



**IONIS PHARMACEUTICALS, INC.**

**ISIS 396443-CS4**

**A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled  
Study to Assess the Clinical Efficacy and Safety of ISIS 396443  
Administered Intrathecally in Patients with Later-onset Spinal  
Muscular Atrophy**

**Protocol Amendment 2 - 30 June 2016**

**EudraCT No: 2014-001947-18**

**Sponsor:**

Ionis Pharmaceuticals, Inc.

2855 Gazelle Court

Carlsbad, CA 92010

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#### **Protocol Amendment 2 – 30 June 2016**

#### **Protocol History:**

Original Protocol:	17 July 2014
Protocol Amendment 1:	26 September 2014

#### **Sponsor:**

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Carlsbad, CA 92010

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## **ISIS 396443**

**Ionis Protocol Number ISIS 396443-CS4**

**Protocol Amendment 2**

**Clinical Phase: 3**

### **A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy**

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#### **Confidentiality Statement**

This document contains confidential information of Ionis Pharmaceuticals, Inc. that must not be disclosed to anyone other than the recipient study staff and members of the institutional review board or authorized regulatory agencies. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

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## Protocol Signature Page

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**Protocol Number:** ISIS 396443-CS4

**Protocol Title:** A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy

**Amendment:** Amendment 2

**Date:** 30 June 2016

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I hereby acknowledge that I have read and understand the attached clinical protocol, entitled “A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy”, dated 30 June 2016, and agree to conduct the study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice (E6).

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

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Investigator's Signature

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Investigator's Name (*please print*)

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Date (DD Month YYYY)

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## PROTOCOL AMENDMENT

**Protocol Number:** ISIS 396443-CS4

**Protocol Title:** A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy

**Amendment Number:** 2

**Amendment Date:** 30 June 2016

Modifications to the Protocol ISIS 396443-CS4 include clarifications of the interim analysis plan, specifically, its timing, subset of the total study population to be included, endpoints and the analytical methods to be utilized, as well as the potential changes to the study conduct that may occur on the basis of a positive benefit-risk assessment of the results of the interim analysis. In addition, minor administrative changes and clarifications (not included in the list of changes below) have also been made to correct errors and/or to improve the overall clarity of the original protocol but these changes do not impact subject safety, exposure, or the overall study design.

The following table provides a summary list of major changes to the protocol:

Protocol Section	Description of Change	Rationale
<a href="#">Protocol Synopsis/ Study Design,</a> <a href="#">Section 3.1 Study Design</a> <a href="#">Section 3.5 End-of-Study</a>	Clarification is made related to the disposition of study subjects under the scenario of a premature study termination based on the result of a positive interim analysis	Clarification is necessary to allow subjects who complete all study assessments to rollover into the long-term extension study under the scenario of the current study being terminated early based on the assessment of risk-benefit of ISIS 396443 as a result of the interim analysis
<a href="#">Section 2.3.4 Clinical Experience</a>	Updated to reflect the up-to-date information provided in the Investigator Brochure, Version 6, January 2016.	Self-evident
<a href="#">Section 4.4 Unblinding of Treatment Assignment</a>	Statement added related to unblinding of certain representatives from the study Sponsor during the conduct of interim analysis, as detailed further in the Statistical Analysis Plan	In order to conduct and review the results of an interim analysis, certain sponsor personnel will need to be unblinded to treatment assignment. Those personnel will no longer be involved in the conduct of the study after they've been unblinded
<a href="#">Section 8.8 Adjustment of Dose and/or Treatment Schedule</a>	Clarification made on the adjustment of visit schedule for subjects who experience dose/sham procedure delays as a result of an illness	Approval of the new visit schedule by the study Medical Monitor is required in advance in order to ensure that adequate time elapses between 2 adjacent doses of Study Drug
<a href="#">Section 10.5 Interim Analysis</a>	Specific description of the timing and methodology for the interim analysis has been added	Self-evident
<a href="#">Section 10.6.3 Efficacy Analysis</a>	Specific description of the methodology for the efficacy endpoints has been added	Self-evident

## PROTOCOL SYNOPSIS

<b>Protocol Title</b>	A Phase 3, Randomized, Double-Blind, Sham Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy
<b>Study Phase</b>	3
<b>Indication</b>	Later-onset Spinal Muscular Atrophy (SMA)
<b>Objectives</b>	<p><b>Primary Objective:</b></p> <p>To examine the efficacy of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p> <p><b>Secondary Objective:</b></p> <p>To examine the safety and tolerability of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p> <p><b>Tertiary Objective:</b></p> <p>To examine the CSF and plasma pharmacokinetics of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p>
<b>Number of Subjects</b>	Approximately 117 subjects will be enrolled into this study
<b>Treatment Groups</b>	12 mg ISIS 396443 or sham procedure control group
<b>Study Design</b>	<p>This randomized, double-blind, sham-procedure controlled study will test the clinical efficacy, safety, tolerability, and pharmacokinetics of intrathecal ISIS 396443 over 15 months. Approximately 117 subjects will be randomized in a 2:1 ratio (78 ISIS 396443: 39 control) to receive ISIS 396443 by intrathecal lumbar puncture (LP) injection or to a sham-procedure control. A dose of 12 mg ISIS 396443 will be given at each of 4 times over the 15 months (i.e., on Study Days 1, 29, 85, and 274).</p> <p>Randomization will be stratified based on:</p> <ol style="list-style-type: none"> <li>1. Age (&lt; 6 years vs. ≥ 6 years at Screening)</li> </ol> <p>After Informed consent/assent is obtained, subjects will undergo a screening evaluation no greater than 28 days prior to first dose administration at which time their eligibility for the study will be examined. Subjects who meet the eligibility criteria will be admitted to the study center on Study Day 1, undergo pre-dose evaluations, and then receive either an LP injection of Study Drug (ISIS 396443) or a sham procedure. Subjects will return to the study center on Days 29, 85 and 274 for follow-up evaluations and subsequent injections/sham procedures. Following the injection/sham procedure on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring. Following the injection/sham procedure on Day 29, 85, and 274 subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. Safety monitoring visits will occur on Days 30, 86, 92, 169, 275, 365, and 456 (through 6 months following the last injection/sham procedure). In addition, the study center will monitor the subject's condition through telephone contact monthly throughout the study. For subjects receiving ISIS 396443, a CSF sample will be taken pre-dose on each injection day in a manner that maintains the blind.</p> <p>Following treatment and the final follow-up evaluation, all eligible subjects may elect to enroll in an open-label treatment extension study. This will be done without unblinding to subject's treatment group.</p> <p>In the event of a decision by the study Sponsor to terminate the study earlier on the grounds that conducting a sham-controlled study is no longer deemed ethical based on an updated risk-benefit assessment of ISIS 396443 from the planned interim analysis, all subjects will be invited for the end-of-double-blind-period (EODBP) study visit, during which all Day 456 assessments will be conducted. After completing the EODBP visit, subjects will be considered study completers and will be allowed to enroll into the open-label extension study.</p>

## PROTOCOL SYNOPSIS *Continued*

<b>Study Design</b> <b><i>Continued</i></b>	If a subject terminates early from the study, they will be encouraged to complete safety assessments per the Day 456 visit. If a subject is randomized but does not complete at least their first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.
<b>Study Population and Main Criteria for Inclusion/ Exclusion</b>	<p><b><u>Inclusion Criteria:</u></b></p> <p>Subjects must meet all of the following criteria at Screening to be eligible:</p> <ol style="list-style-type: none"> <li>1. Signed informed consent of parent or guardian. Signed informed assent of subject, if indicated per subject's age and institutional guidelines</li> <li>2. Genetic documentation of 5q SMA (homozygous gene deletion, mutation, or compound heterozygote)</li> <li>3. Onset of clinical signs and symptoms consistent with SMA at &gt; 6 months of age</li> <li>4. Males and females 2 to 12 years of age</li> <li>5. Can sit independently, but has never had the ability to walk independently</li> <li>6. Motor Function Score (Hammersmith Functional Motor Scale – Expanded) <math>\geq 10</math> and <math>\leq 54</math> at Screening</li> <li>7. Able to complete all study procedures, measurements and visits and parent or guardian/subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator</li> <li>8. Estimated life expectancy &gt; 2 years from Screening, in the opinion of the Investigator</li> <li>9. Meets age-appropriate institutional criteria for use of anesthesia/sedation, if use is planned for study procedures (as assessed by the Site Investigator and either anesthesiologist or pulmonologist)</li> <li>10. For subjects who, in the opinion of the Investigator, have reached reproductive maturity, satisfy 1 of the following:  Females: have a negative pregnancy test at Screening and agrees to employ adequate contraceptive measures for the duration of the study. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products.  Males: be abstinent for the duration of the study</li> </ol> <p><b><u>Exclusion Criteria:</u></b></p> <p>Subjects meeting any of the following criteria are not eligible for the study:</p> <ol style="list-style-type: none"> <li>1. Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for &gt; 6 hours during a 24 hour period, at Screening</li> <li>2. Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator</li> <li>3. Severe contractures or severe scoliosis evident on X-ray examination at Screening</li> <li>4. Hospitalization for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of Screening or planned during the duration of the study</li> <li>5. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period</li> <li>6. History of brain or spinal cord disease, including tumors, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation</li> <li>7. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter</li> <li>8. History of bacterial meningitis</li> <li>9. Dosing with ISIS 396443 in any previous clinical study</li> <li>10. Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline</li> </ol>

## PROTOCOL SYNOPSIS *Continued*

<b>Study Population and Main Criteria for Inclusion/ Exclusion</b> <i>Continued</i>	<b><u>Exclusion Criteria Continued:</u></b> <ol style="list-style-type: none"> <li>Clinically-significant abnormalities in hematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion</li> <li>Treatment with another investigational drug (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, etc.), biological agent, or device within 1-month of Screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation</li> <li>Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anemia, etc.) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures</li> </ol>
<b>Study Drug and Administration</b>	<p>ISIS 396443 (2.4 mg/mL) will be administered as an intrathecal LP injection. The volume of the injection will be 5.0 mL. Details regarding the LP dosing injection procedure and the sham procedure will be provided in the Dosing Administration Manual. Details regarding the Study Drug will be provided in the Study Drug Manual. Per institutional guidelines, anesthesia/sedation may be used for the LP procedure.</p>
<b>Criteria for Evaluation</b>	<p><b><u>Primary Endpoint:</u></b></p> <ul style="list-style-type: none"> <li>Change from baseline in HFMSE (Hammersmith Functional Motor Scale – Expanded) score at 15 months</li> </ul> <p><b><u>Secondary Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>Proportion of subjects who achieve a 3-point or greater increase from baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months</li> <li>Proportion of subjects that achieve any new motor milestone at 15 months</li> <li>Number of motor milestones achieved per subject at 15 months</li> <li>Change from baseline in Upper Limb Module Test at 15 months</li> <li>Proportion of subjects that achieve standing alone at 15 months</li> <li>Proportion of subjects that achieve walking with assistance at 15 months</li> </ul> <p><b><u>Tertiary Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>Change from baseline in CSF SMN protein concentration</li> <li>Clinical Global Impression of Change (Investigator and Caregiver assessment)</li> <li>Change from baseline in PedsQL (Pediatric Quality of Life Inventory)</li> <li>Change from baseline in Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)</li> <li>Disease-related hospitalizations and adverse events</li> </ul> <p><b><u>Safety/Tolerability Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>Adverse events</li> <li>Vital signs and weight</li> <li>Neurological examinations</li> <li>Physical examinations</li> <li>Clinical laboratory tests</li> <li>ECGs</li> <li>Use of concomitant medications</li> </ul>

## PROTOCOL SYNOPSIS *Continued*

<b>Criteria for Evaluation</b> <i>Continued</i>	<p><b><u>Pharmacokinetic Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• CSF levels of ISIS 396443 (sample taken pre-dose at each dosing)</li> <li>• Plasma levels of ISIS 396443</li> </ul> <p><b><u>Immunogenicity Endpoint:</u></b></p> <ul style="list-style-type: none"> <li>• Plasma antibodies to ISIS 396443</li> </ul>
<b>Safety Monitoring</b>	Safety data will be reviewed on an ongoing basis by the Medical Monitor and by an Independent Data Safety and Monitoring Board (DSMB).
<b>Statistical Considerations</b>	The sample size for this study was estimated based on limited available natural history data for the target population and from the ISIS 396443-CS1 and CS2 clinical studies in children with SMA. 70 subjects in the treated group and 35 subjects in the control group will give at least 90% power to detect a 3-point difference between control and treated groups in change on the HFMSE with a standard deviation of 4.4, using a 2-sided t-test with an alpha level of 0.05. The sample size was obtained using the n-Query. 117 subjects will be enrolled to ensure that a small dropout rate will not affect the power of the primary efficacy analysis. An interim analysis may take place when all subjects have completed the 6-month assessment and at least 39 subjects have completed the 15-month assessment.
<b>Sponsor</b>	Ionis Pharmaceuticals, Inc.

## STUDY GLOSSARY

<b><u>Abbreviation/Acronym</u></b>	<b><u>Definition</u></b>
ACEND	Assessment of Caregiver Experience with Neuromuscular Disease
AE	Adverse event/experience
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CGI	Clinical Global Impressions of Change Rating Scale
C <sub>max</sub>	Maximum concentration
CNS	Central nervous system
CPK	Creatinine phosphokinase
CRF	Case report form
CSF	Cerebrospinal fluid
DSMB	Data and Safety Monitoring Board
eCRF	Electronic case report form
ECG	Electrocardiogram
EODBP	End-of-double-blind-period
FL	Full-length
GCP	Good Clinical Practice
HFMSE	Hammersmith Functional Motor Scale - Expanded
hnRNP	Heterogeneous nuclear ribonucleoproteins
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
IT	Intrathecal
IXRS	Interactive Voice/Web-Response System
LP	Lumbar puncture
MAD	Multiple ascending-dose
MedDRA	Medical Dictionary for Regulatory Activities

miRNA	Micro ribonucleic acid (RNA)
MOE	2'- <i>O</i> -(2-methoxyethyl)
mRNA	Messenger ribonucleic acid
NCS	Not clinically-significant
OLE	Open-label extension study
PedsQL	Pediatric Quality of Life Inventory
PK	Pharmacokinetic(s)
PT	Prothrombin Time
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SMA	Spinal muscular atrophy
SMN	Survival motor neuron
snRNA	Small nuclear ribonucleic acid
SUSARs	Suspected unexpected serious adverse reactions
T <sub>max</sub>	Time to maximal concentration
WMA	World Medical Association

## 1. OBJECTIVES

The objectives of this study are to evaluate the clinical efficacy, safety, tolerability, and pharmacokinetics (PK) of ISIS 396443 administered intrathecally to patients with later-onset Spinal Muscular Atrophy (SMA).

### 1.1 Primary Objective

To examine the clinical efficacy of ISIS 396443 administered intrathecally to patients with later-onset SMA.

### 1.2 Secondary Objective

To examine the safety and tolerability of ISIS 396443 administered intrathecally to patients with later-onset SMA.

### 1.3 Tertiary Objective

To examine the cerebral spinal fluid and plasma PK of ISIS 396443 administered intrathecally to patients with later-onset SMA.

## 2. BACKGROUND AND RATIONALE

### 2.1 Spinal Muscular Atrophy

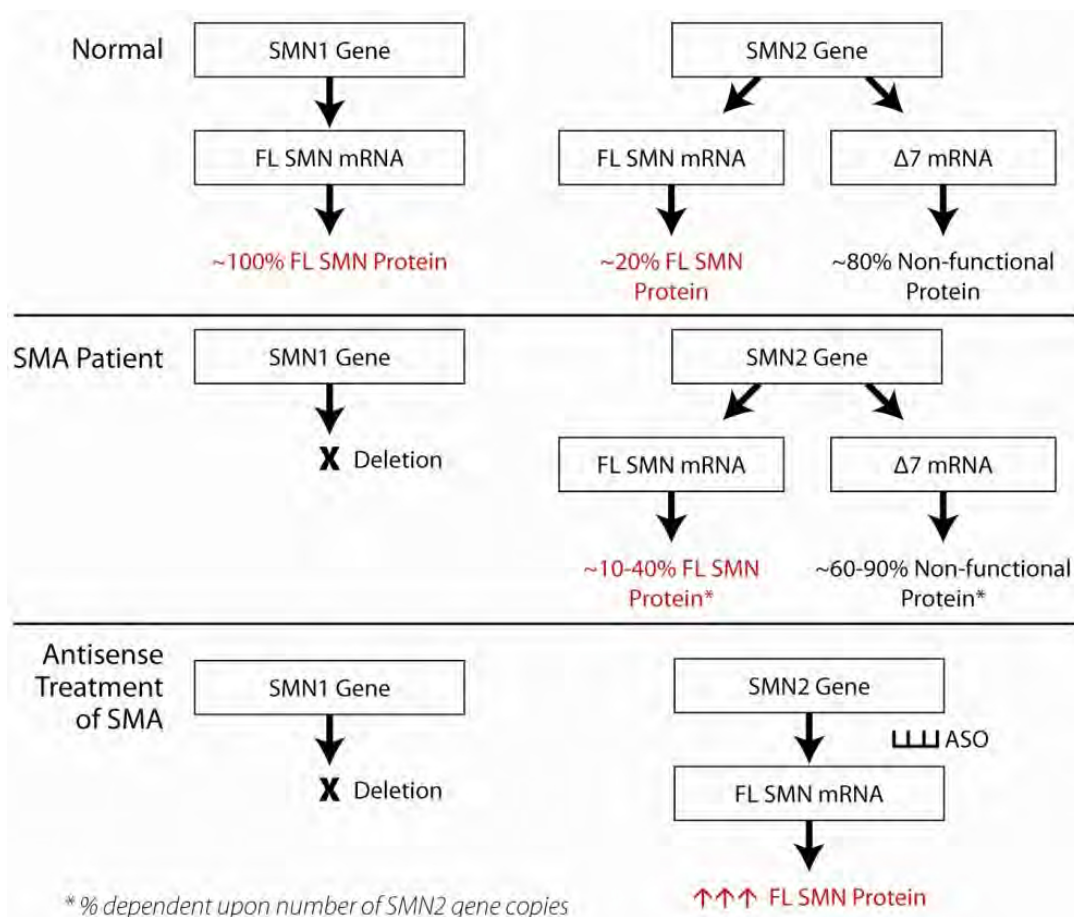
SMA is an autosomal recessive neuromuscular disease characterized by degeneration of the motor neurons in the anterior horn of the spinal cord, resulting in atrophy of the voluntary muscles of the limbs and trunk. With an incidence of 1:6000 to 1:10,000 live births, it is the most common genetic cause of infant mortality, and a major cause of childhood morbidity due to weakness, in the U.S. The natural history of SMA includes 4 major phenotypes that are recognized dependent on age of onset and achieved motor abilities. The most severe form, Type 1 SMA (equivalent to infantile-onset SMA), has a disease onset within the first few months of life; these children are never able to sit or walk and usually die from respiratory failure by the age of 2 years. Later-onset SMA can generally be divided into Type 2 and Type 3 SMA. Type 2 SMA patients are able to sit but never walk unaided, with symptoms presenting between 6-18 months of age. Type 3 SMA patients are able to sit and walk but individuals with this form may become severely and increasingly disabled. Adult-onset SMA patients (Type 4) have an age of onset over 18 years of age and have normal life expectancies.

In 95% of SMA patients, a deletion in the *SMN1* gene on Chromosome 5q11-q13 is found; with the remaining 5% attributable to small mutations in the same gene ([Lefebvre et al. 1995](#); [Helmken et al. 2003](#)). *SMN1* lies in the telomeric portion of an inverted duplication of a region of Chromosome 5. The centromeric half of the duplication contains a homologous gene, named *SMN2* that differs from *SMN1* by 5 nucleotides. The open reading frames for both genes encode for proteins with identical amino acid sequences. Survival motor neuron (SMN) gene transcripts, similar to most mammalian transcripts, undergo alternative splicing in which certain exons are either included or excluded from the mature protein coding transcripts ([Keren et al. 2010](#)). In particular, Exon 7 of the *SMN1* gene is alternatively spliced with 90 to 95% of the mature messenger ribonucleic acid (mRNA) transcripts derived from the *SMN1* gene containing Exon 7, and 5 to 10% of transcripts missing Exon 7. The transcripts missing Exon 7 (often referred to as  $\Delta 7$ ) produce a truncated protein which is defective and unstable

(Choi and Dreyfuss 2010). One (1) of the 5 nucleotide differences between *SMN1* and *SMN2*, a C to T substitution occurs in Exon 7 of the *SMN2* gene resulting in an alternative splicing pattern that favors skipping of Exon 7. The result is that as much as 90% of the transcripts produced from *SMN2* are missing Exon 7. The remainder, *SMN2* transcripts containing Exon 7, produces a full-length (FL) protein product identical to the *SMN1* protein, since the C to T substitution is silent. Humans have a variable copy number of the *SMN2* gene (0-8 copies) (Wirth et al. 2006). The number of *SMN2* copies and the resulting amount of FL-SMN protein expressed in SMA patients (10-40% of normal SMN protein levels) correlates with SMA disease severity and thus *SMN2* is a key modifier of disease phenotype (Covert et al. 1997; Feldkotter et al. 2002; Lefebvre et al. 1997; Prior et al. 2004).

## 2.2 Therapeutic Rationale

Since the number of *SMN2* gene copies and resulting amount of SMN protein is correlated with disease onset and severity, a therapeutic approach predicted to benefit SMA patients is to increase the levels of full length *SMN2* pre-mRNA by restoring the splicing pattern that gives rise to full length *SMN2* mRNA. Increasing inclusion of Exon 7 in the *SMN2* transcript will increase FL-SMN protein levels and SMN protein activity. A therapeutic strategy for promoting Exon 7 inclusion is through the use of antisense oligonucleotides (ASOs) (see Figure 1).



**Figure 1 ASO Therapeutic Approach for Treatment of SMA**

The known potential risks associated with ISIS 396443 are detailed in the Guidance to Investigator section of the Investigator's Brochure. Additional study associated risks related to the lumbar puncture (LP) procedure are also detailed in the Guidance to Investigator section of the Investigator's Brochure.

## 2.3 ISIS 396443

### 2.3.1 Mechanism of Action

ISIS 396443 is a fully modified, 2'-O-2-methoxyethyl (MOE), ASO drug designed to bind to a specific sequence in the intron downstream of Exon 7 of the SMN2 transcript. The region of the pre-mRNA targeted by ISIS 396443 is normally occupied by heterogeneous nuclear ribonucleoproteins (hnRNP) A1/2 proteins, masking the U1 small nuclear ribonucleic acid (snRNA) binding site at the 5'-exon-intron junction of Exon 7, and is referred to as ISS-N1. U1 snRNA base pairs to the sequences that define the 5'-splice site, which is thought to be one of the first steps that initiate splicing of an intron. ISIS 396443 displaces the hnRNP A1/2 proteins from the pre-mRNA binding site, allowing U1 snRNA to bind to the exon-intron junction and promote assembly of the spliceosomal complex, thus promoting inclusion of Exon 7 into the mRNA which results in production of FL-SMN protein.

### 2.3.2 Chemistry

Chemically, ISIS 396443 is a synthetic oligomer of 18 nucleotides (i.e., an 18-mer) that are connected sequentially by phosphorothioate linkages. Each of the 17 internucleotide linkages is a 3'-O to 5'-O phosphorothioate diester. The 18 sugar residues are uniformly modified with 2'-O-(2-methoxyethyl) (MOE). These MOE-modified nucleotides confer (1) increased affinity to the target mRNA (McKay et al. 1999), (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue) (Geary et al. 2003), and (3) amelioration of some of the high dose toxicities associated with ASO containing only the phosphorothioate linkages (Henry et al. 2000).

The sequence of ISIS 396443 is written as follows:



Where A and G are 2'-O-(2-methoxyethyl)nucleosides, <sup>Me</sup>C is 5-methyl-2'-O-(2-methoxyethyl)cytidine and <sup>Me</sup>U designates 5-methyl-2'-O-(2-methoxyethyl)uridine.

### 2.3.3 Preclinical Experience

Detailed information concerning the preclinical studies conducted with ISIS 396443 can be found in the Investigator's Brochure. A summary is included below.

ISIS 396443 was identified after an extensive screen of greater than 500 2'-MOE oligonucleotides in *in vitro* splicing assays, reporter gene assays and in SMA patient fibroblasts (Hua et al. 2007; Hua et al. 2008). Data have shown that ISIS 396443 promotes a concentration-dependent increase in full-length transcripts (including Exon 7) in patient fibroblast cells, achieving greater than 90% full length SMN2 transcripts and forms nuclear structures, called gems, known to contain SMN protein. In a mild mouse model of SMA, ISIS 396443 promoted inclusion of Exon 7 in the SMN2 transgene in a variety of peripheral tissues when dosed

systemically (Hua et al. 2008) and in central nervous system (CNS) tissue, including spinal cord, when injected into the lateral ventricle. ISIS 396443 produced greater than 90% Exon 7 inclusion in the transgenic mice and increased SMN protein production in motor neurons, resulting in the appearance of gems in motor neurons. These studies were extended to a more severe mouse model of SMA (SMA  $\Delta 7$ ) (Le et al. 2005), where the CNS delivery of drug produced a dose-dependent effect on SMN2 Exon 7 inclusion, SMN protein production, and survival. These mice treated with ISIS 396443 demonstrated improved weight gain, improvements in muscle morphology, muscle strength, and motor coordination and improved morphology of the motor neuron junctions (Passini et al. 2011). Further, ISIS 396443 was shown to distribute widely in the CNS following intrathecal (IT) administration in monkey (Passini et al. 2011).

The pharmacokinetics and toxicity of ISIS 396443 were assessed following: 1) single intrathecal (IT) lumbar bolus injections (1 to 7 mg) in adult monkeys 2) following 14 weeks (with a 4-week interim sacrifice) of repeated IT lumbar bolus injections (0.3 to 3 mg/week or every other week) in juvenile monkeys and 3) following 53 weeks of repeated IT lumbar bolus injections in juvenile monkeys. In addition, a dedicated pharmacokinetic study in adult monkeys was performed to assess the half-life of ISIS 396443 in CSF, tissues and plasma. Detailed results from these preclinical studies conducted with ISIS 396443 can be found in the ISIS 396443 Investigator's Brochure.

#### **2.3.4 Clinical Experience**

Excluding the current study ISIS 396443-CS4, 9 additional clinical studies with ISIS 396443 have been initiated: 3 studies have been completed and 6 are ongoing. Four (4) of the clinical studies are in patients with "later-onset SMA," 2 trials are in "infantile-onset (Type I) SMA," 1 trial is in neonates and infants with genetically diagnosed and presymptomatic SMA, and 2 trials are in both "infantile-onset" and "later-onset" populations. The ISIS 396443 clinical studies are described in detail in the Investigator's Brochure and listed here:

- ISIS 396443-CS1 (completed): an open-label, single ascending-dose (SAD) Phase 1 study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with SMA, aged 2 to 14 years
- ISIS 396443-CS2 (completed): an open-label, multiple ascending-dose (MAD) Phase 1/2a study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with SMA, aged 2 to 15 years
- ISIS 396443-CS10 (completed): an open-label Phase 1 study to assess the safety and tolerability of a single IT dose (6 or 9 mg) of ISIS 396443 in patients with SMA who previously participated in ISIS 396443-CS1
- ISIS 396443 CS12 (ongoing): an open-label Phase 2 study to assess the safety and tolerability of a single IT dose (12 mg) of ISIS 396443 in patients with SMA who previously participated in either ISIS 396443 CS2 or ISIS 396443-CS10
- ISIS 396443-CS3A (ongoing): a multiple-dose Phase 2 study designed to assess the safety, tolerability, pharmacokinetics and efficacy of ISIS 396443 in patients with infantile-onset SMA (symptomatic SMA infants < 7 months of age)

- ISIS 396443-CS3B (ongoing): a Phase 3 randomized, double-blind, sham-procedure controlled study to assess the clinical efficacy, safety, tolerability and pharmacokinetics of ISIS 396443 in patients with infantile-onset SMA
- ISIS 396443-CS5 (232SM201: ongoing): an open-label Phase 2 study to assess the efficacy, safety, tolerability, and pharmacokinetics of multiple doses of ISIS 396443 delivered intrathecally to subjects with genetically diagnosed and presymptomatic SMA
- ISIS 396443-CS7 (232SM202: ongoing): a Phase 2, randomized, double-blind, sham-procedure controlled study to assess the safety and tolerability and explore the efficacy of ISIS 396443 administered intrathecally in subjects with SMA who are not eligible to participate in the clinical studies ISIS 396443-CS3B or ISIS 396443-CS4
- ISIS 396443-CS11 (ongoing): an open-label Phase 3 extension study for patients with SMA who previously participated in investigational studies of ISIS 396443. Safety and tolerability will be assessed in this study. Up to 274 patients from ISIS 396443-CS3B, ISIS 396443-CS4, and ISIS 396443-CS12 are eligible to enroll

## 2.4 Rationale for Dose and Schedule of Administration

The proposed study will test the clinical efficacy, safety, tolerability, and PK of multiple doses of ISIS 396443 administered as IT injections to subjects with later-onset SMA. A single dose level of 12 mg ISIS 396443 will be evaluated, delivered as 4 doses administered over 9 months. ISIS 396443 will be administered using a loading regimen (dosings on Study Days 1, 29, 85) followed by maintenance dosing given 6 months thereafter (dosing on Study Day 274).

The ISIS 396443-CS4 dose level and dose interval was selected based on preclinical toxicology and pharmacokinetic observations from monkey studies utilizing single-dose and repeat dosing IT administration, consideration of the target tissue concentration anticipated for drug pharmacology, and safety data in the completed and ongoing clinical studies of ISIS 396443 to date. Based upon pharmacology and pharmacokinetic results in SMA transgenic mice, we estimate that the target tissue concentration to produce 50 to 90% SMN2 Exon 7 inclusion is between 1 and 10 µg/g spinal cord tissue. Nonclinical studies in juvenile monkeys receiving IT doses of ISIS 396443 showed a resulting gradient of distribution of ISIS 396443 along the spinal cord, with mean lumbar spinal cord concentrations ranging from 1.6-2.3 fold and 2.0-3.5 fold higher than thoracic and cervical spinal cord levels, respectively. The dose level selected for this multiple-dose clinical study (12 mg ISIS 396443) is predicted to achieve levels at the high end of this range (approximately 10 µg/g lumbar and 3 µg/g cervical spinal cord tissue concentrations), following the first dose. The loading dose interval was selected based on the nonclinical pharmacokinetic and pharmacology data as the dose interval to achieve and maintain ISIS 396443 spinal cord tissue levels that are predicted to be within the upper end of the pharmacologically active range by Day 85 (predicted to be approximately 24 µg/g lumbar and 8 µg/g cervical tissue concentration), while at the same time considering subject safety and convenience for repeated LP intrathecal injections. The maintenance dose interval (once every 6 months) was selected based on the estimated spinal tissue and CSF drug half-life (4-6 months) and was selected to maintain spinal cord tissue levels of ISIS 396443 at a steady-state level within the estimated pharmacologically active range.

