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PHARMACEUTICALS

Statistical Analysis Plan

ISIS 396443-CS3A

A Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of Nusinersen (ISIS 396443) Delivered Intrathecally to Patients with Infantile-Onset Spinal Muscular Atrophy

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Statistical Analysis Plan Signature Page

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Compound Name: 396443

Protocol: CS3A

Study Title: A Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of Nusinersen (ISIS 396443) Delivered Intrathecally to Patients with Infantile-Onset Spinal Muscular Atrophy

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Section	Title	Change/Rationale
1.2	Objectives	Updated to reflect the changes in protocol amendment 6
1.3	Endpoints	Updated to reflect the changes in protocol amendment 6
3.1.2	Subject Population Analyzed	Added 2 SMN2 Copy Set/To provide additional analysis for the subset of subjects who have 2 copies of the *SMN2* gene
3.1.5	Incomplete or Missing Data	Added missing value imputation method for motor milestone Changed missing value imputation method for CHOP-INTEND assessment/To apply the same imputation method to both primary endpoint and CHOP-INTEND assessment
3.2	Primary Efficacy Analysis	New section/To provide details of the primary efficacy analysis
3.3	Secondary Efficacy Analysis	New section/To provide details of the secondary efficacy analyses
3.6.1	Adverse Events	Added definition for lumber puncture related treatment emergent adverse events and added information about the linked ae records/Clarification
3.6.1.1	Adverse Events over Time	New section
3.6.1.2	Adverse events following Dosing	New section
3.6.5	Lead Electrocardiograms (ECG)	Added outlier analysis

1. INTRODUCTION

This document provides a description of the study organization, study procedures, and the plan for the statistical analysis of the study data. Section 1 discusses study design, objectives, and endpoints; Section 2 provides the study procedures; Section 3 provides the detailed plan for the statistical analyses.

As with any statistical analysis plan (SAP), the proposed methods and approaches to the data analysis should be viewed as flexible. The statistical analysis to some degree is iterative since so much of the planning is based on statistical and other assumptions, which require verification.

1.1 Study Overview

This is a Phase 2, open label study conducted at multiple centers in the United States and Canada. Approximately 8-20 subjects will be enrolled into this multiple-dose study of ISIS 396443. The number of subjects may be higher if some subjects must be replaced and/or if the sizes of some cohorts are expanded in order to obtain further experience with some dose levels.

This study will test the clinical efficacy, safety, tolerability, and pharmacokinetics of multiple doses of ISIS 396443 administered as intrathecal (IT) injections by lumbar puncture (LP).
Two 'loading' dose levels (scaled by infant age to be equivalent to 2 year old doses of 6 mg or 12 mg, based on CSF volume) will be evaluated sequentially. The initial dose level of 6 mg will be studied in a cohort of 4 subjects. The 12 mg dose level will be studied in 4 to approximately 16 subjects. Following this, all subjects will receive 'maintenance' dosing of 12 mg equivalent ISIS 396443 on Days 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261.
Cohort 1 (n=4): 'Loading' dosing of 6 mg equivalent ISIS 396443 on Days 1, 15, 85, IT injection; 'maintenance' dosing of 12 mg equivalent on Days 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261, IT injection
Cohort 2 (n=4-16): 'Loading' dosing of 12 mg equivalent ISIS 396443 on Days 1, 15, 85, IT injection, 'maintenance' dosing of 12 mg equivalent on Days 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261, IT injection

After parental informed consent is obtained, subjects will undergo a Screening evaluation no greater than 21 days prior to first dose administration at which 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 an LP injection of study drug (ISIS 396443). Following the LP injection on Day 1, subjects will remain at the study center for at least 24 hours post injection for safety monitoring. Subjects will return to the study center on Days 15, 85, 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261 for follow-up evaluations of clinical efficacy and safety and subsequent injections. Following LP injections on Study Days 15, 85, 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261 subjects

will not stay overnight in the hospital but will be monitored for at least 6 hours post-injection before leaving the study facility. A CSF sample will be taken pre-dose on each injection day for safety and Pharmacokinetic (PK) analyses. During the treatment period, the study center will monitor the subject's condition through safety monitoring visits on Study Days 16, 29, 86, 92, 169, 254, 337, 442, 568, 694, 820, 946, 1072, and 1198, and by telephone contact on Study Days 8, 43, 57, 71, 106, 127, 134, 148, 189, 197, 218, 239, 274, 295, 316, 358, 380, 400, 421, 463, 484, 506, 526, 547, 589, 610, 632, 652, 673, 715, 736, 758, 778, 799, 841, 862, 884, 904, 925, 967, 988, 1010, 1030, 1051, 1093, 1114, 1136, 1156, 1177, 1219, 1240, 1262 (i.e., approximately every 3 weeks). During the post-treatment follow-up period, the study center will monitor the subject's condition through telephone contact on Study Days 1282, 1303, and 1324 (i.e., every 3 weeks through 13 weeks after the last dose of ISIS 396443). Safety monitoring visit will occur on Day 1352 (through 13 weeks after the last dose of ISIS 396443). If a subject terminates early from the study, they will be encouraged to complete all assessments per the Day 1352 visit.

1.2   Objectives

1.2.1   Primary Objective

To examine the clinical efficacy of multiple doses of ISIS 396443 administered intrathecally to patients with Infantile-Onset SMA.

1.2.2   Secondary Objectivse

To examine the safety and tolerability of multiple doses of ISIS 396443 administered intrathecally to patients with Infantile-Onset SMA.

To examine the cerebral spinal fluid (CSF) and plasma PK of multiple doses of ISIS 396443 administered intrathecally to patients with infantile-onset SMA.

