NCT#: NCT01557400

STATISTICAL ANALYSIS PLAN

AN OPEN-LABEL STUDY FOR PREVIOUSLY TREATED ATALUREN (PTC124®) SUBJECTS WITH NONSENSE MUTATION DYSTROPHINOPATHY

PTC124-GD-019-DMD

Version 1.0

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APPROVAL SIGNATURES

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ABBREVIATIONS

Abbreviation	Definition
6MWD	6-minute walk distance
6MWT	6-minute walk test
ACTH	adrenocorticotropic hormone
ALT	alanine aminotransferase
ATC	Anatomical-Therapeutic-Chemical classification
BUN	blood urea nitrogen
CI	confidence interval
CINRG	Cooperative International Neuromuscular Research Group
CK	blood urea nitrogen
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
D/C	discontinuation
DBP	diastolic blood pressure
DMD	Duchenne muscular dystrophy
ECG	electrocardiogram
EK	Egen Klassifikation
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GGT	gamma glutamyl transferase
LOCF	last observation carried forward
LVEF	Left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	messenger ribonucleic acid
MMRM	mixed model repeated measures
nmDMD	nonsense mutation Duchenne muscular dystrophy
NSAA	North Star Ambulatory Assessment
PCF	peak cough flow
PEF	peak expiratory flow
SAP	statistical analysis plan
SOC	System Organ Class
SBP	systolic blood pressure
TEAE	treatment-emergent adverse event
TFT	Timed function test
TID	ter in die (3 times per day)
Tx	treatment
UE	upper extremity
ULN	upper limit of normal
WHODRUG	World Health Organization Drug Dictionary
WNL	within normal limits

1 OVERVIEW

This statistical analysis plan (SAP) details the statistical methods to be used in the analyses and presentation of the data collected in Study PTC124-GD-019-DMD, also referred to as Study 019. Preparation of this SAP incorporates statistical design elements present in the protocol Version 5.0 (dated 18 November 2015). Details are documented in Sections 1-7.

In addition to the protocol defined analyses, a natural history control using the Cooperative International Neuromuscular Research Group (CINRG) will be utilized in order to help to better understand the long-term ataluren data. The planned analysis will be presented in Section 8.

After the implement of protocol Version 5.0, the sponsor has developed the protocol Version 6.1 (dated 08 March 2017) specifically for Canadian sites only for ethics approval when they finished all visits per Version 5.0 and were waiting to roll into another open-label nmDMD Study PTC124-GD-016-DMD conducted in the United States (US). The maximal scheduled visit in Version 5.0 was extended from week 240 to week 336 in Version 6.1. All data collected based on both Versions will be summarized together.

Where conflicts exist between this SAP and the study protocols, the information contained in this SAP supersedes the protocol.

2 STUDY DESIGN

This study comprises a Phase 3, open-label study of ataluren in subjects with nmDBMD who previously received ataluren at an investigator site in a prior PTC-sponsored clinical study. A separate open-label study (PTC124-GD-016-DMD) is being conducted for nmDBMD subjects who previously received ataluren at an investigator site in the US.

All participating sites must have had at least 1 subject that received ataluren treatment in a prior

PTC-sponsored clinical study in DBMD. It is planned that up to ~96 subjects will be enrolled.

Subjects will receive ataluren 3 times per day (TID) at respective morning, midday, and evening doses of 10 mg/kg, 10 mg/kg, and 20 mg/kg, for approximately 240 weeks. Study assessments will be performed at clinic visits during screening, on the first day of ataluren dosing, and then every 12 weeks during the ataluren treatment period.

The proposed types and timing of data to be recorded are described in Table 1 below based on protocol Version 5.0.

Table 1: Schedule of Events

Period	Screening[a]	Baseline[a]	Ataluren Treatment					Post-Treatment
Study Week (±7 days)	-4 to -1	Week 1	Every 60 Weeks	Every 12 Weeks	Every 24 Weeks	Every 48 Weeks	End of Tx Week 240	6 Week Post D/C
Informed Consent {7.2.2}	X							
Clinical/medication history	X							
Hepatitis screen	X							
Vital signs	X	X		X			X	X
Brooke UE Functional Rating Scale	X							
Height/Ulna length/Arm Span	X				X		X	
Physical examination	X					X	X	
Weight	X	X		X			X	X
Hematology	X	X		X			X	X
Biochemistry	X	X		X			X	X
ACTH and cortisol	X	X		X			X	X
Renin and aldosterone	X	As Indicated						
Urinalysis	X	X		X			X	X
12-lead ECG	X	As Indicated						
Echocardiogram	X		X			X		X[b]
6-minute walk test	X				X		X	
Timed Function Tests	X					X	X	
North Star Ambulatory Assessment	X					X	X	
Egen Klassifikation Scale	X					X	X	
Spirometry	X				X		X	
Disease status survey	X	X		X			X	
Drug administration		X		X				
Drug compliance				X			X	
Adverse events		X		X			X	X
Concomitant medications	X	X		X			X	X

a Ataluren may be initiated as soon as the investigator confirms patient eligibility. Baseline procedures (excluding drug administration) do not need to be performed if Screening procedures have been performed within 7 days of anticipated initiation of ataluren treatment.

b Only applies to patients who discontinue the study after 240 weeks of treatment.