Additional details on dose scaling and expected CSF and tissue concentrations are summarized in the Investigator's Brochure.

### **3. EXPERIMENTAL PLAN**

#### **3.1 Study Design**

This is a Phase 3 multicenter, double-blind, randomized, sham-procedure controlled study of ISIS 396443 in patients with later-onset SMA studied for 15 months. Approximately 117 subjects will be randomized 2:1 to receive 12 mg dose ISIS 396443 or a sham procedure control, respectively. ISIS 396443 will be administered using a loading regime (dosings on Study Days 1, 29, and 85) followed by a maintenance dose 6 months thereafter (dosing on Study Day 274).

Randomization will be stratified based on:

1. Subject's Age at Screening (< 6 years vs.  $\geq$  6 years)

Following treatment and the Day 456 follow-up evaluation, all eligible subjects may elect to enroll in an open-label treatment extension study, pending study approval by the IRB or IEC and the appropriate regulatory authority. This will be done without unblinding to subject's treatment group.

An interim analysis may take place when all subjects have completed the 6-month assessment and at least 39 subjects have completed the 15-month assessment. In the event of a decision by the study Sponsor to terminate the study earlier on the grounds that conducting a sham-controlled study is no longer deemed ethical based on an updated risk benefit assessment of ISIS 396443 from the planned interim analysis, all subjects will be invited for the end-of-double-blind-period (EODBP) study visit, during which all Day 456 assessments will be conducted. After completing the EODBP visit, subjects will be considered study completers and will be allowed to enroll into the open-label extension study.

#### **3.2 Number of Study Centers**

This study will be conducted at multiple centers worldwide.

#### **3.3 Number of Subjects**

Approximately 117 subjects (2 ISIS 396443; 1 sham-procedure) will receive 12 mg dose of ISIS 396443 or a sham procedure control. The total number of subjects randomized may be higher if some subjects do not receive their Day 1 dose/sham procedure. The maximum number of subjects will not exceed 130.

#### **3.4 Overall Study Duration and Follow-up**

The Study will consist of screening, treatment, and post-treatment follow-up-periods. The total duration of participation in the study is approximately 16 months. Please refer to the Schedule of Procedures in [Appendix A](#).

### **3.4.1 Screening**

After informed consent/assent is obtained, subjects will undergo a screening evaluation no greater than 28 days prior to first dose administration at which their eligibility for the study will be examined.

### **3.4.2 Treatment**

Subjects who meet the eligibility criteria will be admitted to the study center on Study Day 1, undergo pre-dose evaluations, be randomized, and then receive either an LP injection of Study Drug (ISIS 396443) or a sham procedure. Subjects will return to the study center on Days 29, 85, and 274 for follow-up evaluations and subsequent injections/sham procedures. Following the injection/sham procedure on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring. Following the injection/sham procedure on Day 29, 85, and 274 subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. Safety monitoring visits will occur Study Days 30, 86, 92, 169, and 275 (through the last injection/sham procedure). In addition, the study center will monitor the subject's condition through telephone contact on a monthly basis.

For subjects receiving ISIS 396443, a CSF sample for PK and SMN protein analyses will be taken pre-dose on each injection day in a manner that protects the blind.

If a subject terminates early from the study, they will be encouraged to complete assessments per the Day 456 visit. If a subject is randomized but does not successfully receive the first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.

### **3.4.3 Post-Treatment Follow-up**

After completion of the Day 275 visit, subjects will enter the 6-month post-treatment evaluation period. This period consists of Study Center visits on Day 365 and 456 and follow-up phone assessments on a monthly basis, as outlined in the Schedule of Procedures ([Appendix A](#)). After completion of the Day 456 visit, subjects may be eligible to participate in an OLE study, pending study approval by the IRB or IEC and the appropriate regulatory authority.

## **3.5 End-of-Study**

The End-of-Study is last subject; last visit (either in-person visit or telephone contact). In the event of a decision by the study Sponsor to terminate the study earlier on the grounds that conducting a sham-controlled study is no longer deemed ethical based on an updated risk-benefit assessment of ISIS 396443 from the planned interim analysis, all subjects will be invited for the EODBP study visit, during which all Day 456 assessments will be conducted. The EODBP visit should occur no less than 2 weeks from the most recent dosing/sham procedure administered. After completing the EODBP visit, subjects will be considered study completers and will be allowed to enroll into the OLE study.

## **3.6 Safety Monitoring and Data Safety Monitoring Board**

Safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor. Safety data will also be reviewed on a quarterly basis by an independent data and safety monitoring board (DSMB). The DSMB will be assembled to review safety, tolerability and efficacy (as needed) data collected on ISIS 396443 during this study. Based on its ongoing

assessment of the data, the DSMB will provide recommendations to the Sponsor for modifying, stopping or continuing the study.

## **4. SUBJECT ENROLLMENT**

### **4.1 Screening**

Before subjects may be enrolled into the study, the Sponsor requires a copy of the Study Center's written IRB or IEC approval of the protocol, informed consent form, informed assent form (if applicable) and all other subject information and/or recruitment material.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent(s) or legal guardian(s) and, in cases where institutional guidelines and the patient's age dictate, informed assent from the subject. At the time of consent/assent, the subject will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including screening procedures, are performed. The screening number must remain constant throughout the entire trial. In the event the subject is re-consented and re-screened the subject must be given a new screening number. Screening numbers, once assigned, will not be re-used.

### **4.2 Randomization**

Subjects will be randomized after all screening assessments have been completed and after the Investigator and the Medical Monitor have verified that they are eligible per criteria in [Sections 5.1](#) and [5.2](#). No subject may begin treatment prior to randomization and assignment of a unique randomization number.

Using an Interactive Voice/Web-Response System (IXRS), eligible subjects will be randomized 2:1 to receive ISIS 396443 or sham-procedure control, respectively. Randomization will be stratified for:

- Subject's Age at Screening (< 6 years vs.  $\geq$  6 years)

The Sponsor Quality Assurance department or designee will hold a copy of the randomization lists generated by the IXRS vendor.

### **4.3 Replacement of Subjects**

If a subject does not successfully receive the first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.

### **4.4 Unblinding of Treatment Assignment**

The Sponsor, parents, and key study site personnel will be blinded to subjects' treatment assignment throughout the study. The DSMB may be unblinded as described in the DSMB charter. Representatives from the Sponsor may be unblinded at the interim analysis as described in the unblinding plan. Those Sponsor representatives will no longer be involved in the conduct of the study after they have been unblinded.

If a subject has experienced an SAE (as defined in [Section 9.3.3](#)), and/or when knowledge of the treatment assignment will impact the clinical management of the subject, the Investigator will

have the ability to unblind the treatment assignment for that subject using the IXRS. However, prior to unblinding, the Investigator should attempt to contact the blinded Medical Monitor to discuss the emergency. The Sponsor or designee will be informed of the unblinding of a subject within 24 hours. Every reasonable attempt should be made to complete the Study Day 456 early termination procedures and observations (see [Appendix A](#)) prior to unblinding, as knowledge of the subject's treatment assignment could influence subsequent assessments. The Investigator must document the reasons for unblinding in the subject's source documents. The Investigator is strongly advised not to divulge the subject's treatment assignment to any individual not directly involved in managing the medical emergency, nor to personnel involved with the analysis and conduct of the study. In cases where there are ethical reasons to have a subject remain on study whose treatment assignment is unblinded for safety reasons, the Site Investigator must obtain specific approval from the Sponsor and the Medical Monitor for the subject to continue in the study.

In addition, all suspected unexpected serious adverse reactions (SUSARs) will be unblinded by the Sponsor's Drug Safety and Quality Assurance personnel for the purpose of regulatory reporting ([Section 9.2](#)).

## 5. SUBJECT ELIGIBILITY

### 5.1 Inclusion Criteria

Subjects must meet all of the following criteria at Screening to be eligible:

1. Signed informed consent of parent or guardian. Signed informed assent of subject, if indicated per subject's age and institutional guidelines
2. Genetic documentation of 5q SMA (homozygous gene deletion, mutation, or compound heterozygote)
3. Onset of clinical signs and symptoms consistent with SMA at > 6 months of age
4. Males and females 2 to 12 years of age
5. Can sit independently, but has never had the ability to walk independently
6. Motor Function Score (Hammersmith Functional Motor Scale – Expanded)  $\geq 10$  and  $\leq 54$  at Screening
7. Able to complete all study procedures, measurements and visits and parent or guardian/subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator
8. Estimated life expectancy > 2 years from screening, in the opinion of the Investigator
9. Meets age-appropriate institutional criteria for use of anesthesia/sedation, if use is planned for study procedures (as assessed by the Site Investigator and either anesthesiologist or pulmonologist)
10. For subjects who, in the opinion of the Investigator, have reached reproductive maturity, satisfy 1 of the following:

Females: have a negative pregnancy test at Screening and agrees to employ adequate contraceptive measures for the duration of the study. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products.

Males: be abstinent for the duration of the study

## 5.2 Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for the study:

1. Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for > 6 hours during a 24 hour period, at Screening
2. Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator
3. Severe contractures or severe scoliosis evident on X-ray examination at Screening
4. Hospitalization for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of screening or planned during the duration of the study
5. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period
6. History of brain or spinal cord disease, including tumors, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation
7. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter
8. History of bacterial meningitis
9. Dosing with ISIS 396443 in any previous clinical study
10. Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline
11. Clinically-significant abnormalities in hematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion
12. Treatment with another investigational drug (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, etc.), biological agent, or device within 1-month of screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation
13. Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anemia, etc.) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures

## 6. STUDY PROCEDURES

### 6.1 Study Schedule

All required study procedures are outlined in [Appendices A, B and C](#).

### 6.2 Study Assessments

#### 6.2.1 Laboratory Analytes

Laboratory measurements of serum chemistry, hematology, urinalysis, coagulation parameters, and plasma antibodies to ISIS 396443 will be performed at the times shown in the Schedule of Procedures (Appendix A). The analytes to be measured are shown in Appendix B.

#### 6.2.2 Neurological Examinations

Neurological examinations include assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes. Neurological examinations will be performed at the times shown in the Schedule of Procedures (Appendix A).

#### 6.2.3 Pharmacokinetics Specimen Collection

Plasma and CSF specimens will be collected as shown in Appendix A (Schedule of Procedures) and Appendix C (Pharmacokinetic Sampling Schedule). The following ISIS 396443 plasma PK parameters (though not necessarily limited to) will be derived when appropriate from the individual subject concentration vs. time profiles using noncompartmental-based methods and based on actual sampling times:

- The maximal observed plasma drug concentration ( $C_{\max}$ )
- The time to reach  $C_{\max}$  in plasma ( $T_{\max}$ )
- The area under the plasma concentrations time curve from the time of the IT dose to the last collected sample
- The apparent terminal elimination half-life ( $t_{1/2}$ ), if possible

#### 6.2.4 Hammersmith Functional Motor Scale - Expanded

Subjects will be evaluated using the Hammersmith Functional Motor Scale – Expanded (HFMSE) at the times shown in the Schedule of Procedures (Appendix A). The HFMSE is a reliable and validated tool used to assess motor function in children with SMA. The scale was originally developed with 20 scored activities and was devised for use in children with SMA Type 2 and Type 3 with limited ambulation to give objective information on motor ability and clinical progression ([Main et al. 2003](#)). The expanded scale includes an additional module of 13 items developed to allow for evaluation of ambulatory SMA patients ([O’Hagen et al. 2007](#)). The HFMSE has been shown to be highly correlated with other clinical assessments and shows good test-retest reliability. The HFMSE is easy to use and quickly administered.

### **6.2.5 Motor Milestones**

Subjects will be evaluated for motor milestones at the times shown in the Schedule of Procedures ([Appendix A](#)). Motor Milestones will be assessed using the WHO Motor Milestone criteria ([WHO Multicentre Growth Reference Study Group 2006](#); [Wijnhoven et al. 2004](#)).

### **6.2.6 PedsQL™ (Generic Core Scales and Neuromuscular Module)**

Subjects will be evaluated using the Pediatric Quality of Life Inventory (PedsQL™) Measurement 4.0 Generic Core Scales and 3.0 Neuromuscular Module ([Varni et al. 1999](#)) at the times shown in the Schedule of Procedures ([Appendix A](#)). This assessment has been validated by the American Spinal Muscular Atrophy Randomized Trials (AmSMART) group for use in SMA patients from age 2 to 18 years ([Iannaccone et al. 2009](#)).

The PedsQL™ Measurement Model is a modular approach to measuring health-related quality of life (HRQOL) in children and adolescents. The PedsQL consists of brief, practical, generic core scales as well as with condition-specific modules for use in designated clinical populations. Pediatric self-report is measured in children and adolescents ages 5-18 years, and parent proxy-report of child HRQOL is measured for children and adolescents ages 2-18 years. The PedsQL™ 4.0 Generic Core Scales include assessment of physical functioning, emotional functioning, social functioning, and school functioning. The PedsQL 3.0 Neuromuscular Module was designed to measure HRQOL dimensions specific to children aged 2 to 18 years with neuromuscular disorders, including SMA.

### **6.2.7 Upper Limb Module Test**

Subjects will be evaluated using the Upper Limb Module Test ([Mazzone et al. 2011](#)) at the times shown in the Schedule of Procedures ([Appendix A](#)). The Upper Limb Module Test is an outcome measure specifically developed to assess upper limb functional abilities in SMA patients, including young children and patients with severe contractures in the lower limbs in whom the possibility to detect functional changes, such as rolling or long sitting, is limited. This test consists of upper limb performance items that are reflective of activities of daily living (i.e., raise a can to mouth as if drinking, take a coin and place it in a box, remove the lid of a container). The Upper Limb Module Test is quickly administered and has been evaluated in SMA patients age 30 months to 27 years ([Mazzone et al. 2011](#)).

### **6.2.8 Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)**

Parents of subjects will complete the Assessment of Caregiver Experience with Neuromuscular Disease (ACEND) questionnaire at the times shown in the Schedule of Procedures ([Appendix A](#)). This assessment instrument has been designed to quantify the caregiver impact experienced by parents of children affected with severe neuromuscular diseases, including children with SMA ([Matsumoto et al. 2011](#)). The ACEND includes domains assessing physical impact (including feeding/grooming/dressing, sitting/play, transfers, and mobility) and general caregiver impact (including time, emotion, and finance).

### **6.2.9 Safety Evaluations**

Safety will be evaluated by assessment of AEs including SAEs as described in [Section 9](#). Additional safety evaluations include the following parameters:

- Vital signs and weight
- Neurological examinations
- Physical examinations
- Clinical laboratory tests (serum chemistry, hematology, urinalysis)
- Electrocardiograms (ECGs)
- Use of concomitant medications

### 6.3 Contraception Requirements

All male subjects must remain abstinent during the study.

All female subjects of childbearing potential must either be abstinent or practice adequate contraception during the study. For the purposes of this study, females of childbearing potential are defined as any female who has experienced menarche. For the purposes of the study, acceptable contraception methods are abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products

## 7. STUDY DRUG

### 7.1 Study Drug Description

Study Drug (ISIS 396443 drug product) characteristics are listed under Table 1.

The Study Drug is contained in 6 mL clear glass vials. The Study Drug and its storage and preparation instructions will be provided by the Sponsor or designee. The Study Drug must be stored securely at 2° to 8° C and protected from light.

**Table 1 Study Drug Characteristics**

Study Drug	ISIS 396443 Drug Product
Strength	2.4 mg/mL
Volume/vial	5.0 mL solution per vial
Route of Administration	IT injection

### 7.2 Packaging and Labeling

The Sponsor will provide the Investigator with packaged Study Drug labeled in accordance with specific country regulatory requirements.

### 7.3 Study Drug Accountability

The study staff is required to document receipt, dispensing and return of Study Drug supplies provided by the Sponsor. Drug accountability documentation and all used and unused Study Drug vials must be returned to the Sponsor or designee.

## 8. TREATMENT OF SUBJECTS

### 8.1 Study Drug Administration

Details regarding the LP dosing injection procedure will be provided in the Dosing Administration Manual. ISIS 396443 will be administered by dedicated study personnel who are unblinded to treatment group; this cannot be any of the key study site personnel (i.e., the Principal Investigator, study coordinator, or outcomes assessors). The Study Drug administration will be performed in a dedicated room and the key study personnel and the parents will not be present during the procedure to ensure blinding.

ISIS 396443 will be administered as an intrathecal slow bolus (1-3 minute) LP injection. ISIS 396443 will be administered using a ‘spinal anesthesia’ needle and syringe. A 22G to 25G spinal anesthesia needle is recommended, but a 21G may be used if indicated by subject size or clinical condition. The target site for needle insertion is the L3/L4 space, but may be 1 segment above or 1-2 segments below this level, if needed. The volume of the injection is 5 mL; prior to the injection 5 mL of CSF fluid is to be collected for analyses. Depending on institutional guidelines, anesthesia or sedation may be used for the LP procedure, following institutional procedures. Subjects will be encouraged to lie flat for 1-hour following dosing, if possible.

Table 2 outlines the dose, ISIS 396443 concentration, and volume for administration of ISIS 396443.

**Table 2 ISIS 396443 Dose, Concentration, and Injection Volume**

Dose (mg)	Concentration (mg/mL)	Injection Volume (mL)
12	2.4	5.0

Prior to each injection on Study Days 1, 29, 85, and 274, 5 mL of CSF fluid is to be collected for analyses. CSF will be used for measurement of ISIS 396443 pharmacokinetic analyses and CSF SMN protein concentration. Extra CSF may be stored for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with CSF constituents. CSF analyses and data presentation will be conducted in a blinded manner.

### 8.2 Sham Procedure

Subjects randomized to the sham-procedure control group will undergo a sham-procedure, rather than Study Drug administration, on Study Days 1, 29, 85, and 274. Details regarding the sham procedure will be provided in the Dosing Administration Manual. The sham procedure will be administered by dedicated study personnel who are unblinded to treatment group; this cannot be any of the key study site personnel (i.e., the Investigator, study coordinator, or outcomes assessors). The sham procedure will be performed in a dedicated room and the key study personnel and the parents will not be present during the procedure to ensure blinding.

In general, the sham procedure will consist of a small needle prick on the lower back at the location where the LP injection is normally made. The needle will break the skin but no LP injection or needle insertion will occur. The needle prick will be covered with the same bandage that is used to cover the LP injection normally, thus simulating the appearance of an LP injection. If anesthesia or sedation is used for the LP procedure in ISIS 396443 treated subjects, then in order to maintain the blind, minimal sedation (i.e. a low dose of an anxiolytic) should be used for the sham procedure, following institutional procedures. The study subject will be kept in the procedure room for the same amount of time that subjects administered Study Drug are kept, thus simulating the time period of a Study Drug administration procedure.

Study Drug and sham kits will be packaged in a blinded fashion. Blinded kits for sham procedure contain artificial CSF (5.0 mL solution per 6 mL vial) that will not be injected but will be used to simulate CSF samples for that subject.

### **8.3 Other Protocol-Required Drugs**

There are no other protocol required drugs.

### **8.4 Other Protocol-Required Procedures**

There are no other protocol-required treatment procedures.

### **8.5 Treatment Precautions**

There are no protocol-required treatment precautions.

### **8.6 Safety Monitoring Rules**

Please refer to the Guidance to Investigator section of the Investigator Brochure.

### **8.7 Stopping Rules**

There are no additional specific stopping rules for this study but the Investigator should discuss significant concerns relating to individual subjects with the Medical Monitor and the Sponsor to ensure that it is appropriate for the subject to continue Study Drug.

### **8.8 Adjustment of Dose and/or Treatment Schedule**

No adjustment of dose is permitted. In the event of a concurrent illness that would prevent the dosing procedure from being performed safely, an adjustment in the dose schedule may be permitted, but must be approved by the Medical Monitor. In general, each scheduled dose may be delayed by up to 4 weeks.

If a delay from the initial study visit schedule occurs, the Medical Monitor must approve the subject-specific make-up schedule in advance. In any schedule adjustments prescribed by the Medical Monitor, dose/sham procedures must occur at least 4 weeks apart with the goal of returning the subject to his/her initial study visit schedule. Any subject whose dosing schedule changes as a result of such recommendations from the Medical Monitor (including skipping a dose/sham procedure) will still be considered as completing treatment and thus potentially eligible to participate in the open-label extension study.

## **8.9 Discontinuation of Study Treatment**

A subject must permanently discontinue study treatment for any of the following:

- The subject's parents/guardians withdraw consent
- The subject experiences an adverse event that necessitates permanent discontinuation of study treatment

The reason for discontinuation of study treatment must be recorded in the Case Report Form (CRF) and source documentation.

Subjects that discontinue treatment will continue follow-up unless consent is withdrawn ([Appendix A](#)).

## **8.10 Withdrawal of Subjects from the Study**

Subjects must be withdrawn from the study for any of the following:

- Withdrawal of consent
- The subject or the subject's parents/guardians is/are unwilling or unable to comply with the protocol
- The subject experiences a medical emergency that necessitates unblinding of the subject's treatment assignment

Other reasons for withdrawal of subjects from the study might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation
- Decision by the Investigator or Sponsor

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from Study, must be recorded in the CRF.

Any subject for whom consent to participate in the study is withdrawn will be removed from further treatment and study observation immediately upon the date of request. It should be encouraged that these subjects complete the early termination study procedures and observations at the time of withdrawal ([Appendix A](#)).

For subjects withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination study procedures and observations at the time of withdrawal ([Appendix A](#)).

## **8.11 Concomitant Therapy and Procedures**

The use of concomitant therapies or procedures defined below must be recorded on the subject's CRF. AEs related to administration of these therapies or procedures must also be documented on the appropriate CRF.

### **8.11.1 Concomitant Therapy**

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications and vitamin supplements) administered between the beginning of screening and last telephone contact or study visit.

Subject's parents/guardians should consult with the Site Investigator or qualified designee prior to initiating any new medication, including non-prescription compounds or any other non-drug therapy.

#### **Allowed Concomitant Therapy**

Throughout the study, Site Investigators or designated licensed physicians involved in the study may prescribe concomitant medications or treatments deemed necessary for adverse events or to provide adequate supportive care.

#### **Disallowed Concomitant Therapy**

Study subjects are prohibited from receiving other experimental agents during the study. This includes marketed agents at experimental doses that are being tested for the treatment of SMA (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, valproate, hydroxyurea).

### **8.11.2 Concomitant Procedures**

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the beginning of screening and last telephone contact or study visit.

## **9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING**

### **9.1 Sponsor Review of Safety Information**

Safety information will be collected, reviewed, and evaluated by the Sponsor in accordance with the Safety Management Plan throughout the conduct of the clinical trial.

### **9.2 Regulatory Requirements**

The Sponsor is responsible for regulatory submissions and reporting to the Investigators of SAEs including suspected unexpected serious adverse reactions (SUSARs) per the International Conference on Harmonization (ICH) guidelines E2A and ICH E6. Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

IRBs/IECs will be notified of any serious adverse event (SAE) according to applicable regulations. The DSMB will be notified of any SAE as specified in the DSMB charter.

## 9.3 Definitions

### 9.3.1 *Adverse Event*

An adverse event is any unfavorable and unintended sign (including a clinically-significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the study or use of investigational drug product, whether or not the AE is considered related to the investigational drug product.

### 9.3.2 *Adverse Reaction and Suspected Adverse Reaction*

An adverse reaction is any adverse event caused by the Study Drug.

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

### 9.3.3 *Serious Adverse Event (SAE)*

A serious adverse event is any adverse event that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event

An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death

- Requires inpatient hospitalization or prolongation of existing hospitalization

Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE

- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

## 9.4 Monitoring and Recording Adverse Events

Any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible.

### 9.4.1 *Serious Adverse Events*

In the interest of subject safety, and in order to fulfill regulatory requirements, all SAEs (regardless of their relationship to Study Drug) should be reported to the Sponsor within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the subject signs the informed consent form and stop at the end of the subject's follow-up period which is defined as the subject's last visit. When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message. An Initial Serious Adverse Event Form should be completed and a copy should be faxed to the Sponsor or designee.

Detailed information should be actively sought and included on Follow-Up Serious Adverse Event Forms as soon as additional information becomes available. All SAEs will be followed until resolution. SAEs that remain ongoing past the subject's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and Sponsor agree the subject's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

### 9.4.2 *Non-Serious Adverse Events*

The recording of non-serious AEs will begin after the subject signs the informed consent form and will stop at the end of the subject's follow-up period, which is defined as subject's last visit. The Investigator will monitor each subject closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

### 9.4.3 *Evaluation of Adverse Events (Serious and Non-Serious)*

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form.

#### 9.4.3.1 *Relationship to the Study Drug*

The event's relationship to the Study Drug is characterized by 1 of the following:

- **Related:** There is clear evidence that the event is related to the use of Study Drug, e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)

- **Not Related:** The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

#### 9.4.3.2 *Severity*

The event's severity is characterized by 1 of the following:

- **Mild:** The event is easily tolerated by the subject and does not affect the subject's usual daily activities
- **Moderate:** The event causes the subject more discomfort and interrupts the subject's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the subject's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in [Section 9.3.3](#)).

#### 9.4.3.3 *Action Taken with Study Drug*

Action taken with Study Drug due to the event is characterized by 1 of the following:

- **None:** No changes were made to Study Drug administration and dose
- **Permanently Discontinued:** Study drug was discontinued and not restarted
- **Temporarily Interrupted, restarted – same dose:** Dosing was temporarily interrupted or delayed due to the AE and restarted at the same dose without unblinding to treatment group

#### 9.4.3.4 *Treatment Given for Adverse Event*

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form (treatment should also be recorded on the concomitant treatment or ancillary procedures CRF as appropriate).

#### 9.4.3.5 *Outcome of the Adverse Event*

If the event is a non-serious AE then the event's outcome is characterized by 1 of the following:

- **AE Persists:** Subject terminates from the trial and the AE continues
- **Recovered:** Subject recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity (if applicable):** AE severity changed

If the event is a SAE then the event's outcome is characterized by 1 of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Subject has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE electronic case report form (eCRF) (the SAE resolution date should be entered as the date of onset of that AE)
- **Recovered:** Subject recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Fatal:** Subject died (the date of death should be entered as the SAE resolution date)

## 9.5 Procedures for Handling Special Situations

### 9.5.1 *Abnormalities of Laboratory Tests*

Clinically-significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically-significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Medical Monitor. Laboratory abnormalities deemed not clinically-significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents.

### 9.5.2 *Prescheduled or Elective Procedures or Routinely Scheduled Treatments*

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

### **9.5.3      *Dosing Errors***

Study Drug errors defined as errors in administration or the administered dose should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic.

Dosing details should be captured on the Dosing CRF.

**Should an overdose occur**, the Investigator or designee should contact the Unblinded Medical Monitor within 24 hours.

### **9.5.4      *Contraception and Pregnancy***

Female subjects that have reached reproductive maturity must have a negative pregnancy test at Screening and must not be able to become pregnant for the duration of the study, as described in [Section 6.3](#).

Male subjects must be abstinent during the duration of the study.

If a subject becomes pregnant or a pregnancy is suspected, or if a male subject makes or believes that he has made someone pregnant during the study, then the site staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported on Follow-up Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the subject's responsibility.

## **10.      STATISTICAL CONSIDERATIONS**

### **10.1      Study Endpoints, Subsets, and Covariates**

#### **10.1.1      *Primary Efficacy Endpoint***

- Change from baseline in HFMSE (Hammersmith Functional Motor Scale – Expanded) score at 15 months

#### **10.1.2      *Secondary Efficacy Endpoints***

- Proportion of subjects who achieve a 3-point or greater increase from baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months
- Proportion of subjects that achieve any new motor milestone at 15 months
- Number of motor milestones achieved per subject at 15 months
- Change from baseline in Upper Limb Module Test at 15 months
- Proportion of subjects that achieve standing alone at 15 months
- Proportion of subjects that achieve walking with assistance at 15 months

### **10.1.3 Tertiary Efficacy Endpoints**

- Change from baseline in CSF SMN protein concentration
- Clinical Global Impression of Change (Investigator and Caregiver assessment)
- Change from baseline in PedsQL (Pediatric Quality of Life Inventory)
- Change from baseline in Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)
- Disease-related hospitalizations and adverse events

### **10.1.4 Safety/Tolerability Endpoints**

- Adverse events
- Vital signs and weight
- Neurological examinations
- Physical examinations
- Clinical laboratory tests (serum chemistry, hematology, urinalysis)
- Electrocardiograms (ECGs)
- Use of concomitant medications

### **10.1.5 Pharmacokinetic Endpoints**

- CSF levels of ISIS 396443
- Plasma levels of ISIS 396443

### **10.1.6 Immunogenicity Endpoint**

- Plasma antibodies to ISIS 396443

## **10.2 Sample Size Considerations**

The sample size for this study was estimated based on limited available natural history data for the target population and data from the ISIS 396443-CS1 and ISIS 396443-CS2 clinical studies. Seventy (70) patients in the treated group and 35 patients in the control group will give at least 90% power to detect a 3-point difference between control and treated groups in change on the HFMSE with a standard deviation of 4.4, using a 2-sided t-test with an alpha level of 0.05. The sample size was estimated using n-Query. One hundred seventeen (117) patients enrolled will ensure that a small dropout rate will not affect the power of the primary efficacy analysis.

## **10.3 Populations**

Intent to Treat (ITT) Set: All patients who are randomized and receive at least 1-dose of Study Drug/sham procedure.

Per-Protocol Set (PPS): PPS will include the subset of the ITT who complete at least the initial 3 doses of Study Drug/sham procedures, have baseline and Day 169 efficacy assessments and who have no significant protocol deviations that would be expected to affect efficacy assessments.

Safety Set: All patients who are randomized and receive at least 1-dose of Study Drug/sham procedure.

Pharmacokinetic Population: All patients who are randomized and for which there is at least 1 evaluable post-dose/post-sham procedure pharmacokinetic sample.

#### **10.4 Definition of Baseline**

The baseline is defined as the last non-missing assessment prior to the first dose of Study Drug.

#### **10.5 Interim Analysis**

An interim analysis may take place when all subjects have completed the 6-month assessment and at least 39 subjects have completed the 15-month assessment. At the time of the interim analysis, it is expected that approximately 52 subjects would have attended the Month 15 assessment and all 126 subjects would have had the opportunity to attend the assessment at Month 6.

