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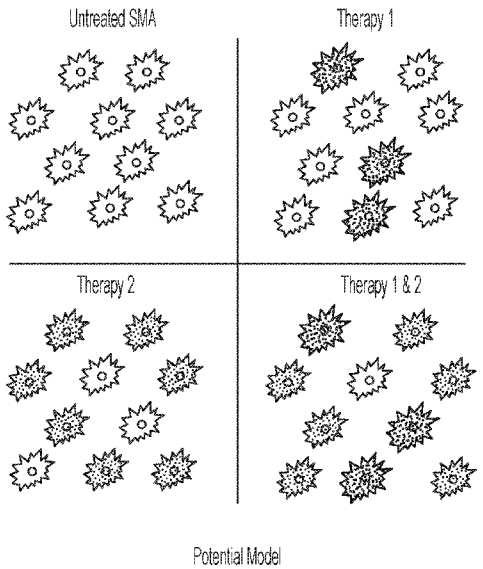
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FIG. 11



(57) Abstract: Aspects of the application relate to compositions and methods for treating spinal muscular atrophy in a subject. In particular, this application provides therapeutic combinations of a recombinant nucleic acid that encodes the survival of motor neuron 1 (SMN1) protein (e.g., in a viral vector), and an antisense oligonucleotide (ASO) that increases full-length survival of motor neuron 2 (SMN2) mRNA (e.g., that is targeted to a nucleic acid molecule encoding the survival of motor neuron 2 (SMN2) and that promotes the inclusion of exon 7 in SMN2 mRNA).

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COMBINATION THERAPY FOR SPINAL MUSCULAR ATROPHY

RELATED APPLICATIONS

This Application claims the benefit under 35 U.S.C. 119(e) of the filing date of U.S. Provisional Application Serial Numbers 62/764,893, filed August 15, 2018, entitled "COMBINATION THERAPY FOR SPINAL MUSCULAR ATROPHY", and 62/783,189, filed December 20, 2018, entitled "COMBINATION THERAPY FOR SPINAL MUSCULAR ATROPHY". The entire contents of each application are incorporated herein by reference.

FIELD

The present application relates to methods and compositions for treating spinal muscular atrophy (SMA).

BACKGROUND

Spinal muscular atrophy (SMA) is a neuromuscular disease caused by mutations in telomeric SMN1, a gene encoding a ubiquitously expressed protein (survival of motor neuron - SMN) involved in spliceosome biogenesis.

The SMN gene product is intracellular and SMN deficiency results in selective toxicity to lower motor neurons, resulting in progressive neuron loss and muscle weakness. The severity of the disease is modified by the copy number of a centromeric duplication of the homologous gene (SMN2), which carries a splice site mutation that results in production of only small amounts of the full length SMN transcript. Patients who carry one to two copies of SMN2 present with the severe form of SMA, characterized by onset in the first few months of life and rapid progression to respiratory failure. Patients with three copies of SMN2 generally exhibit an attenuated form of the disease, typically presenting after six months of age. Though many never gain the ability to walk, they rarely progress to respiratory failure, and often live into adulthood. Patients with four SMN2 copies may not present until adulthood with gradual onset of muscle weakness.

Although several therapies for SMA have been developed, there remains a need for treatments that increase intracellular SMN activity in motor neurons involved in spinal muscular atrophy for patients having different levels of disease severity.

SUMMARY

In some aspects, the present application relates to a treatment for spinal muscular atrophy (SMA) that involves a combined administration, to a subject having SMA, of a recombinant nucleic acid encoding Survival motor neuron 1 (SMN1) and an oligomeric compound that increases full-length Survival motor neuron 2 (SMN2) mRNA. In some aspects, a recombinant nucleic acid encoding SMN1 is provided in a viral vector, for example in a recombinant adeno-associated virus (rAAV). In some aspects, an oligomeric compound is an antisense oligonucleotide (ASO) that increases full-length SMN2 mRNA in a subject (e.g., by modulating SMN2 pre-mRNA splicing to increase the inclusion of exon 7 in SMN2 mRNA).

In some aspects, the present application relates to a treatment for spinal muscular atrophy (SMA) that involves a combined administration, to a subject having SMA, of a recombinant nucleic acid encoding Survival motor neuron 1 (SMN1) and an oligomeric compound that induces exon-skipping in a nucleic acid encoding Survival motor neuron 2 (SMN2). In some aspects, a recombinant nucleic acid encoding SMN1 is provided in a viral vector, for example in a recombinant adeno-associated virus (rAAV). In some aspects, an oligomeric compound that induces exon-skipping in a nucleic acid encoding SMN2 is an antisense oligonucleotide (ASO) that induces exon-skipping in SMN2 pre-mRNA.

In some aspects, the recombinant nucleic acid (e.g., in a viral vector) and the ASO are co-formulated and administered to a subject as a single composition. In some aspects, the recombinant nucleic acid (e.g., in a viral vector) and the ASO are provided as separate compositions, but administered to a subject concurrently (e.g., at the same time or contemporaneously, for example during the same medical visit, for example during the same hour or day). In some aspects, the recombinant nucleic acid (e.g., in a viral vector) and the ASO are provided as separate compositions, and administered to a subject sequentially during separate medical visits (for example, at different times, e.g., on different days) during a course of treatment (e.g., during a treatment regimen over a week, 2-4 weeks, a month, 1-12 months, a year, 2-5 years, or longer). In some aspects, the ASO is administered prior to and/or subsequent to the recombinant nucleic acid. In some aspects, the recombinant nucleic acid (e.g., in a viral vector) and/or ASO are administered at different frequencies. In some aspects, a subject is treated with a combination of a) a composition comprising both the recombinant nucleic acid (e.g., in a viral vector) and the ASO, and b) separate compositions

that comprise either the recombinant nucleic acid (e.g., in a viral vector) or the ASO. In some aspects, two or more different recombinant SMN1 nucleic acids are administered to a subject. In some aspects, two or more different SMN2 ASOs are administered to a subject. In some aspects, different recombinant SMN1 nucleic acids and/or different SMN2 ASOs are administered to a subject during different medical visits.

Accordingly, in some aspects a method of treating SMA in a subject (e.g., a human subject) having SMA involves administering to the subject a recombinant nucleic acid that encodes SMN1 (also referred to as a recombinant SMN1 gene), and an ASO that increases full-length SMN2 mRNA in a subject (also referred to as an SMN2 ASO). In some aspects, a method of treating SMA in a subject comprises administering an effective amount of a recombinant SMN1 gene and an SMN2 ASO to a subject having SMA.

In some aspects, a subject having SMA has one or more symptoms of SMA (e.g., atrophy of the limb muscles, difficulty or inability walking, difficulty breathing, or other symptom of SMA). In some aspects, a subject having SMA has two mutant alleles of the genomic SMN1 gene. In some aspects, the subject has a deletion or a loss of function point mutation in each SMN1 allele. In some aspects, the subject is homozygous for a SMN1 gene mutation. In some aspects, the subject is heterozygous for two different SMN1 gene mutations.

In some aspects, the subject is a human subject. In some aspects, the subject is selected from the pediatric and adult population. In some aspects, the subject is greater than or equal to 18 years of age (e.g., 18 years of age or older). In some aspects, the subject is younger than 18 years of age, younger than 10 years of age, or younger than 6 years of age. In some aspects, the subject is around 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, or 5 years of age.

In some aspects, the recombinant SMN1 gene is operatively linked to a promoter. In some aspects, the SMN1 gene is a human SMN1 gene. In some aspects, the SMN1 gene is codon optimized (e.g., for expression in humans). In some aspects, the recombinant nucleic acid encoding the SMN1 gene is a recombinant AAV genome comprising flanking AAV inverted terminal repeats (ITRs). In some aspects, the recombinant nucleic acid is administered within an AAV particle. In some aspects, the AAV particle comprises AAV capsid proteins (e.g., AAV9, AAVrh10, AAV8 capsid proteins). In some aspects, the AAV

particle comprises AAVhu68 capsid proteins. In some aspects, the AAV particle comprises AAV9 capsid proteins. In some aspects, the ASO alters the splicing pattern of survival of motor neuron 2 (SMN2) pre-mRNA. In some aspects, the ASO promotes the inclusion of exon 7 in survival of motor neuron 2 (SMN2) mRNA. In some aspects, the SMN2 ASO comprises a sequence complementary to intron 6 or intron 7 of a nucleic acid molecule encoding the SMN2 protein. In some aspects, the ASO comprises a sequence complementary to intron 6 of a nucleic acid molecule encoding SMN2 protein. In some aspects, the ASO comprises a sequence complementary to intron 7 of a nucleic acid molecule encoding SMN2 protein. In some aspects, the ASO comprises a sequence of SEQ ID NO: 1. In some aspects, the ASO is nusinersen. In some aspects, the ASO comprises one or more nucleobase or backbone modifications.

In some aspects, a recombinant SMN1 gene (e.g., in a viral vector) is administered (e.g., one or more times) to a subject previously treated with an SMN2 ASO therapy. In some aspects, a recombinant SMN1 gene (e.g., in a viral vector) is administered (e.g., one or more times) to a subject undergoing a current treatment with an SMN2 ASO therapy. In some aspects, a therapy comprising a concurrent or sequential administration of a recombinant SMN1 gene (e.g., in a viral vector) and an SMN2 ASO is initiated for a subject.

In some aspects, an rAAV comprising a recombinant SMN1 gene (also referred to as an SMN1 rAAV) and the SMN2 ASO are administered simultaneously. In some aspects, the SMN1 rAAV and the SMN2 ASO are administered concurrently. In some aspects, the SMN1 rAAV and the SMN2 ASO are administered together in a single composition. In some aspects, the SMN1 rAAV and the SMN2 ASO are administered separately. In some aspects, the SMN1 rAAV and the SMN2 ASO are administered sequentially. In some aspects, the SMN1 rAAV and the SMN2 ASO are administered at different frequencies. In some aspects, the SMN1 rAAV is administered once. In some aspects, the SMN2 ASO is administered 1-6 times per year. In some aspects, two or more subsequent doses of the SMN2 ASO alone are administered following an initial administration of the SMN1 rAAV and the SMN2 ASO. In some aspects, a subject receives one or more additional doses of SMN1 rAAV. In some aspects, first and second administrations of SMN1 rAAV are provided to a subject more than 6 months apart or more than 1 year apart. In some aspects, first and second SMN1 rAAV compositions comprise the same rAAV capsid protein. In

some aspects, first and second SMN1 rAAV compositions comprise different rAAV capsid proteins.

In some aspects, the SMN1 rAAV is administered at a dose from 1 x 10^{10} to 5 x 10^{14} GC. In some aspects, the SMN1 rAAV is administered at a dose from 2 x 10^{10} to 2 x 10^{14} GC. In some aspects, the SMN1 rAAV is administered at a dose from 3 x 10^{13} to 5 x 10^{14} GC. In some aspects, the SMN1 rAAV is administered at a dose of 2 x 10^{14} GC.

In some aspects, a total of 5 mg to 60 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 5 mg to 20 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 50 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 48 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 36 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 28 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg per dose of SMN2 ASO is administered to the subject. In some aspects, the dose volume is 5 mL.

In some aspects, the SMN1 rAAV and the SMN2 ASO are administered into the intrathecal space of the subject. In some aspects, the SMN1 rAAV and the SMN2 ASO are administered into the intracisternal magna space of the subject. In some aspects, initial and/or subsequent doses of the SMN2 ASO are administered intravenously or intramuscularly.

In some aspects, administration of the SMN1 rAAV and the SMN2 ASO increase intracellular SMN protein level in the subject. In some aspects, SMN protein level is increased in the cervical, thoracic, and lumbar spinal cord segments of the subject (e.g., in motor neurons in the brain and/or spinal cord of the subject).

Accordingly, in some aspects, SMN protein expression in a subject having SMA is increased by administering to the subject an effective amount of a composition comprising an SMN1 rAAV and an SMN2 ASO. In some aspects, the subject had previously been administered an SMN1 rAAV. In some aspects, the subject had previously been treated with an SMN2 ASO. In some aspects, SMN protein expression in a subject previously treated with an SMN1 rAAV is increased by administering an effective amount of an SMN2 ASO to the subject. In some aspects, SMN protein expression in a subject previously treated with an SMN2 ASO is increased by administering an effective amount of an SMN1 rAAV to the

subject. In some aspects, the pharmaceutical composition is administered to the CNS or CSF of the subject. In some aspects, the pharmaceutical composition is administered intravenously to the subject.

In some aspects, a composition comprises both an SMN1 rAAV and an SMN2 ASO. In some aspects, a pharmaceutical composition comprises both an SMN1 rAAV and an SMN2 ASO and a pharmaceutically acceptable carrier. In some aspects, a therapeutically effective amount of the pharmaceutical composition is administered to a subject in need thereof.

In some aspects, one or more combinations of an SMN1 rAAV and an SMN2 ASO (e.g., both together in a single compositions or as two separate compositions) are administered to a subject (e.g., a human subject) via an intrathecal route. In some aspects, one or more combinations of an SMN1 rAAV and an SMN2 ASO are administered (e.g., via injection, infusion, using a pump and a catheter, or via other suitable technique) into the spinal canal, subarachnoid space, ventricular or lumbar CSF, by suboccipital puncture, or by other suitable route. In some aspects, one or more combinations of an SMN1 rAAV and an SMN2 ASO (e.g., both together in a single compositions or as two separate compositions) are administered to a subject (e.g., a human subject) via an intracranial, intraventricular, intracerebral, intraparenchymal, intravenous, or other suitable route. Whether administered concurrently or sequentially, each of the SMN1 rAAv and SMN2 ASO may be administered by any suitable or appropriate means known in the art (e.g., intrathecal, intravenous, etc.), and the SMN1 rAAV and SMN2 ASO may be administered by the same or by different means (e.g., via the same or different routes of administration).

In some aspects, an SMN1 rAAV and/or an SMN2 ASO are used 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).

In some aspects, the present disclosure relates to a method of treating spinal muscular atrophy (SMA) in a subject having SMA, comprising administering an effective amount of a composition comprising an rAAV encoding SMN1 to a subject that was previously treated with an ASO that increases full-length SMN2 mRNA.

In some aspects, the present disclosure relates to a method of treating spinal muscular atrophy (SMA) in a subject having SMA, comprising administering an effective amount of a

composition comprising an ASO that increases full-length SMN2 mRNA to a subject that was previously administered an rAAV encoding SMN1.

Other aspects of the present disclosure relates to a composition comprising an rAAV encoding SMN1 and an ASO that is capable of increasing full-length SMN2 mRNA. In some aspects, the rAAV comprises AAV9 capsid proteins. In some aspects, the ASO is nusinersen. In some aspects, the composition is a pharmaceutical composition and comprises a pharmaceutically acceptable carrier.

Other aspects and advantages of the invention will be readily apparent from the following detailed description of the invention.

BRIEF DESCRIPTION OF FIGURES

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present application, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific aspects presented herein.

FIG. 1 illustrates increased levels of SMN activity in a greater number of motor neurons in a subject receiving combined treatment with a recombinant nucleic acid that encodes SMN1 and an antisense oligonucleotide (e.g., nusinersen) that increases full-length SMN2 mRNA (e.g., promotes exon 7 inclusion in SMN2 mRNA);

FIG. 2 is a schematic representation of a non-limiting example of a nucleic acid that encodes SMN1;

FIG. 3 illustrates the chemical structure of nusinersen, a non-limiting example of an antisense oligonucleotide that increases full-length SMN2 mRNA (e.g., promotes exon 7 inclusion in SMN2 mRNA);

FIG. 4 shows the distribution of rAAV following different modes of administration in non-human primates;

FIGs. 5A-5E illustrate the physical and biological compatibility of a recombinant nucleic acid that encodes SMN1 and an antisense oligonucleotide that increases full-length SMN2 mRNA (e.g., promotes exon 7 inclusion in SMN2 mRNA);

FIGs. 6A-6B show that the administration of either an SMN1 gene (e.g., in an rAAV vector) or an SMN2 ASO (e.g., nusinersen, for example in a single dose) partially rescues motor function at postnatal day (PND) 8** with full rescue at PND 16, post dosing. They also show that body weight lags behind the WT control. FIG. 6A is a set of graphs showing the righting reflex (RR) of 4 separate groups after 8 and 16 days of ASO (nusinersen). FIG. 6B is a set of graphs showing the body weight of 4 separate groups after 8 and 16 days of ASO (nusinersen). The partial rescue of RR (PND 7-16) and body weight provides a window for an additional benefit of combination therapy in this pre-clinical model;

FIGs. 7A-7C show the results of a first study with body weight and RR as the primary end points for treatment with a combination of SMN1 gene therapy (in an rAAV vector) and ASO (nusinersen). FIG. 7A is a graph showing the body weight change over time (in days). FIG. 7B is a graph showing the RR change over time (in days). FIG. 7C is a chart outlining conditions for the three testing groups;

FIGs. 8A-8C show the results of a second study with body weight and RR as the primary end points for treatment with a combination of SMN1 gene therapy (in an rAAV vector) and ASO (nusinersen). FIG. 8A is a chart outlining conditions for the three testing groups. FIG. 8B is a graph showing the body weight change over time (in days). FIG. 8C is a graph showing the RR change over time (in days);

FIGs. 9A-9B show the comparison of % change in body weight from PND 7-PND 13. FIG. 9A shows the % change in body weight at a dose of gene therapy (rAAV): 1 x 10^{10} GC/ASO (nusinersen): 1µg. FIG. 9B shows the % change in body weight a dose of gene therapy (rAAV): 3 x 10^{10} GC/ASO (nusinersen): 3µg;

FIGs. 10A-10B show the comparison of % change in RR from PND 7-PND 13. FIG. 10A shows the % change in RR at a dose of gene therapy (rAAV): 1 x 10^{10} GC/ASO (nusinersen): 1µg. FIG. 10B shows the % change in RR at a dose of gene therapy (rAAV): 3 x 10^{10} GC/ASO (nusinersen): 3µg; and,

FIG. 11 illustrates complementarity in neuronal and non-neuronal cells for a combination therapy.

DETAILED DESCRIPTION

The present application relates to compositions and methods for treating spinal muscular atrophy (SMA) in a subject, for example in a human subject having SMA. In some aspects, a treatment comprises administering, to a subject having SMA, both a recombinant nucleic acid that expresses the SMN1 gene (e.g., in a viral vector) and an antisense oligonucleotide (ASO) that increases full-length SMN2 mRNA (e.g., an ASO that promotes the inclusion of exon 7 in SMN2 mRNA) in the subject.

In some aspects, a combination of a recombinant nucleic acid that expresses SMN1 and an antisense oligonucleotide that increases full-length SMN2 mRNA (e.g., an ASO that promotes the inclusion of exon 7 in SMN2 mRNA) can provide enhanced intracellular SMN protein levels in some motor neurons and also increase the number of motor neurons in which intracellular survival-of-motor-neuron (SMN) protein levels are elevated relative to treatment with either the recombinant nucleic acid or the ASO alone, as illustrated in FIGs. 1 and 11. Methods and compositions for combined administration of a recombinant nucleic acid that expressed SMN1 and an ASO that increases full-length SMN2 mRNA (e.g., an ASO that promotes the inclusion of exon 7 in SMN2 mRNA) in SMN2 can be useful to provide therapeutically effective levels of SMN protein in a subject having SMA, and also to treat subjects having different levels of disease severity.

Spinal muscular atrophy or 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 a leading cause of death among infants. The severity of SMA varies among patients and has thus been classified into different types depending on the age of onset and motor development milestones. SMA 0 designation has been proposed to reflect prenatal onset and severe joint contractures, facial diplegia, and respiratory failure. Three types of post-natal form of SMA have been designated. Type I SMA (also called Werdnig-Hoffmann disease) 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 and have serious respiratory dysfunction. Type II SMA is the intermediate form with onset within the first 2 years. Children with Type II SMA are able to sit, but cannot stand or walk. Type III (also called Kugelberg-Welander disease) begins after 18 months to 2 years of age (Lefebvre et al., Hum. Mol. Genet., 1998, 7, 1531-1536) and usually has a chronic evolution. Children with Type III SMA can stand and walk unaided at least in infancy. Adult form (type IV) is the mildest form of SMA, with onset

after 30 years of age, and few cases have been reported. Type III and type IV SMA are also known as later-onset SMA.

The molecular basis of SMA results from 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 SMN delta 7 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.

Although SMA is caused by the homozygous loss of both functional copies of the SMN1 gene, 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.