Abbreviations: ACTH = adrenocorticotropic hormone, D/C = discontinuation, ECG = electrocardiogram, Tx = treatment, UE = upper extremity

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

The primary objective of this study is to assess the long-term safety and tolerability of 10, 10, 20 mg/kg ataluren in subjects with nmDBMD who had prior exposure to ataluren in a PTC-sponsored clinical trial.

3.2 Secondary Objectives

Secondary objectives include the following:

- Ambulatory subjects (able to run/walk 10 meters in ≤30 seconds) - To determine the effect of ataluren on ambulation and other aspects of physical function
- Nonambulatory subjects (unable to run/walk 10 meters in ≤30 seconds) - To assess the effect of ataluren on activities of daily living, upper limb function, and pulmonary function
- All subjects - To assess subject and/or parent/caregiver reports of changes in disease status:
  i. Retrospectively during and after participation in previous studies (Studies 007 and 007e)
  ii. Prospectively during the current study

3.3 Study Endpoints

Primary:

- Safety profile characterized by type, frequency, severity, timing, and relationship to ataluren of any adverse events or laboratory abnormalities

Secondary:

- In ambulatory subjects,
  i. change from baseline in 6MWD as measured by the 6-minute walk test (6MWT)
  ii. change from baseline in physical function as measured by the North Star Ambulatory Assessment (NSAA)
  iii. change from baseline in timed function tests (time to stand from supine and time to run/walk 10 meters)
- In non-ambulatory subjects,
  i. change from baseline in pulmonary function test - % predicated forced vital capacity (FVC) and % predicted forced expiratory volume in 1 second (FEV1) as measured by spirometry
  ii. change from baseline in patient and parent/caregiver-reported activities of daily living, as measured by the Egen Klassifikation (EK) scale

- All subjects,
    i. changes in patient and/or parent/caregiver reports of disease status as measured by a standardized survey administered by site personnel

3.4 Sample Size

The sample size, up to ~96 subjects, was determined by how many subjects satisfy all inclusion/exclusion criteria when applying for participation in this study and not by any formal statistical hypothesis.

4 STUDY POPULATION

The As-Treated population consists of all subjects who receive at least 1 dose of ataluren treatment.

This population will be evaluated in the analyses of safety (the primary endpoint). Subject [redacted] will be excluded from the As-Treated population in the analyses of the secondary endpoints including 6MWT, time to stand from supine, time to run/walk 10 meters, NSAA, EK, spirometry endpoints, disease status survey, and left ventricular ejection fraction (LVEF) because subject [redacted] was re-enrolled into the study as subject [redacted] about 2 years after the discontinuation from the study. All data collected for both subject IDs will be set together chronologically.

Ambulation/Non-ambulation subjects at study entry are defined as follows:

- Ambulation subjects: all subjects who had ≤30 sec for run/walk 10 meters at screening.
- Non-Ambulation subjects: all subjects who had >30 sec for run/walk 10 meters at screening.

5 STATISTICAL ANALYSIS

5.1 General Statistical Considerations

Summary tables for continuous variables will contain the following statistics: n, mean, standard deviation, standard error, 95% confidence intervals (CIs) on the mean, median, minimum, and maximum. Summary tables for categorical variables will include N, n, and percentages. In by-visit summaries, the analysis visits will be derived via visit mapping windows.

By-patient listings will be created for each CRF module. The data will be listed by center/subject ID and visit.

In addition, all analyses will be performed by corticosteroid use (yes or no before or during the study), by ambulatory status (ambulatory or non-ambulatory) at baseline, and overall, unless specified otherwise.

5.2 Subject Disposition

A summary of subjects who discontinued ataluren treatment prematurely or were removed from the study prematurely will be reported for overall subjects, a subgroup of subjects with corticosteroid use (Yes/No), and a subgroup of ambulatory/non-ambulatory subjects at study entry. Reasons for treatment early discontinuations will be described. The number of subjects from PTC124-007 (Study 007) to Study 019 will also be reported.

Since the sponsor has decided to discontinue the Study 019, subjects who discontinue the study due to this reason or complete the study per protocol will be considered as censored in the time to discontinuation analysis. The time to discontinuation (in weeks) is calculated as (discontinuation date - first dose date +1)/7 for subjects who discontinue from the study due to reasons other than sponsor's decision of termination the study or transitioning to commercial drug. For subjects who discontinue due to these reasons or complete the study, the censor time is (discontinuation date - first dose date +1)/7 and (study completion date - first dose date + 1)/7, respectively. Time to discontinuation will be assessed using Kaplan-Meier methods and the Kaplan-Meier curve will be displayed.

The protocol deviations will be summarized by type. A listing of protocol deviations will be provided.