At the interim analysis, the primary efficacy endpoint, change in HFMSE, will be tested at an alpha of 0.02. For the interim analysis, the ITT Set will be used. Since the Month 15 endpoint values will not be available for most of the patients, missing post-baseline HFMSE data will be handled using the multiple imputation method ([Schafer 1997](#); [Schafer 1999](#)).

To control the overall Type I error rate at 0.05 across interim and final analyses for the testing of primary and secondary endpoints, a stage-wise hierarchical strategy utilizing independent alpha spending functions for primary and secondary endpoints ([Glimm et al. 2010](#)) will be applied. In the framework of the stage-wise hierarchical testing, at the final analysis alpha = 0.05 will be used in the sequential testing procedure for the secondary endpoints not tested at the interim, as detailed in the Statistical Analysis Plan (SAP).

In the event that the primary endpoint is not statistically significant at the interim, at the final analysis, the primary endpoint will be tested at an alpha level determined based upon the resampling approach, as detailed in the SAP ([Westfall and Young 1993](#)).

The analysis of safety data for the interim analysis will include all subjects who have received at least 1-dose of study treatment as of the targeted clinical cut-off date.

A DSMB will review the interim analysis results and make recommendations on whether it is appropriate for the trial to continue. During the interim analysis, subjects will continue in the study.

Details of the analysis and controlled access to the unblinded data are contained in the SAP, the unblinding plan and the DSMB Charter.

## **10.6 Planned Methods of Analysis**

Detailed description of the analysis methodology is provided in the SAP.

Data collected on eCRF, lab data transfers, as well as any outcomes derived from the data, will be provided in the patient data listings. Patient data listings will be presented for all patients enrolled into the study.

Descriptive summary statistics including n, mean, median, standard deviation, standard error, interquartile range (25th percentile, 75th percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data by treatment group.

All primary, secondary and tertiary endpoints will be assessed in the ITT Set and PPS, with the former being the basis for the primary efficacy analysis. All safety assessments will be performed on the Safety Set. PK endpoints will be assessed in the PK Set as applicable.

### **10.6.1 Demographic and Baseline Characteristics**

Demographic and baseline characteristics will be summarized using descriptive statistics by treatment group. Patient disposition will be summarized by treatment group. All patients enrolled will be included in a summary of patient disposition.

### **10.6.2 Safety and Tolerability Analysis**

Safety analyses will be conducted in the Safety Set. Treatment duration and amount of Study Drug received will be summarized by treatment group.

All treatment-emergent adverse events and serious adverse events will be summarized for each treatment group using the MedDRA<sup>TM</sup> coding system, by system organ class, preferred term, relationship to Study Drug, and severity. Narratives of deaths, serious adverse events, including early withdrawals from Study Drug and from study due to adverse events, will also be provided.

Laboratory tests including chemistry panel, complete blood count with differential, etc., will be summarized by study visit for each treatment group. These safety variables will also be presented over time after Study Drug administration, as appropriate. Vital sign results will be presented similarly.

Physical and neurological examination findings and results from ECG will be listed for review. As appropriate, results will also be summarized descriptively for each treatment group. Concomitant medication usage for each subject will be listed for review.

### **10.6.3 Efficacy Analysis**

All statistical tests will be 2-sided with a Type 1 error rate of 5%, unless otherwise specified.

The primary analysis of the primary endpoint is to compare the change from baseline in HFMSE score at Month 15 between treatment groups using the ITT Set. The data will be analyzed using an analysis of covariance (ANCOVA) model with treatment group as a factor and age at screening and baseline HFMSE score as covariates. The primary efficacy analysis will take

place after all patients have completed the Day 456/ET visit and the database has been locked. Missing data will be handled by the multiple imputation procedure.

Several sensitivity analyses will also be conducted; the details of these analyses will be outlined in the SAP.

In the framework of the stage wise hierarchical testing, since the secondary endpoints will not be tested at the interim (i.e., no alpha spending), at the final analysis  $\alpha = 0.05$  will be used in the sequential testing.

The secondary endpoints will be tested in the following order:

- Proportion of subjects with a 3-point or greater increase in HFMSE
- Proportion of subjects achieving any new motor milestones at 15 months
- Number of motor milestones achieved at 15 months
- Change from baseline in upper limb module test at 15 months
- Proportion of subjects achieving standing alone at 15 months
- Proportion of subjects achieving walking with assistance at 15 months

The primary analysis of each secondary endpoint will be based on the ITT Set. Analyses based on the PPS are considered sensitivity analyses. Missing data will be handled by the multiple imputation procedure. Additional details of these analyses will be outlined in the SAP.

#### **10.6.4 Pharmacokinetic Analysis**

Plasma pharmacokinetic parameters and ISIS 396443 concentrations in plasma and CSF for the Pharmacokinetic population will be summarized using descriptive statistics and, where warranted, presented graphically.

## **11. INVESTIGATOR'S REGULATORY OBLIGATIONS**

### **11.1 Informed Consent/Assent**

The written informed consent and assent documents should be prepared in the language(s) of the potential subject population, based on an English version provided by the Sponsor and should be easy to understand.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent or legal guardian and, in cases where institutional guidelines and the subject's age dictate, informed assent from the subject, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any Study Drugs are administered. Sufficient time must be given to consider whether to participate in the study.

The acquisition of informed consent/assent and the parent/legal guardian's/subject's agreement or refusal of his/her notification of the primary care physician should be documented in the subject's medical records, and the informed consent/assent form(s) should be signed and

personally dated by the parent/legal guardian/subject and by the study person who conducted the informed consent/assent discussion. The original signed informed consent/assent form(s) should be retained in the Study Master File and in any other locations required by institutional policy, and a copy of the signed consent/assent form(s) should be provided to the parent or guardian.

### **11.2 Ethical Conduct of the Study**

The Guidelines of the World Medical Association (WMA) Declaration of Helsinki dated October 2002 the applicable regulations and guidelines of current Good Clinical Practice (GCP) as well as the demands of national drug and data protection laws and other applicable regulatory requirements will be strictly followed.

### **11.3 Institutional Review Board/Institutional Ethics Committee/Research Ethics Board**

A copy of the protocol, proposed informed consent form, proposed informed assent form (if applicable) other written subject information, and any proposed advertising material must be submitted to the IRB/IEC for written approval. A copy of the written approval of the protocol and informed consent/assent forms must be received by the Sponsor before recruitment of subjects into the study and shipment of Study Drug. A copy of the written approval of any other items/materials that must be approved by the Study Center or IRB/IEC must also be received by the Sponsor before recruitment of subjects into the study and shipment of Study Drug. The Investigator's Brochure must be submitted to the IRB/IEC for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the IRB/IEC for all subsequent protocol amendments and changes to the informed consent/assent documents. The Investigator should notify the IRB/IEC of deviations from the protocol in accordance with ICH GCP Section 4.5.2. The Investigator should also notify the IRB/IEC of serious adverse events occurring at the Study Center and other adverse event reports received from the Sponsor, in accordance with local procedures.

The Investigator will be responsible for obtaining annual IRB/IEC approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all IRB/IEC submissions and the IRB/IEC continuance of approval must be sent to the Sponsor.

### **11.4 Subject Confidentiality**

The Investigator must ensure that the subject's confidentiality is maintained. On the CRFs or other documents submitted to the Sponsor, subjects should be identified by unique, anonymous initials and a subject study number only. Documents that are not for submission to the Sponsor (e.g., signed informed consent/assent forms) should be kept in strict confidence by the Investigator.

In compliance with federal and local regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB/IEC direct access to review the subject's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The Investigator is obligated to inform and obtain the consent of the subject's parent or guardian to permit named representatives to have access to his/her study-related records without violating the confidentiality of the subject.

## **12. ADMINISTRATIVE AND LEGAL OBLIGATIONS**

### **12.1 Protocol Amendments**

Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent/assent documents. The regulatory authority and IRB/IEC must be informed of all amendments and give approval for any amendments likely to affect the safety of the subjects or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the IRB/IEC to the Sponsor.

### **12.2 Study Termination**

The Sponsor reserves the right to terminate the study. The Investigator reserves the right to terminate their participation in the study, according to the terms of the site contract. The Investigator/Sponsor should notify the IRB/IEC in writing of the trial's completion or early termination and send a copy of the termination to the Sponsor.

### **12.3 Study Documentation and Storage**

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the subject's CRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, and correspondence. In this study, CRFs may not be used as source documents.

The Investigator and Study Center staff is responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with Section 8 of the ICH Guidelines (E6), suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. Elements should include:

- Subject files containing completed CRFs, informed consents/assents, and supporting copies of source documentation
- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the IRB and the Sponsor
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction, and all drug-related correspondence

In addition, all original source documents supporting entries in the CRFs must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor.

## **12.4 Study Monitoring**

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., CRFs and other pertinent data) provided that subject confidentiality is respected.

The Sponsor monitor is responsible for inspecting the CRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the CRFs.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department. Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data a clinical data management review will be performed on subject data received by the Sponsor. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor.

The Principal Investigator will sign and date the indicated places on the CRF. These signatures will indicate that the Principal Investigator inspected or reviewed the data on the CRF, the data queries, and the Study Center notifications, and agrees with the content.

## **12.5 Language**

CRFs must be completed in English. Generic names for concomitant medications should be recorded in English if possible, unless it is a combination drug, then record the trade name in English.

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood.

## **12.6 Compensation for Injury**

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Subjects will be treated and/or compensated for any study-related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

### 13. REFERENCES

- Cho S and Dreyfuss G. A degtron created by SMN2 Exon 7 skipping is a principal contributor to spinal muscular atrophy severity. *Genes Dev* 2010; 24: 438-442.
- Coovert DD, Le TT, McAndrew PE, et al. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet* 1997; 6: 1205-1214.
- Feldkötter M, Schwarzer V, Wirth R, et al. Quantitative analyses of SMN1 and SMN2 based on real-time light cycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am J Hum Genet* 2002; 70: 358-368.
- Geary RS, Yu RZ, Watanabe T, et al. Pharmacokinetics of a tumor necrosis factor- $\alpha$  phosphorothioate 2'-O-(2-Methoxyethyl) modified antisense oligonucleotide: comparison across species. *Drug Metab Disp* 2003; 31: 1419-1428.
- Glimm E, Maurer W, Bretz F. Hierarchical testing of multiple endpoints in group-sequential trials. *Stat Med* 2010; 29 (2): 219-228.
- Helmken C, Hofmann Y, Schoenen F, et al. Evidence for a modifying pathway in SMA discordant families: reduced SMN level decreases the amount of its interacting partner and Htra2-beta1. *Hum Genet* 2003; 114: 11-21.
- Henry S, Stecker K, Brooks D, et al. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J Pharmacol Exp Ther* 2000; 292: 468-479.
- Hua Y, Vickers TA, Baker BF, et al. Enhancement of SMN2 Exon 7 inclusion by antisense oligonucleotides targeting the exon. *PLoS Biol* 2007; 5: e73.
- Hua Y, Vickers TA, Okunola HL, et al. Antisense masking of an HnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am J Hum Genet* 2008; 82: 834-848. Epub 2008 Mar 27.
- Iannaccone ST, Hynan LS, Morton A, et al; the AmSMART Group. The PedsQL in pediatric patients with spinal muscular atrophy: feasibility, reliability, and validity of the pediatric quality of life inventory generic core scales and neuromuscular module. *Neuromuscul Disord* 2009; 19: 805-812.
- Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat.Rev.Genet* 2010; 11: 345-355.
- Le TT, Pham LT, Butchbach ME, et al. SMN $\Delta$ 7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. *Hum Mol Genet* 2005; 14: 845-857.
- Lefebvre S, Bürglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995; 80: 155-165.
- Lefebvre S, Burlet P, Liu Q, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet* 1997; 16: 265-269.
- Main M, Kairon H, Mercuri E, Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. *Eur J Paediatr Neurol* 2003; 7(4): 155-159.
- Matsumoto H, Clayton-Krasinski DA, Klinge SA, et al. Development and initial validation of the assessment of caregiver experience with neuromuscular disease. *J Pediatr Orthop* 2011; 31: 284-292.
- Mazzone E, Bianco F, Martinelli D, et al. Assessing upper limb function in nonambulant SMA patients: development of a new module. *Neuromuscul Disord* 2011; 21: 406-412.
- McKay RA, Miraglia LJ, Cummins LL, et al. Characterization of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C- $\alpha$  expression. *J Bio Chem* 1999; 274: 1715-1722.
- O'Hagen JM, Glanzman AM, McDermott MP, et al. An expanded version of the hammersmith functional motor scale for SMA II and III patients. *Neuromuscular Disorders* 2007; 17: 693-697.

Passini MA, Bu J, Richards AM, et al. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Science Trans Med* 2011; 3: 1-12.

Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2 gene. *Am J Med Genet* 2004; 130A: 307-310.

Schafer JL. Analysis of incomplete multivariate data. Boca Raton, FL. Chapman and Hall/CRC: 1997.

Schafer JL. Multiple Imputation: A Primer. *Statistical Methods in Medical Research* 1999; 8: 3-15.

Varni JW, Seid M, Rode CA. Measurement model for the pediatric quality of life inventory. *Medical Care* 1999; 37: 126-139.

Westfall PH and Young SS. Resampling-based multiple testing. New York; Wiley: 1993.

WHO Multicentre Growth Reference Study Group. Assessment of sex differences and heterogeneity in motor milestone attainment among populations in the WHO Multicentre Growth Reference Study. *Acta Pædiatrica*, 2006; Suppl 450: 66/75.

Wijnhoven T, de Onis M, Onyango A, et al. Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food Nutr Bull* 2004; 21: S37-45.

Wirth B, Brichta L, Schrank B, et al. Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number. *Hum Genet*. 2006; 119(4): 422-428.

## **14. APPENDICES**

## **Appendix A      Schedule of Procedures**

## Appendix A Schedule of Procedures

Subjects will also be monitored through phone contact on Study Days 8, 56, 113, 141, 204, 239, 302, 330, 393, 421 (all  $\pm 2$  days)<sup>9</sup>

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 ( ± 1D)			D30	D85 ( ± 2D)			D86	D92 ( ± 1D)	D169 ( ± 2D)	D274 ( ± 7D)			D275	D365 ( ± 7D) and D456 (+ 7D) and Early Term/ EODBP
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
Informed Consent	X																			
Inclusion/Exclusion Criteria	X																			
Medical History	X																			
Screening X-ray	X																			
Urine Pregnancy Test <sup>11</sup>	X																			X <sup>14</sup>
SMN2 copy Number <sup>12</sup>	X																			
Study Drug Injection/Sham Procedure			X				X <sup>7</sup>				X <sup>7</sup>						X <sup>7</sup>			
In-Patient Stay (24 hours)				X																
Vital Signs <sup>1</sup>	X	X		4X <sup>2</sup>	X <sup>3</sup>	X		4X <sup>2</sup>	X	X		4X <sup>2</sup>	X	X	X	X		4X <sup>2</sup>	X	X
Weight	X	X				X				X				X	X	X				X
Height/ulnar length	X																			X <sup>14</sup>
Physical Examination	X	X				X				X				X	X	X				X
Neurological Examination	X	X		X <sup>4</sup>	X <sup>3</sup>	X		X <sup>4</sup>	X	X		X <sup>4</sup>	X	X	X	X		X <sup>4</sup>	X	X
ECG	X				X			X						X						X <sup>14</sup>
Safety Labs <sup>5</sup>	X	X			X	X				X					X	X				X <sup>14</sup>
Coagulation Labs	X																			
Immunogenicity		X				X				X					X	X				X <sup>14</sup>

# Appendix A Schedule of Procedures *Continued*

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 ( ± 1D)			D30	D85 ( ± 2D)			D86	D92 ( ± 1D)	D169 ( ± 2D)	D274 ( ± 7D)			D275	D365 ( ± 7D) and D456 (+ 7D) and Early Term/ EODBP
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
CSF PK <sup>8</sup>		X				X				X						X				
CSF SMN Protein		X				X				X						X				
Plasma PK <sup>8</sup>		X		3X	X	X				X		X			X	X				X <sup>14</sup>
HFMSE	X <sup>10</sup>													X	X	X <sup>13</sup>				X
WHO Motor Milestones	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
Upper Limb	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
PedsQL	X <sup>6</sup>													X	X	X <sup>13</sup>				X
ACEND	X <sup>6</sup>														X					X <sup>14</sup>
CGI														X	X	X <sup>13</sup>				X
Con Med Recording <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Collection <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

- 1 Resting blood pressure, pulse, respiratory rate, and temperature
- 2 Vital signs performed 1, 2, 4, 6 hours after dosing
- 3 Conducted within 20-24 hours after dosing
- 4 Neurological exams at 5 hours after dosing
- 5 Serum chemistry, hematology, urinalysis panels ([Appendix B](#) for analytes)
- 6 Efficacy assessments (with the exception of HFMSE) do not form part of the screening assessment or inclusion/exclusion criteria but are baseline measurements taken during the screening phase of the study
- 7 Overnight stay is optional on Day 29, Day 85, and Day 274
- 8 Refer to [Appendix C](#) for PK sampling schedule
- 9 At telephone contact, changes in concomitant medications and adverse events will be recorded
- 10 To be performed 2 times during the screening period
- 11 Urine pregnancy test performed for females of child-bearing potential, if positive to be confirmed by local serum test
- 12 Only for those subjects who do not have documented evidence of SMN copy number from Athena Diagnostics
- 13 These assessments may be performed up to 7 days prior to dosing, if necessary
- 14 Assessed on Day 456 only

## **Appendix B      Laboratory Analytes**

## Appendix B Laboratory Analytes

CLINICAL SAFETY ASSESSMENTS (minimum requirements)		OTHER ASSESSMENTS
<u><b>Clinical Chemistry</b></u> Sodium Potassium Chloride Total protein Albumin Calcium Phosphorus Bicarbonate Glucose BUN Creatinine Cystatin C Total serum Bilirubin Alkaline phosphatase AST (SGOT) ALT (SGPT) CPK  <u><b>Coagulation</b></u> aPTT PT INR	<u><b>Urinalysis</b></u> Specific gravity pH Protein Glucose Ketones Bilirubin Blood Red blood cells White blood cells Epithelial cells Bacteria Casts Crystals  <u><b>Hematology</b></u> Red blood cells Hemoglobin Hematocrit Platelets White blood cells WBC Differential <ul style="list-style-type: none"> <li>(% and absolute)</li> <li>Neutrophils</li> <li>Eosinophils</li> <li>Basophils</li> <li>Lymphocytes</li> <li>Monocytes</li> </ul>	<u><b>Pregnancy</b></u> Urine hCG  <u><b>PK*</b></u> Plasma ISIS 396443 levels CSF ISIS 396443 levels  <u><b>PD</b></u> CSF SMN protein levels  <u><b>Immunogenicity Evaluation</b></u> ISIS 396443 plasma Abs  <u><b>SMN Genetics</b></u> SMN2 Copy Number

\* Any of the collected PK plasma and CSF samples from the study patients may also be used for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with plasma and CSF constituents.

## **Appendix C      Pharmacokinetic Sampling Schedule**

## Appendix C Pharmacokinetic Sampling Schedule

Treatment Period	Study Day	Timepoints	Blood Collection	CSF Collection
Multiple Dose: LP Injection	D1	Pre-dose	0.35 mL	0.5 mL
		2 hr	0.35 mL	NA
		4 hr	0.35 mL	NA
		8 hr	0.35 mL	NA
	D2	24 hr	0.35 mL	NA
	D29	Pre-dose	0.35 mL	0.5 mL
	D85	Pre-dose	0.35 mL	0.5 mL
		4 hr	0.35 mL	NA
	D169	Anytime	0.35 mL	NA
	D274	Pre-dose	0.35 mL	0.5 mL
	D456	Anytime	0.35 mL	NA

NA Not applicable (No Collection Scheduled)

Details on sampling, preparation, and shipment are included in the study laboratory manual.

Any of the collected PK plasma and CSF samples from the study patients may also be used for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with plasma and CSF constituents.

## Erratum

---

**Protocol Number:** ISIS 396443-CS4

**Protocol Title:** A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy

**Amendment:** 15 July 2016

---

In clinical protocol ISIS 396443-CS4 Amendment 2, Appendix A (Schedule of Procedures), presents in the table heading Day 456 visit as + 7D. Day 456 should have presented as  $\pm 7$  D (see [Appendix A](#) in this Erratum). This is an administrative error only.

□□□

15 July 2016

---

Date (DD Month YYYY)

□□□

---

Name (*please print*)

---

Title

**Appendix A Schedule of Procedures**

Subjects will also be monitored through phone contact on Study Days 8, 56, 113, 141, 204, 239, 302, 330, 393, 421 (all  $\pm 2$  days)<sup>9</sup>

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 ( ± 1D)			D30	D85 ( ± 2D)			D86	D92 ( ± 1D)	D169 ( ± 2D)	D274 ( ± 7D)			D275	D365 ( ± 7D) and D456 ( ± 7D) and Early Term/ EODBP
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
Informed Consent	X																			
Inclusion/Exclusion Criteria	X																			
Medical History	X																			
Screening X-ray	X																			
Urine Pregnancy Test <sup>11</sup>	X																			X <sup>14</sup>
SMN2 copy Number <sup>12</sup>	X																			
Study Drug Injection/Sham Procedure			X				X <sup>7</sup>				X <sup>7</sup>						X <sup>7</sup>			
In-Patient Stay (24 hours)				X																
Vital Signs <sup>1</sup>	X	X		4X <sup>2</sup>	X <sup>3</sup>	X		4X <sup>2</sup>	X	X		4X <sup>2</sup>	X	X	X	X		4X <sup>2</sup>	X	X
Weight	X	X				X				X				X	X	X				X
Height/ulnar length	X																			X <sup>14</sup>
Physical Examination	X	X				X				X				X	X	X				X
Neurological Examination	X	X		X <sup>4</sup>	X <sup>3</sup>	X		X <sup>4</sup>	X	X		X <sup>4</sup>	X	X	X	X		X <sup>4</sup>	X	X
ECG	X				X			X						X						X <sup>14</sup>
Safety Labs <sup>5</sup>	X	X			X	X				X					X	X				X <sup>14</sup>
Coagulation Labs	X																			
Immunogenicity		X				X				X					X	X				X <sup>14</sup>

**Appendix A Schedule of Procedures *Continued***

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 ( ± 1D)			D30	D85 ( ± 2D)			D86	D92 ( ± 1D)	D169 ( ± 2D)	D274 ( ± 7D)			D275	D365 ( ± 7D) and D456 ( ± 7D) and Early Term/ EODBP
		Pre-dose	LP/ SP	Post-dosD ay 456e		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
CSF PK <sup>8</sup>		X				X				X						X				
CSF SMN Protein		X				X				X						X				
Plasma PK <sup>8</sup>		X		3X	X	X				X		X			X	X				X <sup>14</sup>
HFMSE	X <sup>10</sup>													X	X	X <sup>13</sup>				X
WHO Motor Milestones	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
Upper Limb	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
PedsQL	X <sup>6</sup>													X	X	X <sup>13</sup>				X
ACEND	X <sup>6</sup>														X					X <sup>14</sup>
CGI														X	X	X <sup>13</sup>				X
Con Med Recording <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Collection <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 Resting blood pressure, pulse, respiratory rate, and temperature

2 Vital signs performed 1, 2, 4, 6 hours after dosing

3 Conducted within 20-24 hours after dosing

4 Neurological exams at 5 hours after dosing

5 Serum chemistry, hematology, urinalysis panels (Appendix B for analytes)

6 Efficacy assessments (with the exception of HFMSE) do not form part of the screening assessment or inclusion/exclusion criteria but are baseline measurements taken during the screening phase of the study

7 Overnight stay is optional on Day 29, Day 85, and Day 274

8 Refer to Appendix C for PK sampling schedule

9 At telephone contact, changes in concomitant medications and adverse events will be recorded

10 To be performed 2 times during the screening period

11 Urine pregnancy test performed for females of child-bearing potential, if positive to be confirmed by local serum test

12 Only for those subjects who do not have documented evidence of SMN copy number from Athena Diagnostics

13 These assessments may be performed up to 7 days prior to dosing, if necessary

14 Assessed on Day 456 only



**ISIS PHARMACEUTICALS, INC.**

**ISIS 396443-CS4**

**A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled  
Study to Assess the Clinical Efficacy and Safety of ISIS 396443  
Administered Intrathecally in Patients with Later-onset Spinal  
Muscular Atrophy**

**Amendment 1 - 26 September 2014**

**EudraCT No: 2014-001947-18**

**Sponsor:**

Isis Pharmaceuticals, Inc.

2855 Gazelle Court

Carlsbad, CA 92010

## **ISIS 396443-CS4**

### **A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy**

#### **Protocol Amendment 1 – 26 September 2014**

#### **Protocol History:**

Original Protocol: 17 July 2014

#### **Sponsor:**

Isis Pharmaceuticals, Inc.  
2855 Gazelle Court  
Carlsbad, CA 92010

□□□



## **ISIS 396443**

**Isis Protocol Number ISIS 396443-CS4**

**Protocol Amendment 1**

**Clinical Phase: 3**

### **A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy**

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**Date:**

26 September 2014

#### **Confidentiality Statement**

This document contains confidential information of Isis Pharmaceuticals, Inc. that must not be disclosed to anyone other than the recipient study staff and members of the institutional review board or authorized regulatory agencies. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Isis Pharmaceuticals, Inc.

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## Protocol Signature Page

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**Protocol Number:** ISIS 396443-CS4

**Protocol Title:** A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy

**Amendment:** 1

**Date:** 26 September 2014

---

I hereby acknowledge that I have read and understand the attached clinical protocol, entitled “A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy”, dated 26 September 2014, and agree to conduct the study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice (E6).

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Isis Pharmaceuticals, Inc.

---

Investigator's Signature

---

Investigator's Name (*please print*)

---

Date (DD Month YYYY)

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## PROTOCOL AMENDMENT

**Protocol Number:** ISIS 396443-CS4

**Protocol Title:** A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy

**Amendment:** 1

**Amendment Date:** 26 September 2014

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### **Summary of Modifications:**

The purpose of this amendment is to revise the statistical method utilized to perform the interim analysis. A typographical error in the protocol has also been fixed.

The following table provides a summary list of changes to the protocol:

Section	Description of Change
10.5 Interim Analysis	The statistical method utilized to perform the interim analysis has been revised.

## PROTOCOL SYNOPSIS

<b>Protocol Title</b>	A Phase 3, Randomized, Double-Blind, Sham Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy
<b>Study Phase</b>	3
<b>Indication</b>	Later-onset Spinal Muscular Atrophy (SMA)
<b>Objectives</b>	<p><b>Primary Objective:</b></p> <p>To examine the efficacy of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p> <p><b>Secondary Objective:</b></p> <p>To examine the safety and tolerability of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p> <p><b>Tertiary Objective:</b></p> <p>To examine the CSF and plasma pharmacokinetics of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p>
<b>Number of Subjects</b>	Approximately 117 subjects will be enrolled into this study
<b>Treatment Groups</b>	12 mg ISIS 396443 or sham procedure control group
<b>Study Design</b>	<p>This randomized, double-blind, sham-procedure controlled study will test the clinical efficacy, safety, tolerability, and pharmacokinetics of intrathecal ISIS 396443 over 15 months. Approximately 117 subjects will be randomized in a 2:1 ratio (78 ISIS 396443: 39 control) to receive ISIS 396443 by intrathecal lumbar puncture (LP) injection or to a sham-procedure control. A dose of 12 mg ISIS 396443 will be given at each of 4 times over the 15 months (i.e., on Study Days 1, 29, 85, and 274).</p> <p>Randomization will be stratified based on:</p> <p>1) Age (&lt; 6 years versus ≥ 6 years at Screening)</p> <p>After Informed consent/assent is obtained, subjects will undergo a screening evaluation no greater than 28 days prior to first dose administration at which time their eligibility for the study will be examined. Subjects who meet the eligibility criteria will be admitted to the study center on Study Day 1, undergo pre-dose evaluations, and then receive either an LP injection of study drug (ISIS 396443) or a sham procedure. Subjects will return to the study center on Days 29, 85 and 274 for follow-up evaluations and subsequent injections/sham procedures. Following the injection/sham procedure on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring. Following the injection/sham procedure on Day 29, 85, and 274 subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. Safety monitoring visits will occur on Days 30, 86, 92, 169, 275, 365, and 456 (through 6 months following the last injection/sham procedure). In addition, the study center will monitor the subject's condition through telephone contact monthly throughout the study. For subjects receiving ISIS 396443, a CSF sample will be taken pre-dose on each injection day in a manner that maintains the blind.</p> <p>Following treatment and the final follow-up evaluation, all eligible subjects may elect to enroll in an open-label treatment extension study. This will be done without unblinding to subject's treatment group.</p> <p>If a subject terminates early from the study, they will be encouraged to complete safety assessments per the Day 456 visit. If a subject is randomized but does not complete at least their first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.</p>

## Protocol Synopsis *Continued*

<p><b>Study Population and Main Criteria for Inclusion/ Exclusion</b></p>	<p><b><u>Inclusion Criteria:</u></b> Subjects must meet all of the following criteria at Screening to be eligible:</p> <ol style="list-style-type: none"> <li>1. Signed informed consent of parent or guardian. Signed informed assent of subject, if indicated per subject's age and institutional guidelines</li> <li>2. Genetic documentation of 5q SMA (homozygous gene deletion, mutation, or compound heterozygote)</li> <li>3. Onset of clinical signs and symptoms consistent with SMA at &gt; 6 months of age</li> <li>4. Males and females 2 to 12 years of age</li> <li>5. Can sit independently, but has never had the ability to walk independently</li> <li>6. Motor Function Score (Hammersmith Functional Motor Scale – Expanded) <math>\geq 10</math> and <math>\leq 54</math> at Screening</li> <li>7. Able to complete all study procedures, measurements and visits and parent or guardian/subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator</li> <li>8. Estimated life expectancy &gt; 2 years from Screening, in the opinion of the Investigator</li> <li>9. Meets age-appropriate institutional criteria for use of anesthesia/sedation, if use is planned for study procedures (as assessed by the Site Investigator and either anesthesiologist or pulmonologist)</li> <li>10. For subjects who, in the opinion of the Investigator, have reached reproductive maturity, satisfy one of the following:   <div style="margin-left: 40px;"> <p>Females: have a negative pregnancy test at Screening and agrees to employ adequate contraceptive measures for the duration of the study. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products.</p> <p>Males: be abstinent for the duration of the study</p> </div> </li> </ol> <p><b><u>Exclusion Criteria:</u></b> Subjects meeting any of the following criteria are not eligible for the study:</p> <ol style="list-style-type: none"> <li>1. Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for &gt; 6 hours during a 24 hour period, at Screening</li> <li>2. Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator</li> <li>3. Severe contractures or severe scoliosis evident on X-ray examination at Screening</li> <li>4. Hospitalization for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of Screening or planned during the duration of the study</li> <li>5. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period</li> <li>6. History of brain or spinal cord disease, including tumors, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation</li> <li>7. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter</li> <li>8. History of bacterial meningitis</li> <li>9. Dosing with ISIS 396443 in any previous clinical study</li> <li>10. Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline</li> </ol>
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**Protocol Synopsis *Continued***

<b>Study Population and Main Criteria for Inclusion/ Exclusion <i>Continued</i></b>	<p><b><u>Exclusion Criteria <i>Continued</i>:</u></b></p> <ol style="list-style-type: none"> <li>11. Clinically significant abnormalities in hematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion</li> <li>12. Treatment with another investigational drug (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, etc.), biological agent, or device within 1-month of Screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation</li> <li>13. Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anemia, etc.) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures</li> </ol>
<b>Study Drug and Administration</b>	<p>ISIS 396443 (2.4 mg/mL) will be administered as an intrathecal LP injection. The volume of the injection will be 5.0 mL. Details regarding the LP dosing injection procedure and the sham procedure will be provided in the Dosing Administration Manual. Details regarding the study drug will be provided in the Study Drug Manual. Per institutional guidelines, anesthesia/sedation may be used for the LP procedure.</p>
<b>Criteria for Evaluation</b>	<p><b><u>Primary Endpoint:</u></b></p> <ul style="list-style-type: none"> <li>• Change from baseline in HFMSE (Hammersmith Functional Motor Scale – Expanded) score at 15 months</li> </ul> <p><b><u>Secondary Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• Proportion of subjects who achieve a 3-point increase from baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months</li> <li>• Proportion of subjects that achieve any new motor milestone at 15 months</li> <li>• Number of motor milestones achieved per subject at 15 months</li> <li>• Change from baseline in Upper Limb Module Test at 15 months</li> <li>• Proportion of subjects that achieve standing alone at 15 months</li> <li>• Proportion of subjects that achieve walking with assistance at 15 months</li> </ul> <p><b><u>Tertiary Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• Change from baseline in CSF SMN protein concentration</li> <li>• Clinical Global Impression of Change (Investigator and Caregiver assessment)</li> <li>• Change from baseline in PedsQL (Pediatric Quality of Life Inventory)</li> <li>• Change from baseline in Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)</li> <li>• Disease-related hospitalizations and adverse events</li> </ul> <p><b><u>Safety/Tolerability Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• Adverse events</li> <li>• Vital signs and weight</li> <li>• Neurological examinations</li> <li>• Physical examinations</li> <li>• Clinical laboratory tests</li> <li>• ECGs</li> <li>• Use of concomitant medications</li> </ul>

**Protocol Synopsis *Continued***

<b>Criteria for Evaluation <i>Continued</i></b>	<b><u>Pharmacokinetic Endpoints:</u></b> <ul style="list-style-type: none"><li>• CSF levels of ISIS 396443 (sample taken pre-dose at each dosing)</li><li>• Plasma levels of ISIS 396443</li></ul> <b><u>Immunogenicity Endpoint:</u></b> <ul style="list-style-type: none"><li>• Plasma antibodies to ISIS 396443</li></ul>
<b>Safety Monitoring</b>	Safety data will be reviewed on an ongoing basis by the Medical Monitor and by an Independent Data Safety and Monitoring Committee (DSMB).
<b>Statistical Considerations</b>	The sample size for this study was estimated based on limited available natural history data for the target population and from the ISIS 396443-CS1 and CS2 clinical studies in children with SMA. 70 subjects in the treated group and 35 subjects in the control group will give at least 90% power to detect a 3 point difference between control and treated groups in change on the HFMSE with a standard deviation of 4.4, using a two-sided t-test with an alpha level of 0.05. The sample size was obtained using the n-Query. 117 subjects will be enrolled to ensure that a small dropout rate will not affect the power of the primary efficacy analysis. An interim analysis may take place when all subjects have completed the 6 month assessment and at least 39 subjects have completed the 15 month assessment.
<b>Sponsor</b>	Isis Pharmaceuticals, Inc.

