,* 2008, vol. 82, 834-848 **[0007]**
- Carbohydrate Modifications in Antisense Research. American Chemical Society, 1994 **[0045]**
- Remington's Pharmaceutical Sciences. Mack Publishing Co, 1990 **[0045]**
- Antisense Drug Technology, Principles, Strategies, and Applications. CRC Press **[0045]**
- **SAMBROOK et al.** Molecular Cloning, A laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0045]**
- **BAKER et al.** *J. Biol. Chem.,* 1997, vol. 272, 11944-12000 **[0134]**
- **MARTIN, P.** *Helv. Chim. Acta,* 1995, vol. 78, 486-504 **[0134]**
- **ALTMANN et al.** *Chimia,* 1996, vol. 50, 168-176 **[0134]**
- **ALTMANN et al.** *Biochem. Soc. Trans.,* 1996, vol. 24, 630-637 **[0134]**
- **ALTMANN et al.** *Nucleosides Nucleotides,* 1997, vol. 16, 917-926 **[0134]**
- **LETSINGER et al.** *Proc. Natl. Acad. Sci. USA,* 1989, vol. 86, 6553-6556 **[0148]**
- **MANOHARAN et al.** *Bioorg. Med. Chem. Let.,* 1994, vol. 4, 1053-1060 **[0148]**
- **MANOHARAN et al.** *Ann. N.Y. Acad. Sci.,* 1992, vol. 660, 306-309 **[0148]**
- **MANOHARAN et al.** *Bioorg. Med. Chem. Let.,* 1993, vol. 3, 2765-2770 **[0148]**
- **OBERHAUSER et al.** *Nucl. Acids Res.,* 1992, vol. 20, 533-538 **[0148]**
- **SAISON-BEHMOARAS et al.** *EMBO J.,* 1991, vol. 10, 1111-1118 **[0148]**
- **KABANOV et al.** *FEBS Lett.,* 1990, vol. 259, 327-330 **[0148]**
- **SVINARCHUK et al.** *Biochimie,* 1993, vol. 75, 49-54 **[0148]**
- **MANOHARAN et al.** *Tetrahedron Lett.,* 1995, vol. 36, 3651-3654 **[0148]**
- **SHEA et al.** *Nucl. Acids Res.,* 1990, vol. 18, 3777-3783 **[0148]**
- **MANOHARAN et al.** *Nucleosides & Nucleotides,* 1995, vol. 14, 969-973 **[0148]**
- **MISHRA et al.** *Biochim. Biophys. Acta,* 1995, vol. 1264, 229-237 **[0148]**
- **CROOKE et al.** *J. Pharmacol. Exp. Ther.,* 1996, vol. 277, 923-937 **[0148]**
- **BEAUCAGE ; TYER.** *Tetrahedron,* 1993, vol. 49, 1925 **[0153]**
- **FREIER et al.** *Nucleic Acids Research,* 1997, vol. 25 (22), 4429-4443 **[0161]**
- **HUA et al.** *PLoS Biol,* vol. 5 (4), e73 **[0162]**
- **VICKERS et al.** *J. Immunol.,* 15 March 2006, vol. 176 (6), 3652-61 **[0162]**
- **YEO et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 2004, vol. 101 (44), 15700-15705 **[0164]**
- **SCAMBOROVA et al.** *Mol. Cell. Biol.,* 2004, vol. 24 (5), 1855-1869 **[0164]**
- **HOVHANNISYAN ; CARSTENS.** *Mol. Cell. Biol.,* 2005, vol. 25 (1), 250-263 **[0164]**
- **MINOVITSKY et al.** *Nucleic Acids Res.,* 2005, vol. 33 (2), 714-724 **[0164]**
- **AKINC et al.** *Nature Biotechnology,* 01 May 2008, vol. 26, 561-569 **[0171]**
- **GRAVRILINA et al.** *Hum Mol Genet,* 2008, vol. 17 (8), 1063-1075 **[0181]**
- **THURMOND et al.** *J. Med Chem.,* 2008, vol. 51, 449-469 **[0206]**
- **COADY et al.** *PLoS ONE,* 2008, vol. 3 (10), e3468 **[0207]**
- **PASSINI et al.** *J Clin Invest,* 01 April 2010, vol. 120 (4), 1253-64 **[0207]**
- **MONANI et al.** *Hum. Mol. Genet.,* 2000, vol. 9, 333-339 **[0219]**

- **HSIEH-LI et al.** *Nat. Genet.,* 2000, vol. 24, 66-70 **[0219]**
- **PASSINI et al.** *J. Virol.,* 2001, vol. 75, 12382-12392 **[0231]**
- **LE et al.** *Hum. Mol. Genet.,* 2005, vol. 14, 845-857 **[0231]**
- **HSIEH-LI HM et al.** *Nature Genet.,* 2000, vol. 24, 66-70 **[0241]**