1.3   Endpoints

1.3.1   Primary Endpoint

The primary endpoint is the proportion of subjects who achieve improvement in motor milestones as evaluated by Module 2 of the Hammersmith Infant Neurological Examination. Improvement is defined as achievement of at least one of the following:
  (i)   at least a 2-point increase from baseline or achievement of pincer grasp in the category of voluntary grasp, or
  (ii)   at least a 2-point increase in the ability to kick or achievement of touching toes, or
  (iii) a 1-point increase in any of the remaining 6 categories: head control, rolling, sitting, crawling, standing, or walking.

1.3.2   Secondary Endpoints

Secondary efficacy endpoints include:
  • Event-free survival determined by the proportion of subjects who are alive and do not require permanent ventilatory support (defined as tracheostomy or the need for ≥16

hours ventilation/day continuously for at least 2 weeks in the absence of an acute reversible illness)

- Improvement in muscle strength as measured by the Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease (CHOP INTEND)
- Improvement in neuromuscular electrophysiology measured by the Compound Muscle Action Potential (CMAP) of the ulnar and peroneal nerves

The safety and tolerability of multiple doses of ISIS 396443 as assessed by:

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

Pharmacokinetic Measures
- CSF and Plasma pharmacokinetics of ISIS 396443

1.3.3 *Exploratory Endpoints*

The following efficacy evaluations will be performed as exploratory endpoints:

- Measures of respiratory status (number of respiratory events, respiratory infections, respiratory-related hospitalizations, ventilator use and O2 saturation awake)
- Growth parameters (weight for age/length, head circumference, chest circumference, head to chest circumference ratio, arm circumference)

2 PROCEDURES

2.1 General Overview of Procedures

Ionis will review all study data including source documents, CRFs, and laboratory reports. Study site will enter subject source data into the case report form.

2.2 Randomization & Treatment Allocation

This is an open label study. Subjects won't be randomized. Subjects will be registered for the trial before they begin dosing.

2.3 Conduct

The study will be conducted in accordance with current Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) guidelines, the World Medical Association Declaration of Helsinki guidelines, the Food and Drug Administration (FDA) Code of Federal Regulations, and all other local regulatory requirements.

2.4 AE Reporting

Ionis Pharmaceuticals, Inc. is responsible for processing all reported AEs. All serious adverse events, reported to Ionis Pharmaceuticals, Inc. (or designee), will be reviewed according to Ionis standard operating procedures. The Sponsor Medical Monitor and Drug Safety Physician will review all AEs and SAEs on an ongoing basis throughout the study. Ionis Pharmaceuticals, Inc. (or designee) will prepare and submit safety reports to the health authorities. If it becomes necessary to communicate new safety information, Ionis Pharmaceuticals, Inc. (or designee) will also prepare a safety notification letter and transmit it to study sites.

2.5 Data Management

2.5.1 Case Report Form Data

BioClinica (or designee) is responsible for creating the Electronic Data Capture (EDC) data entry screens, database and edit checks using definitions developed by Ionis Pharmaceuticals, Inc. Ionis Pharmaceuticals, Inc. is responsible for the review, data management querying and locking of the database.

Data are single-entered into the EDC system by the investigator site staff. Programmed edit checks (computer logic that checks the validity of the data entered and also prompts for missing data that is expected to be entered) are run and automatic queries are generated. Ionis Pharmaceuticals, Inc. reviews all data for accuracy and validity and generates additional queries in the EDC system when necessary. The data are corrected or an explanation concerning the query is provided in the EDC system. After all data are entered, source data verified, reviewed (by Data Management and Clinical Development) and queried, and all queries resolved, the database is locked.

2.5.2 Laboratory Data

The safety laboratory analysis including chemistry, hematology, urinalysis, coagulation, and CSF safety lab data (CSF WBC, CSF RBC, CSF Protein, CSF Glucose) will be performed at each site's local lab and will be entered on a CRF. These data will be electronically transferred to Ionis Pharmaceuticals, Inc. and stored as SAS data files along with the SAS data files for all other CRFs.

2.5.3 Pharmacokinetics Data

Ionis Pharmaceuticals, Inc. is responsible for the management and review of the plasma and CSF drug concentration data. Final QA'd data will be stored in Documentum.

3 ANALYTICAL PLAN

3.1 General Overview of Analyses

3.1.1 Statistical Methods

Simple descriptive summary statistics, such as n, mean, standard deviation, standard error, median, interquartile range (25th percentile, 75th percentile), minimum, and maximum for continuous variables, and counts and percentages for categorical variables will be used to summarize most data. Where appropriate, *p*-values will be reported. Null hypotheses will be tested using two-sided tests whose Type I error rates are controlled at alpha = 0.05 unless otherwise stated. Since the primary analysis and secondary analyses are not comparative analysis, adjustments for multiplicity of testing will not be used.

Baseline is defined as the last non-missing value prior to the first dose for ISIS 396443 CS3A.

Age (months) at death or permanent ventilation will be calculated using the following SAS code:
yrdif(date1,date2,'ACT/ACT')*12

Subject demographic (e.g., age, gender, race, etc.) and baseline characteristics (e.g., age at onset of symptoms, age at SMA diagnosis, SMN2 copy number, subtype of SMA [Type IB if onset of symptoms ≤ 2 weeks of age, Type IC if onset of symptoms >12 weeks of age], number of hours of BiPAP support, presence/absence of supplemental G-tube or NG tube feeding within 30 days prior to the first dose, etc.) obtained before the first study drug administration will be summarized by cohort and overall using descriptive statistics.

Subject enrollment and disposition will be summarized. Protocol deviations will be listed.

For by-visit data summary, multiple records with the same visit label will be averaged. Unscheduled results will not be included in the summary tables except for determining baseline, but will be presented in data listings.