## STUDY GLOSSARY

<b><u>Abbreviation/Acronym</u></b>	<b><u>Definition</u></b>
ACEND	Assessment of Caregiver Experience with Neuromuscular Disease
AE	Adverse event/experience
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CGI	Clinical Global Impressions of Change Rating Scale
C <sub>max</sub>	Maximum concentration
CNS	Central nervous system
CPK	Creatinine phosphokinase
CRF	Case report form
CSF	Cerebrospinal fluid
DSMB	Data and Safety Monitoring Board
eCRF	Electronic case report form
ECG	Electrocardiogram
FL	Full-length
GCP	Good Clinical Practice
HFMSSE	Hammersmith Functional Motor Scale - Expanded
hnRNP	Heterogeneous nuclear ribonucleoproteins
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
IT	Intrathecal
IXRS	Interactive Voice/Web-Response System
LP	Lumbar puncture
MAD	Multiple ascending-dose
MedDRA	Medical Dictionary for Regulatory Activities
miRNA	Micro ribonucleic acid (RNA)

MOE	2'- <i>O</i> -(2-methoxyethyl)
mRNA	Messenger ribonucleic acid
NCS	Not clinically significant
OLE	Open-label extension study
PedsQL	Pediatric Quality of Life Inventory
PK	Pharmacokinetic(s)
PT	Prothrombin Time
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SMA	Spinal muscular atrophy
SMN	Survival motor neuron
snRNA	Small nuclear ribonucleic acid
SUSARs	Suspected unexpected serious adverse reactions
T <sub>max</sub>	Time to maximal concentration
WMA	World Medical Association

## 1. OBJECTIVES

The objectives of this study are to evaluate the clinical efficacy, safety, tolerability, and pharmacokinetics (PK) of ISIS 396443 administered intrathecally to patients with later-onset Spinal Muscular Atrophy (SMA).

### 1.1 Primary Objective

To examine the clinical efficacy of ISIS 396443 administered intrathecally to patients with later-onset SMA.

### 1.2 Secondary Objective

To examine the safety and tolerability of ISIS 396443 administered intrathecally to patients with later-onset SMA.

### 1.3 Tertiary Objective

To examine the cerebral spinal fluid and plasma PK of ISIS 396443 administered intrathecally to patients with later-onset SMA.

## 2. BACKGROUND AND RATIONALE

### 2.1 Spinal Muscular Atrophy

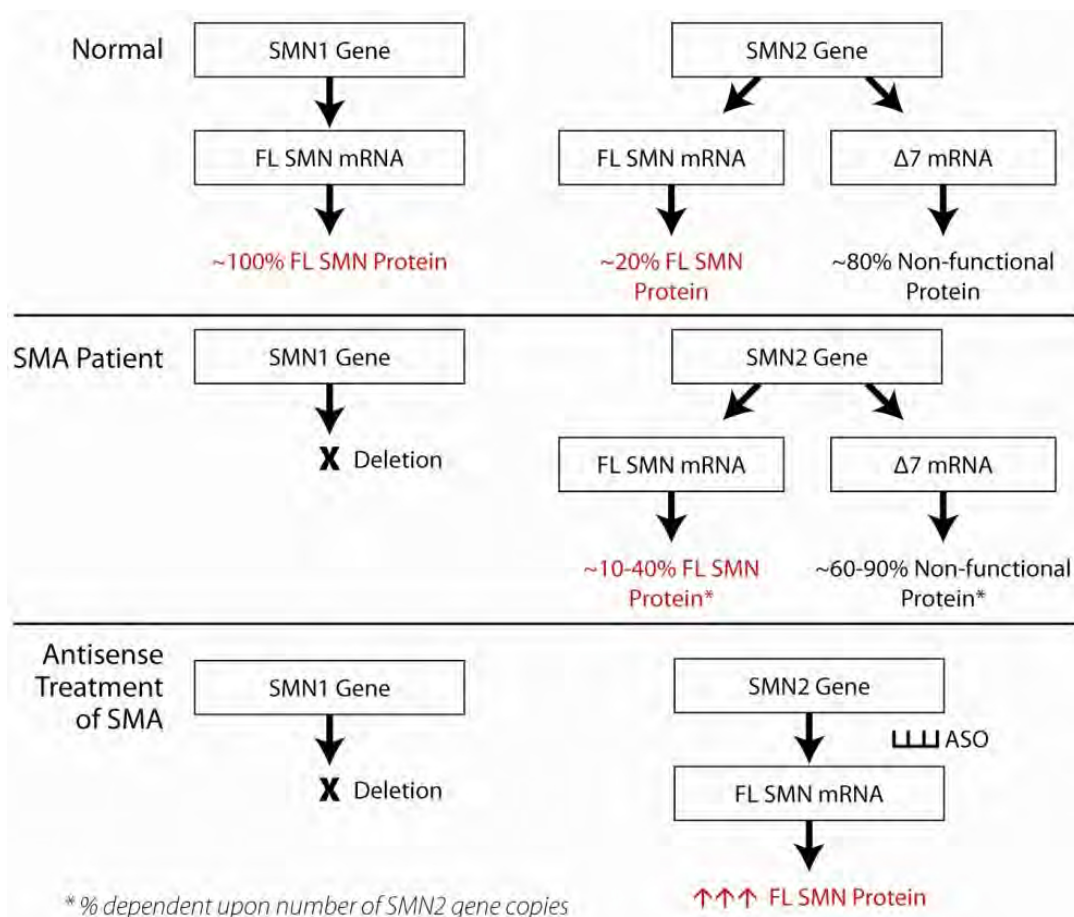
SMA is an autosomal recessive neuromuscular disease characterized by degeneration of the motor neurons in the anterior horn of the spinal cord, resulting in atrophy of the voluntary muscles of the limbs and trunk. With an incidence of 1:6000 to 1:10,000 live births, it is the most common genetic cause of infant mortality, and a major cause of childhood morbidity due to weakness, in the U.S. The natural history of SMA includes four major phenotypes that are recognized dependent on age of onset and achieved motor abilities. The most severe form, Type 1 SMA (equivalent to infantile-onset SMA), has a disease onset within the first few months of life; these children are never able to sit or walk and usually die from respiratory failure by the age of 2 years. Later-onset SMA can generally be divided into Type 2 and Type 3 SMA. Type 2 SMA patients are able to sit but never walk unaided, with symptoms presenting between 6-18 months of age. Type 3 SMA patients are able to sit and walk but individuals with this form may become severely and increasingly disabled. Adult-onset SMA patients (Type 4) have an age of onset over 18 years of age and have normal life expectancies.

In 95% of SMA patients, a deletion in the *SMN1* gene on Chromosome 5q11-q13 is found; with the remaining 5% attributable to small mutations in the same gene ([Lefebvre et al. 1995](#); [Helmken et al. 2003](#)). *SMN1* lies in the telomeric portion of an inverted duplication of a region of Chromosome 5. The centromeric half of the duplication contains a homologous gene, named *SMN2* that differs from *SMN1* by 11 nucleotides. The open reading frames for both genes encode for proteins with identical amino acid sequences. Survival motor neuron (SMN) gene transcripts, similar to most mammalian transcripts, undergo alternative splicing in which certain exons are either included or excluded from the mature protein coding transcripts ([Keren et al. 2010](#)). In particular, Exon 7 of the *SMN1* gene is alternatively spliced with 90 to 95% of the mature messenger ribonucleic acid (mRNA) transcripts derived from the *SMN1* gene containing Exon 7, and 5 to 10% of transcripts missing Exon 7. The transcripts missing Exon 7 (often referred to as  $\Delta 7$ ) produce a truncated protein which is defective and unstable

(Choi and Dreyfuss 2010). One of the 11 nucleotide differences between *SMN1* and *SMN2*, a C to T substitution occurs in Exon 7 of the *SMN2* gene resulting in an alternative splicing pattern that favors skipping of Exon 7. The result is that as much as 90% of the transcripts produced from *SMN2* are missing Exon 7. The remainder, *SMN2* transcripts containing Exon 7, produces a full-length (FL) protein product identical to the *SMN1* protein, since the C to T substitution is silent. Humans have a variable copy number of the *SMN2* gene (0-8 copies). The number of *SMN2* copies and the resulting amount of FL-SMN protein expressed in SMA patients (10-40% of normal SMN protein levels) correlates with SMA disease severity and thus *SMN2* is a key modifier of disease phenotype (Covert et al. 1997; Feldkotter et al. 2002; Lefebvre et al. 1997; Prior et al. 2004).

## 2.2 Therapeutic Rationale

Since the number of *SMN2* gene copies and resulting amount of SMN protein is correlated with disease onset and severity, a therapeutic approach predicted to benefit SMA patients is to increase the levels of full length *SMN2* pre-mRNA by restoring the splicing pattern that gives rise to full length *SMN2* mRNA. Increasing inclusion of Exon 7 in the *SMN2* transcript will increase FL-SMN protein levels and SMN protein activity. A therapeutic strategy for promoting Exon 7 inclusion is through the use of antisense oligonucleotides (ASOs) (see Figure 1).



**Figure 1 ASO Therapeutic Approach for Treatment of SMA**

The known potential risks associated with ISIS 396443 are detailed in the Guidance to Investigator section of the Investigator's Brochure. Additional study associated risks related to the lumbar puncture (LP) procedure are also detailed in the Guidance to Investigator section of the Investigator's Brochure.

## 2.3 ISIS 396443

### 2.3.1 Mechanism of Action

ISIS 396443 is a fully modified, 2'-O-2-methoxyethyl (MOE), ASO drug designed to bind to a specific sequence in the intron downstream of Exon 7 of the SMN2 transcript. The region of the pre-mRNA targeted by ISIS 396443 is normally occupied by heterogeneous nuclear ribonucleoproteins (hnRNP) A1/2 proteins, masking the U1 small nuclear ribonucleic acid (snRNA) binding site at the 5'-exon-intron junction of Exon 7, and is referred to as ISS-N1. U1 snRNA base pairs to the sequences that define the 5'-splice site, which is thought to be one of the first steps that initiate splicing of an intron. ISIS 396443 displaces the hnRNP A1/2 proteins from the pre-mRNA binding site, allowing U1 snRNA to bind to the exon-intron junction and promote assembly of the spliceosomal complex, thus promoting inclusion of Exon 7 into the mRNA which results in production of FL-SMN protein.

### 2.3.2 Chemistry

Chemically, ISIS 396443 is a synthetic oligomer of 18 nucleotides (i.e., an 18-mer) that are connected sequentially by phosphorothioate linkages. Each of the 17 internucleotide linkages is a 3'-O to 5'-O phosphorothioate diester. The 18 sugar residues are uniformly modified with 2'-O-(2-methoxyethyl) (MOE). These MOE-modified nucleotides confer (1) increased affinity to the target mRNA (McKay et al. 1999), (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue) (Geary et al. 2003), and (3) amelioration of some of the high dose toxicities associated with ASO containing only the phosphorothioate linkages (Henry et al. 2000).

The sequence of ISIS 396443 is written as follows:



Where A and G are 2'-O-(2-methoxyethyl)nucleosides, <sup>Me</sup>C is 5-methyl-2'-O-(2-methoxyethyl)cytidine and <sup>Me</sup>U designates 5-methyl-2'-O-(2-methoxyethyl)uridine.

### 2.3.3 Preclinical Experience

Detailed information concerning the preclinical studies conducted with ISIS 396443 can be found in the Investigator's Brochure. A summary is included below.

ISIS 396443 was identified after an extensive screen of greater than 500 2'-MOE oligonucleotides in *in vitro* splicing assays, reporter gene assays and in SMA patient fibroblasts (Hua et al. 2007; Hua et al. 2008). Data have shown that ISIS 396443 promotes a concentration-dependent increase in full-length transcripts (including Exon 7) in patient fibroblast cells, achieving greater than 90% full length SMN2 transcripts and forms nuclear structures, called gems, known to contain SMN protein. In a mild mouse model of SMA, ISIS 396443 promoted inclusion of Exon 7 in the SMN2 transgene in a variety of peripheral tissues when dosed systemically (Hua et al. 2008) and in central nervous system (CNS) tissue, including spinal cord,

when injected into the lateral ventricle. ISIS 396443 produced greater than 90% Exon 7 inclusion in the transgenic mice and increased SMN protein production in motor neurons, resulting in the appearance of gems in motor neurons. These studies were extended to a more severe mouse model of SMA (SMA  $\Delta 7$ ) (Le et al. 2005), where the CNS delivery of drug produced a dose-dependent effect on SMN2 Exon 7 inclusion, SMN protein production, and survival. These mice treated with ISIS 396443 demonstrated improved weight gain, improvements in muscle morphology, muscle strength, and motor coordination and improved morphology of the motor neuron junctions (Passini et al. 2011). Further, ISIS 396443 was shown to distribute widely in the CNS following intrathecal (IT) administration in monkey (Passini et al. 2011).

The pharmacokinetics and toxicity of ISIS 396443 were assessed following: 1) single intrathecal (IT) lumbar bolus injections (1 to 7 mg) in adult monkeys 2) following 14 weeks (with a 4-week interim sacrifice) of repeated IT lumbar bolus injections (0.3 to 3 mg/week or every other week) in juvenile monkeys and 3) following 53 weeks of repeated IT lumbar bolus injections in juvenile monkeys. In addition, a dedicated pharmacokinetic study in adult monkeys was performed to assess the half-life of ISIS 396443 in CSF, tissues and plasma. Detailed results from these preclinical studies conducted with ISIS 396443 can be found in the ISIS 396443 Investigator's Brochure.

#### **2.3.4 Clinical Experience**

Detailed information concerning the clinical studies conducted with ISIS 396443 can be found in the Investigator's Brochure. A summary is included below.

ISIS 396443 has been evaluated in a completed open-label, single ascending-dose (SAD) Phase 1 study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with SMA (ISIS 396443-CS1). A single-dose of ISIS 396443 was administered by IT injection to SMA patients aged 2 to 14 years of age. Four dose levels (1, 3, 6, and 9 mg) were evaluated sequentially. Each dose level was studied in a cohort of 6 or 10 subjects, where all subjects received drug. In this study all subjects completed dosing and the follow-up visits per protocol. Overall, ISIS 396443 was well-tolerated and no safety concerns were identified up to the 9.0 mg dose level, given as a single IT injection. No serious adverse events (SAEs) or dose-limiting toxicities (DLTs) were reported in ISIS 396443-CS1. Adverse events (AEs) reported were mild or moderate in severity and there was no relationship with ISIS 396443 dose level. In addition, no ISIS 396443 related adverse changes in neurological exams were reported, despite intensive monitoring during the immediate post-dosing period. CSF and plasma drug concentrations observed were generally consistent with predictions made from nonclinical monkey studies.

ISIS 396443 is also being evaluated in four ongoing studies: ISIS 396443-CS2, ISIS 396443-CS10, ISIS 396443-CS12, and ISIS 396443-CS3A. ISIS 396443-CS2 is an open-label, multiple ascending-dose (MAD) Phase 1/2a study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with SMA. Multiple doses of ISIS 396443, ranging from 3 mg to 12 mg, are being administered by intrathecal injection to SMA patients aged 2 to 15 years of age. ISIS 396443-CS10 is an open-label, single dose, re-dosing study for SMA patients who previously participated in Cohorts 2, 3 and 4 in ISIS 396443-CS1. ISIS 396443-CS12 is an ongoing open-label study to assess the safety and tolerability of a single (12 mg) intrathecal dose of ISIS 396443 in patients with spinal muscular atrophy who previously participated in ISIS 396443-CS2 or ISIS 396443-CS10.

ISIS 396443 CS3A is an open-label, multiple-dose study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with infantile-onset SMA. Multiple doses of ISIS 396443 are being administered by intrathecal injection to symptomatic SMA infants  $\leq 7$  months of age. Two dose levels (6 and 12 mg dose equivalent scaled by CSF volume) are being evaluated sequentially.

## **2.4 Rationale for Dose and Schedule of Administration**

The proposed study will test the clinical efficacy, safety, tolerability, and PK of multiple doses of ISIS 396443 administered as IT injections to subjects with later-onset SMA. A single dose level of 12 mg ISIS 396443 will be evaluated, delivered as 4 doses administered over 9 months. ISIS 396443 will be administered using a loading regimen (dosings on Study Days 1, 29, 85) followed by maintenance dosing given 6 months thereafter (dosing on Study Day 274).

The ISIS 396443-CS4 dose level and dose interval was selected based on preclinical toxicology and pharmacokinetic observations from monkey studies utilizing single-dose and repeat dosing IT administration, consideration of the target tissue concentration anticipated for drug pharmacology, and safety data in the completed and ongoing clinical studies of ISIS 396443 to date. Based upon pharmacology and pharmacokinetic results in SMA transgenic mice, we estimate that the target tissue concentration to produce 50 to 90% SMN2 Exon 7 inclusion is between 1 and 10  $\mu\text{g/g}$  spinal cord tissue. Nonclinical studies in juvenile monkeys receiving IT doses of ISIS 396443 showed a resulting gradient of distribution of ISIS 396443 along the spinal cord, with mean lumbar spinal cord concentrations ranging from 1.6-2.3 fold and 2.0-3.5 fold higher than thoracic and cervical spinal cord levels, respectively. The dose level selected for this multiple-dose clinical study (12 mg ISIS 396443) is predicted to achieve levels at the high end of this range (approximately 10  $\mu\text{g/g}$  lumbar and 3  $\mu\text{g/g}$  cervical spinal cord tissue concentrations), following the first dose. The loading dose interval was selected based on the nonclinical pharmacokinetic and pharmacology data as the dose interval to achieve and maintain ISIS 396443 spinal cord tissue levels that are predicted to be within the upper end of the pharmacologically active range by Day 85 (predicted to be approximately 24  $\mu\text{g/g}$  lumbar and 8  $\mu\text{g/g}$  cervical tissue concentration), while at the same time considering subject safety and convenience for repeated LP intrathecal injections. The maintenance dose interval (once every 6 months) was selected based on the estimated spinal tissue and CSF drug half-life (4-6 months) and was selected to maintain spinal cord tissue levels of ISIS 396443 at a steady-state level within the estimated pharmacologically active range.

Additional details on dose scaling and expected CSF and tissue concentrations are summarized in the Investigator's Brochure.

## **3. EXPERIMENTAL PLAN**

### **3.1 Study Design**

This is a Phase 3 multicenter, double-blind, randomized, sham-procedure controlled study of ISIS 396443 in patients with later-onset SMA studied for 15 months. Approximately 117 subjects will be randomized 2:1 to receive 12 mg dose ISIS 396443 or a sham procedure control, respectively. ISIS 396443 will be administered using a loading regime (dosings on Study Days 1, 29, and 85) followed by a maintenance dose 6 months thereafter (dosing on Study Day 274).

Randomization will be stratified based on:

- 1) Subject's Age at Screening ( $< 6$  years versus  $\geq 6$  years)

Following treatment and the Day 456 follow-up evaluation, all eligible subjects may elect to enroll in an open-label treatment extension study, pending study approval by the IRB or IEC and the appropriate regulatory authority. This will be done without unblinding to subject's treatment group.

### **3.2 Number of Study Centers**

This study will be conducted at multiple centers worldwide.

### **3.3 Number of Subjects**

Approximately 117 subjects (2 ISIS 396443; 1 sham-procedure) will receive 12 mg dose of ISIS 396443 or a sham procedure control. The total number of subjects randomized may be higher if some subjects do not receive their Day 1 dose/sham procedure. The maximum number of subjects will not exceed 130.

### **3.4 Overall Study Duration and Follow-up**

The Study will consist of screening, treatment, and post-treatment follow-up-periods. The total duration of participation in the study is approximately 16 months. Please refer to the Schedule of Procedures in [Appendix A](#).

#### **3.4.1 Screening**

After informed consent/assent is obtained, subjects will undergo a screening evaluation no greater than 28 days prior to first dose administration at which their eligibility for the study will be examined.

#### **3.4.2 Treatment**

Subjects who meet the eligibility criteria will be admitted to the study center on Study Day 1, undergo pre-dose evaluations, be randomized, and then receive either an LP injection of study drug (ISIS 396443) or a sham procedure. Subjects will return to the study center on Days 29, 85 and 274 for follow-up evaluations and subsequent injections/sham procedures. Following the injection/sham procedure on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring. Following the injection/sham procedure on Day 29, 85, and 274 subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. Safety monitoring visits will occur Study Days 30, 86, 92, 169, and 275 (through the last injection/sham procedure). In addition, the study center will monitor the subject's condition through telephone contact on a monthly basis.

For subjects receiving ISIS 396443, a CSF sample for PK and SMN protein analyses will be taken pre-dose on each injection day in a manner that protects the blind.

If a subject terminates early from the study, they will be encouraged to complete assessments per the Day 456 visit. If a subject is randomized but does not successfully receive the first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.

### **3.4.3 Post-Treatment Follow-up**

After completion of the Day 275 visit, subjects will enter the 6-month post-treatment evaluation period. This period consists of Study Center visits on Day 365 and 456 and follow-up phone assessments on a monthly basis, as outlined in the Schedule of Procedures ([Appendix A](#)). After completion of the Day 456 visit, subjects may be eligible to participate in an OLE study, pending study approval by the IRB or IEC and the appropriate regulatory authority.

### **3.5 End of Study**

The end of study is last subject; last visit (either in-person visit or telephone contact).

### **3.6 Safety Monitoring and Data Safety Monitoring Board**

Safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor. Safety data will also be reviewed on an ongoing basis by an independent DSMB. The DSMB will be assembled to review safety, tolerability and efficacy (as needed) data collected on ISIS 396443 during this study. Based on its ongoing assessment of the data, the DSMB will provide recommendations to the Sponsor for modifying, stopping or continuing the study.

## **4. SUBJECT ENROLLMENT**

### **4.1 Screening**

Before subjects may be enrolled into the study, the Sponsor requires a copy of the Study Center's written IRB or IEC approval of the protocol, informed consent form, informed assent form (if applicable) and all other subject information and/or recruitment material.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent(s) or legal guardian(s) and, in cases where institutional guidelines and the patient's age dictate, informed assent from the subject. At the time of consent/assent, the subject will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including screening procedures, are performed. The screening number must remain constant throughout the entire trial. In the event the subject is re-consented and re-screened the subject must be given a new screening number. Screening numbers, once assigned, will not be re-used.

### **4.2 Randomization**

Subjects will be randomized after all screening assessments have been completed and after the Investigator and the Medical Monitor have verified that they are eligible per criteria in [Sections 5.1](#) and [5.2](#). No subject may begin treatment prior to randomization and assignment of a unique randomization number.

Using an Interactive Voice/Web-Response System (IXRS), eligible subjects will be randomized 2:1 to receive ISIS 396443 or sham-procedure control, respectively. Randomization will be stratified for:

- Subject's Age at Screening (< 6 years versus  $\geq$  6 years)

The Sponsor Quality Assurance department or designee will hold a copy of the randomization lists generated by the IXRS vendor.

### **4.3 Replacement of Subjects**

If a subject does not successfully receive the first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.

### **4.4 Unblinding of Treatment Assignment**

The Sponsor, parents, and key study site personnel will be blinded to subjects' treatment assignment throughout the study. The DSMB may be unblinded as described in the DSMB charter.

If a subject has experienced an SAE (as defined in [Section 9.3.3](#)), and/or when knowledge of the treatment assignment will impact the clinical management of the subject, the Investigator will have the ability to unblind the treatment assignment for that subject using the IXRS. However, prior to unblinding, the Investigator should attempt to contact the blinded Medical Monitor to discuss the emergency. The Sponsor or designee will be informed of the unblinding of a subject within 24 hours. Every reasonable attempt should be made to complete the Study Day 456 early termination procedures and observations (see [Appendix A](#)) prior to unblinding, as knowledge of the subject's treatment assignment could influence subsequent assessments. The Investigator must document the reasons for unblinding in the subject's source documents. The Investigator is strongly advised not to divulge the subject's treatment assignment to any individual not directly involved in managing the medical emergency, nor to personnel involved with the analysis and conduct of the study. In cases where there are ethical reasons to have a subject remain on study whose treatment assignment is unblinded for safety reasons, the Site Investigator must obtain specific approval from the Sponsor and the Medical Monitor for the subject to continue in the study.

In addition, all suspected unexpected serious adverse reactions (SUSARs) will be unblinded by the Sponsor's Drug Safety and Quality Assurance personnel for the purpose of regulatory reporting ([Section 9.2](#)).

## **5. SUBJECT ELIGIBILITY**

### **5.1 Inclusion Criteria**

Subjects must meet all of the following criteria at Screening to be eligible:

1. Signed informed consent of parent or guardian. Signed informed assent of subject, if indicated per subject's age and institutional guidelines
2. Genetic documentation of 5q SMA (homozygous gene deletion, mutation, or compound heterozygote)
3. Onset of clinical signs and symptoms consistent with SMA at > 6 months of age
4. Males and females 2 to 12 years of age
5. Can sit independently, but has never had the ability to walk independently
6. Motor Function Score (Hammersmith Functional Motor Scale – Expanded)  $\geq 10$  and  $\leq 54$  at Screening

7. Able to complete all study procedures, measurements and visits and parent or guardian/subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator
8. Estimated life expectancy > 2 years from screening, in the opinion of the Investigator
9. Meets age-appropriate institutional criteria for use of anesthesia/sedation, if use is planned for study procedures (as assessed by the Site Investigator and either anesthesiologist or pulmonologist)
10. For subjects who, in the opinion of the Investigator, have reached reproductive maturity, satisfy one of the following:  
  
Females: have a negative pregnancy test at Screening and agrees to employ adequate contraceptive measures for the duration of the study. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products.  
  
Males: be abstinent for the duration of the study

## 5.2 Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for the study:

1. Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for > 6 hours during a 24 hour period, at Screening
2. Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator
3. Severe contractures or severe scoliosis evident on X-ray examination at Screening
4. Hospitalization for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of screening or planned during the duration of the study
5. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period
6. History of brain or spinal cord disease, including tumors, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation
7. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter
8. History of bacterial meningitis
9. Dosing with ISIS 396443 in any previous clinical study
10. Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline
11. Clinically significant abnormalities in hematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion

12. Treatment with another investigational drug (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, etc.), biological agent, or device within 1-month of screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation
13. Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anemia, etc.) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures

## 6. STUDY PROCEDURES

### 6.1 Study Schedule

All required study procedures are outlined in [Appendices A, B and C](#).

### 6.2 Study Assessments

#### 6.2.1 *Laboratory Analytes*

Laboratory measurements of serum chemistry, hematology, urinalysis, coagulation parameters, and plasma antibodies to ISIS 396443 will be performed at the times shown in the Schedule of Procedures ([Appendix A](#)). The analytes to be measured are shown in [Appendix B](#).