In some aspects, intracellular SMN protein levels can be increased by contacting motor neurons with both a recombinant nucleic acid that encodes a recombinant SMN1 gene to promote intracellular expression of a recombinant SMN protein, and an ASO that modulates intracellular SMN2 splicing such that the percentage of cellular SMN2 transcripts containing exon 7 is increased thereby resulting in increased expression of full length SMN protein from cellular SMN2 transcripts. In some aspects, a combined treatment with both the recombinant nucleic acid that encodes an SMN1 gene (also referred to herein as a recombinant SMN1 gene) and the ASO that increases full-length SMN2 mRNA (e.g., an ASO that increases the intracellular level of full-length SMN2 mRNA, for example by promoting the inclusion of exon 7 in SMN2 mRNA). In some aspects, increasing

intracellular levels of full-length SMN2 mRNA is useful to target multiple aspects of SMA and can be useful for treating a range of subjects having different disease severities including patients having different types of SMA, including patients having different genomic copy numbers of the SMN2 gene.

In some aspects, the recombinant SMN1 gene (e.g., rAAV encoding SMN1) and the SMN2 ASO are administered concurrently. In some aspects, the recombinant SMN1 gene (e.g., rAAV encoding SMN1) and the SMN2 ASO are administered sequentially.

In some aspects, a combined treatment comprises administering a composition comprising both the recombinant SMN1 gene and the SMN2 ASO co-formulated together. In some aspects, a combined treatment comprises administering a first composition comprising the recombinant SMN1 gene and a separate second composition comprising the SMN2 ASO. In some aspects, the first and second compositions are administered concurrently (e.g., simultaneously or at different times during the same medical visit, for example during the same visit to a hospital, clinic, or other medical center where the subject receives a treatment). In some aspects, the first and second compositions are administered to a subject sequentially, for example during sequential medical visits during which a subject receives either the first or the second composition.

Accordingly, in some aspects, the first and second compositions are administered to the subject separately at different times (e.g., at different times of a day, on different days in the same week, or on different weeks). In some aspects, the first and second compositions are administered at different frequencies. In some aspects, a composition comprising the recombinant SMN1 gene is administered less frequently than a composition comprising the SMN2 ASO.

Accordingly, in some aspects a recombinant SMN1 gene is administered to a subject before the subject is treated with an SMN2 ASO. However, in other aspects a subject is treated with an SMN2 ASO before being administered a recombinant SMN1 gene.

In some aspects, a subject can be treated with both i) a pharmaceutical composition comprising a recombinant SMN1 gene and an SMN2 ASO co-formulated together and ii) separate pharmaceutical compositions comprising either the recombinant SMN1 gene or the SMN2 ASO. For example, in some aspects, a subject is initially treated with a composition comprising both the recombinant SMN1 gene and the SMN2 ASO, and subsequently with a composition comprising either the recombinant SMN1 gene or the SMN2 ASO. In some

aspects, a subject may receive two or more doses of a composition comprising both the recombinant SMN1 gene and the SMN2 ASO, and two or more separate doses of composition comprising either the recombinant SMN1 gene or the SMN2 ASO.

In some aspects, one, two or more subsequent doses of SMN2 ASO alone are administered following an initial administration of a combination of recombinant SMN1 gene and SMN2 ASO. In some aspects, one, two or more subsequent doses of recombinant SMN1 gene are administered following an initial administration of a combination of recombinant SMN1 gene and SMN2 ASO. In some aspects, a combination of recombinant SMN1 gene and SMN2 ASO are administered following an initial administration of either recombinant SMN1 gene alone or SMN2 ASO alone.

The order and frequency of administration of compositions comprising both the recombinant nucleic acid and the ASO and compositions comprising either the recombinant nucleic acid or the ASO can be adjusted for individual treatments.

In some aspects, pharmaceutical compositions comprising both a recombinant SMN1 gene (e.g., in a viral vector) and an SMN2 ASO, or different doses of the recombinant SMN1 gene or the SMN2 ASO are provided.

A variety of assays exist for measuring SMN expression and activity levels in vitro. See, e.g., Tanguy et al, 2015, cited above. The methods described herein can also be combined with any other therapy for treatment of SMA or the symptoms thereof. See, also, Wang et al, Consensus Statement for Standard of Care in Spinal Muscular Atropy, which provides a discussion of the present standard of care for SMA and http://ww v.ncbi.nim.nih.g v/¾oc ^s/ IB 1352/. For example, when nutrition is a concern in SMA, placement of a gastrostomy tube is appropriate. As respiratory function deteriorates, tracheotomy or noninvasive respiratory support is offered. Sleep-disordered breathing can be treated with nighttime use of continuous positive airway pressure. Surgery for scoliosis in individuals with SMA II and SMA III can be carried out safely if the forced vital capacity is greater than 30%-40%. A power chair and other equipment may improve quality of life. See also, US Patent No. 8,211,631, which is incorporated herein by reference.

Recombinant nucleic acids that encode SMN1

In some aspects, a combined therapy for treating SMA includes a recombinant nucleic acid that encodes SMN1 (e.g., administered in a viral vector, such as an rAAV). In some aspects, a recombinant nucleic acid that encodes SMN1 (also referred to herein as a recombinant SMN1 gene) comprises an SMN1 gene operatively linked to a promoter (e.g., to a promoter that is active in motor neuron cells). In some aspects, a recombinant nucleic acid that encodes SMN1 is provided in a non-viral vector (e.g., in a non-viral plasmid). However, in some aspects, a recombinant nucleic acid that encodes SMN1 is provided in a recombinant viral vector (e.g., in a recombinant viral genome packaged within a viral capsid). In some aspects, the recombinant SMN1 gene is provided in a recombinant adeno-associated viral (rAAV) genome and packaged within an AAV capsid particle.

In some aspects a recombinant SMN1 gene is administered to a subject in a viral vector. In some aspects, the recombinant SMN1 gene is administered in a recombinant AAV genome comprising flanking AAV inverted terminal repeats (ITRs). Accordingly, in some aspects a recombinant viral particle (e.g., an rAAV particle) comprising a gene that encodes SMN1 is administered to a subject along with an SMN2 ASO.

FIG. 2 provides a non-limiting example of a recombinant viral genome that comprises an SMN1 gene operably linked to a promoter. FIG. 2 illustrates an SMN1 gene flanked by AAV ITRs. The SMN1 gene comprises a human SMN1 codon optimized SMN1 open reading frame and is operably linked to a CB7 promoter (chicken beta actin promoter with a cytomegalovirus (CMV) enhancer). The recombinant AAV genome also comprises a chicken beta-actin intron, and a rabbit beta-globin poly A signal. The rAAV genome illustrated in FIG. 2 is non-limiting and alternative SMN1 coding sequences, promoters, and other regulatory elements can be used.

In some aspects, the rAAV genome is packaged in a viral capsid. In some aspects, the capsid proteins are hu68 serotype capsid proteins. However, other capsid proteins of other serotypes can be used.

These and other aspects of the recombinant SMN1 gene are described in more detail in the following paragraphs.

SMN1 coding sequences:

In some aspects, a coding sequence that encodes a wild-type human SMN protein (e.g., SMN1 cDNA sequence) is provided. Nucleic acid sequences encoding the human SMN1 are known in the art. See, e.g., GenBank Accession Nos. NM_001297715.1; NM_000344.3; NM_022874.2., DQ894095, NM—000344, NM—022874, and BC062723 for non-limiting examples of nucleic acid sequences of human SMN1. A non-limiting example of an amino acid sequence for wild-type human SMN protein is provided in UniProtKB/Swiss-Prot: Q16637.1. Other publications describing SMN1 coding sequence are, see, e.g., WO2010129021A1, and WO2009151546A2, the entire contents of which are incorporated herein by reference.

In some aspects, a coding sequence that encodes a functional SMN protein is provided. In some aspects, the amino acid sequence of the functional SMN1 is that of a human SMN1 protein or a sequence sharing 95% identity therewith.

In some aspects, a modified hSMN1 coding sequence is provided. In some aspects, the modified hSMN1 coding sequence has less than about 80% identity, preferably about 75% identity or less to a full-length native hSMN1 coding sequence. In some aspects, the modified hSMN1 coding sequence is characterized by an improved translation rate as compared to native hSMN1 following AAV-mediated delivery (e.g., using an rAAV particle). In some aspects, the modified hSMN1 coding sequence shares less than about 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61% or less identity to a full length native hSMN1 coding sequence.

The term "percent (%) identity", "sequence identity", "percent sequence identity", or "percent identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a fragment of at least about 500 to 5000 nucleotides, is desired. However, identity among smaller fragments, e.g., of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired.

"Aligned" sequences or "alignments" refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence.

Alignments can be performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Sequence alignment programs are available for amino acid sequences, e.g., the "Clustal X", "MAP", "PIMA", "MSA", "BLOCKMAKER", "MEME", and "Match-Box" programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. See, e.g., J. D. Thomson et al, Nucl. Acids. Res., "A comprehensive comparison of multiple sequence alignments", 27(13):2682-2690 (1999).

Multiple sequence alignment programs are also available for nucleic acid sequences. Examples of such programs include, "Clustal W", "CAP Sequence Assembly", "BLAST", "MAP", and "MEME", which are accessible through Web Servers on the internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta™, a program in GCG Version 6.1. Fasta™ provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta™ with its default parameters (a word size of 6 and the NOP AM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference.

In some aspects, the modified hSMN1 coding sequence is a codon optimized sequence, optimized for expression in the subject species. As used herein, the "subject" is a mammal, e.g., a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon or gorilla. In some aspects, the subject is a human. Accordingly, in some aspects an SMN1 coding sequence is codon optimized for expression in a human.

Codon-optimized coding regions can be designed by various different methods. This optimization may be performed using methods which are available online (e.g., GeneArt), published methods, or a company which provides codon optimizing services, e.g., DNA2.0 (Menlo Park, CA). One codon optimizing method is described, e.g., in US International Patent Publication No. WO 2015/012924, which is incorporated by reference herein in its entirety. See also, e.g., US Patent Publication No. 2014/0032186 and US Patent Publication No. 2006/0136184.

In some aspects, the entire length of the open reading frame (ORF) is modified. However, in some aspects, only a fragment of the ORF is altered. By using one of these methods, one can apply the frequencies to any given polypeptide sequence, and produce a nucleic acid fragment of a codon-optimized coding region which encodes the polypeptide. Accordingly, in some aspects a codon optimized SMN1 coding sequence is used (e.g., a codon optimized hSMN1 ORF). In some aspects, one or more portions of the SMN1 coding sequence (e.g., up to the entire ORF) are codon optimized for expression in humans.

A number of options are available for performing the actual changes to the codons or for synthesizing the codon-optimized coding regions designed as described herein. Such modifications or synthesis can be performed using standard and routine molecular biological manipulations well known to those of ordinary skill in the art. In one approach, a series of complementary oligonucleotide pairs of 80-90 nucleotides each in length and spanning the length of the desired sequence are synthesized by standard methods. These oligonucleotide pairs are synthesized such that upon annealing, they form double stranded fragments of 80-90 base pairs, containing cohesive ends, e.g., each oligonucleotide in the pair is synthesized to extend 3, 4, 5, 6, 7, 8, 9, 10, or more bases beyond the region that is complementary to the other oligonucleotide in the pair. The single-stranded ends of each pair of oligonucleotides are designed to anneal with the single-stranded end of another pair of oligonucleotides. The oligonucleotide pairs are allowed to anneal, and approximately five to six of these double-stranded fragments are then allowed to anneal together via the cohesive single stranded ends, and then they ligated together and cloned into a standard bacterial cloning vector, for example, a TOPO® vector available from Invitrogen Corporation, Carlsbad, Calif. The construct is then sequenced by standard methods. Several of these constructs consisting of 5 to 6 fragments of 80 to 90 base pair fragments ligated together, i.e., fragments of about 500 base pairs, are prepared, such that the entire desired sequence is represented in a series of

plasmid constructs. The inserts of these plasmids are then cut with appropriate restriction enzymes and ligated together to form the final construct. The final construct is then cloned into a standard bacterial cloning vector, and sequenced. Additional or alternative methods also could be used (including for example commercially available gene synthesis services).

In some aspects, SMNl cDNA sequences can be generated in vitro and synthetically, using techniques known in the art. For example, the PCR-based accurate synthesis (PAS) of long DNA sequence method may be utilized, as described by Xiong et al, PCR-based accurate synthesis of long DNA sequences, Nature Protocols 1, 791 - 797 (2006). A method combining the dual asymmetrical PCR and overlap extension PCR methods is described by Young and Dong, Two-step total gene synthesis method, Nucleic Acids Res. 2004; 32(7): e59. See also, Gordeeva et al, J Microbiol Methods. Improved PCR-based gene synthesis method and its application to the Citrobacter freundii phytase gene codon modification. 2010 May;81(2): 147-52. Epub 2010 Mar 10; see, also, the following patents on oligonucleotide synthesis and gene synthesis, Gene Seq. 2012 Apr;6(l): 10-21 ; US 8008005; and US 7985565. Each of these documents is incorporated herein by reference. In addition, kits and protocols for generating DNA via PCR are available commercially. These include the use of polymerases including, without limitation, Taq polymerase; OneTaq® (New England Biolabs); Q5® High-Fidelity DNA Polymerase (New England Biolabs); and GoTaq® G2 Polymerase (Promega). DNA may also be generated from cells transfected with plasmids containing the hSMN sequences described herein. Kits and protocols are known and commercially available and include, without limitation, QIAGEN plasmid kits; Chargeswitch® Pro Filter Plasmid Kits (Invitrogen); and GenElute™ Plasmid Kits (Sigma Aldrich). Other techniques useful herein include sequence-specific isothermal amplification methods that eliminate the need for thermocycling. Instead of heat, these methods typically employ a strand-displacing DNA polymerase, like Bst DNA Polymerase, Large Fragment (New England Biolabs), to separate duplex DNA. DNA may also be generated from RNA molecules through amplification via the use of Reverse Transcriptases (RT), which are RNA-dependent DNA Polymerases. RTs polymerize a strand of DNA that is complimentary to the original RNA template and is referred to as cDNA. This cDNA can then be further amplified through PCR or isothermal methods as outlined above. Custom DNA can also be generated commercially from companies including, without limitation, GenScript; GENEWIZ®; GeneArt® (Life Technologies); and Integrated DNA Technologies.

By "functional SMN1", is meant a gene which encodes the native SMN protein or another SMN protein which provides at least about 50%, at least about 75%, at least about 80%, at least about 90%, or about the same, or greater than 100% of the biological activity level of the native survival of motor neuron protein, or a natural variant or polymorph thereof which is not associated with disease. Additionally, SMNl homologue- SMN2 also encodes the SMN protein, but processes the functional protein less efficiently. Based on the copy number of SMN2, subjects lacking a functional hSMNl gene demonstrate SMA to varying degrees. Thus, for some subjects, the SMN protein may provide less than 100% of the biological activity of the native SMN protein.

In some aspects, such a functional SMN has a sequence which has about 95% or greater identity to the native protein, or about 97% identity or greater, or about 99% at the amino acid level. Such a functional SMN protein may also encompass natural polymorphs. Identity may be determined by preparing an alignment of the sequences and through the use of a variety of algorithms and/or computer programs known in the art or commercially available (e.g., BLAST, ExPASy; ClustalO; FASTA; using, e.g., Needleman-Wunsch algorithm, Smith-Waterman algorithm).

Percent identity may be readily determined for amino acid sequences over the full-length of a protein, polypeptide, about 32 amino acids, about 330 amino acids, or a peptide fragment thereof or the corresponding nucleic acid sequence coding sequences. A suitable amino acid fragment may be at least about 8 amino acids in length, and may be up to about 700 amino acids. Generally, when referring to "identity", "homology", or "similarity" between two different sequences, "identity", "homology" or "similarity" is determined in reference to "aligned" sequences.

In some aspects, modified SMN1 (e.g., hSMN1) genes described herein are engineered into a suitable genetic element (e.g., vector) useful for generating viral vectors and/or for delivery to a host cell, e.g., naked DNA, phage, transposon, cosmid, episome, etc., which transfers the SMN1 sequences carried thereon. The selected vector may be delivered by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Methods used to make such constructs are known to those of skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic

techniques. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY.

In some aspects, an expression cassette comprising an SMN1 (e.g., a hSMN1) nucleic acid sequence(s) is provided. As used herein, an "expression cassette" refers to a nucleic acid molecule which comprises the SMN1 sequence operably linked to a promoter, and may include other regulatory sequences. In some aspects, the expression cassette is packaged into the capsid of a viral vector (e.g., a viral particle). Typically, such an expression cassette for generating a viral vector contains an SMN1 (e.g., an hSMN1) sequence described herein flanked by packaging signals of the viral genome and other expression control sequences such as those described herein. For example, for an AAV viral vector, the packaging signals are the 5' inverted terminal repeat (ITR) and the 3' ITR. When packaged into the AAV capsid, the ITRs in conjunction with the expression cassette, are referred to herein as the "recombinant AAV (rAAV) genome" or "vector genome" within an rAAV particle or capsid.

The term "expression" is used herein in its broadest meaning and comprises the production of RNA or of RNA and protein. With respect to RNA, the term "expression" or "translation" relates in particular to the production of peptides or proteins. Expression may be transient or may be stable.

The term "translation" in the context of the present invention relates to a process at the ribosome, wherein an mRNA strand controls the assembly of an amino acid sequence to generate a protein or a peptide.

Promoters and regulatory elements:

In some aspects, an expression construct comprises one or more regions comprising a sequence that facilitates expression of the coding sequence of the SMN1 gene, e.g., expression control sequences operably linked to the coding sequence. Non-limiting examples of expression control sequences include promoters, insulators, silencers, response elements, introns, enhancers, initiation sites, termination signals, and poly(A) tails. Any combination of such control sequences is contemplated herein (e.g., a promoter and an enhancer).

In some aspects, an expression cassette contains a promoter sequence as part of the expression control sequences, e.g., located between the 5' ITR sequence and the SMN1 coding sequence. The illustrative plasmid and vector described herein uses the ubiquitous

chicken β-actin promoter (CB) with CMV immediate early enhancer (CMV IE). Alternatively, other neuron-specific promoters may be used (see, e.g., the Lockery Lab neuron-specific promoters database, accessed at http://chinook.uoregon.edu/promoters.html). Such neuron-specific promoters include, without limitation, synapsin I (SYN), calcium/calmodulin-dependent protein kinase II, tubulin alpha I, neuron-specific enolase and platelet-derived growth factor beta chain promoters. See, Hioki et al, Gene Therapy, June 2007, 14(l l):872-82, which is incorporated herein by reference. Other neuron-specific promoters include the 67 kDa glutamic acid decarboxylase (GAD67), homeobox Dlx5/6, glutamate receptor 1 (GluRl), preprotachykinin 1 (Tacl) promoter, neuron-specific enolase (NSE) and dopaminergic receptor 1 (Drdla) promoters. See, e.g., Delzor et al, Human Gene Therapy Methods. August 2012, 23(4): 242-254. In another aspect, the promoter is a GUSb promoter http://www.jci.Org/articles/view/41615#B30.

Other promoters, such as constitutive promoters, regulatable promoters (see, e.g., WO 2011/126808 and WO 2013/04943), or a promoter responsive to physiologic cues may be used. Promoter(s) can be selected from different sources, e.g., human cytomegalovirus (CMV) immediate-early enhancer/promoter, the SV40 early enhancer/promoter, the JC polyomavirus promoter, myelin basic protein (MBP) or glial fibrillary acidic protein (GFAP) promoters, herpes simplex virus (HSV-1) latency associated promoter (LAP), rouse sarcoma virus (RSV) long terminal repeat (LTR) promoter, neuron-specific promoter (NSE), platelet derived growth factor (PDGF) promoter, hSYN, melanin-concentrating hormone (MCH) promoter, chicken beta-actin (CBA) promoter, and the matrix metalloprotein (MPP) promoter.

In addition to a promoter, an expression cassette and/or a vector may contain one or more other appropriate transcription initiation, termination, enhancer sequences, efficient RNA processing signals such as splicing and polyadenylation (poly A) signals; sequences that stabilize cytoplasmic mRNA for example WPRE; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. Examples of suitable polyA sequences include, e.g., SV40, SV50, bovine growth hormone (bGH), human growth hormone, and synthetic poly As. An example of a suitable enhancer is the CMV enhancer. Other suitable enhancers include those that are appropriate for CNS indications. In some aspects, the expression cassette comprises one or more expression enhancers. In some

aspects, the expression cassette contains two or more expression enhancers. These enhancers may be the same or may differ from one another. For example, an enhancer may include a CMV immediate early enhancer. This enhancer may be present in two copies which are located adjacent to one another. Alternatively, the dual copies of the enhancer may be separated by one or more sequences. In still another aspect, the expression cassette further contains an intron, e.g., the chicken beta-actin intron. Other suitable introns include those known in the art, e.g., such as are described in WO 2011/126808. In some aspects, an intron is incorporated upstream of the coding sequence to improve 5' capping and stability of mRNA. Optionally, one or more other sequences may be selected to stabilize mRNA. An example of such a sequence is a modified WPRE sequence, which may be engineered upstream of the polyA sequence and downstream of the coding sequence (see, e.g., MA Zanta-Boussif, et al, Gene Therapy (2009) 16: 605-619).