The presence/absence of supplemental G-tube or NG tube feeding within 30 days prior to the first dose will be manually reviewed by Ionis physicians.

3.1.2 Subject Population Analyzed

Safety Population will include all subjects who are registered and receive at least one dose of study drug.
Pharmacokinetic (PK) Population will include all subjects who are registered and for which there is at least one evaluable post-dose pharmacokinetic sample. The Efficacy Evaluable (Evaluable) Population will include all subjects who are registered, receive all scheduled

loading doses of study drug (i.e., Days 1, 15 and 85), and complete visits through at least Day 92. Two SMN2 Copy Set will include subset of Evaluable Population who have 2 copies of the *SMN2* gene. Safety analyses will be conducted on the Safety Population, PK analyses will be conducted in PK Population, efficacy and biomarker analyses will be conducted on the Evaluable Population and 2 SMN2 Copy Set. In addition to the Safety Population, PK Population and Evaluable Population, it is recognized that some data displays will be provided for "All Screened", "All Enrolled", and "Screening Failures" subjects but no data analysis will be executed in these populations.

3.1.3 Sample Size Consideration

There is no statistical rationale for the selected sample size for the assessment of efficacy, since no data on the rate of motor milestone response, the primary efficacy endpoint of the study, existed at the time of the study design. The sample size was selected to ensure that the safety, tolerability, and pharmacokinetics of ISIS 396443 will be adequately assessed while minimizing unnecessary subject exposure. According to Table 1, the sample size also allows for sufficient power (~90%) to detect a response rate of at least 30%.

Table 1 below provides the power analysis for 20 patients using the proportion of patients who achieved improvement based on the motor milestone endpoint (as defined in section 1.3.1). A two-sided exact test based on the binomial probability distribution with an alpha of 0.05 is used.

Table 1. Power analysis for 20 patients using the proportion of patients who achieved improvement based on the motor milestone

Null hypothesis, H_0	Alternative hypothesis, H_a	Power
5% (1 responder)	20% (4 responders)	58%
5%	25% (5 responders)	77%
5%	30% (6 responders)	89%
5%	35% (7 responders)	95%

3.1.4 Safety Review and Planned Interim Analysis

To ensure subject safety, safety data will be reviewed on an ongoing basis by the Ionis Safety Physician. Safety data will also be reviewed on an ongoing basis by the Data and Safety Monitoring Board (DSMB). After the last patient within Cohort 1 completes the Day 29 visit, safety results for the cohort will be reviewed by the Medical Monitor and the DSMB and a recommendation regarding further enrollment and escalation to Cohort 2 will be made. The Sponsor and DSMB will determine if enrollment of additional patients is required to confirm there is an acceptable toxicity profile prior to its designation as MTD.

Interim efficacy and safety analyses will be performed to provide content for regulatory submissions and to support ISIS 396443 drug development planning and business activities. Specifically, analyses of primary and secondary efficacy endpoints (Sections 3.2 and 3.3), as well as PK (Section 3.5) and safety analyses (Section 3.6), will be conducted at time points that allow for reasonable interpretation of the data, such as all continuing subjects passing a specific age threshold (e.g., 18, 24 or 36 months).

3.1.5 Incomplete or Missing Data

3.1.5.1 Motor Milestones

If not all tests were performed at a visit, the approach described below will be used for the handling of missing data. Missing data will be imputed on an individual item level. If a test is missing at screening, then the missing test will be imputed as the median of the non-missing values of the group to which the subject belongs: age at symptom onset (≤ 12 weeks, >12 weeks) by disease duration (≤ 12 weeks, >12 weeks). If for the subject with the missing test, the corresponding visit is flanked by visits with non-missing tests, the missing test will be imputed using linear interpolation with the result rounded to the nearest integer score. Otherwise, the missing test will be imputed as the lowest score of the visit in the group (age at symptom onset by disease duration) to which the subject belongs. If the group is empty, the missing test will be imputed as the lowest score of all available subjects of the visit.

Disease duration (week) will be calculated as (screening date-birth date)/7- age at symptom onset in week.

3.1.5.2 CHOP-INTEND Total Score

The CHOP-INTEND assessment contains 16 items, 13 of them are tested on both right side and left side. The item score will be equal to the maximum score of both sides for those 13 items. If the score for one side is missing the item score will be equal to the score of the non-missing side.

If not all tests were performed at a visit, the approach described below will be used for the handling of missing data. Missing data will be imputed on an individual item level. If a test is missing at screening, then the missing test will be imputed as the median of the non-missing values of the group to which the subject belongs: age at symptom onset (≤ 12 weeks, >12 weeks) by disease duration (≤ 12 weeks, >12 weeks). If for the subject with the missing test, the corresponding visit is flanked by visits with non-missing tests, the missing test will be imputed using linear interpolation with the result rounded to the nearest integer score. Otherwise, the missing test will be imputed as the lowest score of the visit in the group (age at symptom onset by disease duration) to which the subject belongs. If the group is empty, the missing test will be imputed as the lowest score of all available subjects of the visit.

3.1.5.3 Ventilator Use

If data indicates a patient requires BiPAP or CPAP daily, but the number of hours ventilator use is missing then the missing value will be imputed using the greater requirement of the visits that flank the missing visit(s).

3.2 Primary Efficacy Analysis

Motor milestones will be assessed using Section 2 of the Hammersmith Infant Neurological Exam (HINE) which is comprised of eight independent milestone categories: voluntary grasp, ability to kick in supine position, head control, rolling, sitting, crawling, standing, and walking. Within each of these categories, subjects can progress from complete inability to perform a motor task, for example no grasp, no kicking, unable to maintain head upright, all the way to complete mastery of each category, for example achievement of pincer grasp, touching toes while supine, maintaining their head upright for a maximum of 26 motor milestones (see Table 2 below).