#### 6.2.2 *Neurological Examinations*

Neurological examinations include assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes. Neurological examinations will be performed at the times shown in the Schedule of Procedures ([Appendix A](#)).

#### 6.2.3 *Pharmacokinetics Specimen Collection*

Plasma and CSF specimens will be collected as shown in [Appendix A](#) (Schedule of Procedures) and [Appendix C](#) (Pharmacokinetic Sampling Schedule). The following ISIS 396443 plasma PK parameters (though not necessarily limited to) will be derived when appropriate from the individual subject concentration vs. time profiles using noncompartmental-based methods and based on actual sampling times:

- The maximal observed plasma drug concentration ( $C_{\max}$ )
- The time to reach  $C_{\max}$  in plasma ( $T_{\max}$ )
- The area under the plasma concentrations time curve from the time of the IT dose to the last collected sample
- The apparent terminal elimination half-life ( $t_{1/2}$ ), if possible

#### **6.2.4      *Hammersmith Functional Motor Scale - Expanded***

Subjects will be evaluated using the Hammersmith Functional Motor Scale – Expanded (HFMSE) at the times shown in the Schedule of Procedures ([Appendix A](#)). The HFMSE is a reliable and validated tool used to assess motor function in children with SMA. The scale was originally developed with 20 scored activities and was devised for use in children with SMA Type 2 and Type 3 with limited ambulation to give objective information on motor ability and clinical progression ([Main et al. 2003](#)). The expanded scale includes an additional module of 13 items developed to allow for evaluation of ambulatory SMA patients ([O’Hagen et al. 2007](#)). The HFMSE has been shown to be highly correlated with other clinical assessments and shows good test-retest reliability. The HFMSE is easy to use and quickly administered.

#### **6.2.5      *Motor Milestones***

Subjects will be evaluated for motor milestones at the times shown in the Schedule of Procedures ([Appendix A](#)). Motor Milestones will be assessed using the WHO Motor Milestone criteria ([WHO Multicentre Growth Reference Study Group 2006](#); [Wijnhoven et al. 2004](#)).

#### **6.2.6      *PedsQL™ (Generic Core Scales and Neuromuscular Module)***

Subjects will be evaluated using the Pediatric Quality of Life Inventory (PedsQL™) Measurement 4.0 Generic Core Scales and 3.0 Neuromuscular Module ([Varni et al. 1999](#)) at the times shown in the Schedule of Procedures ([Appendix A](#)). This assessment has been validated by the American Spinal Muscular Atrophy Randomized Trials (AmSMART) group for use in SMA patients from age 2 to 18 years ([Iannaccone et al. 2009](#)).

The PedsQL™ Measurement Model is a modular approach to measuring health-related quality of life (HRQOL) in children and adolescents. The PedsQL consists of brief, practical, generic core scales as well as with condition-specific modules for use in designated clinical populations. Pediatric self-report is measured in children and adolescents ages 5-18 years, and parent proxy-report of child HRQOL is measured for children and adolescents ages 2-18 years. The PedsQL™ 4.0 Generic Core Scales include assessment of physical functioning, emotional functioning, social functioning, and school functioning. The PedsQL 3.0 Neuromuscular Module was designed to measure HRQOL dimensions specific to children aged 2 to 18 years with neuromuscular disorders, including SMA.

#### **6.2.7      *Upper Limb Module Test***

Subjects will be evaluated using the Upper Limb Module Test ([Mazzone et al. 2011](#)) at the times shown in the Schedule of Procedures ([Appendix A](#)). The Upper Limb Module Test is an outcome measure specifically developed to assess upper limb functional abilities in SMA patients, including young children and patients with severe contractures in the lower limbs in whom the possibility to detect functional changes, such as rolling or long sitting, is limited. This test consists of upper limb performance items that are reflective of activities of daily living (i.e., raise a can to mouth as if drinking, take a coin and place it in a box, remove the lid of a container). The Upper Limb Module Test is quickly administered and has been evaluated in SMA patients age 30 months to 27 years ([Mazzone et al. 2011](#)).

### **6.2.8      *Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)***

Parents of subjects will complete the Assessment of Caregiver Experience with Neuromuscular Disease (ACEND) questionnaire at the times shown in the Schedule of Procedures ([Appendix A](#)). This assessment instrument has been designed to quantify the caregiver impact experienced by parents of children affected with severe neuromuscular diseases, including children with SMA ([Matsumoto et al. 2011](#)). The ACEND includes domains assessing physical impact (including feeding/grooming/dressing, sitting/play, transfers, and mobility) and general caregiver impact (including time, emotion, and finance).

### **6.2.9      *Safety Evaluations***

Safety will be evaluated by assessment of AEs including SAEs as described in [Section 9](#). Additional safety evaluations include the following parameters:

- Vital signs and weight
- Neurological examinations
- Physical examinations
- Clinical laboratory tests (serum chemistry, hematology, urinalysis)
- Electrocardiograms (ECGs)
- Use of concomitant medications

## **6.3      Contraception Requirements**

All male subjects must remain abstinent during the study.

All female subjects of childbearing potential must either be abstinent or practice adequate contraception during the study. For the purposes of this study, females of childbearing potential are defined as any female who has experienced menarche. For the purposes of the study, acceptable contraception methods are abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products

## **7.      STUDY DRUG**

### **7.1      Study Drug Description**

Study Drug (ISIS 396443 drug product) characteristics are listed under [Table 1](#).

The Study Drug is contained in 6 mL clear glass vials. The Study Drug and its storage and preparation instructions will be provided by the Sponsor or designee. The Study Drug must be stored securely at 2° to 8° C and protected from light.

**Table 1 Study Drug Characteristics**

Study Drug	ISIS 396443 Drug Product
Strength	2.4 mg/mL
Volume/vial	5.0 mL solution per vial
Route of Administration	IT injection

## 7.2 Packaging and Labeling

The Sponsor will provide the Investigator with packaged Study Drug labeled in accordance with specific country regulatory requirements.

## 7.3 Study Drug Accountability

The study staff is required to document receipt, dispensing and return of Study Drug supplies provided by the Sponsor. Drug accountability documentation and all used and unused Study Drug vials must be returned to the Sponsor or designee.

# 8. TREATMENT OF SUBJECTS

## 8.1 Study Drug Administration

Details regarding the LP dosing injection procedure will be provided in the Dosing Administration Manual. ISIS 396443 will be administered by dedicated study personnel who are unblinded to treatment group; this cannot be any of the key study site personnel (i.e., the Principal Investigator, study coordinator, or outcomes assessors). The study drug administration will be performed in a dedicated room and the key study personnel and the parents will not be present during the procedure to ensure blinding.

ISIS 396443 will be administered as an intrathecal slow bolus (1-3 minute) LP injection. ISIS 396443 will be administered using a ‘spinal anesthesia’ needle and syringe. A 22G to 25G spinal anesthesia needle is recommended, but a 21G may be used if indicated by subject size or clinical condition. The target site for needle insertion is the L3/L4 space, but may be 1 segment above or 1-2 segments below this level, if needed. The volume of the injection is 5 mL; prior to the injection 5 mL of CSF fluid is to be collected for analyses. Depending on institutional guidelines, anesthesia or sedation may be used for the LP procedure, following institutional procedures. Subjects will be encouraged to lie flat for 1 hour following dosing, if possible.

Table 2 outlines the dose, ISIS 396443 concentration, and volume for administration of ISIS 396443.

**Table 2 ISIS 396443 Dose, Concentration, and Injection Volume**

Dose (mg)	Concentration (mg/mL)	Injection Volume (mL)
12	2.4	5.0

Prior to each injection on Study Days 1, 29, 85, and 274, 5 mL of CSF fluid is to be collected for analyses. CSF will be used for measurement of ISIS 396443 pharmacokinetic analyses and CSF SMN protein concentration. Extra CSF may be stored for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with CSF constituents. CSF analyses and data presentation will be conducted in a blinded manner.

## **8.2 Sham Procedure**

Subjects randomized to the sham-procedure control group will undergo a sham-procedure, rather than study drug administration, on Study Days 1, 29, 85, and 274. Details regarding the sham procedure will be provided in the Dosing Administration Manual. The sham procedure will be administered by dedicated study personnel who are unblinded to treatment group; this cannot be any of the key study site personnel (i.e., the Investigator, study coordinator, or outcomes assessors). The sham procedure will be performed in a dedicated room and the key study personnel and the parents will not be present during the procedure to ensure blinding.

In general, the sham procedure will consist of a small needle prick on the lower back at the location where the LP injection is normally made. The needle will break the skin but no LP injection or needle insertion will occur. The needle prick will be covered with the same bandage that is used to cover the LP injection normally, thus simulating the appearance of an LP injection. If anesthesia or sedation is used for the LP procedure in ISIS 396443 treated subjects, then in order to maintain the blind, minimal sedation (i.e. a low dose of an anxiolytic) should be used for the sham procedure, following institutional procedures. The study subject will be kept in the procedure room for the same amount of time that subjects administered study drug are kept, thus simulating the time period of a study drug administration procedure.

Study drug and sham kits will be packaged in a blinded fashion. Blinded kits for sham procedure contain artificial CSF (5.0 mL solution per 6 mL vial) that will not be injected but will be used to simulate CSF samples for that subject.

## **8.3 Other Protocol-Required Drugs**

There are no other protocol required drugs.

## **8.4 Other Protocol-Required Procedures**

There are no other protocol-required treatment procedures.

## **8.5 Treatment Precautions**

There are no protocol-required treatment precautions.

## **8.6 Safety Monitoring Rules**

Please refer to the Guidance to Investigator section of the Investigator Brochure.

### **8.7 Stopping Rules**

There are no additional specific stopping rules for this study but the Investigator should discuss significant concerns relating to individual subjects with the Medical Monitor and the Sponsor to ensure that it is appropriate for the subject to continue Study Drug.

### **8.8 Adjustment of Dose and/or Treatment Schedule**

No adjustment of dose is permitted. In the event of a concurrent illness that would prevent the dosing procedure from being performed safely, an adjustment in the dose schedule may be permitted, but must be approved by the Medical Monitor. In general, each scheduled dose may be delayed by up to 4 weeks.

### **8.9 Discontinuation of Study Treatment**

A subject must permanently discontinue study treatment for any of the following:

- The subject's parents/guardians withdraw consent
- The subject experiences an adverse event that necessitates permanent discontinuation of study treatment

The reason for discontinuation of study treatment must be recorded in the Case Report Form (CRF) and source documentation.

Subjects that discontinue treatment will continue follow-up unless consent is withdrawn ([Appendix A](#)).

### **8.10 Withdrawal of Subjects from the Study**

Subjects must be withdrawn from the study for any of the following:

- Withdrawal of consent
- The subject or the subject's parents/guardians is/are unwilling or unable to comply with the protocol
- The subject experiences a medical emergency that necessitates unblinding of the subject's treatment assignment

Other reasons for withdrawal of subjects from the study might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation
- Decision by the Investigator or Sponsor

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from Study, must be recorded in the CRF.

Any subject for whom consent to participate in the study is withdrawn will be removed from further treatment and study observation immediately upon the date of request. It should be encouraged that these subjects complete the early termination study procedures and observations at the time of withdrawal ([Appendix A](#)).

For subjects withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination study procedures and observations at the time of withdrawal ([Appendix A](#)).

## **8.11 Concomitant Therapy and Procedures**

The use of concomitant therapies or procedures defined below must be recorded on the subject's CRF. AEs related to administration of these therapies or procedures must also be documented on the appropriate CRF.

### **8.11.1 Concomitant Therapy**

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications and vitamin supplements) administered between the beginning of screening and last telephone contact or study visit.

Subject's parents/guardians should consult with the Site Investigator or qualified designee prior to initiating any new medication, including non-prescription compounds or any other non-drug therapy.

#### **Allowed Concomitant Therapy**

Throughout the study, Site Investigators or designated licensed physicians involved in the study may prescribe concomitant medications or treatments deemed necessary for adverse events or to provide adequate supportive care.

#### **Disallowed Concomitant Therapy**

Study subjects are prohibited from receiving other experimental agents during the study. This includes marketed agents at experimental doses that are being tested for the treatment of SMA (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, valproate, hydroxyurea).

### **8.11.2 Concomitant Procedures**

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the beginning of screening and last telephone contact or study visit.

## **9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING**

### **9.1 Sponsor Review of Safety Information**

Safety information will be collected, reviewed, and evaluated by the Sponsor in accordance with the Safety Management Plan throughout the conduct of the clinical trial.

## 9.2 Regulatory Requirements

The Sponsor is responsible for regulatory submissions and reporting to the Investigators of SAEs including suspected unexpected serious adverse reactions (SUSARs) per the International Conference on Harmonization (ICH) guidelines E2A and ICH E6. Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

IRBs/IECs will be notified of any serious adverse event (SAE) according to applicable regulations. The DSMB will be notified of any SAE as specified in the DSMB charter.

## 9.3 Definitions

### 9.3.1 Adverse Event

An adverse event is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the study or use of investigational drug product, whether or not the AE is considered related to the investigational drug product.

### 9.3.2 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction is any adverse event caused by the Study Drug.

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

### 9.3.3 Serious Adverse Event (SAE)

A serious adverse event is any adverse event that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event

An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death

- Requires inpatient hospitalization or prolongation of existing hospitalization

Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE

- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female)

- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

#### **9.4 Monitoring and Recording Adverse Events**

Any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible.

##### **9.4.1 Serious Adverse Events**

In the interest of subject safety, and in order to fulfill regulatory requirements, all SAEs (regardless of their relationship to Study Drug) should be reported to the Sponsor within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the subject signs the informed consent form and stop at the end of the subject's follow-up period which is defined as the subject's last visit. When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message. An Initial Serious Adverse Event Form should be completed and a copy should be faxed to the Sponsor or designee.

Detailed information should be actively sought and included on Follow-Up Serious Adverse Event Forms as soon as additional information becomes available. All SAEs will be followed until resolution. SAEs that remain ongoing past the subject's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and Sponsor agree the subject's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

##### **9.4.2 Non-Serious Adverse Events**

The recording of non-serious AEs will begin after the subject signs the informed consent form and will stop at the end of the subject's follow-up period, which is defined as subject's last visit. The Investigator will monitor each subject closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

##### **9.4.3 Evaluation of Adverse Events (Serious and Non-Serious)**

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form.

#### **9.4.3.1 Relationship to the Study Drug**

The event's relationship to the Study Drug is characterized by one of the following:

- **Related:** There is clear evidence that the event is related to the use of Study Drug e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)
- **Not Related:** The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

#### **9.4.3.2 Severity**

The event's severity is characterized by one of the following:

- **Mild:** The event is easily tolerated by the subject and does not affect the subject's usual daily activities
- **Moderate:** The event causes the subject more discomfort and interrupts the subject's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the subject's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in [Section 9.3.3](#)).

#### **9.4.3.3 Action Taken with Study Drug**

Action taken with Study Drug due to the event is characterized by one of the following:

- **None:** No changes were made to Study Drug administration and dose
- **Permanently Discontinued:** Study drug was discontinued and not restarted
- **Temporarily Interrupted, restarted – same dose:** Dosing was temporarily interrupted or delayed due to the AE and restarted at the same dose without unblinding to treatment group

#### **9.4.3.4 Treatment Given for Adverse Event**

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form (treatment should also be recorded on the concomitant treatment or ancillary procedures CRF as appropriate).

#### **9.4.3.5 Outcome of the Adverse Event**

If the event is a non-serious AE then the event's outcome is characterized by one of the following:

- **AE Persists:** Subject terminates from the trial and the AE continues
- **Recovered:** Subject recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity (if applicable):** AE severity changed

If the event is a SAE then the event's outcome is characterized by one of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Subject has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE electronic case report form (eCRF) (the SAE resolution date should be entered as the date of onset of that AE)
- **Recovered:** Subject recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Fatal:** Subject died (the date of death should be entered as the SAE resolution date)

### **9.5 Procedures for Handling Special Situations**

#### **9.5.1 Abnormalities of Laboratory Tests**

Clinically significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Medical Monitor. Laboratory abnormalities deemed not clinically significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents.

### **9.5.2      *Prescheduled or Elective Procedures or Routinely Scheduled Treatments***

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

### **9.5.3      *Dosing Errors***

Study drug errors defined as errors in administration or the administered dose should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic.

Dosing details should be captured on the Dosing CRF.

**Should an overdose occur**, the Investigator or designee should contact the Unblinded Medical Monitor within 24 hours.

### **9.5.4      *Contraception and Pregnancy***

Female subjects that have reached reproductive maturity must have a negative pregnancy test at Screening and must not be able to become pregnant for the duration of the study, as described in [Section 6.3](#).

Male subjects must be abstinent during the duration of the study.

If a subject becomes pregnant or a pregnancy is suspected, or if a male subject makes or believes that he has made someone pregnant during the study, then the site staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported on Follow-up Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the subject's responsibility.

## **10. STATISTICAL CONSIDERATIONS**

### **10.1 Study Endpoints, Subsets, and Covariates**

#### **10.1.1 Primary Endpoint**

- Change from baseline in HFMSE (Hammersmith Functional Motor Scale – Expanded) score at 15 months

#### **10.1.2 Secondary Efficacy Endpoints**

- Proportion of subjects who achieve a 3-point increase from baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months
- Proportion of subjects that achieve any new motor milestone at 15 months
- Number of motor milestones achieved per subject at 15 months
- Change from baseline in Upper Limb Module Test at 15 months
- Proportion of subjects that achieve standing alone at 15 months
- Proportion of subjects that achieve walking with assistance at 15 months

#### **10.1.3 Tertiary Efficacy Endpoints**

- Change from baseline in CSF SMN protein concentration
- Clinical Global Impression of Change (Investigator and Caregiver assessment)
- Change from baseline in PedsQL (Pediatric Quality of Life Inventory)
- Change from baseline in Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)
- Disease-related hospitalizations and adverse events

#### **10.1.4 Safety/Tolerability Endpoints**

- Adverse events
- Vital signs and weight
- Neurological examinations
- Physical examinations
- Clinical laboratory tests (serum chemistry, hematology, urinalysis)
- Electrocardiograms (ECGs)
- Use of concomitant medications

### **10.1.5 Pharmacokinetic Endpoints**

- CSF levels of ISIS 396443
- Plasma levels of ISIS 396443

### **10.1.6 Immunogenicity Endpoint**

- Plasma antibodies to ISIS 396443

## **10.2 Sample Size Considerations**

The sample size for this study was estimated based on limited available natural history data for the target population and data from the ISIS 396443-CS1 and ISIS 396443-CS2 clinical studies. 70 patients in the treated group and 35 patients in the control group will give at least 90% power to detect a 3 point difference between control and treated groups in change on the HFMSE with a standard deviation of 4.4, using a two-sided t-test with an alpha level of 0.05. The sample size was estimated using n-Query. 117 patients enrolled will ensure that a small dropout rate will not affect the power of the primary efficacy analysis.

## **10.3 Populations**

Intent to Treat (ITT) Set: All patients who are randomized, receive at least one dose of study drug/sham procedure, and have a baseline and at least one post baseline efficacy evaluation.

Per-Protocol Set (PPS): PPS will include the subset of the ITT who complete at least the initial 3 doses of study drug/sham procedures, have baseline and Day 169 efficacy assessments and who have no significant protocol deviations that would be expected to affect efficacy assessments.

Safety Set: All patients who are randomized and receive at least one dose of study drug/sham procedure.

Pharmacokinetic Population: All patients who are randomized and for which there is at least one evaluable post-dose/post-sham procedure pharmacokinetic sample.

## **10.4 Definition of Baseline**

The baseline is defined as the last non-missing assessment prior to the first dose of Study Drug.

## **10.5 Interim Analysis**

An interim analysis may take place when all subjects have completed the 6 month assessment and at least 39 subjects have completed the 15 month assessment. At the interim analysis and final analysis, the same Mixed Effects Repeated Measures (MMRM) model will be used in analyzing the same endpoint (change in Hammersmith Functional Motor Scale Expanded (HFMSE) at month 15 from baseline). At the interim analysis, since the month 15 endpoint values are not available for most of the patients, missing post-baseline HFMSE data will be handled using the multiple imputation method ([Schafer 1997](#); [Schafer 1999](#)).

A DSMB will review the interim analysis results and make determinations on whether it is appropriate for the trial to continue. During the interim analysis, subject accrual will continue.

Details of the analysis and controlled access to the unblinded data are contained in the Statistical Analysis Plan (SAP) and DSMB Charter.

In order to control the Type 1 error, a sequential closed testing procedure will be employed with the sequence of the endpoints as defined in [Sections 10.1.1](#) and [10.1.2](#) at the interim and final analyses.

If the interim analysis is performed, it will be conducted at the significance level of 0.01. The significance level for the final analysis will be determined using an alpha allocation with a multiplicity adjustment method that will increase the significance level for the final analysis above 0.04 by accounting for the correlation between the interim and final analyses. The analyses of the secondary endpoints at the interim and the final analysis will be performed at the same significance level as that for the primary analysis.

If the interim analysis is not performed, significance level for the final analysis will remain at 0.05.

## **10.6 Planned Methods of Analysis**

Data collected on eCRF, lab data transfers, as well as any outcomes derived from the data, will be provided in the patient data listings. Patient data listings will be presented for all patients enrolled into the study.

Descriptive summary statistics including n, mean, median, standard deviation, standard error, interquartile range (25th percentile, 75th percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data by treatment group.

All primary, secondary and tertiary endpoints will be assessed in the ITT Set and PPS, with the former being the basis for the primary efficacy analysis. All safety assessments will be performed on the Safety Set. PK endpoints will be assessed in the PK Set as applicable.

### ***10.6.1 Demographic and Baseline Characteristics***

Demographic and baseline characteristics will be summarized using descriptive statistics by treatment group. Patient disposition will be summarized by treatment group. All patients enrolled will be included in a summary of patient disposition.

### ***10.6.2 Safety and Tolerability Analysis***

Safety analyses will be conducted in the Safety Set. Treatment duration and amount of study drug received will be summarized by treatment group.

All treatment-emergent adverse events and serious adverse events will be summarized for each treatment group using the MedDRA™ coding system, by system organ class, preferred term, relationship to Study Drug, and severity. Narratives of deaths, serious adverse events, including early withdrawals from study drug and from study due to adverse events, will also be provided.

Laboratory tests including chemistry panel, complete blood count with differential, etc. will be summarized by study visit for each treatment group. These safety variables will also be presented over time after study drug administration, as appropriate. Vital sign results will be presented similarly.

Physical and neurological examination findings and results from ECG will be listed for review. As appropriate, results will also be summarized descriptively for each treatment group. Concomitant medication usage for each subject will be listed for review.

### **10.6.3 Efficacy Analysis**

All statistical tests will be 2-sided with a Type 1 error rate of 5%, unless otherwise specified.

Secondary efficacy endpoints have been rank prioritized in the order shown in [Section 10.1.2](#). In order to control the Type 1 error, a sequential closed testing procedure will be employed with the sequence of endpoints defined as above. Testing of secondary endpoints will be performed only if the treatment comparison of the primary endpoint is statistically significant ( $p < 0.050$ , unless interim analysis is performed). Testing of the secondary endpoints will be conducted according to the sequence above: if the first secondary endpoint (proportion of patients that achieve a 3-point increase from baseline in HFMSE at 15 months) is statistically significant ( $p < 0.050$ , unless interim analysis is performed) then the second secondary endpoint (proportion of subjects that achieve any new motor milestone at 15 months) will be tested at the same significance level. However, if the treatment comparison based on the first secondary endpoint is not statistically significant, then all endpoints of a lower rank will not be considered statistically significant. This process is repeated with each subsequent secondary endpoint.

Analyses of tertiary efficacy endpoints will not include adjustments for multiplicity.

The primary analysis of the primary endpoint is to compare the change from baseline in HFMSE score at Month 15 between treatment groups using the ITT Set. The data will be analyzed using a Mixed Effects Model with Repeated Measures (MMRM) model where the treatment group, time, treatment by time interaction, and patient age at Screening will be included in the model as fixed effects; patient will be a random effect; the baseline HFMSE score will be included as a covariate. No imputation for missing data will be made. The unstructured covariance model will be used and test of fixed effects will be carried out. The treatment contrast for Month 15 will be estimated by the model. The primary efficacy analysis will take place after all patients have completed the Day 456/ET visit and the database has been locked.

The following sensitivity analyses will also be conducted, and details of the analyses will be outlined in the SAP:

- The primary analysis will be repeated in the PPS.
- The primary analysis will be repeated in the subset of ITT Set who have non-missing Month 15 HFMSE score.
- The LOCF method will be used to impute missing Month 15 HFMSE score. Stratified Wilcoxon Rank Sum test and ANCOVA with the stratification factor and baseline HFMSE score as covariates will be used to analyze the imputed data.

The primary analysis of each secondary endpoint will be based on the ITT Set. Analyses based on the PPS are considered sensitivity analyses.

- Comparison of proportion of subjects that achieve a 3-point increase from baseline in HFMSE score at Month 15 between the treatment groups. The data will be analyzed using the logistic regression adjusting for each patient's age at screening and baseline HFMSE score. If a patient terminates study before Month 15 due to AE, lack of efficacy or other types of treatment failure, then the patient will be considered as a non-responder. If a patient terminates study before Month 15 or has missing HFMSE score at Month 15 due to any other reason, then the patient will not be included in the analysis.
- Comparison of proportion of subjects that achieve any new motor milestone at Month 15 between the treatment groups. The data will be analyzed using the logistic regression adjusting for each patient's age at Screening and baseline number of motor milestones that a patient has achieved. If a patient terminates study before Month 15 due to AE, lack of efficacy or other types of treatment failure, then the patient will be considered as a non-responder. If a patient terminates study before Month 15 or has missing data at Month 15 due to any other reason, then the patient will not be included in the analysis.
- Comparison of number of motor milestones achieved per subject at Month 15 between treatment groups. The data will be analyzed in a similar way to the primary endpoint.
- Comparison of change from baseline in Upper Limb Module Test at Month 15 between treatment groups. The data will be analyzed in a similar way to the primary endpoint.
- Comparison of proportion of subjects that achieve standing alone milestone at Month 15 between the treatment groups. The data will be analyzed in a similar way to the proportion of subjects that achieve any new motor milestone at Month 15.
- Comparison of proportion of subjects that achieved walking with assistance milestone at Month 15 between the treatment groups. The data will be analyzed in a similar way to the proportion of subjects that achieve any motor milestone at Month 15.

#### **10.6.4 Pharmacokinetic Analysis**

Plasma pharmacokinetic parameters and ISIS 396443 concentrations in plasma and CSF for the Pharmacokinetic population will be summarized using descriptive statistics and, where warranted, presented graphically.

### **11. INVESTIGATOR'S REGULATORY OBLIGATIONS**

#### **11.1 Informed Consent/Assent**

The written informed consent and assent documents should be prepared in the language(s) of the potential subject population, based on an English version provided by the Sponsor and should be easy to understand.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent or legal guardian and, in cases where institutional guidelines and the subject's age dictate, informed assent from the subject, after adequate explanation of the

aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any study drugs are administered. Sufficient time must be given to consider whether to participate in the study.

The acquisition of informed consent/assent and the parent/legal guardian's/subject's agreement or refusal of his/her notification of the primary care physician should be documented in the subject's medical records, and the informed consent/assent form(s) should be signed and personally dated by the parent/legal guardian/subject and by the study person who conducted the informed consent/assent discussion. The original signed informed consent/assent form(s) should be retained in the Study Master File and in any other locations required by institutional policy, and a copy of the signed consent/assent form(s) should be provided to the parent or guardian.

### **11.2 Ethical Conduct of the Study**

The Guidelines of the World Medical Association (WMA) Declaration of Helsinki dated October 2002 the applicable regulations and guidelines of current Good Clinical Practice (GCP) as well as the demands of national drug and data protection laws and other applicable regulatory requirements will be strictly followed.

### **11.3 Institutional Review Board/Institutional Ethics Committee/Research Ethics Board**

A copy of the protocol, proposed informed consent form, proposed informed assent form (if applicable) other written subject information, and any proposed advertising material must be submitted to the IRB/IEC for written approval. A copy of the written approval of the protocol and informed consent/assent forms must be received by the Sponsor before recruitment of subjects into the study and shipment of Study Drug. A copy of the written approval of any other items/materials that must be approved by the Study Center or IRB/IEC must also be received by the Sponsor before recruitment of subjects into the study and shipment of Study Drug. The Investigator's Brochure must be submitted to the IRB/IEC for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the IRB/IEC for all subsequent protocol amendments and changes to the informed consent/assent documents. The Investigator should notify the IRB/IEC of deviations from the protocol in accordance with ICH GCP Section 4.5.2. The Investigator should also notify the IRB/IEC of serious adverse events occurring at the Study Center and other adverse event reports received from the Sponsor, in accordance with local procedures.

The Investigator will be responsible for obtaining annual IRB/IEC approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all IRB/IEC submissions and the IRB/IEC continuance of approval must be sent to the Sponsor.

### **11.4 Subject Confidentiality**

The Investigator must ensure that the subject's confidentiality is maintained. On the CRFs or other documents submitted to the Sponsor, subjects should be identified by unique, anonymous initials and a subject study number only. Documents that are not for submission to the Sponsor (e.g., signed informed consent/assent forms) should be kept in strict confidence by the Investigator.

In compliance with federal and local regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory

agency(s), and the IRB/IEC direct access to review the subject's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The Investigator is obligated to inform and obtain the consent of the subject's parent or guardian to permit named representatives to have access to his/her study-related records without violating the confidentiality of the subject.

## **12. ADMINISTRATIVE AND LEGAL OBLIGATIONS**

### **12.1 Protocol Amendments**

Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent/assent documents. The regulatory authority and IRB/IEC must be informed of all amendments and give approval for any amendments likely to affect the safety of the subjects or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the IRB/IEC to the Sponsor.

### **12.2 Study Termination**

The Sponsor reserves the right to terminate the study. The Investigator reserves the right to terminate their participation in the study, according to the terms of the site contract. The Investigator/Sponsor should notify the IRB/IEC in writing of the trial's completion or early termination and send a copy of the termination to the Sponsor.

### **12.3 Study Documentation and Storage**

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the subject's CRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, and correspondence. In this study, CRFs may not be used as source documents.

The Investigator and Study Center staff is responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with Section 8 of the ICH Guidelines (E6), suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. Elements should include:

- Subject files containing completed CRFs, informed consents/assents, and supporting copies of source documentation
- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the IRB and the Sponsor
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction, and all drug-related correspondence

In addition, all original source documents supporting entries in the CRFs must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor.

#### **12.4 Study Monitoring**

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., CRFs and other pertinent data) provided that subject confidentiality is respected.