In some aspects, these control sequences are "operably linked" to the SMN1 gene sequences. As used herein, the term "operably linked" refers to both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

Recombinant viral vectors:

In some aspects, an adeno-associated viral vector that comprises an AAV capsid and at least one expression cassette is provided. In some aspects, the at least one expression cassette comprises nucleic acid sequences encoding SMN1 and expression control sequences that direct expression of the SMN1 sequences in a host cell. An rAAV vector gene can also comprises AAV ITR sequences. In some aspects, the ITRs are from an AAV serotype that is different from the serotype of the capsid proteins used to package the rAAV genome. In some aspects, the ITR sequences are from AAV2, or the deleted version thereof (AITR), which may be used for convenience and to accelerate regulatory approval. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. Typically, rAAV vector genomes comprise an AAV 5' ITR, the SMN1 coding sequences and any regulatory sequences, and an AAV 3' ITR. However, other configurations of these elements may be suitable. A shortened version of the 5' ITR, termed AITR, has been described in which the D- sequence and terminal resolution site (trs) are deleted. In other aspects, the full-length AAV 5' and 3' ITRs are used.

The ITR sequences of a nucleic acid or nucleic acid vector described herein can be derived from any AAV serotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) or can be derived from more than one serotype. In some aspects, ITR sequences and plasmids containing ITR sequences are known in the art and commercially available (see, e.g., products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; and Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):14082-7; and Curtis A. Machida. Methods in Molecular Medicine™. Viral Vectors for Gene Therapy Methods and Protocols. 10.1385/1-59259-304-6:201 © Humana Press Inc. 2003. Chapter 10. Targeted Integration by Adeno-Associated Virus. Matthew D. Weitzman, Samuel M. Young Jr., Toni Cathomen and Richard Jude Samulski; U.S. Pat. Nos. 5,139,941 and 5,962,313, all of which are incorporated herein by reference).

In some aspects, rAAV nucleic acids or genomes can be single-stranded (ss). However, in some aspects, rAAV nucleic acids or genomes can be self-complementary (sc) AAV nucleic acid vectors. In some aspects, a recombinant AAV particle comprises a nucleic acid vector, such as a single-stranded (ss) or self-complementary (sc) AAV nucleic acid vector. In some aspects, the nucleic acid vector contains an SMN1 gene and one or more regions comprising inverted terminal repeat (ITR) sequences (e.g., wild-type ITR sequences or engineered ITR sequences) flanking the expression construct. In some aspects, the nucleic acid is encapsidated by a viral capsid.

Accordingly, in some aspects, an AAV particle comprises a viral capsid and a nucleic acid vector as described herein, which is encapsidated by the viral capsid. In some aspects, the viral capsid comprises 60 capsid protein subunits comprising VP1, VP2 and VP3. In some aspects, the VP1, VP2, and VP3 subunits are present in the capsid at a ratio of approximately 1:1:10, respectively.

In some aspects, a recombinant adeno-associated virus (rAAV) is an AAV DNase-resistant particle having an AAV protein capsid into which is packaged nucleic acid sequences for delivery to target cells. In some aspects, an AAV capsid is composed of 60 capsid (cap) protein subunits, VP1, VP2, and VP3, that are arranged in an icosahedral symmetry in a ratio of approximately 1 : 1 : 10 to 1 : 1 :20, depending upon the selected

AAV. The AAV capsid may be chosen from those known in the art, including variants thereof. In some aspects, the AAV capsid is chosen from those that effectively transduce neuronal cells. In some aspects, the AAV capsid is selected from AAV1, AAV2, AAV7, AAV 8, AAV9, AAVrh10, AAV5, AAVhu11, AAV8DJ, AAVhu32, AAVhu37, AAVpi2, AAVrh8, AAVhu48R3, AAVhu68 and variants thereof. See, WO2018160585A2, WO2018160582A1, Royo, et al, Brain Res, 2008 Jan, 1190: 15-22; Petrosyan et al, Gene Therapy, 2014 Dec, 21(12):991-1000; Holehonnur et al, BMC Neuroscience, 2014, 15:28; and Cearley et al, Mol Ther. 2008 Oct; 16(10): 1710-1718, each of which is incorporated herein by reference. Other AAV capsids useful herein include AAVrh39, AAVrh20, AAVrh25, AAV10, AAVbb1, and AAVbb2 and variants thereof. Other AAV serotypes may be selected as sources for capsids of AAV viral vectors (DNase resistant viral particles) including, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVrh10, AAVrh64Rl, AAVrh64R2, AAVrh8, and variants of any of the known or mentioned AAVs or AAVs yet to be discovered. See, e.g., US Published Patent Application No. 2007-0036760-A1; US Published Patent Application No. 2009-0197338-A1; EP 1310571. See also, WO 2003/042397 (AAV7 and other simian AAV), US Patent 7790449 and US Patent 7282199 (AAV8), WO 2005/033321 and US 7,906,111 (AAV9), and WO 2006/110689, and WO 2003/042397 (rh10). Alternatively, a recombinant AAV based upon any of the recited AAVs, may be used as a source for the AAV capsid. These documents also describe other AAV which may be selected for generating AAV and are incorporated by reference. In some aspects, an AAV cap for use in the viral vector can be generated by mutagenesis (e.g., by insertions, deletions, or substitutions) of one of the aforementioned AAV Caps or its encoding nucleic acid. In some aspects, the AAV capsid is chimeric, comprising domains from two or three or four or more of the aforementioned AAV capsid proteins. In some aspects, the AAV capsid is a mosaic of Vpl, Vp2, and Vp3 monomers from two or three different AAVs or recombinant AAVs. In some aspects, an rAAV composition comprises more than one of the aforementioned Caps. As used herein, relating to AAV, the term variant means any AAV sequence which is derived from a known AAV sequence, including those sharing at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or greater sequence identity over the amino acid or nucleic acid sequence. In another aspect, the AAV capsid includes variants which may include up to about 10% variation from any described or known AAV capsid sequence. That is, the AAV capsid shares about 90% identity to about 99.9 % identity, about 95% to about 99% identity or about 97% to about 98% identity to an AAV capsid provided herein and/or

known in the art. In some aspects, the AAV capsid shares at least 95% identity with an AAV capsid. When determining the percent identity of an AAV capsid, the comparison may be made over any of the variable proteins (e.g., vpl, vp2, or vp3). In some aspects, the AAV capsid shares at least 95% identity with the AAV8 vp3.

In some aspects, a self-complementary AAV is provided. The abbreviation "sc" in this context refers to self-complementary. "Self-complementary AAV" refers a construct in which a coding region carried by a recombinant AAV nucleic acid sequence has been designed to form an intra-molecular double-stranded DNA template. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. See, e.g., D M McCarty et al, "Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis", Gene Therapy, (August 2001), Vol 8, Number 16, Pages 1248-1254. Self-complementary AAVs are described in, e.g., U.S. Patent Nos. 6,596,535; 7,125,717; and 7,456,683, each of which is incorporated herein by reference in its entirety.

Methods for generating and isolating AAV viral vectors suitable for delivery to a subject are known in the art. See, e.g., US Published Patent Application No. 2007/0036760 (February 15, 2007), US Patent 7790449; US Patent 7282199; WO 2003/042397; WO 2005/033321, WO 2006/110689; and US 7588772 B2. In one system, a producer cell line is transiently transfected with a construct that encodes the transgene flanked by ITRs and a construct(s) that encodes rep and cap. In a second system, a packaging cell line that stably supplies rep and cap is transiently transfected with a construct encoding the transgene flanked by ITRs. In each of these systems, AAV virions are produced in response to infection with helper adenovirus or herpesvirus, requiring the separation of the rAAVs from contaminating virus. Systems also have been developed that do not require infection with helper virus to recover the AAV - the required helper functions (e.g., adenovirus El, E2a, VA, and E4 or herpesvirus UL5, UL8, UL52, and UL29, and herpesvirus polymerase) are also supplied, in trans, by the system. In these systems, the helper functions can be supplied by transient transfection of the cells with constructs that encode the required helper functions, or the cells can be engineered to stably contain genes encoding the helper functions, the expression of which can be controlled at the transcriptional or posttranscriptional level. In yet another system, the transgene flanked by ITRs and rep/cap genes are introduced into insect cells by

infection with baculovirus-based vectors. For reviews on these production systems, see generally, e.g., Zhang et al, 2009, "Adenovirus-adeno-associated virus hybrid for large-scale recombinant adeno-associated virus production," Human Gene Therapy 20:922-929, the contents of each of which is incorporated herein by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following U.S. patents, the contents of each of which is incorporated herein by reference in its entirety: 5,139,941 ; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065.

Optionally, the SMN1 genes described herein may be used to generate viral vectors other than rAAV, and that also can be used in combination therapy with SMN2 ASOs. Such other viral vectors may include any virus suitable for gene therapy may be used, including but not limited to adenovirus; herpes virus; lentivirus; retrovirus; etc. Suitably, where one of these other vectors is generated, it is produced as a replication-defective viral vector.

A "replication-defective virus" or "viral vector" refers to a synthetic or artificial viral particle in which an expression cassette containing a gene of interest is packaged in a viral capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; i.e., they cannot generate progeny virions but retain the ability to infect target cells. In some aspects, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" -containing only the transgene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication. Such replication-defective viruses may be adeno-associated viruses (AAV), adenoviruses, lentiviruses (integrating or non-integrating), or another suitable virus source.

Host cells that comprise at least one of the disclosed AAV particles, expression constructs, or nucleic acid vectors also are provided. Such host cells include mammalian host cells, for example human host cells, and may be either isolated, in cell or tissue culture. In the case of genetically modified animal models (e.g., a mouse), the transformed host cells may be comprised within the body of a non-human animal itself.

Oligomeric compounds that increase full-length SMN2 mRNA production

In some aspects, a combined therapy for treating SMA includes ASOs complementary to a pre-mRNA encoding SMN2 (also referred to as SMN2 ASOs in this application). In some aspects, the ASO increases full-length SMN2 mRNA. In some aspects, the ASO alters splicing of SMN2 pre-mRNA. In some aspects, the ASO promotes exon 7 inclusion in SMN2 mRNA. Some sequences and regions useful for altering splicing of SMN2 may be found in PCT/US06/024469 (published as WO/2007/002390) and WO2018014041A2, which are hereby incorporated by reference in their entirety for any purpose.

In some aspects, SMN2 ASOs 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 is 100% identical to wild-type SMN protein.

ASOs that effectively modulate expression of SMN2 mRNA to produce functional SMN protein are considered active ASOs. 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. The effects of treatment can be assessed by measuring biomarkers associated with the target gene expression in one or more biological fluids, tissues or organs, collected from an animal contacted with one or more compositions described in this application.

In some aspects, an increase in full-length SMN2 mRNA means that the intracellular level of full-length SMN2 mRNA is higher than a reference level, such as the level of full-length SMN2 mRNA in a control (for example in a subject that is not being administered an SMN2 ASO). An increase in intracellular full-length SMN2 mRNA can be measured as an increase in the level of full-length protein and/or mRNA produced from the SMN2 gene. In some aspects, an increase in full-length SMN2 mRNA can be determined by examination of the outward properties of the cell or organism (e.g., as described below in the examples), or by assay techniques such as RNA solution hybridization, nuclease protection, Northern hybridization, reverse transcription, gene expression monitoring with a microarray, antibody binding, enzyme linked immunosorbent assay (ELISA), nucleic acid sequencing, Western blotting, radioimmunoassay (RIA), other immunoassays, fluorescence activated cell analysis

(FACS), or any other technique or combination of techniques that can detect the presence of full-length SMN2 mRNA or protein (e.g., in a subject or a sample obtained from a subject).

In some aspects, by comparing the level of full-length SMN2 mRNA in a sample obtained from a subject receiving an SMN2 ASO treatment to a level of full-length SMN2 mRNA in a subject not treated with the SMN2 ASO, the extent to which the SMN2 ASO increased full-length SMN2 mRNA can be determined. In some aspects, the reference level of full-length SMN2 mRNA is obtained from the same subject prior to receiving SMN2 ASO. In some aspects, the reference level of full-length SMN2 mRNA is a range determined by a population of subjects not receiving SMN2 ASO.

In some aspects, an increased level of full-length SMN2 mRNA is, for example, greater than 1 fold, 1.5-5 fold, 5-10 fold, 10-50 fold, 50-100 fold, about 1.1-, 1.2-, 1.5-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-fold or more higher than a reference value.

In some aspects, by comparing the ratio of full-length SMN2 mRNA to a shorter SMN2 mRNA (e.g., SMN2 mRNA without exon 7) with a reference ratio in a subject receiving SMN2 ASO administration, it can be determined whether the SMN2 ASO resulted in an increase of full-length SMN2 mRNA. In some aspects, the reference ratio is the ratio of the full length SMN2 mRNA to a short SMN2 mRNA (e.g., SMN2 mRNA without exon 7) prior to SMN2 ASO administration. In some aspects, the ratio of the full length SMN2 mRNA to a short SMN2 mRNA (e.g., SMN2 mRNA without exon 7) in a subject receiving SMN2 ASO is, for example, greater than 1 fold, 1.5-5 fold, 5-10 fold, 10-50 fold, 50-100 fold, about 1.1-, 1.2-, 1.5-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-fold or more higher than a reference ratio.

In some aspects, the increase of full-length SMN2 mRNA in a subject can be indicated by the increase of full-length SMN protein as compared to a reference level. In some aspects, the reference level of full-length SMN protein is the full-length SMN protein level obtained from a subject having or at risk of having SMA prior to treatment. In some aspects, exon 7-containing SMN protein production is increased in a subject receiving SMN2 ASO administration with an enhancement of exon 7-containing SMN protein levels of at least about, for example, greater than 1 fold, 1.5-5 fold, 5-10 fold, 10-50 fold, 50-100 fold, about 1.1-, 1.2-, 1.5-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-fold or more higher than a reference value. Methods whereby bodily fluids, organs or tissues

are contacted with an effective amount of one or more compositions described in this application are also contemplated. Bodily fluids, organs or tissues can be contacted with one or more compositions resulting in expression of SMN1 and modulation of SMN2 expression in the cells of bodily fluids, organs or tissues. An effective amount of a composition can be determined by monitoring the effect on functional SMN protein expression of recombinant SMN1 genes and SMN2 ASOs that are administered to a subject or contacted to a cell.

1. Antisense oligonucleotides (ASOs)

In some aspects, an ASO comprising a sequence complementary to a nucleic acid encoding human SMN2 is provided for use in treating (e.g., in combination with a recombinant SMN1 gene) a disease or condition associated with survival motor neuron protein (SMN), such as spinal muscular atrophy (SMA). In some aspects, an ASO comprising a sequence complementary to a nucleic acid encoding human SMN2 is provided for use in treating (e.g., in combination with a recombinant SMN1 gene) a disease or condition associated with survival motor neuron protein (SMN) by administering the ASO directly into the central nervous system (CNS) or CSF.

As used herein, the term "oligomeric compound" refers to a compound comprising an oligonucleotide. In some aspects, an oligomeric compound consists of an oligonucleotide. As used herein, the term "oligonucleotide" refers to a compound comprising a phosphate linking group, a heterocyclic base moiety and a sugar moiety. In some aspects, an oligomeric compound further comprises one or more conjugate and/or terminal groups. In some aspects, oligomeric compounds are antisense oligonucleotides (ASO). As used herein, the terms "antisense oligonucleotide" or "ASO" refer to 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.

In some instances, an antisense oligonucleotide (ASO) increases full-length SMN protein in the subject. In some instances, the ASO increases the full-length SMN2 mRNA in a subject. In some aspects, an ASO that increases the full-length SMN2 mRNA is an antisense oligonucleotide that is complementary to a nucleic acid encoding SMN2. In some aspects, the ASO increases full-length SMN2 mRNA by altering the splicing pattern of SMN2 pre-mRNA. In some aspects the ASO promotes exon skipping during splicing of SMN2 pre-mRNA. In some aspects, the ASO promotes the inclusion of exon 7 in the SMN2 mRNA. In some aspects, the ASO is designed to target, intron 6, intron 7, or the boundary

between exon 7 and an adjacent intron of SMN2 pre-mRNA to promote the inclusion of exon 7 in the SMN2 mRNA. In some aspects, the ASO comprises a nucleobase sequence complementary to intron 6 of SMN2 pre-mRNA. In some aspects, the ASO comprises a nucleobase sequence complementary to exon 6 of SMN2 pre-mRNA. In some aspects, the ASO comprises a nucleobase sequence complementary to intron 7 of SMN2 pre-mRNA. In some aspects, the ASO targeting intron 7 of SMN2 pre-mRNA comprises a nucleotide sequence of SEQ ID NO: 1. In some aspects, the ASO targeting intron 7 of SMN2 pre-mRNA is nusinersen. In some aspects, one or more of the ASOs described herein can be administered to a subject for increased level of full-length SMN protein and/or full-length SMN2 mRNA. Non-limiting examples of sequences and regions useful for altering splicing of SMN2 may be found in PCT/US06/024469, which is hereby incorporated by reference in its entirety for any purpose. In some aspects, an antisense oligonucleotide has a nucleobase sequence that is complementary to intron 7 of SMN2. Non-limiting examples of such nucleobase sequences are exemplified in the table below.

Sequence	Length	SEQ ID NO
TGCTGGCAGACTTAC	15	2
CATAATGCTGGCAGA	15	3
TCATAATGCTGGCAG	15	4
TTCATAATGCTGGCA	15	5
TTTCATAATGCTGGC	15	6
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

In some aspects, the ASO targets intron 7 of SMN2 pre-mRNA. In some aspects, an ASO comprises a nucleobase sequence comprising at least 10 nucleobases of the sequence: TCACTTTCATAATGCTGG (SEQ ID NO: 1). In some aspects, an ASO has a nucleobase sequence comprising at least 11 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising at least 12 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising at least 13 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising at least 14 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising at least 15 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising at least 16 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising at least 17 nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence comprising the nucleobases of SEQ ID NO: 1. In some aspects, an ASO has a nucleobase sequence consisting of the nucleobases of SEQ ID NO: 1. In some aspects, an ASO 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).

In some aspects, SMN2 ASOs are complementary to a nucleic acid molecule encoding the SMN2 protein. In some aspects, the ASOs are complementary to intron 6, exon

7 (or the boundary of exon 7 and an adjacent intron), or intron 7 of a nucleic acid molecule encoding SMN2 protein. In some aspects, the ASO targets intron 7 of SMN2 pre-mRNA. In some aspects, an SMN2 ASO targeting intron 7 of SMN2 pre-mRNA is nusinersen. An exemplary nucleotide sequence for nusinersen is UCACUUUCAUAAUGCUGG-3' (SEQ ID NO: 26). The active substance, nusinersen (also referred to as ISIS 396443), is a uniformly modified 2'-O-(2-methoxyethyl) phosphorothioate antisense oligonucleotide consisting of 18 nucleotide residues having the sequence 5'-
^{Me}U^{Me}CA^{Me}C^{Me}U^{Me}U^{Me}U^{Me}CA^{Me}UAA^{Me}UG^{Me}C^{Me}UGG-3' (SEQ ID NO: 25)

The chemical name of nusinersen sodium is 2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioguanylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioguanylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)guanosine corresponding to the molecular formula C234H323N61O128P17S17Na17 and has a relative molecular mass 7501.0 g/mol and the structure shown in FIG. 3.

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.

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).

In some aspects, antisense oligonucleotides comprise one or more modifications compared to oligonucleotides of naturally occurring oligomers, such as DNA or RNA. Such modified antisense oligonucleotides may possess one or more desirable properties. In some aspects, 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 some aspects, 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. Modified nucleosides

In some aspects, antisense oligonucleotides comprise one or more modified nucleosides. Such modified nucleosides may include a modified sugar and/or a modified nucleobase. In some aspects, 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. Nucleobases

The naturally occurring base portion of nucleosides are heterocyclic bases, 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 some aspects, 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 some aspects, nucleobase mimetics include more complicated structures, such as for example a tricyclic phenoxazine nucleobase mimetic. Methods for preparing modified nucleobases are well known to those skilled in the art.

ii. Modified sugars and sugar surrogates

Antisense oligonucleotides of the present application can optionally contain one or more nucleosides wherein the sugar moiety is modified, compared to a natural sugar. Oligonucleotides comprising 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 Aug. 21, 2008 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 US20050130923, published on Jun. 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on Nov. 22, 2007 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

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 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, OCF_3, $O(CH_2)SCH_3$, $O(CH_2)_2$-O-$N(R_m)(R_n)$, 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.