Table 2. Motor Milestone Achievement (Module 2, HINE)

Motor milestone	Milestone progression				
Voluntary grasp	No grasp	Uses whole hand	Finger and thumb; immature grasp	Pincer grasp	
Ability to kick (in supine)	No kicking	Kicks horizontal; legs do not lift	Upward (vertically)	Touches leg	Touches toes
Head control	Unable to maintain upright	Wobbles	All the time upright		
Rolling	No rolling	Rolling to side	Prone to supine	Supine to prone	
Sitting	Cannot sit	Sit with support at hips	Props	Stable sit	Pivots (rotates)
Crawling	Does not lift head	On elbow	On outstretched hand	Crawling flat on abdomen	On hands and knees
Standing	Does not support weight	Supports weight	Stands with support	Stands unaided	
Walking	No walking	Bouncing	Cruising (holding on)	Walking independently	

For each dosing visit, in addition to the scheduled pre-dose assessment, the HINE including motor milestones are also assessed at 3, 6 hours and 1 day after dosing to monitor patient safety. Since the patient's motor function could be temporarily impacted by the lumbar puncture procedure, only the motor milestone results from the pre-dose assessment will be

used for the efficacy analysis and the results at 3, 6 hours and 1 day after dosing will be excluded.

The improvement in motor milestones is defined as achievement of any of the following :
- (i)   at least a 2-point increase from baseline or achievement of pincer grasp in the category of voluntary grasp, or
- (ii)   at least a 2-point increase in the ability to kick or achievement of touching toes, or
- (iii)   a 1-point increase in any of the remaining 6 categories: head control, rolling, sitting, crawling, standing, or walking.

Subjects who die or withdraw from the study will be counted as non-responders and will be included in the denominator for the calculation of the proportion. As a result, mortality will be accounted for in the motor milestone analysis. For subjects on permanent ventilation, because motor milestone assessment continues, functional scores after permanent ventilation will be used to assess improvement in motor milestones.

The primary analysis will be the proportion of subjects who achieve improvement in motor milestones at their last available visit. It will be tabulated by cohort and overall. The primary analysis will be conducted using the Evaluable Population.

As the sensitivity analyses, the proportion of subjects who achieve improvement in motor milestones at their last available visit will be tabulated
1). by cohort and overall using the 2 SMN2 Copy Set
2). by cohort and overall using the Safety Population
3). by SMA subtype using the Safety Population

In addition, the following analyses will also be conducted to assess effects of treatment with ISIS 396443 on the motor milestone improvement over time:
- The proportion of subjects who achieve improvement in motor milestones will be tabulated by visit.

  Subjects who die or withdraw from the study prior to a visit will be counted as non-responders for the visit and all subsequent visits and will be included in the denominator for the calculation of the proportion. As a result, mortality will be accounted for in the motor milestone analysis. For subjects on permanent ventilation, because motor milestone assessment continues, functional scores after permanent ventilation will be used to assess improvement in motor milestones.

  If a subject misses a visit and has visit(s) beyond the visit, then the subject will be counted as a non-responder and included in the denominator for the calculation of the proportion for the visit.

Subjects who are ongoing in the study and haven't reached a visit will not be included in the denominator for the calculation of the proportion for the visit and all subsequent visits.

- The proportion of subjects who achieve full head control (defined as All the Time Upright), independent sitting (defined as either Prop, Stable Sit or Pivot), rolling (Prone to Supine or Supine to Prone), crawling (defined as crawling On outstretched hand, flat on abdomen, or On hands and knees), standing (defined as Stands with support or Stands unaided), or walking (Cruising (holding on) or Walking independently) at their last available visit. Subjects who die or withdraw from the study will be counted as non-responders and will be included in the denominator for the calculation of the proportion. For subjects on permanent ventilation, because motor milestone assessment continues, functional scores after permanent ventilation will be used to assess improvement in motor milestones.

- The proportion of subjects who achieve full head control (defined as All the Time Upright), independent sitting (defined as either Prop, Stable Sit or Pivot), rolling (Prone to Supine or Supine to Prone), crawling (defined as crawling On outstretched hand, flat on abdomen, or On hands and knees), standing (defined as Stands with support or Stands unaided), or walking (Cruising (holding on) or Walking independently) will be tabulated by visit. Subjects who die or withdraw from the study prior to a visit will be counted as non-responders for the visit and all subsequent visits and will be included in the denominator for the calculation of the proportion. For subjects on permanent ventilation, because motor milestone assessment continues, functional scores after permanent ventilation will be used to assess improvement in motor milestones.

  If a subject misses a visit and has visit(s) beyond the visit, then the subject will be counted as a non-responder and included in the denominator for the calculation of the proportion for the visit.Subjects who are ongoing in the study and haven't reached a visit will not be included in the denominator for the calculation of the proportion for the visit and all subsequent visits.

- Summary of change from baseline in the motor milestone total score at their last available visit. The total motor milestone score equals to the sum of individual scores. For example, if the subject cannot maintain head control this is scored as 0, head wobbles as 1, and all the time upright as 2. Complete inability to perform any motor task will therefore be as zero. The ability to master all motor milestones will be scored as 26.

- Summary of change from baseline in the motor milestone total score by visit.

The above analyses will be tabulated
1). by cohort and overall using the Evaluable Population
2). by cohort and overall using the 2 SMN2 Copy Set

3). by cohort and overall using the Safety Population

4). by SMA subtype using the Safety Population

3.3 Secondary Efficacy Analysis

3.3.1 Event-free Survival

Event-free survival will be estimated using the Kaplan-Meier (K-M) methodology. The proportion of subjects who are alive and do not require permanent ventilatory support at age of 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 months will be estimated from the Kaplan-Meier curve.