5.3 Demographic and Baseline Characteristics

Subject demographic and disease characteristics (age, age group (6-11, 12-17, and ≥18 years), gender, race, ethnicity, height, weight, body mass index, six minute walk distance, six minute walk distance groups (<300 meters, ≥300-<400 meters, ≥400 meters), time to run/walk 10 meter, time to stand from supine, time to stand from supine (<5s, ≥5s), corticosteroid use (Yes/No), and ambulatory/non-ambulatory) will be summarized for overall subjects, a subgroup of subjects with corticosteroid use (Yes/No), baseline six minute walk distance groups (<300 meters, ≥300-<400 meters, ≥400 meters), baseline time to stand from supine groups (<5s, ≥5s), and a subgroup of ambulatory/non-ambulatory subjects at study entry.

5.3.1 Brook Upper Function Rating

The number of subjects with each baseline Brook Upper Function Rating score will be tabulated by the corticosteroid use (Yes/No). Subjects' mean age of each baseline score will be summarized as well.

5.4 Clinical History

Clinical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA Version 20.1). The count and percentage of subjects under each history term, coded by system organ class (SOC) and preferred term (PT), will be summarized.

5.5 Concomitant Medications and Procedures

Prior medications and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODRUG) dictionary dated 2017DEC01 into Anatomical-Therapeutic-Chemical (ATC) classification codes. The prior medications are defined as any medications that subjects started before the first dose date. Concomitant medications are defined as any medications that subjects took after/on the first dose date. The prior and concomitant medications will be summarized by ATC level 3 and preferred terms. The concomitant cardiac medications will be summarized similarly.

Specific attention will be focused on corticosteroid use. The numbers of subjects receiving/not-receiving corticosteroids at baseline or during the study, switching from receiving to not receiving or vice versa, switching in different types of corticosteroids, and duration of corticosteroid use during the study and by type will be summarized.

The prior and concomitant non-drug therapies will be defined similar to the prior and concomitant medications. They will be coded by MedDRA Version 20.1 and summarized by SOC and PT.

5.6 Study Drug Exposure and Compliance

Study drug compliance will be assessed in terms of the percentage of drug actually taken relative to the amount that should have been taken during the study. Treatment duration (weeks) will be calculated as (last dose date – first dose date +1)/7, if date of last study drug intake was not known: (last visit date – first dose date +1)/7. For subject [redacted], the treatment gap between [redacted] will be excluded from the treatment duration. The summary of the overall compliance and treatment duration will be represented for overall subjects and a subgroup of ambulatory/non-ambulatory subjects at study entry.

5.7 Primary Variables

5.7.1 Adverse Events

Adverse events will be classified using MedDRA Version 20.1 classification system. The severity of adverse events will be graded by the investigator according to the CTCAE, Version 3.0 whenever possible. A treatment-emergent adverse event (TEAE) is defined as an adverse event that occurs or worsens in the period extending from the day of a subject's first dose of study drug to 6 weeks after the last dose of study drug in this study.

If a subject has multiple events under any given SOC and PT, the subject will be counted only once under that SOC and PT. If a subject has the same AE on multiple occasions, the highest severity (fetal, life-threatening, severe, moderate, and mild) recorded for the event will be presented and the highest drug relationship (1 = 'Unrelated', 2 = 'Unlikely', 3 = 'Possibly', 4 = 'Probably', 5 = 'Related'), reclassified into Related ('Possibly Related', 'Probably Related', 'Related') or Not Related ('Unrelated' and 'Unlikely'), will be presented on the respective tables.

Total numbers of AEs, TEAEs and serious AEs (SAE) and the number and percentage of subjects experiencing ≥1 TEAE, ≥1 SAE, discontinuation due to AE, and death will be tabulated. The number and percentage of subjects with TEAEs and SAEs will be by relationship to study drug and CTCAE/severity, respectively.

The number and percentage of subjects experiencing a specific TEAE will be tabulated

- by SOC, and PT
- by SOC, PT, and CTCAE/severity grade
- by SOC in the descending order of the SOC frequency in ataluren group
- by PT in the descending order of the PT frequency in ataluren group.

The following TEAEs will be analyzed similarly:

- by SOC, and PT and
- by SOC, PT, and CTCAE/severity grade.
    i. Possibly or probably treatment-related TEAEs
    ii. Possibly treatment-related TEAEs
    iii. Probably treatment-related TEAEs
    iv. Serious TEAEs and possibly or probably treatment-related serious TEAEs
    v. TEAEs leading to discontinuation from treatment
    vi. Hepatic and renal TEAEs and possibly or probably treatment-related hepatic and renal TEAEs leading to special diagnosis evaluation
    vii. TEAEs with CTCAE/severity grade ≥3 and possibly or probably treatment-related TEAEs with CTCAE/severity grade ≥3
    viii. Common TEAEs (subject frequency of ≥5%)

Listings of death, serious AEs, AEs leading to discontinuation from treatment, and hepatic and renal AEs leading to special diagnostic evaluations will be provided.

The drug exposure adjusted TEAE incidence rate will be summarized by SOC and PT, where the incidence rate of the TEAE per 100 patient-year = (number of events/number of patient-years) *100. The number of patient-years is defined as the sum of the number of days on study drug of all patients divided by 336. That is, a year is defined as 48 weeks.