Examples of bicyclic nucleic acids (BNAs) include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In some aspects, antisense compounds provided herein include one or more BNA nucleosides wherein the bridge comprises one of the formulas: 4'-beta-D-(CH_2)—O-2' (beta-D-LNA); 4'-(CH_2)—S-2: 4'-alpha-L-(CH_2)—O-2' (alpha-L-LNA); 4'-(CH_2)$_2$—O-2' (ENA); 4'-C(CH_3)$_2$—O-2' (see PCT/US2008/068922); 4'-CH(CH_3) —O-2' and 4'- C—H(CH_2OCH_3) —O-2' (see U.S. Pat. No. 7,399,845, issued on Jul. 15, 2008): 4'-CH_2—N(OCH_3)-2' (see PCT/US2008/064591); 4'-CH_2—O—N(CH_3)-2' (see published U.S. Patent Application US2004-0171570, published Sep. 2, 2004): 4'-CH_2—N(R) —O-2' (see U.S. Pat. No. 7,427,672, issued on Sep. 23, 2008): 4'-CH_2—C(CH_3)-2' and 4'-CH_2—C(=CH_2)-2' (see PCT/US2008/066154); and wherein R is, independently, H, C_1-C_{12} alkyl, or a protecting group.

In some aspects, modified nucleosides comprising modified sugar moieties are not bicyclic sugar moieties. In some aspects, the sugar ring of a nucleoside may be modified at any position. Examples of useful sugar modifications 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 some aspects, such substituents are at the 2' position of the sugar.

In some aspects, modified nucleosides comprise a substituent at the 2' position of the sugar. In some aspects, 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)$_2$$SCH_3$, O—($CH_2$)$_2$—O—N($R_m$)($R_n$), or O—$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.

In some aspects, modified nucleosides suitable for use in the present invention are: 2-methoxyethoxy, 2'-Omethyl (2'-O CH_3), 2'-fluoro (2'-F).

In some aspects, modified nucleosides having a substituent group at the 2'-position selected from: O[(CH_2)$_n$O]$_m$$CH_3$, O($CH_2$)$_n$$NH_2$, O($CH_2$)$_2$$CH_3$, O($CH_2$)$_n$$ONH_2$, $OCH_2C(=O)N(H)CH_3$, and O(CH_2)$_n$ON[(CH_2)$_n$CH3]$_2$, 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, 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.

In some aspects, modified 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).

In some aspects, 2'-sugar substituent groups are in either the arabino (up) position or ribo (down) position. In some aspects, a 2'-arabino modification is 2'-Farabino (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.

In some aspects, suitable nucleosides 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, each of which is herein incorporated by reference in its entirety.

In some aspects, nucleosides comprise a modification at the 2'-position of the sugar. In some aspects, nucleosides comprise a modification at the 5'-position of the sugar. In some aspects, nucleosides comprise modifications at the 2'-position and the 5'-position of the sugar. In some aspects, modified nucleosides may be useful for incorporation into oligonucleotides. In some aspects, modified nucleosides are incorporated into oligonucleosides at the 5'-end of the oligonucleotide.

b. Internucleoside linkages

Antisense oligonucleotides can optionally contain one or more modified internucleoside linkages. 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 (—CH_2-N(CH_3)-O—CH2), thiodiester (—O—C(O)—S—), thionocarbamate (—O—C(O)(NH)—S—); siloxane (—O—Si(H)$_2$-O—); and N,N'-dimethylhydrazine (—CH_2-N(CH_3)-N(CH_3)-). 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 some aspects, 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.

The antisense oligonucleotides described herein can 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. Antisense compounds provided herein can include all such possible isomers, as well as their racemic and optically pure forms.

In some aspects, antisense oligonucleotides have at least one modified internucleoside linkage. In some aspects, antisense oligonucleotides have at least 2 modified internucleoside linkages. In some aspects, antisense oligonucleotides have at least 3 modified internucleoside linkages. In some aspects, antisense oligonucleotides have at least 10 modified internucleoside linkages. In some aspects, each internucleoside linkage of an antisense oligonucleotide is a modified internucleoside linkage. In some aspects, such modified internucleoside linkages are phosphorothioate linkages.

c. Lengths

In some aspects, the present invention provides antisense oligonucleotides of any of a variety of ranges of lengths. In some aspects, antisense compounds or antisense

oligonucleotides comprise or consist 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 some aspects, antisense compounds or antisense oligonucleotides comprise or consist 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.

In some aspects, antisense compounds or antisense oligonucleotides are 15 nucleosides in length. In some aspects, antisense compounds or antisense oligonucleotides are 16 nucleosides in length. In some aspects, antisense compounds or antisense oligonucleotides are 17 nucleosides in length. In some aspects, antisense compounds or antisense oligonucleotides are 18 nucleosides in length. In some aspects, antisense compounds or antisense oligonucleotides are 19 nucleosides in length. In some aspects, antisense compounds or antisense oligonucleotides are 20 nucleosides in length.

d. Oligonucleotide motifs

In some aspects, antisense oligonucleotides have chemically modified subunits arranged in specific orientations along their length. In some aspects, antisense oligonucleotides are fully modified. In some aspects, antisense oligonucleotides are uniformly modified. In some aspects, antisense oligonucleotides are uniformly modified and each nucleoside comprises a 2-MOE sugar moiety. In some aspects, antisense oligonucleotides are uniformly modified and each nucleoside comprises a 2'-OMe sugar moiety. In some aspects, antisense oligonucleotides are uniformly modified and each nucleoside comprises a morpholino sugar moiety.

In some aspects, oligonucleotides comprise an alternating motif. In some aspects, the alternating modification types are selected from among 2'-MOE, 2'-F, a bicyclic sugar-modified nucleoside, and DNA (unmodified 2'-deoxy). In some aspects, each alternating region comprises a single nucleoside.

In some aspects, oligonucleotides comprise one or more block of nucleosides of a first type and one or more block of nucleosides of a second type.

In some aspects, one or more alternating regions in an alternating motif include more than a single nucleoside of a type. For example, oligomeric compounds may include one or more regions of any of the following nucleoside motifs:

Nu1 Nu1 Nu2 Nu2 Nu1 Nu1;

Nu1 Nu2 Nu2 Nu1 Nu2 Nu2;

Nu1 Nu1 Nu2 Nu1 Nu1 Nu2;

Nu1 Nu2 Nu2 Nu1 Nu2 Nu1 Nu1 Nu2 Nu2;

Nu1 Nu2 Nu1 Nu2 Nu1 Nu1;

Nu1 Nu1 Nu2 Nu1 NU2 Nu1 Nu2;

Nu1 Nu2 Nu1 Nu2 Nu1 Nu1;

Nu1 Nu2 Nu2 Nu1 Nu1 Nu2 Nu2 Nu1 Nu2 Nu1 Nu2 Nu1 Nu1;

Nu2 Nu1 Nu2 Nu2 Nu1 Nu1 Nu2 Nu2 Nu1 Nu2 Nu1 Nu2 Nu1 Nu1; or

Nu1 Nu2 Nu1 Nu2 Nu2 Nu1 Nu1 Nu2 Nu2 Nu1 Nu2 Nu1 Nu2 Nu1 Nu1;

wherein Nu1 is a nucleoside of a first type and Nu2 is a nucleoside of a second type. In some aspects, one of Nu1 and Nu2 is a 2'-MOE nucleoside and the other of Nu1 and Nu2 is selected from: a 2'-OMe modified nucleoside, BNA, and an unmodified DNA or RNA nucleoside.

2. Oligomeric compounds

In some aspects, oligomeric compounds are comprised only of an oligonucleotide. In some aspects, an oligomeric compound comprises an oligonucleotide and one or more conjugate and/or terminal groups. Such conjugate and/or terminal groups may be added to oligonucleotides having any of the chemical motifs described in this application. Thus, for example, an oligomeric compound comprising an oligonucleotide having one or more regions of alternating nucleosides may comprise a terminal group.

a. Conjugate groups

In some aspects, oligonucleotides 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 can include 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), athiocholesterol (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).

In some aspects, 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 Ser. No. 09/334,130.

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. 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

In some aspects, oligomeric compounds comprise terminal groups at one or both ends. In some aspects, a terminal group may comprise any of the conjugate groups described in this application. In some aspects, terminal groups may comprise additional nucleosides and/or inverted abasic nucleosides. In some aspects, a terminal group is a stabilizing group.

In some aspects, oligomeric compounds comprise one or more terminal stabilizing groups that enhance 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 terminal modifications protect 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 non-limiting 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).

In some aspects, one or more additional nucleosides are 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 some aspects, 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

In some aspects, oligomeric compounds comprise a motif: T-$(Nu_1)_{n1}$,-$(Nu_2)_{n2}$-$(Nu_1)_{n3}$-$(Nu_2)_{n4}$-$(Nu_1)_{n5}$-T2, 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.

In some aspects, the Sum of n2 and n4 is 13 or 14; n1 is 2; n3 is 2 or 3; and n5 is 2. In some aspects, oligomeric compounds comprise a motif selected from Table A.

TABLE A

nl	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

3. Antisense

In some aspects, oligomeric compounds are antisense compounds. Accordingly, in some aspects oligomeric compounds hybridize with a target nucleic acid (e.g., a target pre-mRNA or a target mRNA) resulting in an antisense activity.

a. Hybridization

In some aspects, antisense compounds 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 (e.g., 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).

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.

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 ATm 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

In some aspects, antisense compounds provided herein are complementary to a pre-mRNA. In some aspects, such antisense compounds alter splicing of the pre-mRNA. In some aspects, 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 some aspects, 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 U.S. Pat. No. 6,210,892; U.S. Pat. No. 5,627,274; U.S. Pat. Nos. 5,665,593; 5,916,808; U.S. Pat. No. 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, each

of which is hereby incorporated by reference in its entirety for any purpose. In some aspects antisense sequences that alter splicing are modified according to motifs described in this application.

In some aspects, ASOs or oligomeric compounds may include one or more modifications described in WO/2018/014043 (PCT/US2017/042465), WO/2018/014042 (PCT/US2017/042464), WO/2018/014041 (PCT/US2017/042463), the contents of which are incorporated herein in their entirety.

Combined administration and treatment

In some aspects, a "therapeutically effective" amount of a recombinant SMN1 gene (e.g., in a viral vector, for example an rAAV), an SMN2 ASO, or a combination thereof, is delivered to a subject as described herein (e.g., via concurrent or sequential administration) to achieve a desired result, for example, treatment of SMA or one or more symptoms thereof. In some aspects, a desired result includes reducing muscle weakness, increasing muscle strength and tone, preventing or reducing scoliosis, or maintaining or increasing respiratory health, or reducing tremors or twitching. Other desired endpoints can be determined by a physician.

In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to increase body weight. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to prevent or reduce muscle weakness. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to increase muscle strength. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to increase muscle tone. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to prevent or reduce scoliosis. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to reduce tremors or twitching. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to maintain or increase respiratory health. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to prevent or reduce neuron loss. In some aspects, a combination of a recombinant SMN1 gene and an SMN2 ASO is delivered to a subject to prevent or reduce motor neuron loss.

In some aspects, administration of a combination of a recombinant SMN1 gene and an SMN2 ASO produces a synergistic effect. In some aspects, the combination potentiates the effect of the recombinant SMN1 gene and allows for a lower dose (e.g., a lower dose of rAAV encoding a recombinant SMN1 gene) to be delivered to a subject. In some aspects, the combination potentiates the effect of the SMN2 ASO and allows for a lower dose of ASO to be administered to a subject. In some aspects, a lower dose of rAAV encoding a recombinant SMN1 gene is less than 1×10^{10} GC. In some aspects, a lower dose of rAAV encoding a recombinant SMN1 gene is 1.0×10^{8} to 1.0×10^{10} GC. In some aspects, a lower dose of rAAV encoding a recombinant SMN1 gene is 1.0×10^{9} to 1.0×10^{10} GC. In some aspects, a lower dose of rAAV encoding a recombinant SMN1 gene is 1.0×10^{10} to 1.0×10^{13} GC. In some aspects, a lower dose of rAAV encoding a recombinant SMN1 gene administered to a human subject is 3×10^{13} GC. In some aspects, a lower dose of rAAV encoding a recombinant SMN1 gene administered to a human subject is less than 1×10^{14} GC, for example 1×10^{13} to 1×10^{14} GC, 1×10^{12} to 1×10^{13} GC, 1×10^{11} to 1×10^{12} GC, 1×10^{10} to 1×10^{11} GC, or 1×10^{9} to 1×10^{10} GC, or less per dose administered to the human subject.

In some aspects, a lower dose of SMN2 ASO is 12 mg. A total of 5 mg to 60 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 48 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 36 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg per dose of SMN2 ASO is administered to the subject.

In some instances, SMA is detected in a fetus at around 30 to 36 weeks of pregnancy. In this situation, it may be desirable to treat the neonate as soon as possible after delivery. It also may be desirable to treat the fetus in utero. Thus, a method of rescuing and/or treating a neonatal subject having SMA is provided, comprising the step of delivering a combination of a recombinant SNM1 gene and an SMN2 ASO to the neuronal cells of a fetus and/or a newborn subject (e.g., a human fetus and/or newborn). In some aspects, a method of rescuing and/or treating a fetus having SMA is provided, comprising the step of delivering a combination of a recombinant SNM1 gene and an SMN2 ASO to the neuronal cells of the fetus in utero. In some aspects, the combination is delivered in one or more compositions described herein via intrathecal injection. In some aspects, treatment in utero is defined as administering a combination of a recombinant SNM1 gene and an SMN2 ASO as described herein after detection of SMA in the fetus. See, e.g., David et al, Recombinant adeno-

associated virus-mediated in utero gene transfer gives therapeutic transgene expression in the sheep, Hum Gene Ther. 2011 Apr;22(4):419-26. doi: 10.1089/hum.2010.007. Epub 2011 Feb 2, which is incorporated herein by reference.

In some aspects, neonatal treatment involves delivering at least one dose of one or both of a recombinant SNM1 gene and an SMN2 ASO within 8 hours, the first 12 hours, the first 24 hours, or the first 48 hours of delivery. In another aspect, particularly for a primate (human or non-human), neonatal delivery is within the period of about 12 hours to about 1 week, 2 weeks, 3 weeks, or about 1 month, or after about 24 hours to about 48 hours.

In some aspects, for late onset SMA, one or both of a recombinant SNM1 gene and an SMN2 ASO are delivered after onset of symptoms. In some aspects, treatment of the patient (e.g., a first injection) is initiated prior to the first year of life. In another aspect, treatment is initiated after the first 1 year, or after the first 2 to 3 years of age, after 5 years of age, after 11 years of age, or at an older age.

In some aspects, one or both of a recombinant SNM1 gene and an SMN2 ASO are re-administered at a later date.

In some aspects, more than one re-administration is provided. Such re-administration may involve re-administering a recombinant SMN1 gene in the same type of viral vector, a different viral vector (e.g., using AAV capsid proteins of a different serotype), or via non-viral delivery. For example, in the event a patient was treated with a first rAAV (e.g., rAAV9) encoding SMN1 and requires a second treatment with a recombinant SMN1 gene (e.g., in addition to receiving an SMN2 ASO), a second different rAAV (e.g., rAAVhu68) encoding the recombinant SMN1 gene can be subsequently administered, and vice-versa. Also, if a patient has neutralizing antibodies to a first rAAV serotype, then a second different rAAV serotype can be used to deliver a second dose of a recombinant SMN1 gene to a subject.

In some aspects, treatment of SMA patients with a combination of a recombinant SMN1 gene (e.g., in a viral vector such as an rAAV) may require a further therapy, such as transient co-treatment with an immunosuppressant before, during and/or after treatment with compositions described in this application. Immunosuppressant for such co-therapy include, but are not limited to, steroids, antimetabolites, T-cell inhibitors, and alkylating agents, or procedures to remove circulating antibodies such as plasmapheresis. For example, such

transient treatment may include a steroid (e.g., prednisone, or prednisolone) dosed once daily for 7 days at a decreasing dose, in an amount starting at about 60 mg, and decreasing by 10 mg/day (day 7 no dose). Other doses and immunosuppressants may be selected.

In some aspects, a subject has one or more indicators of SMA. In some aspects, the subject has reduced electrical activity of one or more muscles. In some aspects, the subject has a mutant SMN1 gene (e.g., two mutant alleles of the SMN1 gene). In some aspects, the subject's SMN1 gene (e.g., both alleles of the SMN1 gene) is absent or incapable of producing functional SMN protein. In some aspects the subject has a deletion or a loss of function point mutation in each SMN1 allele. In some aspects the subject is homozygous for a SMN1 gene mutation. In some aspects, the subject is diagnosed by a genetic test. In some aspects, the subject is identified by muscle biopsy. In some aspects, a subject is unable to sit upright. In some aspects, a subject is unable to stand or walk. In some aspects, a subject requires assistance to breathe and/or eat. In some aspects, a subject is identified by electrophysiological measurement of muscle and/or muscle biopsy.

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

In some aspects, the first dose of a pharmaceutical composition (e.g., of a recombinant SMN1 gene, an SMN2 ASO, or a combination of both) is administered in utero. In some such aspects, the first dose is administered before complete development of the blood-brain-barrier. In some aspects, the first dose is administered to the subject in utero systemically. In some aspects, the first dose is administered in utero after formation of the blood-brain-barrier. In some aspects, the first dose is administered to the CSF.

In some aspects, the first dose of a pharmaceutical composition (e.g., of a recombinant SMN1 gene, an SMN2 ASO, or a combination of both) is administered when the

subject is less than one week old. In some aspects, the first dose is administered when the subject is less than one month old. In some aspects, the first dose is administered when the subject is less than 3 months old. In some aspects, the first dose is administered when the subject is less than 6 months old. In some aspects, the first dose is administered when the subject is less than one year old. In some aspects, the first dose is administered when the subject is less than 2 years old. In some aspects, the first dose is administered when the subject is less than 15 years old. In some aspects, the first dose is administered when the subject is older than 15 years old.

In some aspects, an SMN2 ASO is administered 1-6 times per year, and the recombinant SMN1 gene (e.g., in an rAAV) is administered once initially. In some aspects, two or more subsequent doses of SMN2 ASO alone are administered following an initial administration of SMN2 ASO and recombinant SMN1 gene. In some aspects, the SMN2 ASO is administered twice monthly. In some aspects, such doses are administered every month. In some aspects, the SMN2 ASO is administered every 2 months. In some aspects, the SMN2 ASO is administered every 6 months. In some aspects, the recombinant SMN1 gene (e.g., in an rAAV) is re-administered, for example 1 or more years (e.g., 2-5 years, 5-10 years, 10-15 years, 15-20 years on longer) after an initial administration.

In some aspects, administration of at least one pharmaceutical composition (e.g., of a recombinant SMN1 gene, an SMN2 ASO, or a combination of both) results in a phenotypic change in the subject. In some aspects, such phenotypic changes include, but are not limited to: increased absolute amount of recombinant SMN mRNA and/or cellular 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;; improved muscle strength; improved electrical activity in at least one muscle; improved respiration; improved weight gain; decreased fatigue; and increased survival. In some aspects, at least one phenotypic change is detected in a motor neuron of the subject. In some aspects, administration of at least one pharmaceutical composition described in this application results in a subject being able to sit-up, to stand, and/or to walk. In some aspects, administration of at least one pharmaceutical composition results in a subject being able to eat, drink, and/or breathe without assistance. In some aspects, efficacy of treatment is assessed by electrophysiological assessment of muscle. In some aspects, administration of a pharmaceutical composition improves at least one symptom of SMA. In some aspects, administration of a pharmaceutical composition improves at least one symptom of SMA and has little or no inflammatory effect.

In some aspects, absence of inflammatory effect is determined by the absence of significant increase in Aif1 levels upon treatment.

In some aspects, administration of at least one pharmaceutical composition delays the onset of at least one symptom of SMA. In some aspects, administration of at least one pharmaceutical composition slows the progression of at least one symptom of SMA. In some aspects, administration of at least one pharmaceutical composition reduces the severity of at least one symptom of SMA. In some aspects, administration of at least one pharmaceutical composition results in an undesired side-effect. In some aspects, a treatment regimen is identified that results in desired amelioration of symptoms while avoiding undesired side-effects.