The Sponsor monitor is responsible for inspecting the CRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the CRFs.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department. Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data a clinical data management review will be performed on subject data received by the Sponsor. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor.

The Principal Investigator will sign and date the indicated places on the CRF. These signatures will indicate that the Principal Investigator inspected or reviewed the data on the CRF, the data queries, and the Study Center notifications, and agrees with the content.

#### **12.5 Language**

CRFs must be completed in English. Generic names for concomitant medications should be recorded in English if possible, unless it is a combination drug, then record the trade name in English.

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood.

## **12.6 Compensation for Injury**

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Subjects will be treated and/or compensated for any study-related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

### 13. REFERENCES

- Cho S and Dreyfuss G. A degtron created by SMN2 Exon 7 skipping is a principal contributor to spinal muscular atrophy severity. *Genes Dev* 2010; 24: 438-442.
- Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO, Mendell JR, Coulson SE, Androphy EJ, Prior TW, Burghes AH. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet* 1997; 6: 1205-1214.
- Feldkötter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time light cycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am J Hum Genet* 2002; 70: 358-368.
- Geary RS, Yu RZ, Watanabe T, Henry SP, Hardee GE, Chappell A, Matson J, Sasnor H, Cummins L, Levin AA. Pharmacokinetics of a tumor necrosis factor- $\alpha$  phosphorothioate 2'-O-(2-Methoxyethyl) modified antisense oligonucleotide: comparison across species. *Drug Metab Disp* 2003; 31: 1419-1428.
- Helmken C, Hofmann Y, Schoenen F, Oprea G, Raschke H, Rudnik-Schöneborn S, Zerres K, Wirth B. Evidence for a modifying pathway in SMA discordant families: reduced SMN level decreases the amount of its interacting partner and Htra2-beta1. *Hum Genet* 2003; 114: 11-21.
- Henry S, Stecker K, Brooks D, Monteith D, Concklin B, Bennett CF. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J Pharmacol Exp Ther* 2000; 292: 468-479.
- Hua Y, Vickers TA, Baker BF, Bennett CF, Krainer AR. Enhancement of SMN2 Exon 7 inclusion by antisense oligonucleotides targeting the exon. *PLoS Biol* 2007; 5: e73.
- Hua Y, Vickers TA, Okunola HL, Bennett CF, Krainer AR. Antisense masking of an HnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am J Hum Genet* 2008; 82: 834-848. Epub 2008 Mar 27.
- Iannaccone ST, Hynan LS, Morton A, Buchanan R, Limbers CA, Varni JW, the AmSMART Group. The PedsQL in pediatric patients with spinal muscular atrophy: feasibility, reliability, and validity of the pediatric quality of life inventory generic core scales and neuromuscular module. *Neuromuscul Disord* 2009; 19: 805-812.
- Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat.Rev.Genet* 2010; 11: 345-355.
- Le TT, Pham LT, Butchbach ME, Zhang HL, Monani UR, Coovert DD, Gavrilina TO, Xing L, Bassell GJ, Burghes AH. SMN $\Delta$ 7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. *Hum Mol Genet* 2005; 14: 845-857. Epub 2005 Feb 9.
- Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M, Le Paslier D, Frézal J, Cohen D, Weissenbach J, Munnich A, Melki J. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995; 80: 155-165.
- Lefebvre S, Burlet P, Liu Q, Bertrand S, Clermont O, Munnich A, Dreyfuss G, Melki J. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet* 1997; 16: 265-269.
- Main M, Kairon H, Mercuri E, Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. *Eur J Paediatr Neurol* 2003; 7(4): 155-159.
- Matsumoto H, Clayton-Krasinski DA, Klinge SA, Gomex JA, Booker WA, Hman JE, Roye Jr. DP, Vitale MG. Development and initial validation of the assessment of caregiver experience with neuromuscular disease. *J Pediatr Orthop* 2011; 31: 284-292.
- Mazzone E, Bianco F, Martinelli D, Glanzman A, Messina S, De Sanctis R, Main M, Eagle M, Florence J, Krosschell K, Vasco G, Pelliccioni M, Lombardo M, Pane M, Finkel R, Muntoni F, Bertini E, Mercuri E. Assessing upper limb function in nonambulant SMA patients: development of a new module. *Neuromuscul Disord* 2011; 21: 406-412.

McKay RA, Miraglia LJ, Cummins LL, Owens SR, Sasmor H, Dean NM. Characterization of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C- $\alpha$  expression. *J Bio Chem* 1999; 274: 1715-1722.

O'Hagen JM, Glanzman AM, McDermott MP, Ryan PA, Flickinger J, Quigley J, Riley S, Sanborn E, Irvine C, Martens WB, Annis C, Tawil R, Oskoui M, Darras BT, Finkel RS, De Vivo DC. An expanded version of the hammersmith functional motor scale for SMA II and III patients. *Neuromuscular Disorders* 2007; 17: 693-697.

Passini MA, Bu J, Richards AM, Kinnecom C, Sardi SP, Stanek LM, Hua Y, Rigo F, Matson J, Hung G, Kaye EM, Shihabuddin LS, Krainer AR, Bennett CF, Cheng SH. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Science Trans Med* 2011; 3: 1-12.

Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2 gene. *Am J Med Genet* 2004; 130A: 307-310.

Schafer JL. Analysis of incomplete multivariate data. Boca Raton, FL. Chapman and Hall/CRC: 1997.

Schafer JL. Multiple Imputation: A Primer. *Statistical Methods in Medical Research* 1999; 8: 3-15.

Varni JW, Seid M, Rode CA. Measurement model for the pediatric quality of life inventory. *Medical Care* 1999; 37: 126-139.

WHO Multicentre Growth Reference Study Group. Assessment of sex differences and heterogeneity in motor milestone attainment among populations in the WHO Multicentre Growth Reference Study. *Acta Pædiatrica*, 2006; Suppl 450: 66/75.

Wijnhoven T, de Onis M, Onyango A, Wang T, Bjoerneboe G, Bhandari N, Lartey A, Al Rashidi B. Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food Nutr Bull* 2004; 21: S37-45.

## **14. APPENDICES**

## **Appendix A     Schedule of Procedures**

## Appendix A Schedule of Procedures

Subjects will also be monitored through phone contact on Study Days 8, 56, 113, 141, 204, 239, 302, 330, 393, 421 (all  $\pm 2$  days)<sup>9</sup>

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 (±1D)			D30	D85 (±2D)			D86	D92 (±1D)	D169 (±2D)	D274 (±7D)			D275	D365 (±7D) and D456 (+ 7D) and Early Term
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
Informed Consent	X																			
Inclusion/Exclusion Criteria	X																			
Medical History	X																			
Screening X-ray	X																			
Urine Pregnancy Test <sup>11</sup>	X																			X <sup>14</sup>
SMN2 copy Number <sup>12</sup>	X																			
Study Drug Injection/Sham Procedure			X				X <sup>7</sup>				X <sup>7</sup>						X <sup>7</sup>			
In-Patient Stay (24 hours)				X																
Vital Signs <sup>1</sup>	X	X		4X <sup>2</sup>	X <sup>3</sup>	X		4X <sup>2</sup>	X	X		4X <sup>2</sup>	X	X	X	X		4X <sup>2</sup>	X	X
Weight	X	X				X				X				X	X	X				X
Height/ulnar length	X																			X <sup>14</sup>
Physical Examination	X	X				X				X				X	X	X				X
Neurological Examination	X	X		X <sup>4</sup>	X <sup>3</sup>	X		X <sup>4</sup>	X	X		X <sup>4</sup>	X	X	X	X		X <sup>4</sup>	X	X
ECG	X				X			X						X						X <sup>14</sup>
Safety Labs <sup>5</sup>	X	X			X	X				X					X	X				X <sup>14</sup>
Coagulation Labs	X																			
Immunogenicity		X				X				X					X	X				X <sup>14</sup>

## Appendix A Schedule of Procedures *Continued*

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 (±1D)			D30	D85 (±2D)			D86	D92 (±1D)	D169 (±2D)	D274 (±7D)			D275	D365 (±7D) and D456 (+ 7D) and Early Term
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
CSF PK <sup>8</sup>		X				X				X						X				
CSF SMN Protein		X				X				X						X				
Plasma PK <sup>8</sup>		X		3X	X	X				X		X			X	X				X <sup>14</sup>
HFMSE	X <sup>10</sup>													X	X	X <sup>13</sup>				X
WHO Motor Milestones	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
Upper Limb	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
PedsQL	X <sup>6</sup>													X	X	X <sup>13</sup>				X
ACEND	X <sup>6</sup>														X					X <sup>14</sup>
CGI														X	X	X <sup>13</sup>				X
Con Med Recording <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Collection <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

- 1 Resting blood pressure, pulse, respiratory rate, and temperature
- 2 Vital signs performed 1, 2, 4, 6 hours after dosing
- 3 Conducted within 20-24 hours after dosing
- 4 Neurological exams at 5 hours after dosing
- 5 Serum chemistry, hematology, urinalysis panels ([Appendix B](#) for analytes)
- 6 Efficacy assessments (with the exception of HFMSE) do not form part of the screening assessment or inclusion/exclusion criteria but are baseline measurements taken during the screening phase of the study
- 7 Overnight stay is optional on Day 29, Day 85, and Day 274
- 8 Refer to [Appendix C](#) for PK sampling schedule
- 9 At telephone contact, changes in concomitant medications and adverse events will be recorded
- 10 To be performed 2 times during the screening period
- 11 Urine pregnancy test performed for females of child-bearing potential, if positive to be confirmed by local serum test
- 12 Only for those subjects who do not have documented evidence of SMN copy number from Athena Diagnostics
- 13 These assessments may be performed up to 7 days prior to dosing, if necessary
- 14 Assessed on Day 456 only

## **Appendix B      Laboratory Analytes**

## Appendix B Laboratory Analytes

CLINICAL SAFETY ASSESSMENTS (minimum requirements)		OTHER ASSESSMENTS
<u><b>Clinical Chemistry</b></u> Sodium Potassium Chloride Total protein Albumin Calcium Phosphorus Bicarbonate Glucose BUN Creatinine Cystatin C Total serum Bilirubin Alkaline phosphatase AST (SGOT) ALT (SGPT) CPK  <u><b>Coagulation</b></u> aPTT PT INR	<u><b>Urinalysis</b></u> Specific gravity pH Protein Glucose Ketones Bilirubin Blood Red blood cells White blood cells Epithelial cells Bacteria Casts Crystals  <u><b>Hematology</b></u> Red blood cells Hemoglobin Hematocrit Platelets White blood cells WBC Differential <ul style="list-style-type: none"> <li>(% and absolute)</li> <li>Neutrophils</li> <li>Eosinophils</li> <li>Basophils</li> <li>Lymphocytes</li> <li>Monocytes</li> </ul>	<u><b>Pregnancy</b></u> Urine hCG  <u><b>PK*</b></u> Plasma ISIS 396443 levels CSF ISIS 396443 levels  <u><b>PD</b></u> CSF SMN protein levels  <u><b>Immunogenicity Evaluation</b></u> ISIS 396443 plasma Abs  <u><b>SMN Genetics</b></u> SMN2 Copy Number

\* Any of the collected PK plasma and CSF samples from the study patients may also be used for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with plasma and CSF constituents.

## **Appendix C      Pharmacokinetic Sampling Schedule**

## Appendix C Pharmacokinetic Sampling Schedule

Treatment Period	Study Day	Timepoints	Blood Collection	CSF Collection
Multiple Dose: LP Injection	D1	Predose	0.35 mL	0.5 mL
		2 hr	0.35 mL	NA
		4 hr	0.35 mL	NA
		8 hr	0.35 mL	NA
	D2	24 hr	0.35 mL	NA
	D29	Predose	0.35 mL	0.5 mL
	D85	Predose	0.35 mL	0.5 mL
		4 hr	0.35 mL	NA
	D169	Anytime	0.35 mL	NA
	D274	Predose	0.35 mL	0.5 mL
	D456	Anytime	0.35 mL	NA

NA Not applicable (No Collection Scheduled)

Details on sampling, preparation, and shipment are included in the study laboratory manual.

Any of the collected PK plasma and CSF samples from the study patients may also be used for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with plasma and CSF constituents.



**ISIS PHARMACEUTICALS, INC.**

**ISIS 396443-CS4**

**A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled  
Study to Assess the Clinical Efficacy and Safety of ISIS 396443  
Administered Intrathecally in Patients with Later-onset Spinal  
Muscular Atrophy**

**Original Protocol 17 July 2014**

**EudraCT No: 2014-001947-18**

**Sponsor:**

Isis Pharmaceuticals, Inc.

2855 Gazelle Court

Carlsbad, CA 92010

## **ISIS 396443-CS4**

### **A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy**

**Original Protocol – 17 July 2014**

**Sponsor:**

Isis Pharmaceuticals, Inc.  
2855 Gazelle Court  
Carlsbad, CA 92010

□□□



## **ISIS 396443**

**Isis Protocol Number ISIS 396443-CS4**

**Original Protocol**

**Clinical Phase: 3**

### **A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy**

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**Date:**

17 July 2014

#### **Confidentiality Statement**

This document contains confidential information of Isis Pharmaceuticals, Inc. that must not be disclosed to anyone other than the recipient study staff and members of the institutional review board or authorized regulatory agencies. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Isis Pharmaceuticals, Inc.

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## Protocol Signature Page

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**Protocol Number:** ISIS 396443-CS4

**Protocol Title:** A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy

**Amendment:** Original Protocol

**Date:** 17 July 2014

---

I hereby acknowledge that I have read and understand the attached clinical protocol, entitled “A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy”, dated 17 July 2014, and agree to conduct the study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice (E6).

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Isis Pharmaceuticals, Inc.

---

Investigator's Signature

---

Investigator's Name (*please print*)

---

Date (DD Month YYYY)

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## PROTOCOL SYNOPSIS

<b>Protocol Title</b>	A Phase 3, Randomized, Double-Blind, Sham Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy
<b>Study Phase</b>	3
<b>Indication</b>	Later-onset Spinal Muscular Atrophy (SMA)
<b>Objectives</b>	<p><b>Primary Objective:</b></p> <p>To examine the efficacy of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p> <p><b>Secondary Objective:</b></p> <p>To examine the safety and tolerability of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p> <p><b>Tertiary Objective:</b></p> <p>To examine the CSF and plasma pharmacokinetics of ISIS 396443 administered intrathecally to patients with later-onset SMA.</p>
<b>Number of Subjects</b>	Approximately 117 subjects will be enrolled into this study
<b>Treatment Groups</b>	12 mg ISIS 396443 or sham procedure control group
<b>Study Design</b>	<p>This randomized, double-blind, sham-procedure controlled study will test the clinical efficacy, safety, tolerability, and pharmacokinetics of intrathecal ISIS 396443 over 15 months. Approximately 117 subjects will be randomized in a 2:1 ratio (78 ISIS 396443: 39 control) to receive ISIS 396443 by intrathecal lumbar puncture (LP) injection or to a sham-procedure control. A dose of 12 mg ISIS 396443 will be given at each of 4 times over the 15 months (i.e., on Study Days 1, 29, 85, and 274).</p> <p>Randomization will be stratified based on:</p> <p>1) Age (&lt;6 years versus ≥6 years at Screening)</p> <p>After Informed consent/assent is obtained, subjects will undergo a screening evaluation no greater than 28 days prior to first dose administration at which time their eligibility for the study will be examined. Subjects who meet the eligibility criteria will be admitted to the study center on Study Day 1, undergo pre-dose evaluations, and then receive either an LP injection of study drug (ISIS 396443) or a sham procedure. Subjects will return to the study center on Days 29, 85 and 274 for follow-up evaluations and subsequent injections/sham procedures. Following the injection/sham procedure on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring. Following the injection/sham procedure on Day 29, 85, and 274 subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. Safety monitoring visits will occur on Days 30, 86, 92, 169, 275, 365, and 456 (through 6 months following the last injection/sham procedure). In addition, the study center will monitor the subject's condition through telephone contact monthly throughout the study. For subjects receiving ISIS 396443, a CSF sample will be taken pre-dose on each injection day in a manner that maintains the blind.</p> <p>Following treatment and the final follow-up evaluation, all eligible subjects may elect to enroll in an open-label treatment extension study. This will be done without unblinding to subject's treatment group.</p> <p>If a subject terminates early from the study, they will be encouraged to complete safety assessments per the Day 456 visit. If a subject is randomized but does not complete at least their first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.</p>

## Protocol Synopsis *Continued*

<p><b>Study Population and Main Criteria for Inclusion/ Exclusion</b></p>	<p><b><u>Inclusion Criteria:</u></b> Subjects must meet all of the following criteria at Screening to be eligible:</p> <ol style="list-style-type: none"> <li>1. Signed informed consent of parent or guardian. Signed informed assent of subject, if indicated per subject's age and institutional guidelines</li> <li>2. Genetic documentation of 5q SMA (homozygous gene deletion, mutation, or compound heterozygote)</li> <li>3. Onset of clinical signs and symptoms consistent with SMA at &gt;6 months of age</li> <li>4. Males and females 2 to 12 years of age</li> <li>5. Can sit independently, but has never had the ability to walk independently</li> <li>6. Motor Function Score (Hammersmith Functional Motor Scale – Expanded) <math>\geq 10</math> and <math>\leq 54</math> at Screening</li> <li>7. Able to complete all study procedures, measurements and visits and parent or guardian/subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator</li> <li>8. Estimated life expectancy &gt;2 years from Screening, in the opinion of the Investigator</li> <li>9. Meets age-appropriate institutional criteria for use of anesthesia/sedation, if use is planned for study procedures (as assessed by the Site Investigator and either anesthesiologist or pulmonologist)</li> <li>10. For subjects who, in the opinion of the Investigator, have reached reproductive maturity, satisfy one of the following:  <p style="margin-left: 40px;">Females: have a negative pregnancy test at Screening and agrees to employ adequate contraceptive measures for the duration of the study. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products.</p> <p style="margin-left: 40px;">Males: be abstinent for the duration of the study</p> </li> </ol> <p><b><u>Exclusion Criteria:</u></b> Subjects meeting any of the following criteria are not eligible for the study:</p> <ol style="list-style-type: none"> <li>1. Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for &gt;6 hours during a 24 hour period, at Screening</li> <li>2. Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator</li> <li>3. Severe contractures or severe scoliosis evident on X-ray examination at Screening</li> <li>4. Hospitalization for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of Screening or planned during the duration of the study</li> <li>5. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period</li> <li>6. History of brain or spinal cord disease, including tumors, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation</li> <li>7. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter</li> <li>8. History of bacterial meningitis</li> <li>9. Dosing with ISIS 396443 in any previous clinical study</li> <li>10. Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline</li> </ol>
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**Protocol Synopsis *Continued***

<b>Study Population and Main Criteria for Inclusion/ Exclusion <i>Continued</i></b>	<p><b><u>Exclusion Criteria <i>Continued</i>:</u></b></p> <ol style="list-style-type: none"> <li>11. Clinically significant abnormalities in hematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion</li> <li>12. Treatment with another investigational drug (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, etc.), biological agent, or device within 1-month of Screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation</li> <li>13. Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anemia, etc.) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures</li> </ol>
<b>Study Drug and Administration</b>	<p>ISIS 396443 (2.4 mg/mL) will be administered as an intrathecal LP injection. The volume of the injection will be 5.0 mL. Details regarding the LP dosing injection procedure and the sham procedure will be provided in the Dosing Administration Manual. Details regarding the study drug will be provided in the Study Drug Manual. Per institutional guidelines, anesthesia/sedation may be used for the LP procedure.</p>
<b>Criteria for Evaluation</b>	<p><b><u>Primary Endpoint:</u></b></p> <ul style="list-style-type: none"> <li>• Change from baseline in HFMSE (Hammersmith Functional Motor Scale – Expanded) score at 15 months</li> </ul> <p><b><u>Secondary Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• Proportion of subjects who achieve a 3-point increase from baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months</li> <li>• Proportion of subjects that achieve any new motor milestone at 15 months</li> <li>• Number of motor milestones achieved per subject at 15 months</li> <li>• Change from baseline in Upper Limb Module Test at 15 months</li> <li>• Proportion of subjects that achieve standing alone at 15 months</li> <li>• Proportion of subjects that achieve walking with assistance at 15 months</li> </ul> <p><b><u>Tertiary Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• Change from baseline in CSF SMN protein concentration</li> <li>• Clinical Global Impression of Change (Investigator and Caregiver assessment)</li> <li>• Change from baseline in PedsQL (Pediatric Quality of Life Inventory)</li> <li>• Change from baseline in Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)</li> <li>• Disease-related hospitalizations and adverse events</li> </ul> <p><b><u>Safety/Tolerability Endpoints:</u></b></p> <ul style="list-style-type: none"> <li>• Adverse events</li> <li>• Vital signs and weight</li> <li>• Neurological examinations</li> <li>• Physical examinations</li> <li>• Clinical laboratory tests</li> <li>• ECGs</li> <li>• Use of concomitant medications</li> </ul>

**Protocol Synopsis *Continued***

<b>Criteria for Evaluation <i>Continued</i></b>	<b><u>Pharmacokinetic Endpoints:</u></b> <ul style="list-style-type: none"><li>• CSF levels of ISIS 396443 (sample taken pre-dose at each dosing)</li><li>• Plasma levels of ISIS 396443</li></ul> <b><u>Immunogenicity Endpoint:</u></b> <ul style="list-style-type: none"><li>• Plasma antibodies to ISIS 396443</li></ul>
<b>Safety Monitoring</b>	Safety data will be reviewed on an ongoing basis by the Medical Monitor and by an Independent Data Safety and Monitoring Committee (DSMB).
<b>Statistical Considerations</b>	The sample size for this study was estimated based on limited available natural history data for the target population and from the ISIS 396443-CS1 and CS2 clinical studies in children with SMA. 70 subjects in the treated group and 35 subjects in the control group will give at least 90% power to detect a 3 point difference between control and treated groups in change on the HFMSE with a standard deviation of 4.4, using a two-sided t-test with an alpha level of 0.05. The sample size was obtained using the n-Query. 117 subjects will be enrolled to ensure that a small dropout rate will not affect the power of the primary efficacy analysis. An interim analysis may take place when all subjects have completed the 6 month assessment and at least 39 subjects have completed the 15 month assessment.
<b>Sponsor</b>	Isis Pharmaceuticals, Inc.

## STUDY GLOSSARY

<b><u>Abbreviation/Acronym</u></b>	<b><u>Definition</u></b>
ACEND	Assessment of Caregiver Experience with Neuromuscular Disease
AE	Adverse event/experience
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CGI	Clinical Global Impressions of Change Rating Scale
C <sub>max</sub>	Maximum concentration
CNS	Central nervous system
CPK	Creatinine phosphokinase
CRF	Case report form
CSF	Cerebrospinal fluid
DSMB	Data and Safety Monitoring Board
eCRF	Electronic case report form
ECG	Electrocardiogram
FL	Full-length
GCP	Good Clinical Practice
HFMSE	Hammersmith Functional Motor Scale - Expanded
hnRNP	Heterogeneous nuclear ribonucleoproteins
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
IT	Intrathecal
IXRS	Interactive Voice/Web-Response System
LP	Lumbar puncture
MAD	Multiple ascending-dose
MedDRA	Medical Dictionary for Regulatory Activities
miRNA	Micro ribonucleic acid (RNA)

MOE	2'- <i>O</i> -(2-methoxyethyl)
mRNA	Messenger ribonucleic acid
NCS	Not clinically significant
OLE	Open-label extension study
PedsQL	Pediatric Quality of Life Inventory
PK	Pharmacokinetic(s)
PT	Prothrombin Time
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SMA	Spinal muscular atrophy
SMN	Survival motor neuron
snRNA	Small nuclear ribonucleic acid
SUSARs	Suspected unexpected serious adverse reactions
T <sub>max</sub>	Time to maximal concentration
WMA	World Medical Association

## 1. OBJECTIVES

The objectives of this study are to evaluate the clinical efficacy, safety, tolerability, and pharmacokinetics (PK) of ISIS 396443 administered intrathecally to patients with later-onset Spinal Muscular Atrophy (SMA).

### 1.1 Primary Objective

To examine the clinical efficacy of ISIS 396443 administered intrathecally to patients with later-onset SMA.

### 1.2 Secondary Objective

To examine the safety and tolerability of ISIS 396443 administered intrathecally to patients with later-onset SMA.

### 1.3 Tertiary Objective

To examine the cerebral spinal fluid and plasma PK of ISIS 396443 administered intrathecally to patients with later-onset SMA.

## 2. BACKGROUND AND RATIONALE

### 2.1 Spinal Muscular Atrophy

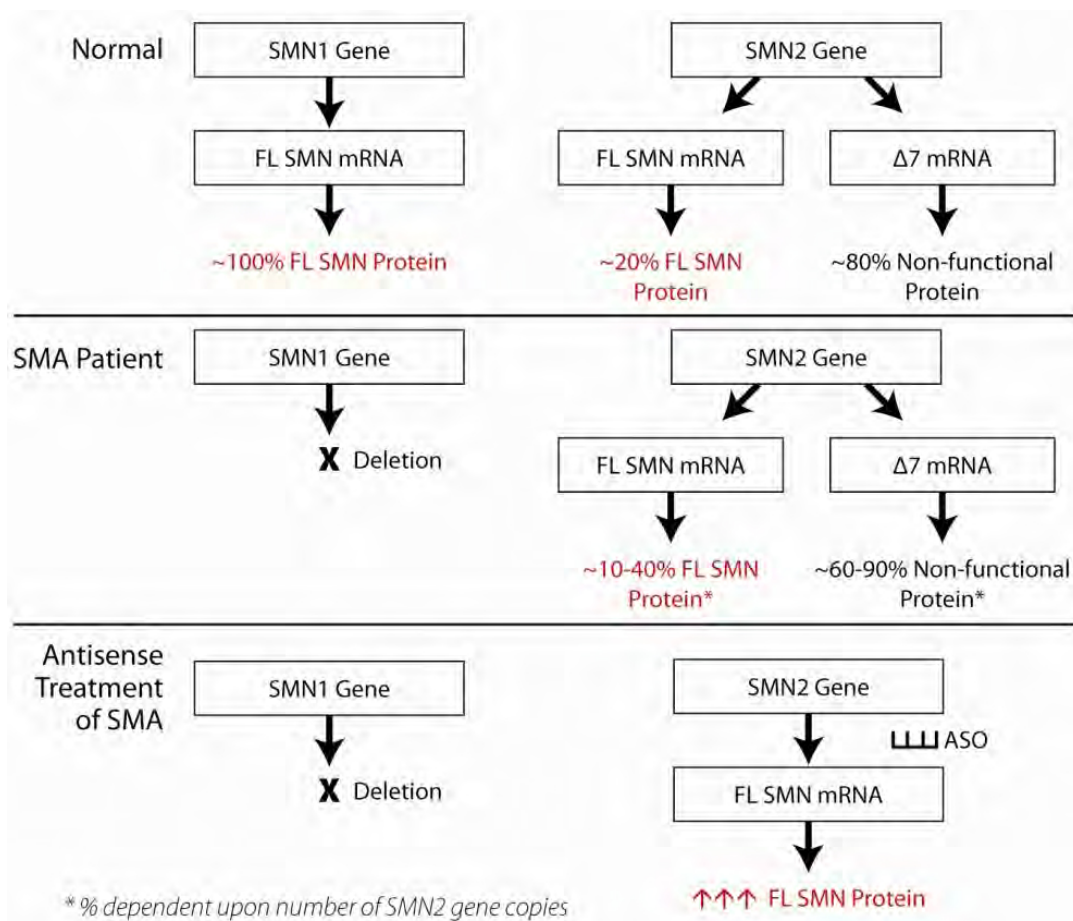
SMA is an autosomal recessive neuromuscular disease characterized by degeneration of the motor neurons in the anterior horn of the spinal cord, resulting in atrophy of the voluntary muscles of the limbs and trunk. With an incidence of 1:6000 to 1:10,000 live births, it is the most common genetic cause of infant mortality, and a major cause of childhood morbidity due to weakness, in the U.S. The natural history of SMA includes four major phenotypes that are recognized dependent on age of onset and achieved motor abilities. The most severe form, Type 1 SMA (equivalent to infantile-onset SMA), has a disease onset within the first few months of life; these children are never able to sit or walk and usually die from respiratory failure by the age of 2 years. Later-onset SMA can generally be divided into Type 2 and Type 3 SMA. Type 2 SMA patients are able to sit but never walk unaided, with symptoms presenting between 6-18 months of age. Type 3 SMA patients are able to sit and walk but individuals with this form may become severely and increasingly disabled. Adult-onset SMA patients (Type 4) have an age of onset over 18 years of age and have normal life expectancies.

In 95% of SMA patients, a deletion in the *SMN1* gene on Chromosome 5q11-q13 is found; with the remaining 5% attributable to small mutations in the same gene ([Lefebvre et al. 1995](#); [Helmken et al. 2003](#)). *SMN1* lies in the telomeric portion of an inverted duplication of a region of Chromosome 5. The centromeric half of the duplication contains a homologous gene, named *SMN2* that differs from *SMN1* by 11 nucleotides. The open reading frames for both genes encode for proteins with identical amino acid sequences. Survival motor neuron (SMN) gene transcripts, similar to most mammalian transcripts, undergo alternative splicing in which certain exons are either included or excluded from the mature protein coding transcripts ([Keren et al. 2010](#)). In particular, Exon 7 of the *SMN1* gene is alternatively spliced with 90 to 95% of the mature messenger ribonucleic acid (mRNA) transcripts derived from the *SMN1* gene containing Exon 7, and 5 to 10% of transcripts missing Exon 7. The transcripts missing Exon 7 (often referred to as  $\Delta 7$ ) produce a truncated protein which is defective and unstable

(Cho and Dreyfuss 2010). One of the 11 nucleotide differences between *SMN1* and *SMN2*, a C to T substitution occurs in Exon 7 of the *SMN2* gene resulting in an alternative splicing pattern that favors skipping of Exon 7. The result is that as much as 90% of the transcripts produced from *SMN2* are missing Exon 7. The remainder, *SMN2* transcripts containing Exon 7, produces a full-length (FL) protein product identical to the *SMN1* protein, since the C to T substitution is silent. Humans have a variable copy number of the *SMN2* gene (0-8 copies). The number of *SMN2* copies and the resulting amount of FL-SMN protein expressed in SMA patients (10-40% of normal SMN protein levels) correlates with SMA disease severity and thus *SMN2* is a key modifier of disease phenotype (Coovert et al. 1997; Feldkötter et al. 2002; Lefebvre et al. 1997; Prior et al. 2004).