In addition, the number of subjects with clinical laboratory abnormalities, abnormal vital signs, and abnormal electrocardiogram reported as TEAE will be summarized.

Non-serious TEAE will be displayed by SOC and PT. The common non-serious TEAE (subject frequency of ≥5%) will also be displayed by SOC and PT.

Numbers of occurrence of serious TEAE and the numbers of occurrence of non-serious TEAE will be summarized by SOC and PT, separately.

5.7.2 Laboratory Tests

Hematological, serum biochemistry, adrenal laboratories, and urine data and their changes (only for continuous laboratory parameters) from baseline will be summarized by visit.

Shift tables for hematology and biochemistry data will also be presented showing change in CTCAE severity grade from baseline to each visit. For parameters for which a CTCAE scale does not exist, shift tables will be presented showing change in results from baseline (normal, low and high [or abnormal]) to each visit (normal, low and high [or abnormal]).

Summary of abnormalities in laboratory variables pre-defined for safety monitoring will be done per the following Table 2.

Table 2: Safety Monitoring Parameters and Actions to be Taken

Organ System and Laboratory Parameter	Stop Study Drug Immediately, Confirm Abnormal Value, and Then Start Work-Up	Stop Study Drug After Confirming[a] Abnormal Value, and Then Start Work-Up	Continue Study Drug, Confirm Abnormal Value, and then Start Work-Up[a]
Hepatic			
Serum total bilirubin	≥Grade 3 (≥3.0 x ULN)	Grade 2 (1.5 – 3.0 x ULN)	---
Serum GGT	≥Grade 3 (≥5.0 x ULN)	Grade 2 (>2.5 – 5.0 x ULN)	---
Serum ALT			↑ of >150 U/L with stable or ↓ CK
Adrenal			
Plasma ACTH	---	>ULN (and plasma cortisol <LLN)	>ULN (and cortisol WNL)
Renal			
Serum cystatin C	>2.00 mg/L	>1.33 – 2.00 mg/L	---
Serum creatinine	≥Grade 2 (≥1.5 x ULN for age)	Grade 1 (>ULN – 1.5 x ULN for age)	---
Serum BUN	≥3.0 x ULN	≥1.5 – 3.0 x ULN	---
Urine protein: urine creatinine (spot)	---	>0.40 mg:mg	---
Urine protein: urine osmolality (spot)	---	>0.30 mg/L:mOsm/kg	---
Urine blood (by dipstick)	4+ (Large)	3+ (Moderate)	2+ (Small)

Organ System and Laboratory Parameter	Stop Study Drug Immediately, Confirm Abnormal Value, and Then Start Work-Up	Stop Study Drug After Confirming[a] Abnormal Value, and Then Start Work-Up	Continue Study Drug, Confirm Abnormal Value, and then Start Work-Up[a]
Serum electrolytes	**Grade 3-4**	**Grade 2**	**Grade 1**
Serum Na+, high	>155 mmol/L	>150 – 155 mmol/L	---
Serum Na+, low	<130 mmol/L	---	---
Serum K+, high	>6.0 mmol/L	>5.5 – 6.0 mmol/L	---
Serum K+, low	<3.0 mmol/L	---	---
Serum Mg^{2+}, high	>1.23 mmol/L	---	---
Serum Mg^{2+}, low	<0.4 mmol/L	<0.5 – 0.4 mmol/L	---
Total serum Ca^{2+}, high	>3.1 mmol/L	>2.9 – 3.1 mmol/L	---
Total serum Ca^{2+}, low	<1.75 mmol/L	<2.0 – 1.75 mmol/L	---
Serum phosphorous	<0.6 mmol/L	<0.8 – 0.6 mmol/L	---
Serum HCO_3-	<11 mmol/L	<16 – 11 mmol/L	---

a Laboratory abnormalities may be confirmed immediately or at the next scheduled clinic visit based on investigator judgment.

Abbreviations: ACTH = adrenocorticotropic hormone, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Ca^{2+} = calcium, CK = creatine kinase, GGT = gamma glutamyl transferase, HCO_3- = bicarbonate, K+ = potassium, Mg^{2+} = magnesium, Na+ = sodium, ULN = upper limit of normal, WNL = within normal limits

5.7.3 Vital Signs

Vital signs (systolic and diastolic blood pressure, pulse rate, body weight, height, ulna length, arm span, and body temperature) will summarized by visit for overall subjects.

Number (%) of subjects with meeting hypertension criteria as the following will be summarized.

- If age <18 years old, the hypertension criteria are based on age, gender, and height-adjusted systolic blood pressure (SBP) and diastolic blood pressure (DBP) percentile results [USDHHS 2005] (Hypertensive: ≥95th percentile; Pre-hypertensive: 90 - <95th percentile; Normal: <90th percentile).
- If age ≥18 years old, hypertensive: SBP ≥140 mmHg or DBP ≥90 mmHg; pre-hypertensive: SBP 120 - 139 mmHg or DBP 80 - 89 mmHg; Normal: SBP 90 -119 mmHg and DBP 60 - 79 mmHg.