Dosage and formulation

Accordingly, in some aspects, a therapeutically effective amount of an SMN2 ASO is administered to a subject that has SMA. In some aspects the SMN2 ASO is administered alone to the subject. In some aspects, the SMN2 ASO is administered to the subject along with other compounds and/or pharmaceutical compositions. In some aspects, an SMN2 ASO and a recombinant nucleic acid (e.g., in an rAAV) are administered to the subject. In some aspects, the SMN2 ASO and the recombinant nucleic acid encoding SMN1 (e.g., in an rAAV) are administered concurrently (e.g., simultaneously or during the same medical visit), or sequentially (e.g., during different medical visits) to the subject. In some aspects, the SMN2 ASO and the recombinant nucleic acid are administered together in a single composition to the subject. In some aspects, the SMN2 ASO and the recombinant nucleic acid are administered separately to the subject.

In some aspects, the SMN2 ASO and the recombinant nucleic acid encoding SMN1 are administered to a subject concurrently (e.g., either simultaneously or at different times during a visit to a hospital, clinic, or other medical center, for example at different times during the same day of a medical visit). Accordingly, in some aspects, administering the SMN2 ASO and the recombinant nucleic acid encoding SMN1 concurrently means administration during the same medical visit (e.g., during the same clinic day). In some aspects, administering the SMN2 ASO and the recombinant nucleic acid encoding SMN1 concurrently means administration at different times during the same visit (e.g., during the same clinic day). In some aspects, the concurrent administration of SMN1 gene (e.g., rAAV encoding SMN1) and the SMN2 ASO is an initiation of a new therapy. In other aspects, the

concurrent administration of SMN1 gene (e.g., rAAV encoding SMN1) and the SMN2 ASO is an additional therapy for a subject currently being treated with a different composition or a single composition (e.g., an SMN1 gene therapy or SMN2 ASO therapy alone).

In some aspects, the SMN2 ASO and the recombinant nucleic acid encoding SMN1 are administered to a subject sequentially during different visits (e.g., different clinic days). In some aspects, administering the SMN2 ASO and the recombinant nucleic acid encoding SMN1 sequentially means administration of recombinant nucleic acid encoding SMN1 during a first visit, followed by administration of SMN2 ASO during a different visit (e.g., different clinic days). In some aspects, administering the SMN2 ASO and the recombinant nucleic acid encoding SMN1 sequentially means administration of SMN2 ASO during a first visit, followed by administration of recombinant nucleic acid encoding SMN1 during a different visit (e.g., different clinic days). In some aspects, the recombinant nucleic acid encoding SMN1 and the SMN2 ASO are administered at different frequencies. As used herein, a sequential administration can include an administration protocol wherein an administration of a first therapy (e.g., a recombinant nucleic acid encoding SMN1) during a medical visit can follow or precede one or more administrations of a second therapy (e.g., an SMN2 ASO) during one or more different medical visits.

In some aspects, the SMN2 ASO and the recombinant nucleic acid are administered at different frequencies. In some aspects, the SMN2 ASO is administered to the subject 1-6 times per year. In some aspects, the recombinant nucleic acid is administered once. In some aspects, two or more subsequent doses of the SMN2 ASO alone are administered following an initial administration of the SMN2 ASO and recombinant nucleic acid. In some aspects, the SMN2 ASO is administered to the subject prior to the administration of a combination of SMN2 ASO and recombinant nucleic acid in the same composition. In some aspects, the SMN2 ASO is administered to the subject at a dose of 0.01 to 25 milligrams (e.g., 0.01 to 10 milligrams, 0.05 to 5 milligrams, 0.1 to 2 milligrams, or 0.5 to 1 milligrams) per kilogram of body weight of the subject, and the recombinant nucleic acid is administered in an rAAV at a dose from 2×10^{10} to 2×10^{14} GC (e.g., from 1.0×10^{13} to 1.0×10^{14} GC, or for example for IT dosing from about 1.0×10^{13} to 5.0×10^{14} GC). In some aspects, the SMN2 ASO is administered to the subject at a dose of 0.001 to 25 milligrams (e.g., 0.001 to 10 milligrams, 0.005 to 5 milligrams, 0.01 to 2 milligrams, or 0.05 to 1 milligrams) per kilogram of body weight of the subject, and the recombinant nucleic acid is administered in an rAAV at a dose

from 1 x 10^{10} to 2 x 10^{14} GC (e.g., from 1.0 x 10^{13} to 1.0 x 10^{14} GC, or for example for IT dosing from about 1.0 x 10^{13} to 5.0 x 10^{14} GC or for example for IV dosing from about 3 x 10^{13} to 5 x 10^{14} GC. In some aspects, the SMN2 ASO is administered at a dose from 0.01 to 10 milligrams per kilogram of body weight of the subject. In some aspects, the SMN2 ASO is administered at a dose from 0.001 to 10 milligrams per kilogram of body weight of the subject. In some aspects, the SMN2 ASO is administered at a dose of less than 0.001 milligrams per kilogram of body weight of the subject.

In some aspects, a total of 5 mg to 60 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 5 mg to 20 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 48 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg to 36 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 28 mg per dose of SMN2 ASO is administered to the subject. In some aspects, a total of 12 mg per dose of SMN2 ASO is administered to the subject. In some aspects, the SMN2 ASO and/or the recombinant SMN1 gene is administered to the subject intravenously or intramuscularly. In some aspects, the SMN2 ASO and/or the recombinant SMN1 gene is administered into the intrathecal space of the subject. In some aspects, the SMN2 ASO and/or the recombinant SMN1 gene is administered into the intracisternal magna space of the subject. In some aspects, administration of the SMN2 ASO and the recombinant nucleic acid increase intracellular SMN protein level in the subject. In some aspects, administration of the SMN2 ASO and the recombinant nucleic acid increase intracellular SMN protein level in the cervical, thoracic, and lumbar spinal cord segments of motor neurons in the subject.

In some aspects, doses of recombinant SMN1 gene (e.g., in an rAAV) and SMN2 ASO are administered by bolus injection into the CSF. In some aspects, doses are administered by LP and/or ICM bolus injection. In some aspects, doses are administered by bolus systemic injection (e.g., subcutaneous, intramuscular, or intravenous injection). In some aspects, subjects receive bolus injections into the CSF and bolus systemic injections. In some aspects, the doses of the CSF bolus and the systemic bolus may be the same or different from one another. In some aspects, the CSF and systemic doses are administered at different frequencies.

In some aspects, pharmaceutical compositions comprising a recombinant SMN1 gene (e.g., in an rAAV), an SMN2 ASO, or a combination thereof are provided. Pharmaceutical

compositions can be designed for delivery to subjects in need thereof by any suitable route or a combination of different routes. For example, one or more compositions may be administered to human subjects using routes comprising intracerebroventricular (ICV), intravenous (IV), and intrathecal (IT) (e.g., via lumbar puncture (LP), and/or intracisternal magna (ICM) delivery).

In some aspects, direct delivery to the CNS is desired and may be performed via intrathecal injection. The term "intrathecal administration" refers to delivery that targets the cerebrospinal fluid (CSF). This may be done by direct injection into the ventricular or lumbar CSF, by suboccipital puncture, or by other suitable means. Meyer et al, Molecular Therapy (31 October 2014), demonstrated the efficacy of direct CSF injection which resulted in widespread transgene expression throughout the spinal cord in mice and nonhuman primates when using a 10 times lower dose compared to the IV application. This document is incorporated herein by reference. In some aspects, a recombinant SMN1 gene is delivered via intracerebroventricular viral injection (see, e.g., Kim et al, J Vis Exp. 2014 Sep 15;(91):51863, which is incorporated herein by reference). See also, Passini et al, Hum Gene Ther. 2014 Jul;25(7):619-30, which is incorporated herein by reference. In some aspects, a composition is delivered via lumbar injection.

In some aspects, delivery means and formulations are designed to avoid direct systemic delivery of a suspension containing AAV composition(s) described in this application. Suitably, this may have the benefit of reducing systemic exposure as compared to systemic administration, reducing toxicity and/or reducing undesirable immune responses to the AAV and/or transgene product.

Compositions comprising a recombinant SMN1 gene (e.g., in an rAAV) and/or SMN2 ASO may be formulated for any suitable route of administration (e.g., oral, inhalation, intranasal, intratracheal, intraarterial, intraocular, intravenous, intramuscular, and other parenteral routes).

In some aspects, recombinant SMN1 gene delivery constructs described in this application may be delivered in a single composition or multiple compositions. In some aspects, two or more different AAV may be delivered (see, e.g., WO 2011/126808 and WO 2013/049493). In some aspects, such multiple viruses may contain different replication-defective viruses (e.g., AAV, adenovirus, and/or lentivirus). Alternatively, delivery may be

mediated by non-viral constructs, e.g., "naked DNA", "naked plasmid DNA", RNA, and mRNA, coupled with various delivery compositions and nanoparticles, including, e.g., micelles, liposomes, cationic lipid - nucleic acid compositions, poly-glycan compositions and other polymers, lipid and/or cholesterol-based - nucleic acid conjugates, and other constructs such as described in this application or known in the art. See, e.g., X. Su et al, Mol. Pharmaceutics, 2011, 8 (3), pp 774-787; web publication: March 21, 2011; WO2013/182683, WO 2010/053572 and WO 2012/170930, both of which are incorporated herein by reference. Non-viral SMN1 delivery constructs also may be formulated for any suitable route of administration.

Viral vectors, or non-viral DNA or RNA transfer moieties, can be formulated with a physiologically acceptable carrier for use in gene transfer and gene therapy applications. A number of suitable purification methods may be selected. Examples of suitable purification methods for separating empty capsids from vector particles are described, e.g., the process described in International Patent Application No. PCT/US 16/65976, filed December 9, 2016 and its priority documents US Patent Application Nos. 62/322,098, filed April 13, 2016 and US Patent Appln No. 62/266,341, filed on December 11, 2015, and entitled "Scalable Purification Method for AAV8", which is incorporated by reference herein. See, also, purification methods described in International Patent Application No. PCT/US 16/65974, filed December 9, 2016, and its priority documents, US Patent Applications No.62/322,083, filed April 13, 2016 and 62/266,351, filed December 11, 2015 (AAV1); International Patent Appln No. PCT/US16/66013, filed December 9, 2016 and its priority documents US Provisional Applications No. 62/322,055, filed April 13, 2016 and 62/266,347, filed December 11, 2015 (AAVrhlO); and International Patent Application No. PCT/US 16/65970, filed December 9, 2016, and its priority applications US Provisional Application Nos. 62/266,357 and 62/266,357 (AAV9), which are incorporated by reference herein. Briefly, a two-step purification scheme is described which selectively captures and isolates the genome-containing rAAV vector particles from the clarified, concentrated supernatant of a rAAV production cell culture. The process utilizes an affinity capture method performed at a high salt concentration followed by an anion exchange resin method performed at high pH to provide rAAV vector particles which are substantially free of rAAV intermediates.

In the case of AAV viral vectors, quantification of the genome copies ("GC") may be used as the measure of the dose contained in the formulation. Any method known in the art

can be used to determine the genome copy (GC) number of the replication-defective virus compositions of the invention. One method for performing AAV GC number titration is as follows: Purified AAV vector samples are first treated with DNase to eliminate contaminating host DNA from the production process. The DNase resistant particles are then subjected to heat treatment to release the genome from the capsid. The released genomes are then quantitated by real-time PCR using primer/probe sets targeting specific region of the viral genome (for example poly A signal). Another suitable method for determining genome copies are the quantitative-PCR (qPCR), particularly the optimized qPCR or digital droplet PCR (Lock Martin, et al, Human Gene Therapy Methods. April 2014, 25(2): 115-125. doi: 10.1089/hgtb.2013.131, published online ahead of editing December 13, 2013).

In some aspects, replication-defective virus compositions can be formulated either alone or co-formulated with an ASO in dosage units to contain an amount of replication-defective virus that is in the range of about 1.0 x 10^9 GC to about 1.0 x 10^{15} GC (e.g., to treat an average subject of 70 kg in body weight) including all integers or fractional amounts within the range, and preferably 1.0 x 10^{12} GC to 1.0 x 10^{14} GC for a human patient. The total dose administered to a subject may depend on the route of administration. In some aspects, the compositions are formulated to contain at least 1 x 10^9, 2 x 10^9, 3 x 10^9, 4 x 10^9, 5 x 10^9, 6 x 10^9, 7 x 10^9, 8 x 10^9, or 9 x 10^9 GC per dose including all integers or fractional amounts within the range. In another aspect, the compositions are formulated to contain at least l x 10^{10}, 2 x 10^{10}, 3 x 10^{10}, 4 x 10^{10}, 5 x 10^{10}, 6 x 10^{10}, 7 x 10^{10}, 8 x 10^{10}, or 9 x 10^{10} GC per dose including all integers or fractional amounts within the range. In another aspect, the compositions are formulated to contain at least l x 10^{11}, 2 x 10^{11}, 3 x 10^{11}, 4 x 10^{11}, 5 x 10^{11}, 6 x 10^{11}, 7 x 10^{11}, 8 x 10^{11}, or 9 x 10^{11} GC per dose including all integers or fractional amounts within the range. In another aspect, the compositions are formulated to contain at least 1 x 10^{12}, 2 x 10^{12}, 3 x 10^{12}, 4 x 10^{12}, 5 x 10^{12}, 6 x 10^{12}, 7 x 10^{12}, 8 x 10^{12}, or 9 x 10^{12} GC per dose including all integers or fractional amounts within the range. In another aspect, the compositions are formulated to contain at least l x 10^{13}, 2 x 10^{13}, 3 x 10^{13}, 4 x 10^{13}, 5 x 10^{13}, 6 x 10^{13}, 7 x 10^{13}, 8 x 10^{13}, or 9 x 10^{13} GC per dose including all integers or fractional amounts within the range. In another aspect, the compositions are formulated to contain at least l x 10^{14}, 2 x 10^{14}, 3 x 10^{14}, 4 x 10^{14}, 5 x 10^{14}, 6 x 10^{14}, 7 x 10^{14}, 8 x 10^{14}, or 9 x 10^{14} GC per dose including all integers or fractional amounts within the range. In another aspect, the compositions are formulated to contain at least l x 10^{15}, 2 x 10^{15}, 3 x 10^{15}, 4 x 10^{15}, 5 x 10^{15}, 6 x 10^{15}, 7 x 10^{15}, 8 x 10^{15}, or 9 x 10^{15} GC per dose including all integers or fractional amounts

within the range. In some aspects, for human application the dose of a virus (e.g., of an rAAV) can range from 1 x 10^{10} to about 1 x 10^{12} GC per dose including all integers or fractional amounts within the range.

These above doses may be administered in a variety of volumes of carrier, excipient or buffer formulation, ranging from about 25 microliters to about 1,000 microliters, or to about 10 milliliters, or up to 20 milliliters, including all numbers within the range, depending on the size of the area to be treated, the viral titer used, the route of administration, and the desired effect of the method. In some aspects, the volume of carrier, excipient or buffer is at least about 25 µl. In some aspects, the volume is about 50 µl. In another aspect, the volume is about 75 µl. In another aspect, the volume is about 100 µl. In another aspect, the volume is about 125 µl. In another aspect, the volume is about 150 µl. In another aspect, the volume is about 175 µl. In yet another aspect, the volume is about 200 µl. In another aspect, the volume is about 225 µl. In yet another aspect, the volume is about 250 µl. In yet another aspect, the volume is about 275 µl. In yet another aspect, the volume is about 300 µl. In yet another aspect, the volume is about 325 µl. In another aspect, the volume is about 350 µl. In another aspect, the volume is about 375 µl. In another aspect, the volume is about 400 µl. In another aspect, the volume is about 450 µl. In another aspect, the volume is about 500 µl. In another aspect, the volume is about 550 µl. In another aspect, the volume is about 600 µl. In another aspect, the volume is about 650 µl. In another aspect, the volume is about 700 µl. In another aspect, the volume is between about 700 and 1000 µl.

In other aspects, volumes of about 1 µl to 150 mL may be selected, with the higher volumes being selected for adults. Typically, for newborn infants a suitable volume is about 0.5 mL to about 10 mL. For older infants, about 0.5 mL to about 15 mL may be selected. For toddlers, a volume of about 0.5 mL to about 20 mL may be selected. For children, volumes of up to about 30 mL may be selected. For pre-teens and teens, volumes up to about 50 mL may be selected. In still other aspects, a patient may receive an intrathecal administration in a volume of about 5 mL to about 15 mL are selected, or about 7.5 mL to about 10 mL. Other suitable volumes and dosages may be determined. The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed.

Recombinant SMN1 genes, for example in viral vectors (e.g., packaged in an rAAV), may be delivered to host cells using suitable methods. The rAAV, preferably suspended in a

physiologically compatible carrier, may be administered to a human or non-human mammalian patient. In some aspects, the composition includes a carrier, diluent, excipient and/or adjuvant. Suitable carriers may be selected for the route of administration. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water.

In some aspects, compositions may contain, in addition to the rAAV and/or ASO and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

In some aspects, compositions comprising an rAAV and/or an ASO may comprise a pharmaceutically acceptable carrier and/or be admixed with suitable excipients designed for delivery to a subject via injection, osmotic pump, intrathecal catheter, or for delivery by another device or route. In one example, a composition is formulated for intrathecal delivery. In some aspects, intrathecal delivery encompasses an injection into the spinal canal, e.g., the subarachnoid space.

Viral vectors described in this application may be used in preparing a medicament for delivering SMN1 to a subject (e.g., a human patient) in need thereof, supplying functional SMN to a subject, and/or for treating spinal muscular atrophy in combination therapies with one or more SMN2 ASOs.

In some aspects, buffers, carriers, and/or other components of a pharmaceutical formulation comprising an rAAV are selected to include one or more components that prevent rAAV from sticking to infusion tubing but does not interfere with the rAAV binding activity in vivo. For co-formulation with an ASO, buffers, carriers, and/or other components also may be selected to avoid unwanted interaction with the ASO.

In some aspects, SMN2 ASOs are formulated for delivery alone or co-formulated with a recombinant SMN1 gene, for example co-formulated with an rAAV comprising a recombinant nucleic acid encoding an SMN1 gene. In some such aspects, ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for

systemic administration) in amounts ranging from 5 mg to 60 mg of ASO per dose. In some such aspects, ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) in amounts ranging 5 mg to 20 mg of ASO per dose. In some such aspects, ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) in amounts ranging 12 mg to 50 mg of ASO per dose. In some such aspects ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) in amounts ranging 12 mg to 48 mg of ASO per dose. In some such aspects, ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) in amounts ranging from 12 mg to 36 mg of ASO per dose. In some such aspects, ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) in amounts of 28 mg of ASO per dose. In some such aspects ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) in amounts of 12 mg of ASO per dose. In some such aspects, the dose volume is 5 mL.

In some such aspects, ASOs (e.g., SMN2 ASO) (alone or with a recombinant SMN1 gene) are formulated for delivery (e.g., for systemic administration) ranging from 0.1 mg/kg to 200 mg/kg (ASO/patient weight). In some aspects, the dose is from 0.1 mg/kg to 100 mg/kg. In some aspects, the dose is from 0.5 mg/kg to 100 mg/kg. In some aspects, the dose is from 1 mg/kg to 100 mg/kg. In some aspects, the dose is from 1 mg/kg to 50 mg/kg. In some aspects, the dose is from 1 mg/kg to 25 mg/kg. In some aspects, the dose is from 0.1 mg/kg to 25 mg/kg. In some aspects, the dose is from 0.1 mg/kg to 10 mg/kg. In some aspects, the dose is from 1 mg/kg to 10 mg/kg. In some aspects, the dose is from 1 mg/kg to 5 mg/kg.

In some aspects, dosing a subject with an ASO is divided into an induction phase and a maintenance phase. In some such aspects, the dose administered during the induction phase is greater than the dose administered during the maintenance phase. In some aspects, the dose administered during the induction phase is less than the dose administered during the maintenance phase. In some aspects, the induction phase is achieved by bolus injection and the maintenance phase is achieved by continuous infusion. In some aspects, a combination formulation is used during the induction phase.

In some aspects, pharmaceutical compositions are administered as a bolus injection. In some such aspects, the dose of the bolus injection contains a total of 5 mg to 60 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose of the bolus injection contains a total of 5 mg to 20 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose of the bolus injection contains a total of 12 mg to 50 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose of the bolus injection contains a total of 12 mg to 48 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose of the bolus injection contains a total of 12 mg to 36 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose of the bolus injection contains a total of 28 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose of the bolus injection contains a total of 12 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO). In some such aspects, the dose volume is 5 mL.