The medians and 95% CIs will be estimated if possible. The data will be tabulated

1). by cohort and overall using the Evaluable Population

2). by cohort and overall using the 2 SMN2 Copy Set

3). by cohort and overall using the Safety Population

4). by SMA subtype using the Safety Population

Event-free survival will be calculated from a patient's birth date to death date or permanent ventilation date. Permanent ventilation is defined as tracheostomy OR ≥ 16 hours of ventilation support/day continuously for ≥ 14 days in the absence of an acute reversible illness. The endpoint was determined by the investigator using chart review of all available patient data. The date of the permanent ventilation event will be the date of reaching 14 days of continuous BiPAP support for at least 16 hours; i.e., at the end of the 14-day period.

If a patient is not known to have died or have had permanent ventilation, the data will be censored at the latest visit date with non-missing ventilation use assessment, either as an in-person visit or by telephone contact, whether or not the patient had received the full course of treatment, and whether the patient completed the study or withdrew prematurely.

The calculation will be as follows:

If a patient is not on permanent ventilation but died, event-free survival = number of months between birth date and death date, censor=0;

If a patient is on permanent ventilation, event-free survival = number of months between birth date and the date when the patient is first time on permanent ventilation, censor=0;

If a patient is alive and not on permanent ventilation, event-free survival = number of months between birth date and last ventilation assessment date, censor=1. Where last ventilation assessment date equals the latest date from the last visit date from Ventilation CRFs and last phone contact date from Phone Contact CRFs where BiPAP or CPAP assessment is recorded.

3.3.2 CHOP-INTEND Infant Motor Function Scale

The CHOP-INTEND assessment contains 16 items, the total score ranges from worst possible score of 0 to a best possible score of 64

The CHOP-INTEND infant motor function scale total score, change, and percent change from baseline will be summarized by visit.

In addition, the CHOP-INTEND infant motor function scale total score, change, and percent change from baseline to the last available visit for each patient will also be summarized.

The proportion of subjects who achieve improvement in CHOP INTEND total scores will be tabulated by visit. Improvement is defined as at least a four-point increase from baseline. Subjects who die or withdraw from the study prior to a visit will be counted as non-responders for the visit and all subsequent visits and will be included in the denominator for the calculation of the proportion. For subjects on permanent ventilation, because CHOP INTEND assessment continues, functional scores after permanent ventilation will be used to assess improvement in CHOP-INTEND. If a subject misses a visit and has visit(s) beyond the visit, then the subject will be counted as a non-responder and included in the denominator for the calculation of the proportion for the visit.
Subjects who are ongoing in the study and haven't reached a visit will not be included in the denominator for the calculation of the proportion for the visit and all subsequent visits.

The proportion of subjects who achieve improvement in CHOP INTEND total scores at their last available visit will be tabulated. Subjects who die or withdraw from the study will be counted as non-responders and will be included in the denominator for the calculation of the proportion. As a result, mortality will be accounted for in the CHOP INTEND analysis. For subjects on permanent ventilation, because CHOP INTEND assessment continues, functional scores after permanent ventilation will be used to assess improvement in CHOP INTEND.

The above analyses will be tabulated
1). by cohort and overall using the Evaluable Population
2). by cohort and overall using the 2 SMN2 Copy Set
3). by cohort and overall using the Safety Population
4). by SMA subtype using the Safety Population

3.3.3 CMAP Ulnar and Peroneal Nerves

CMAP is an electrophysiological measure used to determine the approximate number of motor neurons in a muscle or group of muscles. CMAP amplitude and CMAP area will be evaluated at two sites, right side ulnar nerve and right side peroneal nerve.

The absolute values for CMAP amplitude and CMAP area, as well as changes and percent changes from baseline will be summarized by visit.

In addition, the absolute values for CMAP amplitude and CMAP area, as well as changes and percent change from baseline to the last available visit for each patient will also be summarized.

The proportion of subjects with a change from baseline CMAP amplitude at the peroneal nerve greater than 0.5 mV will be tabulated by visit. Subjects who die or withdraw from the study prior to a visit will be counted as non-responders for the visit and all subsequent visits and will be included in the denominator for the calculation of the proportion. As a result, mortality will be accounted for in the CMAP analysis. For subjects on permanent ventilation, because CMAP assessment continues, the scores after permanent ventilation will be used to assess improvement in CMAP. If a subject misses a visit and has visit(s) beyond the visit, then the subject will be counted as a non-responder and included in the denominator for the calculation of the proportion for the visit.Subjects who are ongoing in the study and haven't reached a visit will not be included in the denominator for the calculation of the proportion for the visit and all subsequent visits.

The proportion of subjects with a change from baseline CMAP amplitude at the peroneal nerve greater than 0.5 mV at their last available visit will be tabulated. Subjects who die or withdraw from the study will be counted as non-responders and will be included in the denominator for the calculation of the proportion. As a result, mortality will be accounted for in the CMAP analysis. For subjects on permanent ventilation, because CMAP assessment continues, the assessments after permanent ventilation will be used to assess improvement in CMAP.

The above analyses will be tabulated
1). by cohort and overall using the Evaluable Population
2). by cohort and overall using the 2 SMN2 Copy Set
3). by cohort and overall using the Safety Population
4). by SMA subtype using the Safety Population

3.4   Exploratory Analysis

3.4.1   Measures of Respiratory Status

Measures of respiratory status include number of respiratory events, respiratory infections, respiratory-related hospitalizations, as well as number of hours per day ventilator use and O2 saturation awake.

All serious adverse events that are coded into the System Organ Class (SOC) Respiratory, thoracic, and mediastinal disorders, will be considered respiratory events.