5.7.4 Electrocardiogram

Number (%) of subjects experiencing a normal/abnormal electrocardiogram (ECG) assessment will be tabulated by visit for overall subjects.

5.8 Secondary Variables

Subject [redacted] will be excluded from the As-Treated population in the analyses of all secondary endpoints.

5.8.1 6-Minute Walk Test

The distance walked at each visit and the change from baseline at each post baseline visit, as obtained from the 6-minute walk test (6MWT), will be summarized descriptively for ambulatory subjects with non-missing baseline 6MWT assessments. The distance walked at each will also be summarized for each actual age.

The endpoint will also be analyzed by means of a mixed model repeated measures (MMRM). An unstructured variance-covariance will be assumed and the estimation method will be the default, ReML. The model will include the factors for treatment, visit, age (6-11, 12-17, and ≥18 years), use of corticosteroids at baseline (yes vs no), and baseline 6MWD category (<300 meters, ≥300-<400 meters, ≥400 meters), and baseline 6MWD as a covariate. Visit is considered as a categorical variable. From this analysis the mean change at each visit will be evaluated. If the model cannot converge, compound symmetry variance-covariance structure will be assumed.

5.8.2 Timed Function Tests

Timed function test data (ie, time to stand from supine and time to run/walk 10 meters) and change from baseline to each post baseline visit will be summarized descriptively by visit for ambulatory subjects with non-missing baseline timed function tests assessments. Similarly the time to stand from supine and time to run/walk 10 meters will also be summarized for each actual age.

Timed function test (TFT) data imputation rules as the following will be used in the summary.

- If time to run/walk 10 meters >30 sec, then set to 30 sec.
- If time to stand from supine >30 sec, then set to 30 sec.

Similar to 6MWT, the time to stand from supine and time to run/walk 10 meters of will also be analyzed by means of a mixed model repeated measures (MMRM) where baseline is baseline time taken to stand from supine and time taken to run/walk 10 meters, respectively.

The Kaplan-Meier method will be applied to the analysis of age at loss of ambulation. The median age at loss of ambulation will be reported by corticosteroids use at baseline. The Kaplan-Meier curves will also be displayed. The age at loss of ambulation is defined as age of the disease progression reported as the adverse event or age at the time to run/walk 10 meters >30 seconds, whichever occurs earlier. The subjects who are ambulatory at the end of study or discontinuation will be censored on the last valid timed function tests assessment date. Age on that date will be used in the analysis. A sensitivity analysis will be performing based on the loss of ambulation defined only by disease progression AE.

5.8.3 North Star Ambulatory Assessment

The NSAA consists of 17 activities, each scored as 0, 1, or 2. The sum of these 17 scores will be used to form a total score. If fewer than 13 of the 17 activities are performed, the total score will be considered missing. If from 13 to 16 activities are performed, the total score will be calculated by multiplying the sum of the scores in the x activities that were performed by 17/x. If an activity cannot be performed due to disease progression/loss of ambulation, a score of zero will be assigned. The linear score is the linear transformation of the NSAA score to a scale of 0 to 100 [Mayhew 2013].

Total scores and linear scores from the North Star Ambulatory Assessment will be summarized by visit and change from baseline to each post baseline visit will be summarized descriptively for ambulatory subjects with non-missing baseline NSAA assessments. Similarly the total and linear scores will also be summarized for each actual age.

The number and proportion of loss of function of each North Star Ambulatory Assessment item at week 48 will be tabulated. The function loss defined as a shift from non-zero at baseline to zero at week 48. The missing data will be handled by last observation carried forward (LOCF).

5.8.4 Egen Klassifikation Scale

The total score of EK scale and change from baseline to each post baseline visit will be summarized descriptively by visit for non- ambulatory subjects and As-Treated population. Similarly the total score will also be summarized for each actual age.

5.8.5 Spirometry

Pulmonary function parameters of %-predicated FVC, %-predicted FEV1 (adjusted using ulna length and age), peak expiratory flow (PEF), and peak cough flow (PCF) and their absolute and relative changes from baseline will be summarized by visit for non- ambulatory subjects and As-Treated population. Similarly the observed values of the endpoints will also be summarized for each actual age.

The Kaplan-Meier method will be applied to the analysis of age at FVC <1 liter. The median age at FVC<1 liter will be reported by corticosteroids use at baseline. The Kaplan-Meier curves will also be displayed. The age at FVC<1 liter is the one at the first time FVC<1 liter. If subjects do not have FVC<1 liter, the age at the last non-missing FVC assessment will be chosen as the censor age.

5.8.6 Disease Status Survey

For change in disease status of prospective survey data, number (%) of subjects with changes from baseline to each visit will be summarized by each category for overall subjects and a subgroup of subjects with corticosteroid use (Yes/No). If there are multiple symptoms under a category, the highest score/worse case of all available symptoms was selected for the category in the summary.

Data from the retrospective survey in studies 007 and 007e will be summarized separately.

5.8.7 Echocardiogram

The LVEF will be summarized by visit and change from baseline to each post baseline visit will be summarized descriptively for overall subjects. Similarly the LVEF will be summarized for each actual age.