In some aspects, pharmaceutical compositions are administered as a bolus injection. In some such aspects, the dose of the bolus injection is from 0.01 to 25 milligrams of antisense compound per kilogram body weight of the subject. In some such aspects, the dose of the bolus injection is from 0.01 to 10 milligrams of antisense compound per kilogram body weight of the subject. In some aspects, the dose is from 0.05 to 5 milligrams of antisense compound per kilogram body weight of the subject. In some aspects, the dose is from 0.1 to 2 milligrams of antisense compound per kilogram body weight of the subject. In some aspects, the dose is from 0.5 to 1 milligrams of antisense compound per kilogram body weight of the subject.

In some aspects, such doses are administered twice monthly. In some aspects, such doses are administered every month. In some aspects, such doses are administered every 2 months. In some aspects, such doses are administered every 6 months. In some aspects, such doses are administered by bolus injection into the CSF. In some aspects, such doses are administered by intrathecal bolus injection. In some aspects, such doses are administered by bolus systemic injection (e.g., subcutaneous, intramuscular, or intravenous injection). In some aspects, subjects receive bolus injections into the CSF and bolus systemic injections. In such aspects, the doses of the CSF bolus and the systemic bolus may be the same or different from one another. In some aspects, the CSF and systemic doses are administered at different frequencies. In some aspects, the invention provides a dosing regimen comprising at least one bolus intrathecal injection and at least one bolus subcutaneous injection.

In some aspects, pharmaceutical compositions are administered by continuous infusion (e.g., wherein a dose can be administered over a period time, for example, a 24 hour period). Such continuous infusion may be accomplished by an infusion pump that delivers pharmaceutical compositions to the CSF. In some aspects, such infusion pump delivers pharmaceutical composition IT or ICV. In some such aspects, the dose administered is between 5 mg to 60 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose administered is between 5 mg to 20 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose administered is between 12 mg to 50 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose administered is between 12 mg to 48 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose administered is between 12 mg to 36 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose administered is 28 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose administered is 12 mg per dose of an antisense oligonucleotide (e.g., SMN2 ASO) per day. In some such aspects, the dose volume is 5 mL.

In other aspects, the dose administered is between 0.05 and 25 milligrams of antisense compound per kilogram body weight of the subject per day. In some aspects, the dose administered is from 0.1 to 10 milligrams of antisense compound per kilogram body weight of the subject per day. In some aspects, the dose administered is from 0.5 to 10 milligrams of antisense compound per kilogram body weight of the subject per day. In some aspects, the dose administered is from 0.5 to 5 milligrams of antisense compound per kilogram body weight of the subject per day. In some aspects, the dose administered is from 1 to 5 milligrams of antisense compound per kilogram body weight of the subject per day. In some aspects, the invention provides a dosing regimen comprising infusion into the CNS and at least one bolus systemic injection. In some aspects, the invention provides a dosing regimen comprising infusion into the CNS and at least one bolus subcutaneous injection. In some aspects, 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 some aspects, 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 some aspects, 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.

In some aspects, the invention provides a dosing regimen comprising infusion into the CNS and at least one bolus systemic injection. In some aspects, the invention provides a dosing regimen comprising infusion into the CNS and at least one bolus subcutaneous injection. In some aspects, 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 some aspects, 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 some aspects, 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.

Accordingly, in some aspects, the present invention provides pharmaceutical compositions comprising one or more therapeutic molecules, for example one or more recombinant nucleic acids (e.g., in a viral vector, for example packaged in an rAAV) and/or antisense compounds. In some aspects, such pharmaceutical composition comprises a sterile saline solution and one or more therapeutic molecules. In some aspects, such pharmaceutical compositions consist of a sterile saline solution and one or more therapeutic molecules. In some aspects, therapeutic molecules 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. In some aspects, therapeutic molecules can be utilized in pharmaceutical compositions by combining such therapeutic molecules with a suitable pharmaceutically acceptable diluent or carrier. In some aspects, a pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in some aspects, employed in the methods described herein is a pharmaceutical composition comprising one or more therapeutic molecules and a pharmaceutically acceptable diluent. In some aspects, the pharmaceutically acceptable diluent is PBS. Pharmaceutical compositions comprising one or more therapeutic molecules described in this application encompass any pharmaceutically acceptable salts, esters, or salts of such esters. In some aspects, pharmaceutical compositions comprising ASOs 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, in some aspects

pharmaceutically acceptable salts of ASOs, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents are provided. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

In some aspects, 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. 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. Some preparations are described in Akinc et al., Nature Biotechnology 26, 561-569 (1 May 2008), which is herein incorporated by reference in its entirety.

Kits

In some aspects, kits are provided comprising a recombinant SMN1 gene (e.g., in an rAAV) and/or an SMN2 ASO, e.g., in a pharmaceutical composition. In some aspects, such kits further comprise additional therapeutic agents such as one or more immunosuppressive agents. In some aspects, such kits further comprise a means of delivery, for example a syringe or infusion pump.

The following examples are illustrative only and are not intended to limit the present invention.

EXAMPLES

Example 1: rAAV vectors containing an hSMN1 gene

A recombinant neurotropic AAV virus was constructed bearing a codon-optimized human SMN1 cDNA.

Example 2: ASOs that increases full-length SMN2 mRNA (e.g., by promoting exon 7 inclusion in hSMN2 mRNA)

An ASO that increases full-length SMN2 mRNA (e.g., that promotes exon 7 inclusion in SMN2 mRNA) was prepared (FIG. 3).

Example 3: Administration and bio-distribution of rAAV vectors containing an hSMN1 gene with an ASO that increases full-length SMN2 mRNA (e.g., that promotes exon 7 inclusion in SMN2 mRNA). The rAAV of Example 1 and the ASO of Example 2 are administered to animal SMA disease models and control animals, including mice, pig, and non-human primate (e.g., macaque), SMA disease and control animal models.

The rAAV and ASO are administered via different routes, including via intrathecal and systemic routes (e.g., via lumbar puncture, intra-cisterna magna, and intravenous delivery).

The distribution of rAAV and ASO is evaluated in the animal models. In particular, distribution within the spinal cord is evaluated, for example to determine the relative amount of rAAV and/or ASO in the cervical, thoracic, and lumbar regions of the spinal cord.

FIG. 4 illustrates results using 3 x 10^{13} GC rAAV administered via lumbar puncture or intra-cisterna magna delivery, and using 2 x 10^{14} GC administered intra-venously.

Example 4: Co-formulation of rAAV vectors containing an hSMN1 gene with an ASO that increases full-length SMN2 mRNA (e.g., that promotes exon 7 inclusion in SMN2 mRNA)

FIG. 5 illustrates non-limiting examples of physical and biological characterizations of a composition comprising both an rAAV vector an hSMN1 gene and an ASO that increases full-length SMN2 mRNA (e.g., that promotes exon 7 inclusion in SMN2 mRNA).

FIG. 5A shows an SEC-HPLC profile of the rAAV vector alone. FIG. 5B shows an SEC-HPLC profile of the ASO alone. FIG. 5C shows an SEC-HPLC profile of the rAAV vector and the ASO when they are combined together in the same formulation. The HPLC profiles of the rAAV vector and ASO remain the same in FIG. 5C, showing that there is no significant incompatibility when the rAAV and the ASO are co-formulated.

FIG. 5D provides data for rAAV infectivity in cells in vitro upon delivery of either the rAAV vector alone or in combination with the ASO. The results show that rAAV infectivity is not significantly affected by the presence of the ASO in a co-formulation.

FIG. 5E shows intracellular SMN protein expression level and GEM formation in cells following treatment with rAAV, ASO, or both.

Example 5: Intracerebroventricular (ICV) administration of nusinersen and AAV-SMN1

Using a micro-osmotic pump (ALZET Osmotic Pumps, Cupertino, Calif., USA), Nusinersen and AAV-SMN1 are delivered into cerebrospinal fluid (CSF) through the right lateral ventricle in neonatal (P0-P1) SMA mice with a human SMN2 transgene. A low or high dose of nusinersen (1 µg and 4 µg respectively) is administered to the mice along with a low or high dose of AAV-SMN1 (1×10^{10} GC or 8×10^{10} GC respectively) at birth (P0-P1). The mice body weight and righting reflex is measured and compared to the body weight and righting reflex of control mice of the same genotype having received either nusinersen or AAV-SMN1 alone.

Mice administered both nusinersen and AAV-SMN1 will have a significantly higher body weight and faster righting reflex compared to controls.

Studies will reveal that intracerebroventricular (ICV) administration of nusinersen and AAV-SMN1 increases SMN2 exon 7 inclusion in the spinal cord. Further studies will show that a greater number of spinal-cord motor neurons have increased SMN expression compared to controls.

Example 6: Administration of compositions of nusinersen and AAV-SMN1

Using a micro-osmotic pump (ALZET Osmotic Pumps, Cupertino, Calif., USA), compositions of nusinersen and AAV-SMN1 are delivered into cerebrospinal fluid (CSF) through the right lateral ventricle in neonatal (P0-P1) SMA mice with a human SMN2 transgene. Compositions of a low dose of nusinersen (1 µg) and a low dose of AAV-SMN1 (1×10^{10} GC), or a low dose of nusinersen (1 µg) and a high dose of AAV-SMN1 (8×10^{10} GC), or a high dose of nusinersen (4 µg) and a low dose of AAV-SMN1 (1×10^{10} GC), or a high dose of nusinersen (4 µg) and a high dose of AAV-SMN1 (8×10^{10} GC) are administered to the mice at birth (P0-P1). The mice body weight and righting reflex is measured and compared to the body weight and righting reflex of control mice of the same genotype having received either nusinersen or AAV-SMN1 alone.

Mice administered a composition of nusinersen and AAV-SMN1 will have a significantly higher body weight and faster righting reflex compared to controls.

Studies will reveal that intracerebroventricular (ICV) administration of the composition of nusinersen and AAV-SMN1 increases SMN2 exon 7 inclusion in the spinal cord. Further studies will show that a greater number of spinal-cord motor neurons have increased SMN expression compared to controls.

Example 7: Administration and analysis of nusinersen and AAV-SMN1 distribution in non-human mammals

SMA mice, Rhesus Macaques and Cynomolgus monkeys are used to assess distribution of nusinersen and AAV-SMN1 compositions at different doses and routes of administration. Nusinersen and AAV-SMN1 compositions are administered to some mice and some monkeys at a dose of about 1 mg/kg by intracerebroventricular (ICV) infusion or by intrathecal (IT) infusion over a 24 hour period. The animals are sacrificed and tissues harvested 96 hours after the end of the infusion period. The concentration of nusinersen and AAV-SMN1 are measured in samples from Cervical, Thoracic, and Lumbar sections of the spinal cord.

Additional mice, Rhesus Macaques and Cynomolgus monkeys of the same genotype as above, are administered nusinersen and AAV-SMN1 compositions at the same dose of about 1 mg/kg by ICV infusion or by IT infusion. The animals are administered the nusinersen and AAV-SMN1 compositions over a period 3 days, 7 days, or 14 days prior to being sacrificed 5 days after the end of the infusion period.

Example 8: Administration of nusinersen and AAV-SMN1 to human subjects

Nusinersen and AAV-SMN1 are administered to human subjects using routes comprising intracerebroventricular (ICV), intravenous (IV), and intrathecal (IP) (e.g., via lumbar puncture (LP), and/or intracisternal magna (ICM) delivery). The compositions are tested in both children and adults.

In some aspects, rAAV-SMN1 compositions are administered to children (e.g., having SMA) at a dose of about 1 x 10^{14} GC, for example by lumbar puncture (LP) infusion (e.g.,

over a 24 hour period). In some aspects, rAAV-SMN1 compositions are administered to adults (e.g., having SMA) at a dose of about 1.5 x 10^{14} GC, for example by intracisternal magna (ICM) infusion (e.g., over a 24 hour period).

In some aspects, other rAAV-SMN1 doses can be used, for example about 5-6 x 10^{13} GC, or higher, for example, around 1.2 x 10^{14} GC, or 1.5-1.8 x 10^{14} GC. Any suitable route of administration can be used, for example via IT delivery (e.g., infusion over a 24 hour period), for example via LP or ICM delivery.

Example 9: Intracerebroventricular (ICV) administration of nusinersen and AAV-SMN1

Nusinersen and AAV-SMN1 were administered to neonatal (P0-P1) SMA mice having a human SMN2 transgene. A low or high dose of nusinersen (1 μg and 3 μg respectively) was administered to the mice along with a low or high dose of AAV-SMN1 (1 x 10^{10} GC or 3 x 10^{10} GC respectively) at birth (P0-P1). The mice body weight and righting reflex were measured and compared to the body weight and righting reflex of control mice of the same genotype having received either nusinersen or AAV-SMN1 alone.

Mice administered both nusinersen and AAV-SMN1 have a significantly higher body weight and faster righting reflex compared to controls.

FIGs. 6A-6B either an SMN1 gene (e.g., in an rAAV vector) or an ASO such as nusinersen (e.g., in a single dose). The experiments show partial rescue of motor function at postnatal day (PND) 8** with full rescue at PND 16, post dosing. FIG. 6A shows the righting reflex (RR) of 4 separate groups of mice after 8 and 16 days of nusinersen. FIG. 6B shows the body weight of 4 separate groups of mice after 8 and 16 days of nusinersen. A combination therapy can improve on the partial rescue of RR (PND 7-16) and body weight seen with monotherapy.

FIGs. 7A-7C show the results of a first combination therapy study showing the effect of a combination of SMN1 gene therapy and nusinersen on body weight and RR. FIG. 7A shows body weight change over time. FIG. 7B shows RR change over time. FIG. 7C is a chart outlining conditions for the three groups of animals that were tested.

FIGs. 8A-8C show the results of a second combination therapy showing the effect of a combination of SMN1 gene therapy and nusinersen on body weight and RR. FIG. 8A is a

chart outlining conditions for the three groups of animals that were tested. FIG. 8B shows the body weight change over time, and FIG. 8C shows the RR change over time (in days).

FIGs. 9A-9B show the comparison of % change in body weight from PND 7-PND 13. FIG. 9A shows the % change in body weight at a dose of gene therapy (rAAV): 1 x 10^{10} GC/ASO (nusinersen): 1 µg. FIG. 9B shows the % change in body weight a dose of gene therapy (rAAV): 3 x 10^{10} GC/ASO (nusinersen): 3 µg. FIGs. 10A-10B show the comparison of % change in RR from PND 7-PND 13. FIG. 10A shows the % change in RR at a dose of gene therapy (rAAV): 1 x 10^{10} GC/ASO (nusinersen): 1 µg. FIG. 10B shows the % change in RR at a dose of gene therapy (rAAV): 3 x 10^{10} GC/ASO (nusinersen): 3 µg.

OTHER ASPECTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, other aspects are also within the claims.

EQUIVALENTS

While several inventive aspects have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive aspects described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive

aspects described herein. It is, therefore, to be understood that the foregoing aspects are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive aspects may be practiced otherwise than as specifically described and claimed. Inventive aspects of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally,

additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03. It should be

appreciated that aspects described in this document using an open-ended transitional phrase (e.g., "comprising") are also contemplated, in alternative aspects, as "consisting of" and "consisting essentially of" the feature described by the open-ended transitional phrase. For example, if the disclosure describes "a composition comprising A and B", the disclosure also contemplates the alternative aspects "a composition consisting of A and B" and "a composition consisting essentially of A and B".

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 some 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).

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"CGAUCG," wherein "C indicates a cytosine base comprising a methyl group at the 5-position.

CLAIMS

1. A method of treating spinal muscular atrophy (SMA) in a subject having SMA, the method comprising administering to the subject:

a) a recombinant nucleic acid that encodes the survival of motor neuron 1 (SMN1) protein, and

b) an antisense oligonucleotide (ASO) that increases full-length survival of motor neuron 2 (SMN2) mRNA.

2. The method of claim 1, wherein the subject has one or more symptoms of SMA.

3. The method of claim 2, wherein the symptoms comprise atrophy of the limb muscles, difficulty or inability walking, or difficulty breathing.

4. The method of any one of claims 1-3, wherein the subject is a human subject selected from the pediatric and adult population.

5. The method of claim 4, wherein the subject is greater than or equal to 18 years of age.

6. The method of claim 5, wherein the subject is younger than 18 years of age.

7. The method of claim 6, wherein the subject is around 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, or 5 years of age.

8. The method of any one of claims 1-7, wherein the ASO alters the splicing pattern of survival of motor neuron 2 (SMN2) pre-mRNA.

9. The method of claim 8, wherein the ASO is promotes the inclusion of exon 7 in survival of motor neuron 2 (SMN2) mRNA

10. The method of any one of claims 1-9, wherein the ASO comprises a sequence complementary to intron 6, or intron 7 of a nucleic acid molecule encoding SMN2 protein.

11. The method of claim 10, wherein the ASO comprises a sequence complementary to intron 6 of a nucleic acid molecule encoding SMN2 protein.

12. The method of claim 10, wherein the ASO comprises a sequence complementary to intron 7 of a nucleic acid molecule encoding SMN2 protein.

13. The method of any one of claims 1-12, wherein the ASO comprises a nucleic acid sequence of SEQ ID NO: 1.

14. The method of claim 13, wherein the ASO is nusinersen.

15. The method of any one of claims 1-14, wherein the ASO comprises one or more nucleobase or backbone modifications.

16. The method of any any one of claims 1-15, wherein the recombinant nucleic acid comprises a promoter operatively linked to the SMN1 gene.

17. The method of any one of claims 1-16, wherein the recombinant nucleic acid is a recombinant AAV (rAAV) genome comprising flanking AAV inverted terminal repeats (ITRs).

18. The method of claim 17, wherein the recombinant nucleic acid is packaged in an rAAV particle and the rAAV particle is administered to the subject.

19. The method of claim 18, wherein the rAAV particle comprises AAV9 capsid proteins.

20. The method of any one of claims 1-19, wherein the rAAV and the ASO are administered simultaneously.

21. The method of any one of claims 1-19, wherein the rAAV and the ASO are administered concurrently.

22. The method of claims 20 or 21, wherein the rAAV and the ASO are administered together in a single composition.

23. The method of claims 20 or 21, wherein the rAAV and the ASO are administered in separate compositions.

24. The method of any one of claims 1-19, wherein the rAAV and the ASO are administered at different frequencies.

25. The method of any one of claims 1-19 or 24, wherein the rAAV and the ASO are administered sequentially.

26. The method of any one of claims 1-25, wherein the ASO is administered 1-6 times per year.

27. The method of any one of claims 1-26, wherein the rAAV is administered once.

28. The method of any one of claims 24-27, wherein two or more subsequent doses of the ASO alone are administered following an initial administration of the rAAV and the ASO.

29. The method of any one of claims 1-28, wherein the SMN1 rAAV is administered at a dose from 2 x 10^{10} to 2 x 10^{14} GC, and the ASO is administered at a dose from 0.01 to 10 milligrams per kilogram of body weight of the subject.

30. The method of claim 29, wherein a total of 5 mg to 20 mg per dose of ASO is administered to the subject.

31. The method of claim 30, wherein 12 mg per dose of ASO is administered to the subject.

32. The method of any one of claims 1-31, wherein the rAAV and the ASO are administered into the intrathecal space of the subject.

33. The method of any one of claims 1-31, wherein the rAAV and the ASO are administered into the intracisternal magna space of the subject.

34. The method of any one of claims 1-31, wherein initial and/or subsequent doses of the ASO are administered intravenously or intramuscularly.

35. The method of any one of claims 1-34, wherein administration of the rAAV and the ASO increase intracellular SMN protein levels in motor neurons in the subject.

36. The method of claim 35, wherein SMN protein level is increased in the cervical, thoracic, and lumbar spinal cord segments of the subject.

37. The method of any one of claims 1-36, wherein the subject has a deletion or a loss of function point mutation in each SMN1 allele.

38. The method of claim 37, wherein the subject is homozygous for a SMN1 gene mutation.

39. A method of treating spinal muscular atrophy (SMA) in a subject having SMA, the method comprising administering an effective amount of a composition comprising an rAAV encoding SMN1 to a subject that was previously treated with an ASO that increases full-length SMN2 mRNA.

40. A method of treating spinal muscular atrophy (SMA) in a subject having SMA, the method comprising administering an effective amount of a composition comprising an ASO that increases full-length SMN2 mRNA to a subject that was previously administered an rAAV encoding SMN1.

41. A composition comprising an rAAV encoding SMN1 and an ASO that is capable of increasing full-length SMN2 mRNA.

42. The composition of claim 41, wherein the rAAV comprises AAV9 capsid proteins.

43. The composition of claims 41 or 42 wherein the ASO is nusinersen.

44. A pharmaceutical composition comprising a composition of any of claims 41-43 and a pharmaceutically acceptable carrier.