Respiratory infections will include those serious adverse events that are coded under the System Organ Class (SOC) of infections and infestations as well as those in the System Organ Class (SOC) of investigations. For the sake of completeness, respiratory infections will be identified via a two-step process: first, all serious adverse events that code to the High Level Group Term of Respiratory tract infections under the SOC Respiratory, thoracic, and mediastinal disorders will be extracted programmatically; secondly, all remaining SAEs of infections will be manually reviewed by a Safety Physician who will identify those that are respiratory in nature.

Respiratory-related hospitalizations will combine the above two categories of respiratory events and respiratory infections where the SAE had resulted in an inpatient hospitalization.

The number of respiratory events, respiratory infections, and respiratory-related hospitalizations during the study will be analyzed using the rate at which they occur. For descriptive purposes, the aggregate event rate will be calculated by dividing the total number of respiratory events that occurred in the group by the total number of subject-years on study. The aggregate rates for respiratory infections and respiratory-related hospitalizations will be calculated similarly. The aggregate rates will be tabulated.

If a patient doesn't require BiPAP or CPAP daily, then the number of hours per day ventilator use will be equal to 0. If ventilattor use is reported as a range, e.g, 6 to 12 hours, the maximum will be used. The handling of missing values is described in Section 3.1.5.2.

The absolute values for number of hours per day ventilator use and O2 saturation awake, as well as changes and percent changes from baseline will be summarized by visit.

The above analyses will be tabulated
1). by cohort and overall using the Evaluable Population
2). by cohort and overall using the 2 SMN2 Copy Set
3). by cohort and overall using the Safety Population
4). by SMA subtype using the Safety Population.

3.4.2 Growth Parameters

The growth parameters will include weight to age ratio, weight to length ratio, head circumference, chest circumference, head to chest circumference ratio, arm circumference, and weight for age percentile, height for age percentile, and head circumference percentile using the WHO child growth standards (WHO Child Growth Standards, 2006). The absolute values as well as changes, and percent changes from baseline will be summarized by visit.

The above analyses will be tabulated

1). by cohort and overall using the Evaluable Population
2). by cohort and overall using the 2 SMN2 Copy Set
3). by cohort and overall using the Safety Population
4). by SMA subtype using the Safety Population.

3.4.3 *Hammersmith Functional Motor Scale – Expanded*

All subjects who have maintained a CHOP INTEND total score of ≥50 for two consecutive study visits will be evaluated using the Hammersmith Functional Motor Scale Expanded (HFMSE). This evaluation will be performed in addition to the CHOP INTEND. Since the data is only collected for a subset of patients, the data will be provided in a data listing.

3.5 Pharmacokinetic Analyses

CSF and plasma samples will be collected at protocol designated times for ISIS 396443 pharmacokinetic assessments from all treatment groups (cohorts).

3.5.1 *CSF Concentration Data*

CSF concentrations of ISIS 396443, along with the scheduled (nominal) and actual samples times (i.e., time from IT dosing) will be listed (when applicable) for each patient, treatment group (cohort), nominal dose, and day. Differences between scheduled and actual sampling days will also be listed for all patients. Percent differences between actual administered dose and nominal dose will also be listed.

CSF concentrations below the lower limit of quantification (LLOQ) will be indicated by "BLQ". For the purpose of calculating typical descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for CSF concentrations, all BLQ values will be set to zero. Mean CSF concentrations that are BLQ will be presented as BLQ, and the SD and %CV will be reported as not applicable. Summary statistics of the ISIS 396443 CSF concentrations will be tabulated by treatment group (cohort), nominal dose, day, and scheduled time point. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling days or times, or large deviations between actual dose and nominal dose.

ISIS 396443 CSF concentration versus time (actual) profiles from Day 1 to Day 673, for each patient, as well as the mean (±SE) CSF concentration versus time (scheduled) profiles for each treatment cohort, will be presented graphically. Samples may be excluded from the mean plots if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

Due to the limited CSF samples collected no CSF pharmacokinetic parameters will be calculated.

3.5.2 Plasma Pharmacokinetics

3.5.2.1 Plasma Concentration Data

Plasma concentrations of ISIS 396443, along with the scheduled (nominal) and actual samples times (i.e., time from IT dosing) will be listed (when applicable) for each patient, treatment group (cohort), nominal dose, and day. Percent differences between scheduled and actual sampling times will also be listed for all patients. Percent differences between actual administered dose and nominal dose will also be listed.

Plasma concentrations below the lower limit of quantification (LLOQ) will be indicated by "BLQ". For the purpose of calculating typical descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for plasma concentrations, all BLQ values will be set to zero. Mean plasma concentrations that are BLQ will be presented as BLQ, and the SD and %CV will be reported as not applicable. Summary statistics of the ISIS 396443 plasma concentrations will be tabulated by treatment group (cohort), nominal dose, day, and scheduled time point. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

ISIS 396443 plasma concentration versus time (actual) profiles from Day 1 to Day 15 (Cohorts 1 and 2), for each patient, as well as the mean (±SE) plasma concentration versus time (scheduled) profiles for each treatment cohort, will be presented graphically on linear and semilogarithmic scales. Additionally, ISIS 396443 plasma concentration versus time (actual) profiles (Day 1) from 0 to 24 hours for all patients , as well as the mean (±SE) plasma concentration versus time (scheduled) profiles (0 to 24 hours) for each applicable treatment cohort will be presented graphically on linear and semilogarithmic scales. Samples may be excluded from the mean plots if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

ISIS 396443 plasma trough (predose) concentration versus time (actual) profiles from Day 1 to Day 673, for each patient, as well as the mean (±SE) plasma concentration versus time (scheduled) profiles for each treatment cohort, will be presented graphically. Samples may be excluded from the mean plots if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

3.5.2.2 Plasma Pharmacokinetic Parameters

Non-compartmental pharmacokinetic analysis of ISIS 396443 will be carried out on each individual subject data set using Phoenix WinNonlin version 6.0 or higher (Pharsight

Corporation, Mountain View, CA). Plasma pharmacokinetic parameters in each patient (when applicable) will be determined. For calculation of PK parameters, all BLQ values will be set to zero. The following plasma PK parameters will be calculated (when applicable) and based on actual sampling times:

- C_{max}: the maximum observed drug concentration in plasma.
- T_{max}: the time at which C_{max} occurs.