## 2.2 Therapeutic Rationale

Since the number of *SMN2* gene copies and resulting amount of SMN protein is correlated with disease onset and severity, a therapeutic approach predicted to benefit SMA patients is to increase the levels of full length *SMN2* pre-mRNA by restoring the splicing pattern that gives rise to full length *SMN2* mRNA. Increasing inclusion of Exon 7 in the *SMN2* transcript will increase FL-SMN protein levels and SMN protein activity. A therapeutic strategy for promoting Exon 7 inclusion is through the use of antisense oligonucleotides (ASOs) (see Figure 1).



**Figure 1 ASO Therapeutic Approach for Treatment of SMA**

The known potential risks associated with ISIS 396443 are detailed in the Guidance to Investigator section of the Investigator's Brochure. Additional study associated risks related to the lumbar puncture (LP) procedure are also detailed in the Guidance to Investigator section of the Investigator's Brochure.

## 2.3 ISIS 396443

### 2.3.1 Mechanism of Action

ISIS 396443 is a fully modified, 2'-O-2-methoxyethyl (MOE), ASO drug designed to bind to a specific sequence in the intron downstream of Exon 7 of the SMN2 transcript. The region of the pre-mRNA targeted by ISIS 396443 is normally occupied by heterogeneous nuclear ribonucleoproteins (hnRNP) A1/2 proteins, masking the U1 small nuclear ribonucleic acid (snRNA) binding site at the 5'-exon-intron junction of Exon 7, and is referred to as ISS-N1. U1 snRNA base pairs to the sequences that define the 5'-splice site, which is thought to be one of the first steps that initiate splicing of an intron. ISIS 396443 displaces the hnRNP A1/2 proteins from the pre-mRNA binding site, allowing U1 snRNA to bind to the exon-intron junction and promote assembly of the spliceosomal complex, thus promoting inclusion of Exon 7 into the mRNA which results in production of FL-SMN protein.

### 2.3.2 Chemistry

Chemically, ISIS 396443 is a synthetic oligomer of 18 nucleotides (i.e., an 18-mer) that are connected sequentially by phosphorothioate linkages. Each of the 17 internucleotide linkages is a 3'-O to 5'-O phosphorothioate diester. The 18 sugar residues are uniformly modified with 2'-O-(2-methoxyethyl) (MOE). These MOE-modified nucleotides confer (1) increased affinity to the target mRNA (McKay et al. 1999), (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue) (Geary et al. 2003), and (3) amelioration of some of the high dose toxicities associated with ASO containing only the phosphorothioate linkages (Henry et al. 2000).

The sequence of ISIS 396443 is written as follows:



Where A and G are 2'-O-(2-methoxyethyl)nucleosides, <sup>Me</sup>C is 5-methyl-2'-O-(2-methoxyethyl)cytidine and <sup>Me</sup>U designates 5-methyl-2'-O-(2-methoxyethyl)uridine.

### 2.3.3 Preclinical Experience

Detailed information concerning the preclinical studies conducted with ISIS 396443 can be found in the Investigator's Brochure. A summary is included below.

ISIS 396443 was identified after an extensive screen of greater than 500 2'-MOE oligonucleotides in *in vitro* splicing assays, reporter gene assays and in SMA patient fibroblasts (Hua et al. 2007; Hua et al. 2008). Data have shown that ISIS 396443 promotes a concentration-dependent increase in full-length transcripts (including Exon 7) in patient fibroblast cells, achieving greater than 90% full length SMN2 transcripts and forms nuclear structures, called gems, known to contain SMN protein. In a mild mouse model of SMA, ISIS 396443 promoted inclusion of Exon 7 in the SMN2 transgene in a variety of peripheral tissues when dosed systemically (Hua et al. 2008) and in central nervous system (CNS) tissue, including spinal cord,

when injected into the lateral ventricle. ISIS 396443 produced greater than 90% Exon 7 inclusion in the transgenic mice and increased SMN protein production in motor neurons, resulting in the appearance of gems in motor neurons. These studies were extended to a more severe mouse model of SMA (SMA  $\Delta 7$ ) (Le et al. 2005), where the CNS delivery of drug produced a dose-dependent effect on SMN2 Exon 7 inclusion, SMN protein production, and survival. These mice treated with ISIS 396443 demonstrated improved weight gain, improvements in muscle morphology, muscle strength, and motor coordination and improved morphology of the motor neuron junctions (Passini et al. 2011). Further, ISIS 396443 was shown to distribute widely in the CNS following intrathecal (IT) administration in monkey (Passini et al. 2011).

The pharmacokinetics and toxicity of ISIS 396443 were assessed following: 1) single intrathecal (IT) lumbar bolus injections (1 to 7 mg) in adult monkeys 2) following 14 weeks (with a 4-week interim sacrifice) of repeated IT lumbar bolus injections (0.3 to 3 mg/week or every other week) in juvenile monkeys and 3) following 53 weeks of repeated IT lumbar bolus injections in juvenile monkeys. In addition, a dedicated pharmacokinetic study in adult monkeys was performed to assess the half-life of ISIS 396443 in CSF, tissues and plasma. Detailed results from these preclinical studies conducted with ISIS 396443 can be found in the ISIS 396443 Investigator's Brochure.

#### **2.3.4 Clinical Experience**

Detailed information concerning the clinical studies conducted with ISIS 396443 can be found in the Investigator's Brochure. A summary is included below.

ISIS 396443 has been evaluated in a completed open-label, single ascending-dose (SAD) Phase 1 study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with SMA (ISIS 396443-CS1). A single-dose of ISIS 396443 was administered by IT injection to SMA patients aged 2 to 14 years of age. Four dose levels (1, 3, 6, and 9 mg) were evaluated sequentially. Each dose level was studied in a cohort of 6 or 10 subjects, where all subjects received drug. In this study all subjects completed dosing and the follow-up visits per protocol. Overall, ISIS 396443 was well-tolerated and no safety concerns were identified up to the 9.0 mg dose level, given as a single IT injection. No serious adverse events (SAEs) or dose-limiting toxicities (DLTs) were reported in ISIS 396443-CS1. Adverse events (AEs) reported were mild or moderate in severity and there was no relationship with ISIS 396443 dose level. In addition, no ISIS 396443 related adverse changes in neurological exams were reported, despite intensive monitoring during the immediate post-dosing period. CSF and plasma drug concentrations observed were generally consistent with predictions made from nonclinical monkey studies.

ISIS 396443 is also being evaluated in four ongoing studies: ISIS 396443-CS2, ISIS 396443-CS10, ISIS 396443-CS12, and ISIS 396443-CS3A. ISIS 396443-CS2 is an open-label, multiple ascending-dose (MAD) Phase 1/2a study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with SMA. Multiple doses of ISIS 396443, ranging from 3 mg to 12 mg, are being administered by intrathecal injection to SMA patients aged 2 to 15 years of age. ISIS 396443-CS10 is an open-label, single dose, re-dosing study for SMA patients who previously participated in Cohorts 2, 3 and 4 in ISIS 396443-CS1. ISIS 396443-CS12 is an ongoing open-label study to assess the safety and tolerability of a single (12 mg) intrathecal dose of ISIS 396443 in patients with spinal muscular atrophy who previously participated in ISIS 396443-CS2 or ISIS 396443-CS10.

ISIS 396443 CS3A is an open-label, multiple-dose study designed to assess the safety, tolerability and pharmacokinetics of ISIS 396443 in patients with infantile-onset SMA. Multiple doses of ISIS 396443 are being administered by intrathecal injection to symptomatic SMA infants  $\leq 7$  months of age. Two dose levels (6 and 12 mg dose equivalent scaled by CSF volume) are being evaluated sequentially.

## **2.4 Rationale for Dose and Schedule of Administration**

The proposed study will test the clinical efficacy, safety, tolerability, and PK of multiple doses of ISIS 396443 administered as IT injections to subjects with later-onset SMA. A single dose level of 12 mg ISIS 396443 will be evaluated, delivered as 4 doses administered over 9 months. ISIS 396443 will be administered using a loading regimen (dosings on Study Days 1, 29, 85) followed by maintenance dosing given 6 months thereafter (dosing on Study Day 274).

The ISIS 396443-CS4 dose level and dose interval was selected based on preclinical toxicology and pharmacokinetic observations from monkey studies utilizing single-dose and repeat dosing IT administration, consideration of the target tissue concentration anticipated for drug pharmacology, and safety data in the completed and ongoing clinical studies of ISIS 396443 to date. Based upon pharmacology and pharmacokinetic results in SMA transgenic mice, we estimate that the target tissue concentration to produce 50 to 90% SMN2 Exon 7 inclusion is between 1 and 10  $\mu\text{g/g}$  spinal cord tissue. Nonclinical studies in juvenile monkeys receiving IT doses of ISIS 396443 showed a resulting gradient of distribution of ISIS 396443 along the spinal cord, with mean lumbar spinal cord concentrations ranging from 1.6-2.3 fold and 2.0-3.5 fold higher than thoracic and cervical spinal cord levels, respectively. The dose level selected for this multiple-dose clinical study (12 mg ISIS 396443) is predicted to achieve levels at the high end of this range (approximately 10  $\mu\text{g/g}$  lumbar and 3  $\mu\text{g/g}$  cervical spinal cord tissue concentrations), following the first dose. The loading dose interval was selected based on the nonclinical pharmacokinetic and pharmacology data as the dose interval to achieve and maintain ISIS 396443 spinal cord tissue levels that are predicted to be within the upper end of the pharmacologically active range by Day 85 (predicted to be approximately 24  $\mu\text{g/g}$  lumbar and 8  $\mu\text{g/g}$  cervical tissue concentration), while at the same time considering subject safety and convenience for repeated LP intrathecal injections. The maintenance dose interval (once every 6 months) was selected based on the estimated spinal tissue and CSF drug half-life (4-6 months) and was selected to maintain spinal cord tissue levels of ISIS 396443 at a steady-state level within the estimated pharmacologically active range.

Additional details on dose scaling and expected CSF and tissue concentrations are summarized in the Investigator's Brochure.

## **3. EXPERIMENTAL PLAN**

### **3.1 Study Design**

This is a Phase 3 multicenter, double-blind, randomized, sham-procedure controlled study of ISIS 396443 in patients with later-onset SMA studied for 15 months. Approximately 117 subjects will be randomized 2:1 to receive 12 mg dose ISIS 396443 or a sham procedure control, respectively. ISIS 396443 will be administered using a loading regime (dosings on Study Days 1, 29, and 85) followed by a maintenance dose 6 months thereafter (dosing on Study Day 274).

Randomization will be stratified based on:

- 1) Subject's Age at Screening (<6 years versus  $\geq 6$  years)

Following treatment and the Day 456 follow-up evaluation, all eligible subjects may elect to enroll in an open-label treatment extension study, pending study approval by the IRB or IEC and the appropriate regulatory authority. This will be done without unblinding to subject's treatment group.

### **3.2 Number of Study Centers**

This study will be conducted at multiple centers worldwide.

### **3.3 Number of Subjects**

Approximately 117 subjects (2 ISIS 396443; 1 sham-procedure) will receive 12 mg dose of ISIS 396443 or a sham procedure control. The total number of subjects randomized may be higher if some subjects do not receive their Day 1 dose/sham procedure. The maximum number of subjects will not exceed 130.

### **3.4 Overall Study Duration and Follow-up**

The Study will consist of screening, treatment, and post-treatment follow-up-periods. The total duration of participation in the study is approximately 16 months. Please refer to the Schedule of Procedures in [Appendix A](#).

#### **3.4.1 Screening**

After informed consent/assent is obtained, subjects will undergo a screening evaluation no greater than 28 days prior to first dose administration at which their eligibility for the study will be examined.

#### **3.4.2 Treatment**

Subjects who meet the eligibility criteria will be admitted to the study center on Study Day 1, undergo pre-dose evaluations, be randomized, and then receive either an LP injection of study drug (ISIS 396443) or a sham procedure. Subjects will return to the study center on Days 29, 85 and 274 for follow-up evaluations and subsequent injections/sham procedures. Following the injection/sham procedure on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring. Following the injection/sham procedure on Day 29, 85, and 274 subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. Safety monitoring visits will occur Study Days 30, 86, 92, 169, and 275 (through the last injection/sham procedure). In addition, the study center will monitor the subject's condition through telephone contact on a monthly basis.

For subjects receiving ISIS 396443, a CSF sample for PK and SMN protein analyses will be taken pre-dose on each injection day in a manner that protects the blind.

If a subject terminates early from the study, they will be encouraged to complete assessments per the Day 456 visit. If a subject is randomized but does not successfully receive the first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.

### **3.4.3 Post-Treatment Follow-up**

After completion of the Day 275 visit, subjects will enter the 6-month post-treatment evaluation period. This period consists of Study Center visits on Day 365 and 456 and follow-up phone assessments on a monthly basis, as outlined in the Schedule of Procedures ([Appendix A](#)). After completion of the Day 456 visit, subjects may be eligible to participate in an OLE study, pending study approval by the IRB or IEC and the appropriate regulatory authority.

### **3.5 End of Study**

The end of study is last subject; last visit (either in-person visit or telephone contact).

### **3.6 Safety Monitoring and Data Safety Monitoring Board**

Safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor. Safety data will also be reviewed on an ongoing basis by an independent DSMB. The DSMB will be assembled to review safety, tolerability and efficacy (as needed) data collected on ISIS 396443 during this study. Based on its ongoing assessment of the data, the DSMB will provide recommendations to the Sponsor for modifying, stopping or continuing the study.

## **4. SUBJECT ENROLLMENT**

### **4.1 Screening**

Before subjects may be enrolled into the study, the Sponsor requires a copy of the Study Center's written IRB or IEC approval of the protocol, informed consent form, informed assent form (if applicable) and all other subject information and/or recruitment material.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent(s) or legal guardian(s) and, in cases where institutional guidelines and the patient's age dictate, informed assent from the subject. At the time of consent/assent, the subject will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including screening procedures, are performed. The screening number must remain constant throughout the entire trial. In the event the subject is re-consented and re-screened the subject must be given a new screening number. Screening numbers, once assigned, will not be re-used.

### **4.2 Randomization**

Subjects will be randomized after all screening assessments have been completed and after the Investigator and the Medical Monitor have verified that they are eligible per criteria in [Sections 5.1](#) and [5.2](#). No subject may begin treatment prior to randomization and assignment of a unique randomization number.

Using an Interactive Voice/Web-Response System (IXRS), eligible subjects will be randomized 2:1 to receive ISIS 396443 or sham-procedure control, respectively. Randomization will be stratified for:

- Subject's Age at Screening (<6 years versus ≥6 years)

The Sponsor Quality Assurance department or designee will hold a copy of the randomization lists generated by the IXRS vendor.

The Sponsor Quality Assurance department or designee will hold a copy of the randomization lists generated by the IXRS vendor.

#### **4.3 Replacement of Subjects**

If a subject does not successfully receive the first dose of ISIS 396443/undergo the first sham procedure, they will be replaced.

#### **4.4 Unblinding of Treatment Assignment**

The Sponsor, parents, and key study site personnel will be blinded to subjects' treatment assignment throughout the study. The DSMB may be unblinded as described in the DSMB charter.

If a subject has experienced an SAE (as defined in [Section 9.3.3](#)), and/or when knowledge of the treatment assignment will impact the clinical management of the subject, the Investigator will have the ability to unblind the treatment assignment for that subject using the IXRS. However, prior to unblinding, the Investigator should attempt to contact the blinded Medical Monitor to discuss the emergency. The Sponsor or designee will be informed of the unblinding of a subject within 24 hours. Every reasonable attempt should be made to complete the Study Day 456 early termination procedures and observations (see [Appendix A](#)) prior to unblinding, as knowledge of the subject's treatment assignment could influence subsequent assessments. The Investigator must document the reasons for unblinding in the subject's source documents. The Investigator is strongly advised not to divulge the subject's treatment assignment to any individual not directly involved in managing the medical emergency, nor to personnel involved with the analysis and conduct of the study. In cases where there are ethical reasons to have a subject remain on study whose treatment assignment is unblinded for safety reasons, the Site Investigator must obtain specific approval from the Sponsor and the Medical Monitor for the subject to continue in the study.

In addition, all suspected unexpected serious adverse reactions (SUSARs) will be unblinded by the Sponsor's Drug Safety and Quality Assurance personnel for the purpose of regulatory reporting ([Section 9.2](#)).

### **5. SUBJECT ELIGIBILITY**

#### **5.1 Inclusion Criteria**

Subjects must meet all of the following criteria at Screening to be eligible:

1. Signed informed consent of parent or guardian. Signed informed assent of subject, if indicated per subject's age and institutional guidelines
2. Genetic documentation of 5q SMA (homozygous gene deletion, mutation, or compound heterozygote)
3. Onset of clinical signs and symptoms consistent with SMA at >6 months of age
4. Males and females 2 to 12 years of age
5. Can sit independently, but has never had the ability to walk independently

6. Motor Function Score (Hammersmith Functional Motor Scale – Expanded)  $\geq 10$  and  $\leq 54$  at Screening
7. Able to complete all study procedures, measurements and visits and parent or guardian/subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator
8. Estimated life expectancy  $> 2$  years from screening, in the opinion of the Investigator
9. Meets age-appropriate institutional criteria for use of anesthesia/sedation, if use is planned for study procedures (as assessed by the Site Investigator and either anesthesiologist or pulmonologist)
10. For subjects who, in the opinion of the Investigator, have reached reproductive maturity, satisfy one of the following:
  - Females: have a negative pregnancy test at Screening and agrees to employ adequate contraceptive measures for the duration of the study. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products.
  - Males: be abstinent for the duration of the study

## 5.2 Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for the study:

1. Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for  $> 6$  hours during a 24 hour period, at Screening
2. Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator
3. Severe contractures or severe scoliosis evident on X-ray examination at Screening
4. Hospitalization for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of screening or planned during the duration of the study
5. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period
6. History of brain or spinal cord disease, including tumors, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation
7. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter
8. History of bacterial meningitis
9. Dosing with ISIS 396443 in any previous clinical study
10. Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline

11. Clinically significant abnormalities in hematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion
12. Treatment with another investigational drug (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, etc.), biological agent, or device within 1-month of screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation
13. Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anemia, etc.) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures

## **6. STUDY PROCEDURES**

### **6.1 Study Schedule**

All required study procedures are outlined in [Appendices A, B and C](#).

### **6.2 Study Assessments**

#### **6.2.1 Laboratory Analytes**

Laboratory measurements of serum chemistry, hematology, urinalysis, coagulation parameters, and plasma antibodies to ISIS 396443 will be performed at the times shown in the Schedule of Procedures ([Appendix A](#)). The analytes to be measured are shown in [Appendix B](#).

#### **6.2.2 Neurological Examinations**

Neurological examinations include assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes. Neurological examinations will be performed at the times shown in the Schedule of Procedures ([Appendix A](#)).

#### **6.2.3 Pharmacokinetics Specimen Collection**

Plasma and CSF specimens will be collected as shown in [Appendix A](#) (Schedule of Procedures) and [Appendix C](#) (Pharmacokinetic Sampling Schedule). The following ISIS 396443 plasma PK parameters (though not necessarily limited to) will be derived when appropriate from the individual subject concentration vs. time profiles using noncompartmental-based methods and based on actual sampling times:

- The maximal observed plasma drug concentration ( $C_{\max}$ )
- The time to reach  $C_{\max}$  in plasma ( $T_{\max}$ )
- The area under the plasma concentrations time curve from the time of the IT dose to the last collected sample
- The apparent terminal elimination half-life ( $t_{1/2}$ ), if possible

#### **6.2.4      *Hammersmith Functional Motor Scale - Expanded***

Subjects will be evaluated using the Hammersmith Functional Motor Scale – Expanded (HFMSE) at the times shown in the Schedule of Procedures ([Appendix A](#)). The HFMSE is a reliable and validated tool used to assess motor function in children with SMA. The scale was originally developed with 20 scored activities and was devised for use in children with SMA Type 2 and Type 3 with limited ambulation to give objective information on motor ability and clinical progression ([Main et al. 2003](#)). The expanded scale includes an additional module of 13 items developed to allow for evaluation of ambulatory SMA patients ([O’Hagen et al. 2007](#)). The HFMSE has been shown to be highly correlated with other clinical assessments and shows good test-retest reliability. The HFMSE is easy to use and quickly administered.

#### **6.2.5      *Motor Milestones***

Subjects will be evaluated for motor milestones at the times shown in the Schedule of Procedures ([Appendix A](#)). Motor Milestones will be assessed using the WHO Motor Milestone criteria ([WHO Multicentre Growth Reference Study Group 2006](#); [Wijnhoven et al. 2004](#)).

#### **6.2.6      *PedsQL™ (Generic Core Scales and Neuromuscular Module)***

Subjects will be evaluated using the Pediatric Quality of Life Inventory (PedsQL™) Measurement 4.0 Generic Core Scales and 3.0 Neuromuscular Module ([Varni et al. 1999](#)) at the times shown in the Schedule of Procedures ([Appendix A](#)). This assessment has been validated by the American Spinal Muscular Atrophy Randomized Trials (AmSMART) group for use in SMA patients from age 2 to 18 years ([Iannaccone et al. 2009](#)).

The PedsQL™ Measurement Model is a modular approach to measuring health-related quality of life (HRQOL) in children and adolescents. The PedsQL consists of brief, practical, generic core scales as well as with condition-specific modules for use in designated clinical populations. Pediatric self-report is measured in children and adolescents ages 5-18 years, and parent proxy-report of child HRQOL is measured for children and adolescents ages 2-18 years. The PedsQL™ 4.0 Generic Core Scales include assessment of physical functioning, emotional functioning, social functioning, and school functioning. The PedsQL 3.0 Neuromuscular Module was designed to measure HRQOL dimensions specific to children aged 2 to 18 years with neuromuscular disorders, including SMA.

#### **6.2.7      *Upper Limb Module Test***

Subjects will be evaluated using the Upper Limb Module Test ([Mazzone et al. 2011](#)) at the times shown in the Schedule of Procedures ([Appendix A](#)). The Upper Limb Module Test is an outcome measure specifically developed to assess upper limb functional abilities in SMA patients, including young children and patients with severe contractures in the lower limbs in whom the possibility to detect functional changes, such as rolling or long sitting, is limited. This test consists of upper limb performance items that are reflective of activities of daily living (i.e., raise a can to mouth as if drinking, take a coin and place it in a box, remove the lid of a container). The Upper Limb Module Test is quickly administered and has been evaluated in SMA patients age 30 months to 27 years ([Mazzone et al. 2011](#)).

### **6.2.8      *Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)***

Parents of subjects will complete the Assessment of Caregiver Experience with Neuromuscular Disease (ACEND) questionnaire at the times shown in the Schedule of Procedures ([Appendix A](#)). This assessment instrument has been designed to quantify the caregiver impact experienced by parents of children affected with severe neuromuscular diseases, including children with SMA ([Matsumoto et al. 2011](#)). The ACEND includes domains assessing physical impact (including feeding/grooming/dressing, sitting/play, transfers, and mobility) and general caregiver impact (including time, emotion, and finance).

### **6.2.9      *Safety Evaluations***

Safety will be evaluated by assessment of AEs including SAEs as described in [Section 9](#). Additional safety evaluations include the following parameters:

- Vital signs and weight
- Neurological examinations
- Physical examinations
- Clinical laboratory tests (serum chemistry, hematology, urinalysis)
- Electrocardiograms (ECGs)
- Use of concomitant medications

## **6.3      Contraception Requirements**

All male subjects must remain abstinent during the study.

All female subjects of childbearing potential must either be abstinent or practice adequate contraception during the study. For the purposes of this study, females of childbearing potential are defined as any female who has experienced menarche. For the purposes of the study, acceptable contraception methods are abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products

## **7.      STUDY DRUG**

### **7.1      Study Drug Description**

Study Drug (ISIS 396443 drug product) characteristics are listed under [Table 1](#).

The Study Drug is contained in 6 mL clear glass vials. The Study Drug and its storage and preparation instructions will be provided by the Sponsor or designee. The Study Drug must be stored securely at 2° to 8° C and protected from light.

**Table 1 Study Drug Characteristics**

Study Drug	ISIS 396443 Drug Product
Strength	2.4 mg/mL
Volume/vial	5.0 mL solution per vial
Route of Administration	IT injection

## 7.2 Packaging and Labeling

The Sponsor will provide the Investigator with packaged Study Drug labeled in accordance with specific country regulatory requirements.

## 7.3 Study Drug Accountability

The study staff is required to document receipt, dispensing and return of Study Drug supplies provided by the Sponsor. Drug accountability documentation and all used and unused Study Drug vials must be returned to the Sponsor or designee.

# 8. TREATMENT OF SUBJECTS

## 8.1 Study Drug Administration

Details regarding the LP dosing injection procedure will be provided in the Dosing Administration Manual. ISIS 396443 will be administered by dedicated study personnel who are unblinded to treatment group; this cannot be any of the key study site personnel (i.e., the Principal Investigator, study coordinator, or outcomes assessors). The study drug administration will be performed in a dedicated room and the key study personnel and the parents will not be present during the procedure to ensure blinding.

ISIS 396443 will be administered as an intrathecal slow bolus (1-3 minute) LP injection. ISIS 396443 will be administered using a ‘spinal anesthesia’ needle and syringe. A 22G to 25G spinal anesthesia needle is recommended, but a 21G may be used if indicated by subject size or clinical condition. The target site for needle insertion is the L3/L4 space, but may be 1 segment above or 1-2 segments below this level, if needed. The volume of the injection is 5 mL; prior to the injection 5 mL of CSF fluid is to be collected for analyses. Depending on institutional guidelines, anesthesia or sedation may be used for the LP procedure, following institutional procedures. Subjects will be encouraged to lie flat for 1 hour following dosing, if possible.

Table 2 outlines the dose, ISIS 396443 concentration, and volume for administration of ISIS 396443.

**Table 2 ISIS 396443 Dose, Concentration, and Injection Volume**

Dose (mg)	Concentration (mg/mL)	Injection Volume (mL)
12	2.4	5.0

Prior to each injection on Study Days 1, 29, 85, and 274, 5 mL of CSF fluid is to be collected for analyses. CSF will be used for measurement of ISIS 396443 pharmacokinetic analyses and CSF SMN protein concentration. Extra CSF may be stored for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with CSF constituents. CSF analyses and data presentation will be conducted in a blinded manner.

## **8.2 Sham Procedure**

Subjects randomized to the sham-procedure control group will undergo a sham-procedure, rather than study drug administration, on Study Days 1, 29, 85, and 274. Details regarding the sham procedure will be provided in the Dosing Administration Manual. The sham procedure will be administered by dedicated study personnel who are unblinded to treatment group; this cannot be any of the key study site personnel (i.e., the Investigator, study coordinator, or outcomes assessors). The sham procedure will be performed in a dedicated room and the key study personnel and the parents will not be present during the procedure to ensure blinding.

In general, the sham procedure will consist of a small needle prick on the lower back at the location where the LP injection is normally made. The needle will break the skin but no LP injection or needle insertion will occur. The needle prick will be covered with the same bandage that is used to cover the LP injection normally, thus simulating the appearance of an LP injection. If anesthesia or sedation is used for the LP procedure in ISIS 396443 treated subjects, then in order to maintain the blind, minimal sedation (i.e. a low dose of an anxiolytic) should be used for the sham procedure, following institutional procedures. The study subject will be kept in the procedure room for the same amount of time that subjects administered study drug are kept, thus simulating the time period of a study drug administration procedure.

Study drug and sham kits will be packaged in a blinded fashion. Blinded kits for sham procedure contain artificial CSF (5.0 mL solution per 6 mL vial) that will not be injected but will be used to simulate CSF samples for that subject.

## **8.3 Other Protocol-Required Drugs**

There are no other protocol required drugs.

## **8.4 Other Protocol-Required Procedures**

There are no other protocol-required treatment procedures.

## **8.5 Treatment Precautions**

There are no protocol-required treatment precautions.

## **8.6 Safety Monitoring Rules**

Please refer to the Guidance to Investigator section of the Investigator Brochure.

### **8.7 Stopping Rules**

There are no additional specific stopping rules for this study but the Investigator should discuss significant concerns relating to individual subjects with the Medical Monitor and the Sponsor to ensure that it is appropriate for the subject to continue Study Drug.

### **8.8 Adjustment of Dose and/or Treatment Schedule**

No adjustment of dose is permitted. In the event of a concurrent illness that would prevent the dosing procedure from being performed safely, an adjustment in the dose schedule may be permitted, but must be approved by the Medical Monitor. In general, each scheduled dose may be delayed by up to 4 weeks.

### **8.9 Discontinuation of Study Treatment**

A subject must permanently discontinue study treatment for any of the following:

- The subject's parents/guardians withdraw consent
- The subject experiences an adverse event that necessitates permanent discontinuation of study treatment

The reason for discontinuation of study treatment must be recorded in the Case Report Form (CRF) and source documentation.

Subjects that discontinue treatment will continue follow-up unless consent is withdrawn ([Appendix A](#)).

### **8.10 Withdrawal of Subjects from the Study**

Subjects must be withdrawn from the study for any of the following:

- Withdrawal of consent
- The subject or the subject's parents/guardians is/are unwilling or unable to comply with the protocol
- The subject experiences a medical emergency that necessitates unblinding of the subject's treatment assignment

Other reasons for withdrawal of subjects from the study might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation
- Decision by the Investigator or Sponsor

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from Study, must be recorded in the CRF.

Any subject for whom consent to participate in the study is withdrawn will be removed from further treatment and study observation immediately upon the date of request. It should be encouraged that these subjects complete the early termination study procedures and observations at the time of withdrawal ([Appendix A](#)).

For subjects withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination study procedures and observations at the time of withdrawal ([Appendix A](#)).

## **8.11 Concomitant Therapy and Procedures**

The use of concomitant therapies or procedures defined below must be recorded on the subject's CRF. AEs related to administration of these therapies or procedures must also be documented on the appropriate CRF.

### **8.11.1 Concomitant Therapy**

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications and vitamin supplements) administered between the beginning of screening and last telephone contact or study visit.

Subject's parents/guardians should consult with the Site Investigator or qualified designee prior to initiating any new medication, including non-prescription compounds or any other non-drug therapy.

### **Allowed Concomitant Therapy**

Throughout the study, Site Investigators or designated licensed physicians involved in the study may prescribe concomitant medications or treatments deemed necessary for adverse events or to provide adequate supportive care.

### **Disallowed Concomitant Therapy**

Study subjects are prohibited from receiving other experimental agents during the study. This includes marketed agents at experimental doses that are being tested for the treatment of SMA (e.g., oral albuterol/salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate, valproate, hydroxyurea).

### **8.11.2 Concomitant Procedures**

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the beginning of screening and last telephone contact or study visit.

## **9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING**

### **9.1 Sponsor Review of Safety Information**

Safety information will be collected, reviewed, and evaluated by the Sponsor in accordance with the Safety Management Plan throughout the conduct of the clinical trial.