FIG. 1

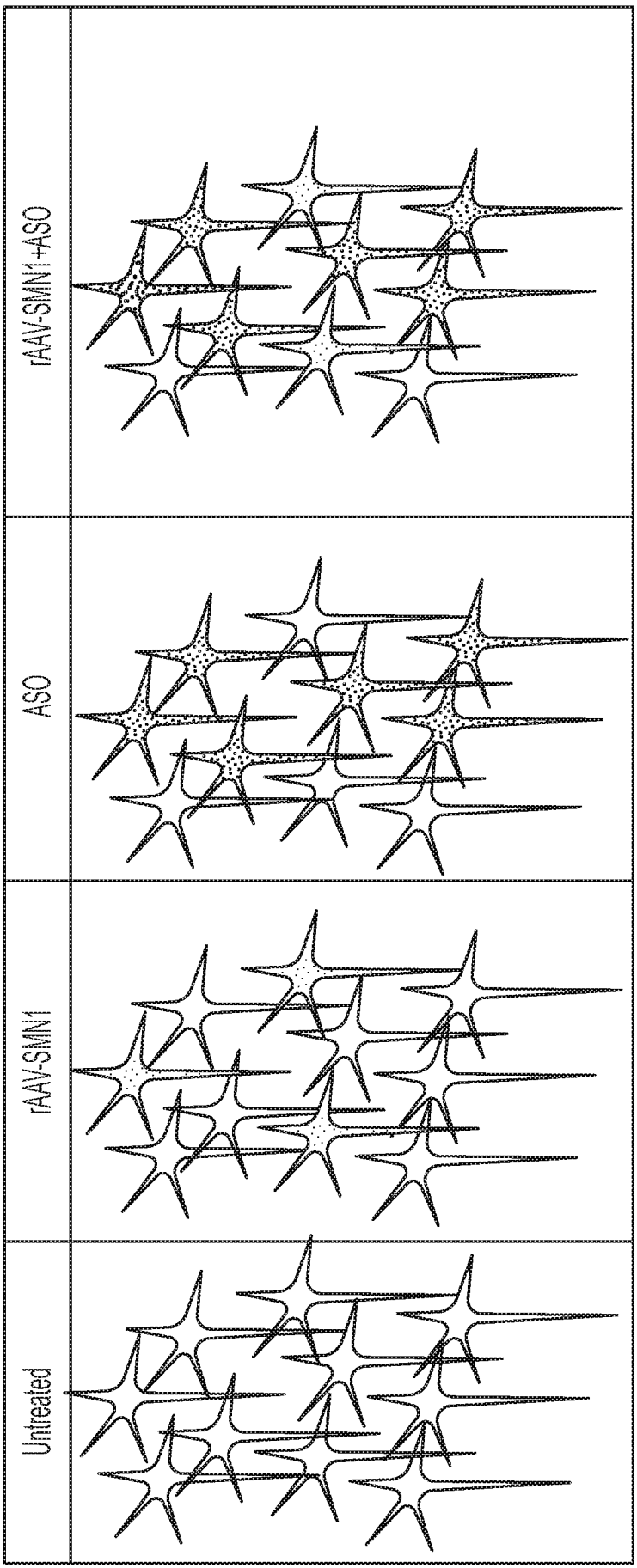


FIG. 2

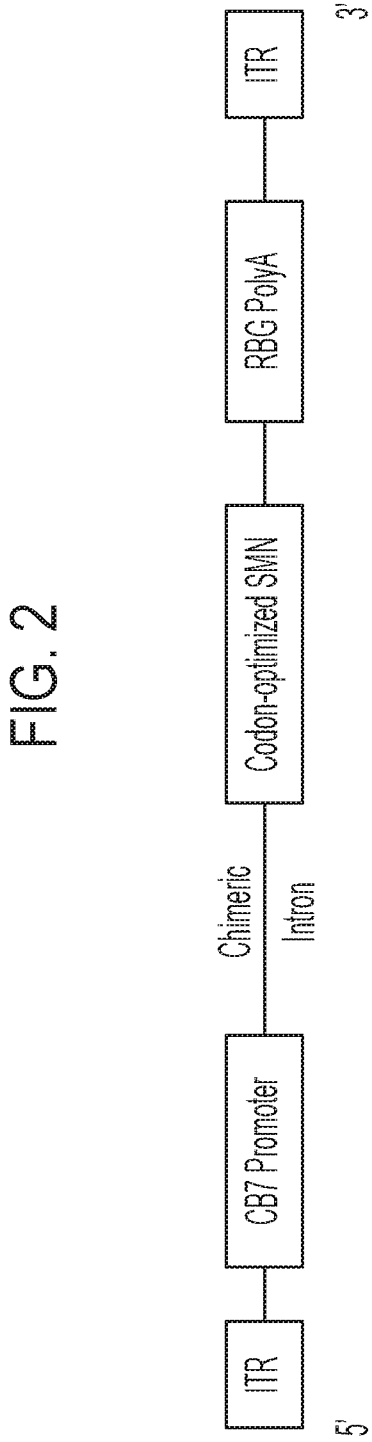

5'
ITR
CB7 Promoter
Chimeric
Intron
Codon-optimized SMN
RBG PolyA
ITR
3'

FIG. 3

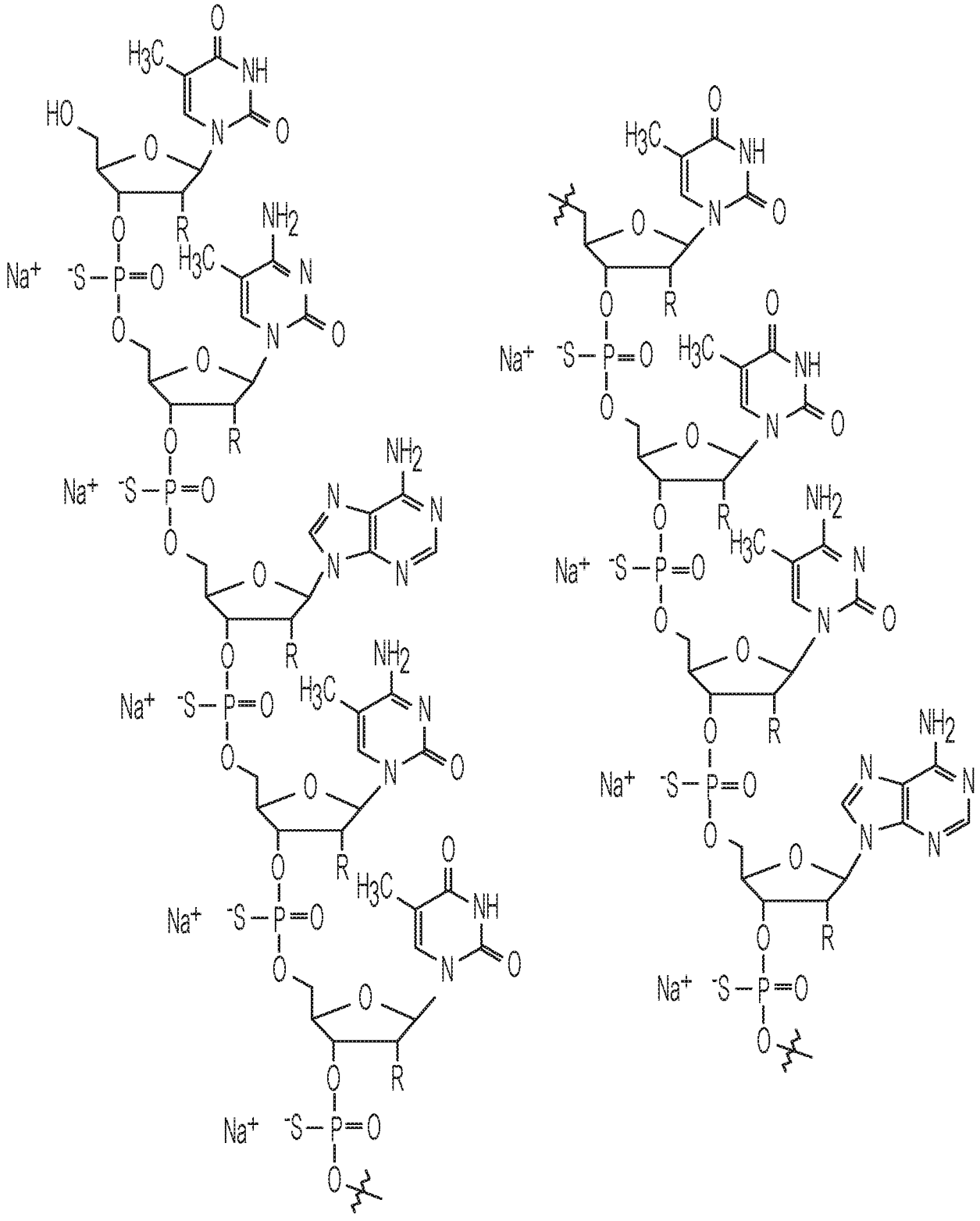


FIG. 3
Continued

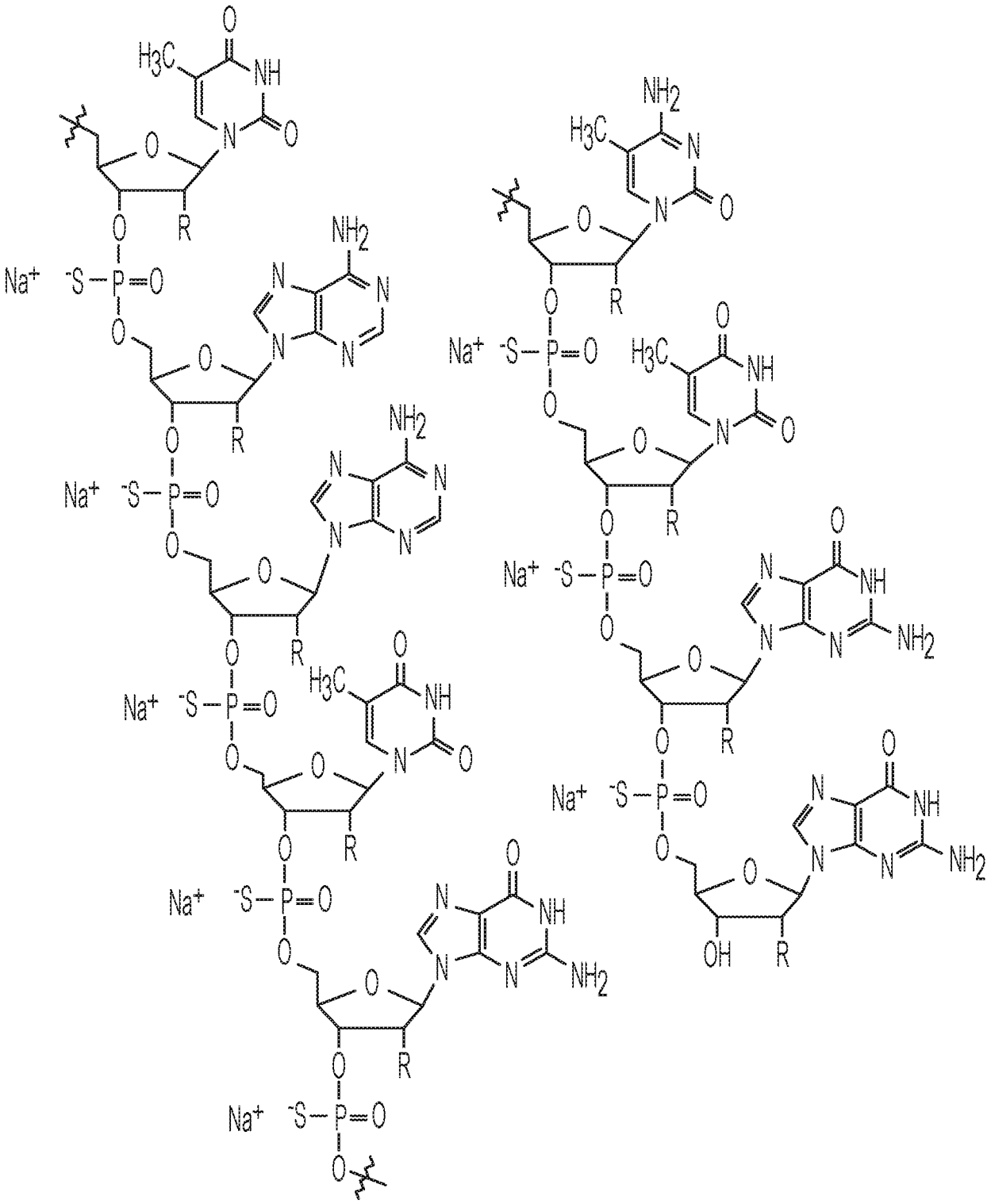


FIG. 4B

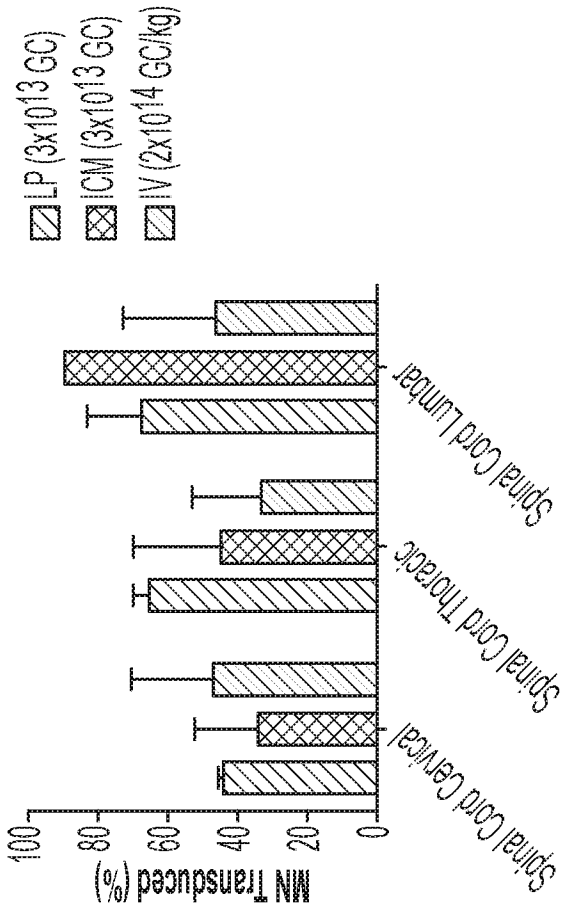

LP (3x10^13 GC)
ICM (3x10^13 GC)
IV (2x10^14 GC/kg)
MN Transduced (%)
100
80
60
40
20
0
Spinal Cord Cervical
Spinal Cord Thoracic
Spinal Cord Lumbar

FIG. 4A

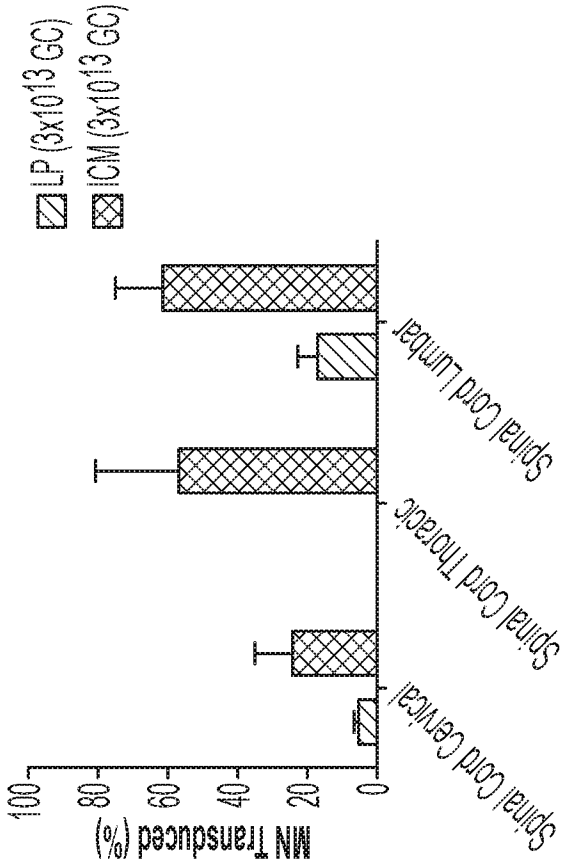

LP (3x10^13 GC)
ICM (3x10^13 GC)
MN Transduced (%)
100
80
60
40
20
0
Spinal Cord Cervical
Spinal Cord Thoracic
Spinal Cord Lumbar

FIG. 5A

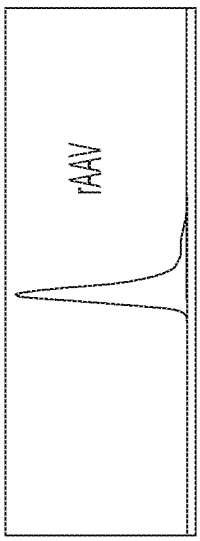


FIG. 5B

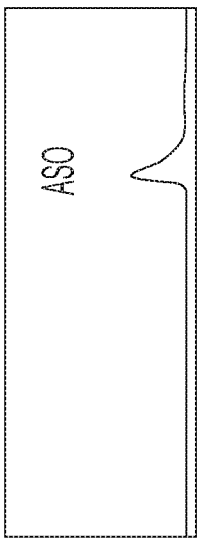


FIG. 5C

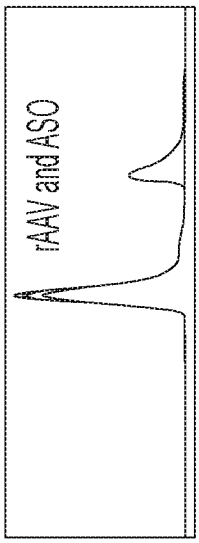


FIG. 5D

Sample	Vector Titer (vg/mL)	Infectivity-TCID50/mL	P:I
rAAV	2.77e13	1.12e10	2463
rAAV + ASO	2.24e13	8.43E09	2656
rAAV + ASO	2.39E13	1.50E10	1593

FIG. 5E

Sample	SMN expression (%RP)	GEM Formation (%RP)
rAAV	133	121
rAAV + ASO	168	127
rAAV + ASO	180	Not tested
ASO	Not detected	Not tested

FIG. 6A

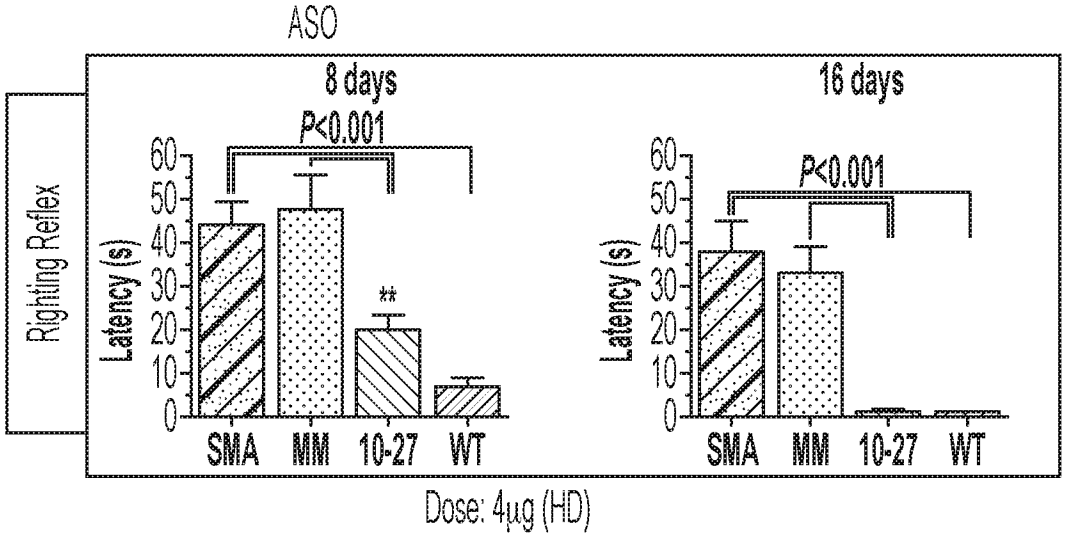

ASO
Righting Reflex
8 days
P<0.001
16 days
P<0.001
Latency (s)
60
50
40
30
20
10
0
**
SMA
MM
10-27
WT
Dose: 4μg (HD)