The following PK parameters may be calculated using actual sampling times where appropriate data exists at the discretion of the pharmacokinetic scientist:

- AUC_{0-4h}: areas under the plasma concentration-time curve from zero time (pre-dose) to 4 hours after IT administration will be calculated using the linear trapezoidal rule.
- Additional partial AUC values (e.g., AUC_{0-24h}) may be calculated depending upon the samples collected during the study and the number of patients for which additional samples are collected via an indwelling catheter, as well as when warranted and at the discretion of the pharmacokinetic scientist.
- Though outlined in the study protocol, apparent terminal elimination half-life ($t_{1/2}$) values will not be determined as the limited post-treatment sampling schedule precludes this evaluation.

Plasma pharmacokinetic parameters (if applicable) will be summarized using descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) by treatment cohort, nominal dose, and day.

3.5.2.3 Immunogenicity Analyses

Immunogenicity (IM) testing (anti-ISIS 396443 antibody positivity), using designated plasma samples collected from each study subject, is planned to be conducted and reported. Initially, designated available immunogenicity plasma samples collected only on Days 1 (predose only), 85 (predose only), 169, 253, 337, 421, 505, 589, and 673 are planned to undergo immunogenicity testing. Plasma samples collected at other time points for ISIS 396443 concentration determinations may also be potentially evaluated for IM testing if of further interest and deemed warranted by the pharmacokinetic scientist. An individual sample result will be designated 'antibody positive' based on both positive screening and confirmation assay results (i.e., confirmed positive result), and otherwise will be deemed 'antibody negative'. A study subject will be given 'antibody positive' status if they have at least one confirmed positive sample result at any time during the treatment or post-treatment evaluation periods. A study subject will be given 'antibody negative' status if all evaluated IM sample results are antibody negative and they have at least one evaluable IM result from Day 85 or later during the treatment and post-treatment evaluation periods. Otherwise, a study subject will be given 'antibody inconclusive' status.

The IM incidence and IM incidence rate at each evaluated study timepoint, and for the overall treatment and post-treatment evaluation period, will be determined and appropriately summarized, by treatment, as the number of and percent (%) of evaluated subjects with antibody negative, antibody positive, and antibody inconclusive status. In addition, in antibody positive study subjects, antibody titers of any antibody positive samples will be reported (listed) and also appropriately summarized across subjects and by treatment (e.g., at each evaluated timepoint, or by observed peak titer values, etc.) at the discretion of the designated study pharmacokineticist and/or statistician.

At this time, antibody positivity in any antibody positive study subjects from this study is not yet planned to be designated as being either 'persistent' or 'transient'. However, the IM results from this study may be evaluated at a later date (and possibly in combination with IM results from other studies potentially involving these patients) to determine these designations. At that time, criteria for persistent versus transient positive antibody responses will also be defined.

When and where warranted, PK and selected safety and efficacy results may be further summarized (stratified) by antibody status (antibody negative vs. positive subjects) and treatment.

3.6 Safety Analyses

Safety will be assessed by summarizing the incidence and type of AEs and SAEs. Laboratory parameters will be provided in the data listings.

Days of exposure, number of doses and total amount of drug received for each subject will be summarized by cohort and overall.

Time on study will be categorized into intervals and summarized by cohort and overall.

The time on study will be defined as the total number of days a subject is known to be followed on study calculated as follows:

Time on study = Last date on study − Date of first dose + 1.

Where the last date on study is defined as the date of the latest visit or evaluation or telephone contact, or time of death from all available data for a given subject.

3.6.1 Adverse Events

The incidence of AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ class for:

- Any treatment emergent adverse events
- Related treatment emergent adverse events. Related adverse event is defined as those events with related or possible related relationship to study drug.
- Any treatment emergent adverse events by severity
- Related treatment emergent adverse events by severity
- Serious treatment emergent adverse events
- Serious and related treatment emergent adverse events.
- Lumber puncture related treatment emergent adverse events. Lumber puncture related adverse event is defined as those events with related or possible related relationship to study procedure/other study drug.

AEs that lead to treatment discontinuation will be listed separately. Early dosing termination and early study termination will be summarized by termination reason.

A treatment emergent adverse event is defined as an event occurring after the initiation of study treatment and before the end of the follow-up period. Non-treatment emergent adverse event will be flagged in the data listing.

If there is no "Formlink" link, and the AE (start date/time) occurs after the subject's first dosing date/time, then the AE is treatment-emergent. Otherwise, if the AE (start date/time) occurs prior to the subject's first dosing date/time, then the AE is not treatment-emergent.

If there is a "Formlink" link between two AE records, then we compare them pairwise, and consider two cases, where we compare the AE severity (mild/moderate/severe) between the two records in the pair. We chronologically order the 2 records (by AE start date) and refer to the "first" and "second" AEs.

Case 1: The first AE record in the pair occurs before first dosing, and the second record occurs after dosing.

If the AE severity on the second record is worse than the severity on the first record, then only count the second AE as treatment-emergent. But, if the severity improves (second record severity is less severe than the first record severity), then neither record is counted as treatment-emergent.