5.9 Subgroup analysis

5.9.1 Six minute walk distance subgroups

The by-visit summary on the observed values and change from baseline to each post baseline visit values will be performed descriptively for each 6MWT subgroup (<300 meters, ≥300-<400 meters, ≥400 meters) on 6MWT, TFT, and NSAA endpoints.

5.9.2 Corticosteroids type subgroups

The by-visit summary on the observed values and change from baseline to each post baseline visit values will be performed descriptively for each corticosteroid type prior to study entry (deflazacort vs prednisone/prednisolone) on 6MWT and TFTs. The Kaplan-Meier method will be applied to the analysis of age at loss of ambulation and age at FVC <1 liter each subgroup.

5.9.3 Cumulative duration of corticosteroids use subgroups

The cumulative duration of corticosteroid use prior to study entry will be categorized as <12 months vs ≥12 months. The by-visit summary on the observed values and change from baseline to each post baseline visit values will be performed descriptively for each subgroup on 6MWT and TFTs. The Kaplan-Meier method will be applied to the analysis of age at loss of ambulation and age at FVC <1 liter for each subgroup.

5.9.4 Other subgroups

Corticosteroids use in the study will be reviewed. If any other corticosteroids subgroup is clinically meaningful, the subgroup analyses on it will be performed on all efficacy endpoints.

6 DATA HANDLING

6.1 Analysis Visits based on Visit Windows

Since some subjects had early terminations or unscheduled visits in during, the derived analysis visits will be generated by visit window defined as follows.

The derived visits will be generated by visit window defined as follows.

Let ADY=assessment date –first dose date +1.

IF .<= ADY <=1 THEN AVISIT= BASELINE AND AVISITN=1;

IF 42<= ADY <=126 THEN AVISIT=WEEK 12 AND AVISITN=2;

IF 126<ADY <=210 THEN AVISIT=WEEK 24 AND AVISITN=3;

IF 210<ADY <=294 THEN AVISIT=WEEK 36 AND AVISITN=4;

IF 294<ADY <=378 THEN AVISIT=WEEK 48 AND AVISITN=5;

IF 378<ADY <=462 THEN AVISIT=WEEK 60 AND AVISITN=6;

IF 462<ADY <=546 THEN AVISIT=WEEK 72 AND AVISITN=7;

IF 546<ADY <=630 THEN AVISIT=WEEK 84 AND AVISITN=8;

IF 630<ADY <=714 THEN AVISIT=WEEK 96 AND AVISITN=9;

IF 714<ADY <=798 THEN AVISIT=WEEK 108 AND AVISITN=10;

IF 798<ADY <=882 THEN AVISIT=WEEK 120 AND AVISITN=11;

IF 882<ADY <=966 THEN AVISIT=WEEK 132 AND AVISITN=12;

IF 966<ADY <=1050 THEN AVISIT=WEEK 144 AND AVISITN=13;

IF 1050<ADY <=1134 THEN AVISIT=WEEK 156 AND AVISITN=14;

IF 1134<ADY <=1218 THEN AVISIT=WEEK 168 AND AVISITN=15;

IF 1218<ADY <=1302 THEN AVISIT=WEEK 180 AND AVISITN=16;

IF 1302<ADY <=1386 THEN AVISIT=WEEK 192 AND AVISITN=17;

IF 1470<ADY <=1554 THEN AVISIT=WEEK 216 AND AVISITN=19;

IF 1554<ADY <=1638 THEN AVISIT=WEEK 228 AND AVISITN=20;

IF 1638<ADY <=1722 THEN AVISIT=WEEK 240 AND AVISITN=21

IF 1722<ADY <=1806 THEN AVISIT=WEEK 252 AND AVISITN=22

IF 1806<ADY <=1890 THEN AVISIT=WEEK 264 AND AVISITN=23

IF 1890<ADY <=1974 THEN AVISIT=WEEK 276 AND AVISITN=24

IF 1974<ADY <=2058 THEN AVISIT=WEEK 288 AND AVISITN=25

IF 2058<ADY <=2142 THEN AVISIT=WEEK 300 AND AVISITN=26

IF 2142<ADY <=2226 THEN AVISIT=WEEK 312 AND AVISITN=27

IF 2226<ADY <=2310 THEN AVISIT=WEEK 324 AND AVISITN=28

IF 2310<ADY <=2394 THEN AVISIT=WEEK 336 AND AVISITN=29.

If there are more than 1 valid assessment within one visit window, the last one will be chosen as the analysis value.

6.2 Baseline

In general, Baseline is defined as the last non-missing valid assessment prior to or on the date of the first dose.

6.3 Absolute and Relative Change from Baseline

The absolute change from baseline at each post-baseline visit is calculated as (post-baseline value – Baseline), while the relative change (%) from baseline at each post-baseline visit is calculated as (post-baseline value – Baseline)/Baseline×100.

6.4 Missing Dates of AE and Prior and Concomitant Medications

6.4.1 Missing Date Information for Adverse Events

The following imputation rules only apply to cases in which the start date is incomplete (ie, partially missing) for adverse events.