## 9.2 Regulatory Requirements

The Sponsor is responsible for regulatory submissions and reporting to the Investigators of SAEs including suspected unexpected serious adverse reactions (SUSARs) per the International Conference on Harmonization (ICH) guidelines E2A and ICH E6. Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

IRBs/IECs will be notified of any serious adverse event (SAE) according to applicable regulations. The DSMB will be notified of any SAE as specified in the DSMB charter.

## 9.3 Definitions

### 9.3.1 Adverse Event

An adverse event is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the study or use of investigational drug product, whether or not the AE is considered related to the investigational drug product.

### 9.3.2 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction is any adverse event caused by the Study Drug.

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

### 9.3.3 Serious Adverse Event (SAE)

A serious adverse event is any adverse event that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event

An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death

- Requires inpatient hospitalization or prolongation of existing hospitalization

Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE

- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female)

- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

#### **9.4 Monitoring and Recording Adverse Events**

Any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible.

##### **9.4.1 Serious Adverse Events**

In the interest of subject safety, and in order to fulfill regulatory requirements, all SAEs (regardless of their relationship to Study Drug) should be reported to the Sponsor within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the subject signs the informed consent form and stop at the end of the subject's follow-up period which is defined as the subject's last visit. When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message. An Initial Serious Adverse Event Form should be completed and a copy should be faxed to the Sponsor or designee.

Detailed information should be actively sought and included on Follow-Up Serious Adverse Event Forms as soon as additional information becomes available. All SAEs will be followed until resolution. SAEs that remain ongoing past the subject's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and Sponsor agree the subject's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

##### **9.4.2 Non-Serious Adverse Events**

The recording of non-serious AEs will begin after the subject signs the informed consent form and will stop at the end of the subject's follow-up period, which is defined as subject's last visit. The Investigator will monitor each subject closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

##### **9.4.3 Evaluation of Adverse Events (Serious and Non-Serious)**

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form.

#### **9.4.3.1 Relationship to the Study Drug**

The event's relationship to the Study Drug is characterized by one of the following:

- **Related:** There is clear evidence that the event is related to the use of Study Drug e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)
- **Not Related:** The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

#### **9.4.3.2 Severity**

The event's severity is characterized by one of the following:

- **Mild:** The event is easily tolerated by the subject and does not affect the subject's usual daily activities
- **Moderate:** The event causes the subject more discomfort and interrupts the subject's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the subject's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in [Section 9.3.3](#)).

#### **9.4.3.3 Action Taken with Study Drug**

Action taken with Study Drug due to the event is characterized by one of the following:

- **None:** No changes were made to Study Drug administration and dose
- **Permanently Discontinued:** Study drug was discontinued and not restarted
- **Temporarily Interrupted, restarted – same dose:** Dosing was temporarily interrupted or delayed due to the AE and restarted at the same dose without unblinding to treatment group

#### **9.4.3.4 Treatment Given for Adverse Event**

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form (treatment should also be recorded on the concomitant treatment or ancillary procedures CRF as appropriate).

#### 9.4.3.5 *Outcome of the Adverse Event*

If the event is a non-serious AE then the event's outcome is characterized by one of the following:

- **AE Persists:** Subject terminates from the trial and the AE continues
- **Recovered:** Subject recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity (if applicable):** AE severity changed

If the event is a SAE then the event's outcome is characterized by one of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Subject has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE electronic case report form (eCRF) (the SAE resolution date should be entered as the date of onset of that AE)
- **Recovered:** Subject recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Fatal:** Subject died (the date of death should be entered as the SAE resolution date)

### 9.5 *Procedures for Handling Special Situations*

#### 9.5.1 *Abnormalities of Laboratory Tests*

Clinically significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Medical Monitor. Laboratory abnormalities deemed not clinically significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents.

### **9.5.2      *Prescheduled or Elective Procedures or Routinely Scheduled Treatments***

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

### **9.5.3      *Dosing Errors***

Study drug errors defined as errors in administration or the administered dose should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic.

Dosing details should be captured on the Dosing CRF.

**Should an overdose occur**, the Investigator or designee should contact the Unblinded Medical Monitor within 24 hours.

### **9.5.4      *Contraception and Pregnancy***

Female subjects that have reached reproductive maturity must have a negative pregnancy test at Screening and must not be able to become pregnant for the duration of the study, as described in [Section 6.3](#).

Male subjects must be abstinent during the duration of the study.

If a subject becomes pregnant or a pregnancy is suspected, or if a male subject makes or believes that he has made someone pregnant during the study, then the site staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported on Follow-up Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the subject's responsibility.

## **10. STATISTICAL CONSIDERATIONS**

### **10.1 Study Endpoints, Subsets, and Covariates**

#### **10.1.1 Primary Endpoint**

- Change from baseline in HFMSE (Hammersmith Functional Motor Scale – Expanded) score at 15 months

#### **10.1.2 Secondary Efficacy Endpoints**

- Proportion of subjects who achieve a 3-point increase from baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months
- Proportion of subjects that achieve any new motor milestone at 15 months
- Number of motor milestones achieved per subject at 15 months
- Change from baseline in Upper Limb Module Test at 15 months
- Proportion of subjects that achieve standing alone at 15 months
- Proportion of subjects that achieve walking with assistance at 15 months

#### **10.1.3 Tertiary Efficacy Endpoints**

- Change from baseline in CSF SMN protein concentration
- Clinical Global Impression of Change (Investigator and Caregiver assessment)
- Change from baseline in PedsQL (Pediatric Quality of Life Inventory)
- Change from baseline in Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)
- Disease-related hospitalizations and adverse events

#### **10.1.4 Safety/Tolerability Endpoints**

- Adverse events
- Vital signs and weight
- Neurological examinations
- Physical examinations
- Clinical laboratory tests (serum chemistry, hematology, urinalysis)
- Electrocardiograms (ECGs)
- Use of concomitant medications

### **10.1.5    *Pharmacokinetic Endpoints***

- CSF levels of ISIS 396443
- Plasma levels of ISIS 396443

### **10.1.6    *Immunogenicity Endpoint***

- Plasma antibodies to ISIS 396443

## **10.2    Sample Size Considerations**

The sample size for this study was estimated based on limited available natural history data for the target population and data from the ISIS 396443-CS1 and ISIS 396443-CS2 clinical studies. 70 patients in the treated group and 35 patients in the control group will give at least 90% power to detect a 3 point difference between control and treated groups in change on the HFMSE with a standard deviation of 4.4, using a two-sided t-test with an alpha level of 0.05. The sample size was estimated using n-Query. 117 patients enrolled will ensure that a small dropout rate will not affect the power of the primary efficacy analysis.

## **10.3    Populations**

Intent to Treat (ITT) Set: All patients who are randomized, receive at least one dose of study drug/sham procedure, and have a baseline and at least one post baseline efficacy evaluation.

Per-Protocol Set (PPS): PPS will include the subset of the ITT who complete at least the initial 3 doses of study drug/sham procedures, have baseline and Day 169 efficacy assessments and who have no significant protocol deviations that would be expected to affect efficacy assessments.

Safety Set: All patients who are randomized and receive at least one dose of study drug/sham procedure.

Pharmacokinetic Population: All patients who are randomized and for which there is at least one evaluable post-dose/post-sham procedure pharmacokinetic sample.

## **10.4    Definition of Baseline**

The baseline is defined as the last non-missing assessment prior to the first dose of Study Drug.

## **10.5    Interim Analysis**

An interim analysis may take place when all subjects have completed the 6 month assessment and at least 39 subjects have completed the 15 month assessment. At the interim analysis, the last recorded observation for each subject (i.e., at 6 to 15 months) will be used in the analysis. The data will be analyzed using ANCOVA with patient age at screening and baseline value as covariates.

A DSMB will review the interim analysis results and make determinations on whether it is appropriate for the trial to continue. During the interim analysis, subject accrual will continue. Details of the analysis and controlled access to the unblinded data are contained in the Statistical Analysis Plan (SAP) and DSMB Charter.

If the interim analysis is performed, based on the Lan-DeMets method for group sequential trials using O'Brien-Fleming boundaries ([Reboussin et al. 2000](#)), for a single interim analysis conducted using approximately 60% of the information, the levels of significance for the interim and final analyses are 0.01 and 0.0468, respectively. In order to control the Type 1 error, a sequential closed testing procedure with significance level of 0.01 will be employed with the sequence of the endpoints as defined in [Sections 10.1.1](#) and [10.1.2](#) at the interim analysis. A significance level of 0.0468 will be used for sequential testing procedure at the final analysis. However, if the interim analysis is not performed, significance level for the final analysis will remain at 0.05.

## **10.6 Planned Methods of Analysis**

Data collected on eCRF, lab data transfers, as well as any outcomes derived from the data, will be provided in the patient data listings. Patient data listings will be presented for all patients enrolled into the study.

Descriptive summary statistics including n, mean, median, standard deviation, standard error, interquartile range (25th percentile, 75th percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data by treatment group.

All primary, secondary and tertiary endpoints will be assessed in the ITT Set and PPS, with the former being the basis for the primary efficacy analysis. All safety assessments will be performed on the Safety Set. PK endpoints will be assessed in the PK Set as applicable.

### **10.6.1 Demographic and Baseline Characteristics**

Demographic and baseline characteristics will be summarized using descriptive statistics by treatment group. Patient disposition will be summarized by treatment group. All patients enrolled will be included in a summary of patient disposition.

### **10.6.2 Safety and Tolerability Analysis**

Safety analyses will be conducted in the Safety Set. Treatment duration and amount of study drug received will be summarized by treatment group.

All treatment-emergent adverse events and serious adverse events will be summarized for each treatment group using the MedDRA™ coding system, by system organ class, preferred term, relationship to Study Drug, and severity. Narratives of deaths, serious adverse events, including early withdrawals from study drug and from study due to adverse events, will also be provided.

Laboratory tests including chemistry panel, complete blood count with differential, etc. will be summarized by study visit for each treatment group. These safety variables will also be presented over time after study drug administration, as appropriate. Vital sign results will be presented similarly.

Physical and neurological examination findings and results from ECG will be listed for review. As appropriate, results will also be summarized descriptively for each treatment group. Concomitant medication usage for each subject will be listed for review.

### 10.6.3 Efficacy Analysis

All statistical tests will be 2-sided with a Type 1 error rate of 5%, unless otherwise specified.

Secondary efficacy endpoints have been rank prioritized in the order shown in [Section 10.1.2](#). In order to control the Type 1 error, a sequential closed testing procedure will be employed with the sequence of endpoints defined as above. Testing of secondary endpoints will be performed only if the treatment comparison of the primary endpoint is statistically significant ( $p < 0.050$ , unless interim analysis is performed). Testing of the secondary endpoints will be conducted according to the sequence above: if the first secondary endpoint (proportion of patients that achieve a 3-point increase from baseline in HFMSE at 15 months) is statistically significant ( $p < 0.050$ , unless interim analysis is performed) then the second secondary endpoint (proportion of subjects that achieve any new motor milestone at 15 months) will be tested at the same significance level. However, if the treatment comparison based on the first secondary endpoint is not statistically significant, then all endpoints of a lower rank will not be considered statistically significant. This process is repeated with each subsequent secondary endpoint.

Analyses of tertiary efficacy endpoints will not include adjustments for multiplicity.

The primary analysis of the primary endpoint is to compare the change from baseline in HFMSE score at Month 15 between treatment groups using the ITT Set. The data will be analyzed using a Mixed Effects Model with Repeated Measures (MMRM) model where the treatment group, time, treatment by time interaction, and patient age at Screening will be included in the model as fixed effects; patient will be a random effect; the baseline HFMSE score will be included as a covariate. No imputation for missing data will be made. The unstructured covariance model will be used and test of fixed effects will be carried out. The treatment contrast for Month 15 will be estimated by the model. The primary efficacy analysis will take place after all patients have completed the Day 456/ET visit and the database has been locked.

The following sensitivity analyses will also be conducted, and details of the analyses will be outlined in the SAP:

- The primary analysis will be repeated in the PPS.
- The primary analysis will be repeated in the subset of ITT Set who have non-missing Month 15 HFMSE score.
- The LOCF method will be used to impute missing Month 15 HFMSE score. Stratified Wilcoxon Rank Sum test and ANCOVA with the stratification factor and baseline HFMSE score as covariates will be used to analyze the imputed data.

The primary analysis of each secondary endpoint will be based on the ITT Set. Analyses based on the PPS are considered sensitivity analyses.

- Comparison of proportion of subjects that achieve a 3-point increase from baseline in HFMSE score at Month 15 between the treatment groups. The data will be analyzed using the logistic regression adjusting for each patient's age at screening and baseline HFMSE score. If a patient terminates study before Month 15 due to AE, lack of efficacy or other types of treatment failure, then the patient will be considered as a non-responder.

If a patient terminates study before Month 15 or has missing HFMSE score at Month 15 due to any other reason, then the patient will not be included in the analysis.

- Comparison of proportion of subjects that achieve any new motor milestone at Month 15 between the treatment groups. The data will be analyzed using the logistic regression adjusting for each patient's age at Screening and baseline number of motor milestones that a patient has achieved. If a patient terminates study before Month 15 due to AE, lack of efficacy or other types of treatment failure, then the patient will be considered as a non-responder. If a patient terminates study before Month 15 or has missing data at Month 15 due to any other reason, then the patient will not be included in the analysis.
- Comparison of number of motor milestones achieved per subject at Month 15 between treatment groups. The data will be analyzed in a similar way to the primary endpoint.
- Comparison of change from baseline in Upper Limb Module Test at Month 15 between treatment groups. The data will be analyzed in a similar way to the primary endpoint.
- Comparison of proportion of subjects that achieve standing alone milestone at Month 15 between the treatment groups. The data will be analyzed in a similar way to the proportion of subjects that achieve any new motor milestone at Month 15.
- Comparison of proportion of subjects that achieved walking with assistance milestone at Month 15 between the treatment groups. The data will be analyzed in a similar way to the proportion of subjects that achieve any motor milestone at Month 15.

#### **10.6.4     *Pharmacokinetic Analysis***

Plasma pharmacokinetic parameters and ISIS 396443 concentrations in plasma and CSF for the Pharmacokinetic population will be summarized using descriptive statistics and, where warranted, presented graphically.

### **11.     INVESTIGATOR'S REGULATORY OBLIGATIONS**

#### **11.1     Informed Consent/Assent**

The written informed consent and assent documents should be prepared in the language(s) of the potential subject population, based on an English version provided by the Sponsor and should be easy to understand.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent or legal guardian and, in cases where institutional guidelines and the subject's age dictate, informed assent from the subject, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any study drugs are administered. Sufficient time must be given to consider whether to participate in the study.

The acquisition of informed consent/assent and the parent/legal guardian's/subject's agreement or refusal of his/her notification of the primary care physician should be documented in the subject's medical records, and the informed consent/assent form(s) should be signed and personally dated by the parent/legal guardian/subject and by the study person who conducted the

informed consent/assent discussion. The original signed informed consent/assent form(s) should be retained in the Study Master File and in any other locations required by institutional policy, and a copy of the signed consent/assent form(s) should be provided to the parent or guardian.

### **11.2 Ethical Conduct of the Study**

The Guidelines of the World Medical Association (WMA) Declaration of Helsinki dated October 2002 the applicable regulations and guidelines of current Good Clinical Practice (GCP) as well as the demands of national drug and data protection laws and other applicable regulatory requirements will be strictly followed.

### **11.3 Institutional Review Board/Institutional Ethics Committee/Research Ethics Board**

A copy of the protocol, proposed informed consent form, proposed informed assent form (if applicable) other written subject information, and any proposed advertising material must be submitted to the IRB/IEC for written approval. A copy of the written approval of the protocol and informed consent/assent forms must be received by the Sponsor before recruitment of subjects into the study and shipment of Study Drug. A copy of the written approval of any other items/materials that must be approved by the Study Center or IRB/IEC must also be received by the Sponsor before recruitment of subjects into the study and shipment of Study Drug. The Investigator's Brochure must be submitted to the IRB/IEC for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the IRB/IEC for all subsequent protocol amendments and changes to the informed consent/assent documents. The Investigator should notify the IRB/IEC of deviations from the protocol in accordance with ICH GCP Section 4.5.2. The Investigator should also notify the IRB/IEC of serious adverse events occurring at the Study Center and other adverse event reports received from the Sponsor, in accordance with local procedures.

The Investigator will be responsible for obtaining annual IRB/IEC approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all IRB/IEC submissions and the IRB/IEC continuance of approval must be sent to the Sponsor.

### **11.4 Subject Confidentiality**

The Investigator must ensure that the subject's confidentiality is maintained. On the CRFs or other documents submitted to the Sponsor, subjects should be identified by unique, anonymous initials and a subject study number only. Documents that are not for submission to the Sponsor (e.g., signed informed consent/assent forms) should be kept in strict confidence by the Investigator.

In compliance with federal and local regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB/IEC direct access to review the subject's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The Investigator is obligated to inform and obtain the consent of the subject's parent or guardian to permit named representatives to have access to his/her study-related records without violating the confidentiality of the subject.

## **12. ADMINISTRATIVE AND LEGAL OBLIGATIONS**

### **12.1 Protocol Amendments**

Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent/assent documents. The regulatory authority and IRB/IEC must be informed of all amendments and give approval for any amendments likely to affect the safety of the subjects or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the IRB/IEC to the Sponsor.

### **12.2 Study Termination**

The Sponsor reserves the right to terminate the study. The Investigator reserves the right to terminate their participation in the study, according to the terms of the site contract. The Investigator/Sponsor should notify the IRB/IEC in writing of the trial's completion or early termination and send a copy of the termination to the Sponsor.

### **12.3 Study Documentation and Storage**

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the subject's CRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, and correspondence. In this study, CRFs may not be used as source documents.

The Investigator and Study Center staff is responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with Section 8 of the ICH Guidelines (E6), suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. Elements should include:

- Subject files containing completed CRFs, informed consents/assents, and supporting copies of source documentation
- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the IRB and the Sponsor
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction, and all drug-related correspondence

In addition, all original source documents supporting entries in the CRFs must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor.

## **12.4 Study Monitoring**

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., CRFs and other pertinent data) provided that subject confidentiality is respected.

The Sponsor monitor is responsible for inspecting the CRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the CRFs.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department. Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data a clinical data management review will be performed on subject data received by the Sponsor. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor.

The Principal Investigator will sign and date the indicated places on the CRF. These signatures will indicate that the Principal Investigator inspected or reviewed the data on the CRF, the data queries, and the Study Center notifications, and agrees with the content.

## **12.5 Language**

CRFs must be completed in English. Generic names for concomitant medications should be recorded in English if possible, unless it is a combination drug, then record the trade name in English.

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood.

## **12.6 Compensation for Injury**

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Subjects will be treated and/or compensated for any study-related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

### 13. REFERENCES

- Cho S and Dreyfuss G. A degtron created by SMN2 Exon 7 skipping is a principal contributor to spinal muscular atrophy severity. *Genes Dev* 2010; 24: 438-442.
- Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO, Mendell JR, Coulson SE, Androphy EJ, Prior TW, Burghes AH. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet* 1997; 6: 1205-1214.
- Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time light cycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am J Hum Genet* 2002; 70: 358-368.
- Geary RS, Yu RZ, Watanabe T, Henry SP, Hardee GE, Chappell A, Matson J, Sasnor H, Cummins L, Levin AA. Pharmacokinetics of a tumor necrosis factor- $\alpha$  phosphorothioate 2'-O-(2-Methoxyethyl) modified antisense oligonucleotide: comparison across species. *Drug Metab Disp* 2003; 31: 1419-1428.
- Helmken C, Hofmann Y, Schoenen F, Oprea G, Raschke H, Rudnik-Schöneborn S, Zerres K, Wirth B. Evidence for a modifying pathway in SMA discordant families: reduced SMN level decreases the amount of its interacting partner and Htra2-beta1. *Hum Genet* 2003; 114: 11-21.
- Henry S, Stecker K, Brooks D, Monteith D, Concklin B, Bennett CF. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J Pharmacol Exp Ther* 2000; 292: 468-479.
- Hua Y, Vickers TA, Baker BF, Bennett CF, Krainer AR. Enhancement of SMN2 Exon 7 inclusion by antisense oligonucleotides targeting the exon. *PLoS Biol* 2007; 5: e73.
- Hua Y, Vickers TA, Okunola HL, Bennett CF, Krainer AR. Antisense masking of an HnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am J Hum Genet* 2008; 82: 834-848. Epub 2008 Mar 27.
- Iannaccone ST, Hynan LS, Morton A, Buchanan R, Limbers CA, Varni JW, the AmSMART Group. The PedsQL in pediatric patients with spinal muscular atrophy: feasibility, reliability, and validity of the pediatric quality of life inventory generic core scales and neuromuscular module. *Neuromuscul Disord* 2009; 19: 805-812.
- Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat.Rev.Genet* 2010; 11: 345-355.
- Le TT, Pham LT, Butchbach ME, Zhang HL, Monani UR, Coovert DD, Gavrilina TO, Xing L, Bassell GJ, Burghes AH. SMN $\Delta$ 7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. *Hum Mol Genet* 2005; 14: 845-857. Epub 2005 Feb 9.
- Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M, Le Paslier D, Frézal J, Cohen D, Weissenbach J, Munnich A, Melki J. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995; 80: 155-165.
- Lefebvre S, Burlet P, Liu Q, Bertrand S, Clermont O, Munnich A, Dreyfuss G, Melki J. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet* 1997; 16: 265-269.
- Main M, Kairon H, Mercuri E, Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. *Eur J Paediatr Neurol* 2003; 7(4): 155-159.
- Matsumoto H, Clayton-Krasinski DA, Klinge SA, Gomex JA, Booker WA, Hman JE, Roye Jr. DP, Vitale MG. Development and initial validation of the assessment of caregiver experience with neuromuscular disease. *J Pediatr Orthop* 2011; 31: 284-292.
- Mazzone E, Bianco F, Martinelli D, Glanzman A, Messina S, De Sanctis R, Main M, Eagle M, Florence J, Krosschell K, Vasco G, Pelliccioni M, Lombardo M, Pane M, Finkel R, Muntoni F, Bertini E, Mercuri E. Assessing upper limb function in nonambulant SMA patients: development of a new module. *Neuromuscul Disord* 2011; 21: 406-412.

McKay RA, Miraglia LJ, Cummins LL, Owens SR, Sasmor H, Dean NM. Characterization of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C- $\alpha$  expression. *J Bio Chem* 1999; 274: 1715-1722.

O'Hagen JM, Glanzman AM, McDermott MP, Ryan PA, Flickinger J, Quigley J, Riley S, Sanborn E, Irvine C, Martens WB, Annis C, Tawil R, Oskoui M, Darras BT, Finkel RS, De Vivo DC. An expanded version of the hammersmith functional motor scale for SMA II and III patients. *Neuromuscular Disorders* 2007; 17: 693-697.

Passini MA, Bu J, Richards AM, Kinnecom C, Sardi SP, Stanek LM, Hua Y, Rigo F, Matson J, Hung G, Kaye EM, Shihabuddin LS, Krainer AR, Bennett CF, Cheng SH. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Science Trans Med* 2011; 3: 1-12.

Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2 gene. *Am J Med Genet* 2004; 130A: 307-310.

Reboussin DM, DeMets DL, Kim K, Lan KKG. Computations for group sequential boundaries using the Lan-DeMets spending function method. *Controlled Clinical Trials* 2000; 21: 190-207.

Varni JW, Seid M, Rode CA. Measurement model for the pediatric quality of life inventory. *Medical Care* 1999; 37: 126-139.

WHO Multicentre Growth Reference Study Group. Assessment of sex differences and heterogeneity in motor milestone attainment among populations in the WHO Multicentre Growth Reference Study. *Acta Pædiatrica*, 2006; Suppl 450: 66/75.

Wijnhoven T, de Onis M, Onyango A, Wang T, Bjoerneboe G, Bhandari N, Lartey A, Al Rashidi B. Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food Nutr Bull* 2004; 21: S37-45.

## **14. APPENDICES**

## **Appendix A      Schedule of Procedures**

## Appendix A Schedule of Procedures

Subjects will also be monitored through phone contact on Study Days 8, 56, 113, 141, 204, 239, 302, 330, 393, 421 (all  $\pm 2$  days)<sup>9</sup>

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 (±1D)			D30	D85 (±2D)			D86	D92 (±1D)	D169 (±2D)	D274 (±7D)			D275	D365 (±7D) and D456 (+ 7D) and Early Term
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
Informed Consent	X																			
Inclusion/Exclusion Criteria	X																			
Medical History	X																			
Screening X-ray	X																			
Urine Pregnancy Test <sup>11</sup>	X																			X <sup>14</sup>
SMN2 copy Number <sup>12</sup>	X																			
Study Drug Injection/Sham Procedure			X				X <sup>7</sup>				X <sup>7</sup>						X <sup>7</sup>			
In-Patient Stay (24 hours)				X																
Vital Signs <sup>1</sup>	X	X		4X <sup>2</sup>	X <sup>3</sup>	X		4X <sup>2</sup>	X	X		4X <sup>2</sup>	X	X	X	X		4X <sup>2</sup>	X	X
Weight	X	X				X				X				X	X	X				X
Height/ulnar length	X																			X <sup>14</sup>
Physical Examination	X	X				X				X				X	X	X				X
Neurological Examination	X	X		X <sup>4</sup>	X <sup>3</sup>	X		X <sup>4</sup>	X	X		X <sup>4</sup>	X	X	X	X		X <sup>4</sup>	X	X
ECG	X				X			X						X						X <sup>14</sup>
Safety Labs <sup>5</sup>	X	X			X	X				X					X	X				X <sup>14</sup>
Coagulation Labs	X																			
Immunogenicity		X				X				X					X	X				X <sup>14</sup>

## Appendix A Schedule of Procedures *Continued*

Study Period	Screen	Treatment/Follow-up																		
Study Day	D -28 to D -1	D1			D2	D29 (±1D)			D30	D85 (±2D)			D86	D92 (±1D)	D169 (±2D)	D274 (±7D)			D275	D365 (±7D) and D456 (+ 7D) and Early Term
		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose		Pre-dose	LP/ SP	Post-dose				Pre-dose	LP/ SP	Post-dose		
CSF PK <sup>8</sup>		X				X				X						X				
CSF SMN Protein		X				X				X						X				
Plasma PK <sup>8</sup>		X		3X	X	X				X		X			X	X				X <sup>14</sup>
HFMSE	X <sup>10</sup>													X	X	X <sup>13</sup>				X
WHO Motor Milestones	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
Upper Limb	X <sup>6, 10</sup>													X	X	X <sup>13</sup>				X
PedsQL	X <sup>6</sup>													X	X	X <sup>13</sup>				X
ACEND	X <sup>6</sup>														X					X <sup>14</sup>
CGI														X	X	X <sup>13</sup>				X
Con Med Recording <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Collection <sup>9</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

- 1 Resting blood pressure, pulse, respiratory rate, and temperature
- 2 Vital signs performed 1, 2, 4, 6 hours after dosing
- 3 Conducted within 20-24 hours after dosing
- 4 Neurological exams at 5 hours after dosing
- 5 Serum chemistry, hematology, urinalysis panels ([Appendix B](#) for analytes)
- 6 Efficacy assessments (with the exception of HFMSE) do not form part of the screening assessment or inclusion/exclusion criteria but are baseline measurements taken during the screening phase of the study
- 7 Overnight stay is optional on Day 29, Day 85, and Day 274
- 8 Refer to [Appendix C](#) for PK sampling schedule
- 9 At telephone contact, changes in concomitant medications and adverse events will be recorded
- 10 To be performed 2 times during the screening period
- 11 Urine pregnancy test performed for females of child-bearing potential, if positive to be confirmed by local serum test
- 12 Only for those subjects who do not have documented evidence of SMN copy number from Athena Diagnostics
- 13 These assessments may be performed up to 7 days prior to dosing, if necessary
- 14 Assessed on Day 456 only

## **Appendix B      Laboratory Analytes**

## Appendix B Laboratory Analytes

CLINICAL SAFETY ASSESSMENTS (minimum requirements)		OTHER ASSESSMENTS
<u><b>Clinical Chemistry</b></u> Sodium Potassium Chloride Total protein Albumin Calcium Phosphorus Bicarbonate Glucose BUN Creatinine Cystatin C Total serum Bilirubin Alkaline phosphatase AST (SGOT) ALT (SGPT) CPK  <u><b>Coagulation</b></u> aPTT PT INR	<u><b>Urinalysis</b></u> Specific gravity pH Protein Glucose Ketones Bilirubin Blood Red blood cells White blood cells Epithelial cells Bacteria Casts Crystals  <u><b>Hematology</b></u> Red blood cells Hemoglobin Hematocrit Platelets White blood cells WBC Differential <ul style="list-style-type: none"> <li>(% and absolute)</li> <li>Neutrophils</li> <li>Eosinophils</li> <li>Basophils</li> <li>Lymphocytes</li> <li>Monocytes</li> </ul>	<u><b>Pregnancy</b></u> Urine hCG  <u><b>PK*</b></u> Plasma ISIS 396443 levels CSF ISIS 396443 levels  <u><b>PD</b></u> CSF SMN protein levels  <u><b>Immunogenicity Evaluation</b></u> ISIS 396443 plasma Abs  <u><b>SMN Genetics</b></u> SMN2 Copy Number

\* Any of the collected PK plasma and CSF samples from the study patients may also be used for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with plasma and CSF constituents.

## **Appendix C      Pharmacokinetic Sampling Schedule**

## Appendix C Pharmacokinetic Sampling Schedule

Treatment Period	Study Day	Timepoints	Blood Collection	CSF Collection
Multiple Dose: LP Injection	D1	Predose	0.35 mL	0.5 mL
		2 hr	0.35 mL	NA
		4 hr	0.35 mL	NA
		8 hr	0.35 mL	NA
	D2	24 hr	0.35 mL	NA
	D29	Predose	0.35 mL	0.5 mL
	D85	Predose	0.35 mL	0.5 mL
		4 hr	0.35 mL	NA
	D169	Anytime	0.35 mL	NA
	D274	Predose	0.35 mL	0.5 mL
	D456	Anytime	0.35 mL	NA

NA Not applicable (No Collection Scheduled)

Details on sampling, preparation, and shipment are included in the study laboratory manual.

Any of the collected PK plasma and CSF samples from the study patients may also be used for investigation of possible biomarkers of SMA disease or the pharmacodynamic effects of ISIS 396443 (e.g., CSF mRNA, CSF protein, CSF miRNA panel) or for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity assessments (including assay development and validation purposes), or to assess other actions of ISIS 396443 with plasma and CSF constituents.