Case 2: Both AE records in the pair occur after first dosing.

If the AE severity on the second record is worse than the severity on the first record, then count both records as treatment-emergent. But, if the severity improves, then only count the first record as treatment-emergent.

When counting the total number of treatment-emergent events, events linked together through change in severity will still be counted as separate events.

The most conservative approach will be used to determine if the event occurs during the treatment or follow-up periods. For example,

- AEs that have onset dates or resolution dates prior to the first study treatment dates will be considered to have occurred prior to the study period. If the onset or resolution date of an AE is a partial date with only month or year available or completely missing, then the event is assumed to be within the study period unless the year is prior to the year of the first study treatment date, or if in the same year, the month is prior to the month of the first study treatment date.

- AEs that have onset dates after study termination date will be assumed to have occurred after the study period. If the onset of an AE is a partial date with only month or year available or complete missing, then similar approach as above will be used.

3.6.1.1 Adverse Events over Time
The incidence of AEs will be evaluated by time of onset by 90-day time intervals. For a given time interval, the number of subjects who were followed for adverse events during that time interval will be presented along with the incidence of adverse events during that time interval. Therefore, for a given System Organ Class (SOC) or preferred term (PT), subjects will be counted only once for a given time interval but may be counted more than once across time intervals.

3.6.1.2 Adverse events following Dosing

To examine the onset of any adverse events following dosing, the incidence of events that occur during loading and maintenance dosing periods will be presented by treatment group and overall. In addition, presentations of events that occurred in the first 24 hours and the first 72 hours following dosing will be provided.

3.6.2 Laboratory Measurements

The following is the list of lab analytes that will be collected throughout the study:
- Chemistry: Sodium, Potassium, Chloride, Total protein, Albumin, Calcium, Phosphorus, Bicarbonate, Glucose, BUN, Creatinine, Cystatin C, Total serum bilirubin, ALT, AST, Alkaline phosphatase, CPK
- Hematology: Hematocrit, Hemoglobin, Platelets, RBC, WBC, and WBC differential (Basophils, Eosinophils, Lymphocytes, Monocytes, and Neutrophils)
- Coagulation: PT, aPTT, INR
- Urinalysis: Specific gravity, pH, Protein, Glucose, Ketones, Bilirubin, Blood, Red blood cells, White blood cells, Epithelial cell, Bacteria, Casts, Crystals

- CSF safety: Red blood cells, White blood cells, Glucose, Protein.
- SMN Genetics: SMN2 copy number and SMN gene sequencing

If WBC differential absolute counts are missing, and percentages are available, then the absolute counts will be calculated by multiplying the percentage by total WBC count. Conversely, if absolute counts are available, percentages are missing, percentages will be calculated by dividing absolute count by the total WBC count. If neutrophils counts and percentages are missing, and segmented neutrophils and bands neutrophils results are available, then neutrophils will be calculated by adding segmented neutrophils and bands neutrophils.

Each subject's laboratory values will be classified according to whether the test result is "low" (i.e., below the lower limit of normal [LLN]), "normal" (within the normal range), or "high" (i.e., above the upper limit of normal [ULN]). If a subject is missing a baseline value but had a post-baseline value, then the baseline assessment is labeled as "unknown". Likewise, if a subject had a baseline value but had no post-baseline values, then the minimum and maximum are labeled as "unknown". Post-baseline laboratory results are defined as any assessment taken after the first dose. The shifts (relative to the normal range) from baseline to the minimum and maximum post-baseline values will be presented. Should a treatment affect a laboratory parameter, that parameter could be affected at different times for different subjects. Therefore, these analyses present the most extreme values for each subject. For many laboratory parameters, the effect could be in either direction, (i.e., an increase or a decrease), so both the maximum and minimum values have been analyzed. From these, the shifts (relative to the normal range) from baseline to low and high will be calculated. If a subject's value shifts, it can change from normal to either low or high, from low to normal or high, from high to normal or low, or from unknown to low, normal, or high. For each parameter, the incidence of shift to low will be summarized using the minimum post-baseline values. Shift to low includes subjects with a normal, high, or unknown baseline value and at least one post-baseline value of the given test. Similarly, the incidence of shift to high will be summarized using the maximum post-baseline values. Shift to high includes subjects with a low, normal, or unknown baseline value and at least one post-baseline value.

3.6.3 Vital Signs

Vital signs will include body temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure, pulse oximetry awake and pulse oximetry asleep. The vital sign data will be provided in a data listing.

3.6.4 Physical Examinations

Adverse changes in physical examinations that are deemed clinically significant by the investigator will be classified as adverse events. All physical examination data will be provided in a data listing.

3.6.5 Lead Electrocardiograms (ECG)

The ECG data will include ventricular rate (VR), PR interval, QRS duration, QT/QTC, and Overall interpretation.

Outlier analyses will be performed for the corrected Fridericia QT interval (QTcF). This includes summaries of the number and percentage of subjects with a post-baseline QTcF greater than 450 msec, 480 msec, and 500 msec) and the number and percentage of subjects with an increase of 30 msec and 60 msec from baseline in corrected QT interval (QTc). QTcF will be calculated based on the subject's reportable ECG data at each visit using the formula described below:

$$QTcF = QT / (RR)^{1/3}, \text{ where } RR = 60/VR$$

The ECG data will also be provided in a data listing.

3.6.6 Concomitant Medications

Concomitant medications will be coded using WHO Drug dictionary and summarized by ATC class, generic name and treatment group.

3.6.7 Hammersmith Infant Neurological Examinations – Sections 1 and 3

Adverse changes in neurological examinations that are deemed clinically significant by the investigator will be classified as adverse events. Data collected for HINE sections 1 and 3 will be provided in a data listing.