Missing day and month

- If the year is same as the year of the date of the first dose of double-blind study drug, then the day and month of the date of the first dose of double-blind study drug will be assigned to the missing fields.
- If the year is prior to the year of the date of the first dose double-blind study drug, then December 31 will be assigned to the missing fields.
- If the year is after the year of the date of the first dose double-blind study drug, then January 1 will be assigned to the missing fields.

Missing month only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

Missing day only

- If the month and year are same as the year and month of the date of the first dose double-blind study drug, then the date of the first dose double-blind study drug will be assigned to the missing day.
- If either the year is before the year of the date of the first dose of double-blind study drug or if both years are the same but the month is before the month of the date of the first dose of double-blind study drug, then the last day of the month will be assigned to the missing day.

- If either the year is after the year of the date of the first dose of double-blind study drug or if both years are the same but the month is after the month of the date of the first dose of double-blind study drug, then the first day of the month will be assigned to the missing day.

If the stop date is complete and the imputed start date as above is after the stop date, the start date will be imputed by the stop date.

6.4.2 Missing Date Information for Prior or Concomitant Medications

For prior or concomitant medications, including rescue medications, incomplete (ie, partial missing) start date and/or stop date will be imputed. When the start date and the stop date are both incomplete for a subject, impute the start date first.

6.4.2.1 Incomplete Start Date

The following rules will be applied to impute the missing numerical fields. If the stop date is complete and the imputed start date is after the stop date, then the start date will be imputed using the stop date.

Missing day and month

- If the year of the incomplete start date is the same as the year of the date of the first dose of double-blind study drug, then the day and month of the date of the first dose of double-blind study drug will be assigned to the missing fields.
- If the year of the incomplete start date is prior to the year of the date of the first dose of double-blind study drug, then December 31 will be assigned to the missing fields.
- If the year of the incomplete start date is after the year of the date of the first dose of double-blind study drug, then January 1 will be assigned to the missing fields.

Missing month only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

Missing day only

- If the month and year of the incomplete start date are the same as the month and year of the date of the first dose of double-blind study drug, then the day of the date of the first dose of double-blind study drug will be assigned to the missing day.
- If either the year is before the year of the date of the first dose of double-blind study drug or if both years are the same but the month is before the month of the date of the first dose of double-blind study drug, then the last day of the month will be assigned to the missing day.
- If either the year is after the year of the date of the first dose of double-blind study drug or if both years are the same but the month is after the month of the date of the first dose of double-blind study drug, then the first day of the month will be assigned to the missing day.

6.4.2.2 Incomplete Stop Date

The following rules will be applied to impute the missing numerical fields. If the date of the last dose of double-blind study drug is missing, replace it with the last visit date or data cut-off date if the subject is on-going. If the imputed stop date is before the start date (imputed or non-imputed start date), then the imputed stop date will be equal to the start date.

Missing day and month

- If the year of the incomplete stop date is the same as the year of the date of the last dose of double-blind study drug, then the day and month of the date of the last dose of double-blind study drug will be assigned to the missing fields.
- If the year of the incomplete stop date is prior to the year of the date of the last dose of double-blind study drug, then December 31 will be assigned to the missing fields.
- If the year of the incomplete stop date is after the year of the date of the last dose of double-blind study drug, then January 1 will be assigned to the missing fields.

Missing month only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

Missing day only

- If the month and year of the incomplete stop date are the same as the month and year of the date of the last dose of double-blind study drug, then the day of the date of the last dose of double-blind study drug will be assigned to the missing day.
- If either the year is before the year of the date of the last dose of double-blind study drug or if both years are the same but the month is before the month of the date of the last dose of double-blind study drug, then the last day of the month will be assigned to the missing day.
- If either the year is after the year of the date of the last dose of double-blind study drug or if both years are the same but the month is after the month of the date of the last dose of double-blind study drug, then the first day of the month will be assigned to the missing day.

7 ANALYSIS CHANGES FROM PROTOCOL

The paired t-test is removed from the analysis on change from baseline in 6-minute walk test, timed function tests, North Star Ambulatory Assessment, Egen Klassifikation Scale, and spirometry endpoints. Subgroup analyses based on 6-minute walk test, corticosteroid type, and the cumulative duration of corticosteroid use are added.

8 CINRG DATA

8.1 Overview of CINRG Data

CINRG is a natural history registry database which collects DMD data using consortium of medical and scientific investigators from academic and research centers on neuromuscular disease. Demographics and baseline characteristics, corticosteroids medication use (and type), efficacy endpoints including 6MWT, time taken to stand from supine, time taken to run/walk 10 meters, NSAA, spirometry, and EK will be analyzed. Other endpoints collected by CINRG but not in Study 019 will not be included into this analysis plan. The CINRG natural history control will be compared to ataluren's efficacy data to assess the ataluren's long- term treatment benefit.

8.2 Matched Populations

To compare CINRG with Study 019, the matched populations are defined in the following table for different endpoints and analyses. Matching criteria will be adjusted if imbalance demographics and baseline characteristics are observed.