FIG. 6B

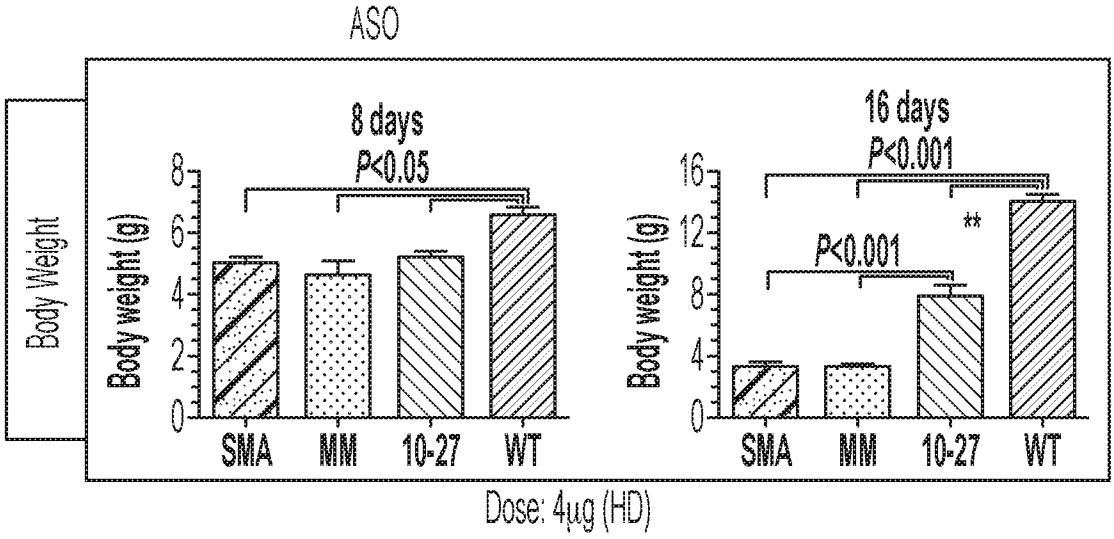

ASO
Body Weight
8 days
P<0.05
16 days
P<0.001
P<0.001
Body weight (g)
8
6
4
2
0
16
12
8
4
0
**
SMA
MM
10-27
WT
Dose: 4μg (HD)

FIG. 7A

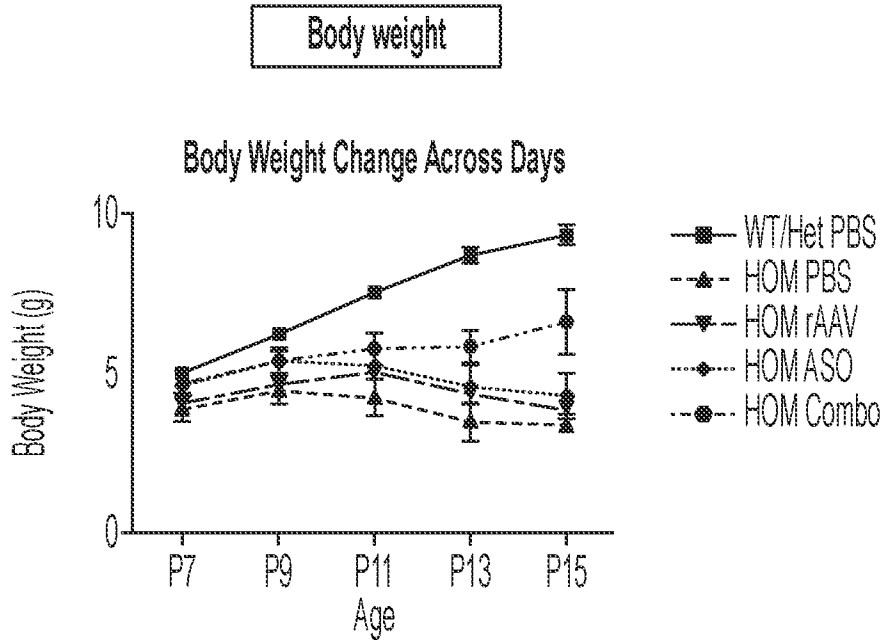

Body weight
Body Weight Change Across Days
Body Weight (g)
10
5
0
P7
P9
P11
P13
P15
Age
WT/Het PBS
HOM PBS
HOM rAAV
HOM ASO
HOM Combo

FIG. 7B

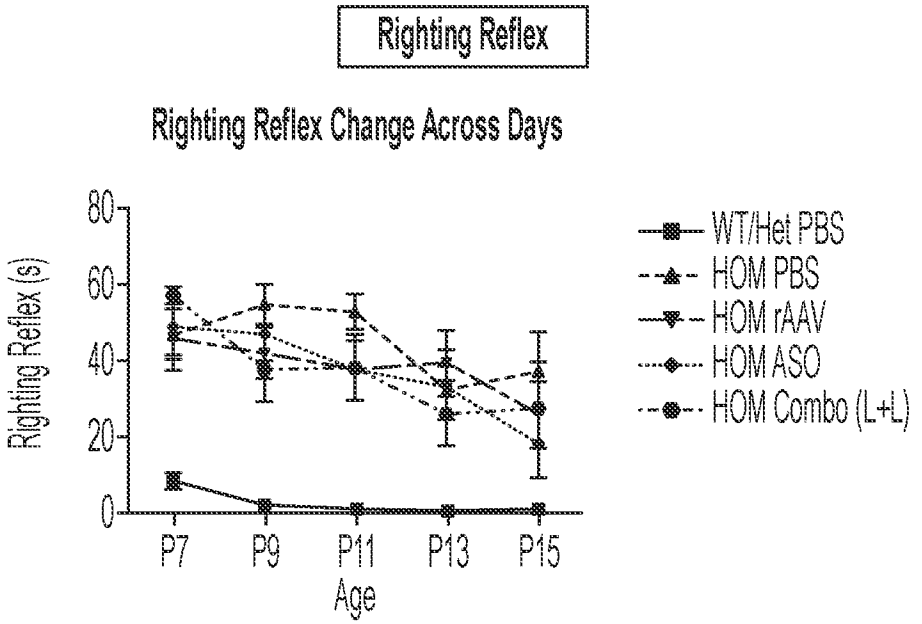

Righting Reflex
Righting Reflex Change Across Days
Righting Reflex (s)
80
60
40
20
0
P7
P9
P11
P13
P15
Age
WT/Het PBS
HOM PBS
HOM rAAV
HOM ASO
HOM Combo (L+L)

FIG. 7C

Group	#mice	Genotype	Treatment	Dose	ROA	Dose	Dosing	Assays
1	8 (F+M)	SMN2*delta7	ASO	1μg	ICV	**Low**	1x at P0-P1	body weight, RR
2	8 (F+M)	SMN2*delta7	rAAV	$1x10^{10}$ GC	ICV	**Low**	1x at P0-P1	body weight, RR
3	8 (F+M)	SMN2*delta7	rAAV+ASO	1+2	ICV	Combo	1x at P0-P1	body weight, RR

FIG. 8A

Group	#mice	Genotype	Treatment	Dose	ROA	Dose	Dosing	Assay
1	8 (F+M)	SMN2*delta7	ASO	3μg	ICV	**3XLow**	1x at P0-P1	body weight, RR
2	8 (F+M)	SMN2*delta7	rAAV	$3x10^{10}$ GC	ICV	**3XLow**	1x at P0-P1	body weight, RR
3	8 (F+M)	SMN2*delta7	rAAV+ASO	1+2	ICV	Combo	1x at P0-P1	body weight, RR

FIG. 8B

Body weight

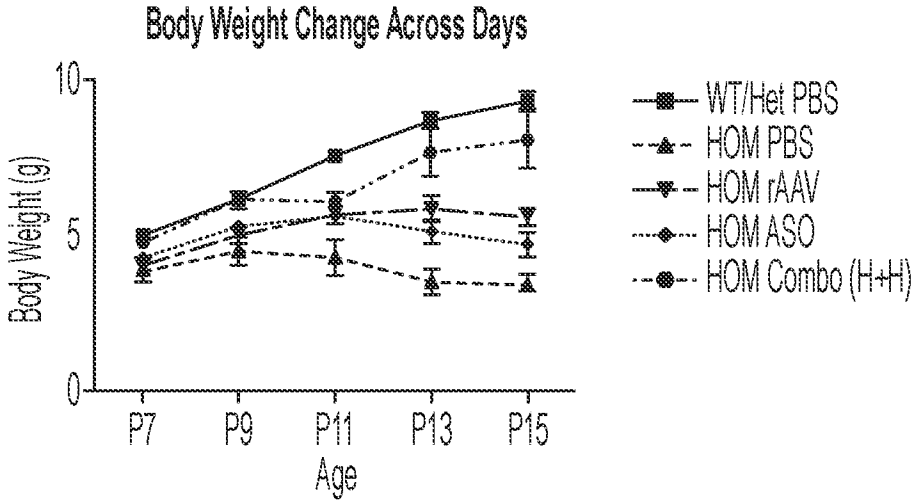


FIG. 8C

Righting Reflex

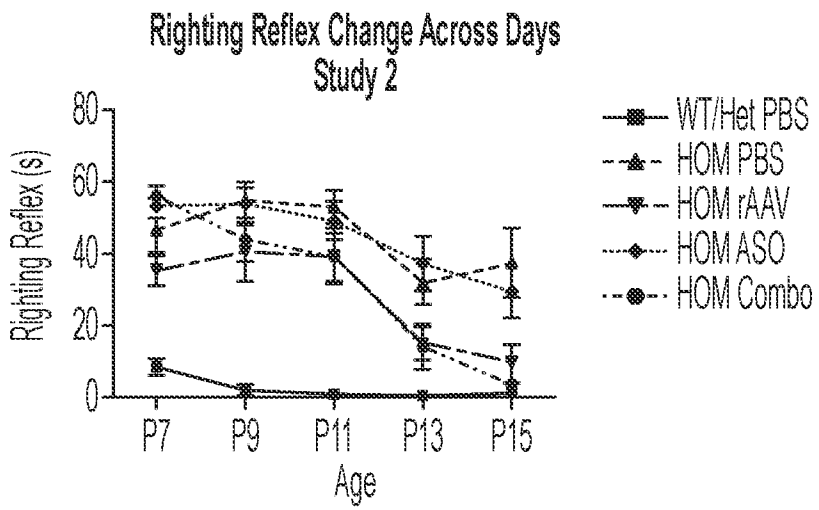


FIG. 9A

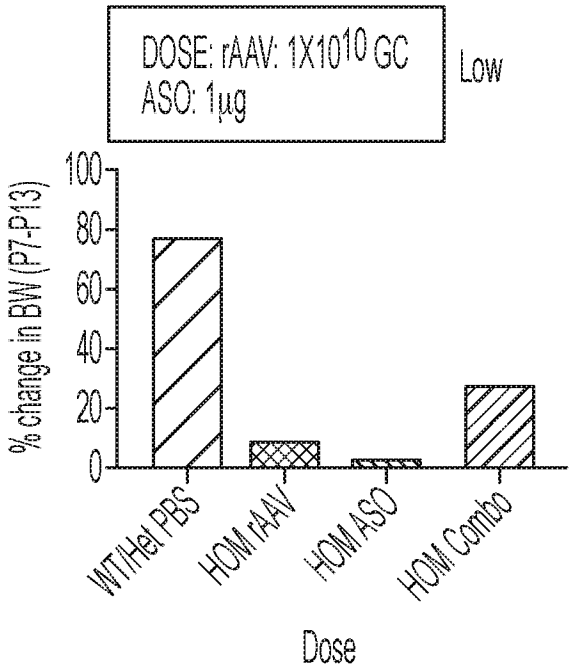

DOSE: rAAV: 1X10^10 GC
ASO: 1µg
Low
% change in BW (P7-P13)
100
80
60
40
20
0
WT/Het PBS
HOM rAAV
HOM ASO
HOM Combo
Dose

FIG. 9B

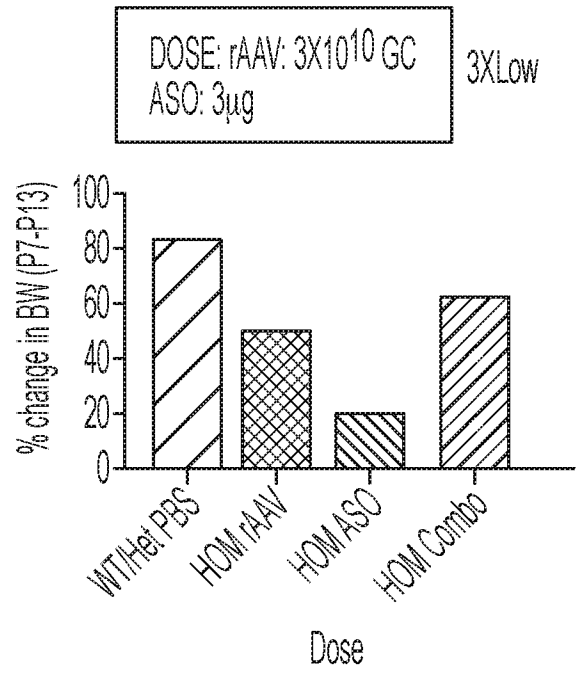

DOSE: rAAV: 3X10^10 GC
ASO: 3µg
3XLow
% change in BW (P7-P13)
100
80
60
40
20
0
WT/Het PBS
HOM rAAV
HOM ASO
HOM Combo
Dose

FIG. 10A

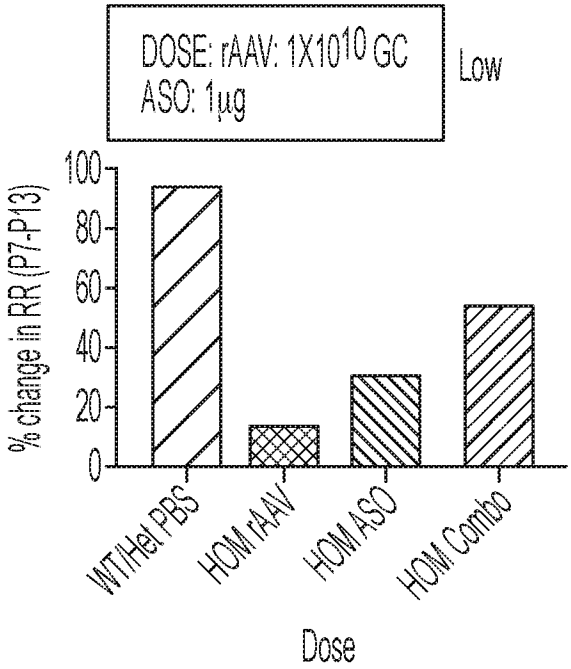


FIG. 10B

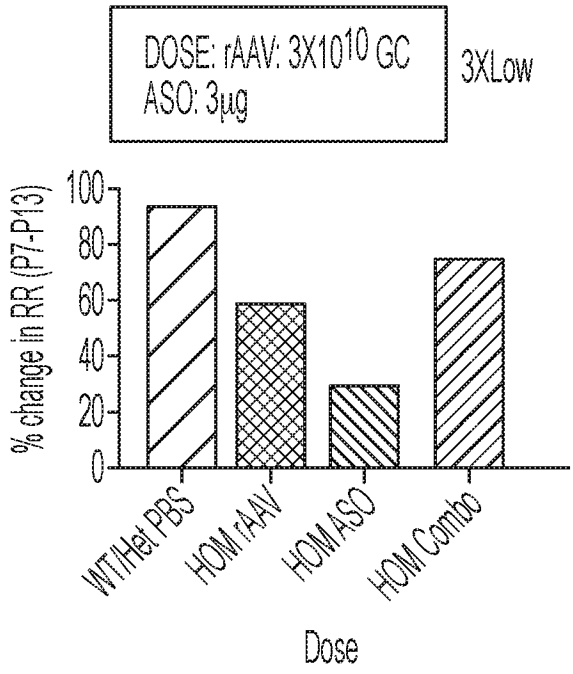
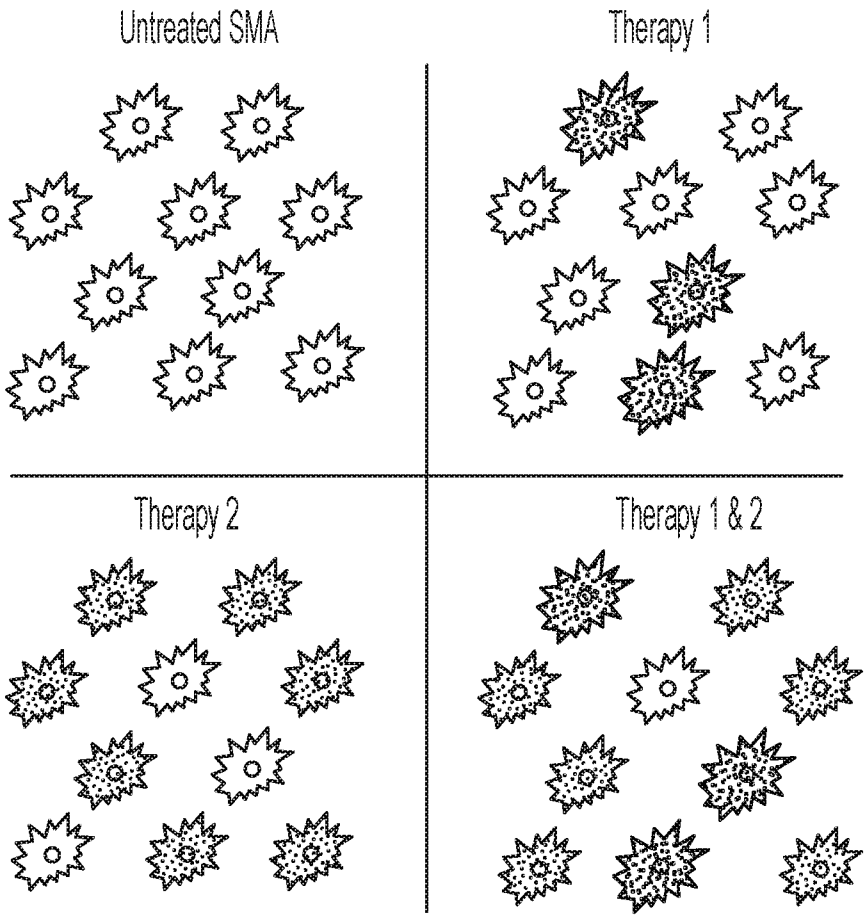


FIG. 11


Untreated SMA
Therapy 1
Therapy 2
Therapy 1 & 2
Potential Model

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/46720

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/864; A61K 48/00; A61K 31/713; A61P 21/00 (2019.01)

CPC - A61K 48/0066; A61K 38/1709; A61K 9/0085; A61K 31/713; A61P 21/00; C07K 14/4702; C12N 2750/14143; C12N 2310/11

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/106354 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 22 June 2017 (22.06.2017). Especially pg 1 ln 20-24; pg 2 ln 6-7; pg 5 ln 12-20; claims 1, 4.	1-7, 39, 40
A	US 2012/0190728 A1 (BENNETT et al.) 26 July 2012 (26.07.2012). Especially claim 108.	1-7, 39, 40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2019	27 DEC 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/46720

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-38, 44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
------Go to Extra Sheet for continuation----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 39-40

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 19/46720

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-7, 39-40, drawn to a method of treating spinal muscular atrophy.

Group II: Claims 41-43, drawn to a composition comprising an rAAV encoding SMN1 and an antisense oligonucleotide capable of increasing full-length SMN2 mRNA.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of administering a composition to a subject, not required by Group II.

Group II has the special technical feature of a composition comprising an rAAV encoding SMN1 and an antisense oligonucleotide capable of increasing full-length SMN2 mRNA.

Common Technical Features:

Groups I and II share the common technical features of:

1. an rAAV encoding SMN1
2. an ASO [i.e. anti-sense oligonucleotide] that is capable of increasing full-length SMN2 mRNA.

However, said common technical features do not represent a contribution over the prior art and are disclosed by WO 2017/106354 A1 to The Trustees of the University of Pennsylvania (hereinafter "UPenn").

As to common technical feature #1, UPenn discloses an rAAV encoding SMN1 (claims 1 and 4; "1. A recombinant adeno-associated viral (AAV) vector comprising an AAVrh10 capsid and a vector genome comprising a nucleic acid sequence encoding a functional SMN protein and expression control sequences that direct expression of the SMN sequences in a host cell. 4. The AAV vector of claim 1 or 3, wherein the nucleic acid sequence encoding SMN is the SMN1 sequence of SEQ ID NO: 2, or a sequence sharing at least 70% identity therewith").

As to common technical feature #2, UPenn discloses an ASO that is capable of increasing full-length SMN2 mRNA (pg 2 ln 6-7; "treatment studied the use of antisense oligonucleotides injected into the mouse CNS to redirect the splicing of SMN2 and boost production of SMN protein").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (cont.): Claims 8-38, 44 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).