Endpoint/ Analysis	Age (Years) at Study Entry	Age (Years) at Assessment	Steroid Use	Ambulation status	Exclude EXON 51 and 45	Baseline value	Visit Year
Age at loss of ambulation	9-18	Not applicable	Cumulative Steroid use duration at study entry ≥12 months	Ambulatory at study entry	Yes	time taken to run/walk 10 meters 3.5-26.4 seconds2	Not applicable
Age at FVC <1 liter	9-18	Not applicable	Not applicable	Non-ambulatory at study entry	Yes	1-3.08	Not applicable
Piece-wise regression based on FVC	Not applicable	≤22	Cumulative Steroid use duration at each assessment ≥24 months	Non-ambulatory at each assessment	Yes	Not applicable	≥2012
%-predicted FVC	9-21	Not applicable	Not applicable	Non-ambulatory at study entry	Yes	32.6-110.3	Not applicable
%-predicted FEV1	9-21	Not applicable	Not applicable	Non-ambulatory at study entry	Yes	28.1-104.0	Not applicable
PEF	9-21	Not applicable	Not applicable	Non-ambulatory at study entry	Yes	1.3-218.0	Not applicable
EK	9-18	Not applicable	Not applicable	Ambulatory at study entry	Yes	Score 0-15	Not applicable
6MWT	9-18	Not applicable	Cumulative Steroid use duration at study entry ≥12 months s	Ambulatory at study entry	Yes	36-553 meters	Not applicable
time taken to run/walk 10 meters	9-18	Not applicable	Cumulative Steroid use duration at study entry ≥12 months	Ambulatory at study entry	Yes	3.5-26.4 seconds	Not applicable
time taken to stand from supine	9-18	Not applicable	Cumulative Steroid use duration at study entry ≥12 months	Ambulatory at study entry	Yes	2.3-30 seconds	Not applicable
NSAA	9-18	Not applicable	Cumulative Steroid use duration at study entry ≥12 months s	Ambulatory at study entry	Yes	Total score 3-34	Not applicable

8.2.1 Analyses Based on Matched Populations of CINRG and Study 019

Demographics and baseline characteristics (age, race, ambulation status, corticosteroid use (yes or no), duration of corticosteroid use, corticosteroid type, baseline 6MWD, baseline time to run/walk 10 meters, baseline time to stand from supine, baseline %-predicted FEV1, %-predicted FVC, PEF, EK score, NSAA total score) will be summarized descriptively for CINRG and Study 019 based on each matched population. The summary will be displayed by corticosteroid use (yes vs no) and cumulative steroid use (≥12 months vs <12 months) for baseline ambulatory and non-ambulatory subjects separately. In general, the baseline is defined as the first visit in CINRG data. The baseline age in the FVC analysis using piece-wise regression models is the age at the first FVC assessments for both CINRG and Study 019.

The comparison to the natural history data (ie, CINRG data) based on matched subjects will be performed in FVC using piece-wise regression models, age to loss of ambulation, and age to FVC <1 liter using Kaplan-Meier method.

Piece-wise regression models will be applied to log FVC in CINRG and Study 019 data, separately, using different ages as the changepoint. The most possible changepoint in terms of age will be chosen at the best model fit (ie, AICC value is the maximal). Scatter plots of log FVC and the most fitted piece-wise regression line will be generated for CINRG and Study 019, separately. Comparison between the observed and predicted FVC in Study 019 will be performed using repeated measures analysis of variance to account for within-subject correlation, where the predicted values are based on the regression equation estimated by the best fit regression model based on CINRG data.

The Kaplan-Meier method will be applied to the analysis of age to loss of ambulation and age to FVC <1 liter. The median age to loss of ambulation and median age to FVC<1 liter will be reported. The comparison between Study 019 and CINRG will be conducted via log-rank test by corticosteroid use at baseline (yes or no) and overall. The Kaplan-Meier curves will also be displayed.

In Study 019, the loss of ambulation is defined as the disease progression reported as the adverse event or the time to run/walk 10 meters >30 seconds, whichever occurs earlier. The event age is the one on the AE start date. The subjects who are ambulatory at the end of study will be censored on the last valid timed function tests assessment date. Age on that date will be used in the analysis. In the CINRG data, the age at the earliest report of the non-ambulation or the time to run/walk 10 meters >30 seconds, whichever earlier, will be picked as the event age. If subjects in CINRG data do not report non-ambulation, the age at the last report of ambulation will be chosen as the censor age. A sensitivity analysis will be performing based on the loss of ambulation defined only by disease progression AE.

Similarly, the age at FVC<1 liter is the one at the first time FVC<1 liter. If subjects do not have FVC<1 liter, the age at the last non-missing FVC assessment will be chosen as the censor age.

For CINRG data only, the observed values will be summarized descriptively for each actual age on 6MWT, time to run/walk 10 meters, time to stand from supine, NSAA total and linear scores, %-predicted FVC, %-predicted FEV1, PEF and EK total scores by corticosteroid use at baseline (yes or no) and overall based on the matched populations.

NSAA score derivation algorithms in Study 019 will be applied to CINRG data.

9 BIBLIOGRAPHY

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