NCT #NCT03796637

STATISTICAL ANALYSIS PLAN

PHASE 2, NON-INTERVENTIONAL, CLINICAL STUDY TO ASSESS DYSTROPHIN LEVELS IN SUBJECTS WITH NONSENSE MUTATION DUCHENNE MUSCULAR DYSTROPHY WHO HAVE BEEN TREATED WITH ATALUREN FOR ≥9 MONTHS

PTC124-GD-046-DMD

VERSION 1.0

12 FEBRUARY 2020

PTC THERAPEUTICS, INC.
100 CORPORATE COURT
SOUTH PLAINFIELD, NJ 07080 USA

APPROVAL SIGNATURES

[redacted]

PTC Therapeutics, Inc.

Date

[redacted]

PTC Therapeutics, Inc.

Date

[redacted]

PTC Therapeutics, Inc.

Date

TABLE OF CONTENTS

APPROVAL SIGNATURES 2
TABLE OF CONTENTS 3
LIST OF ABBREVIATIONS AND DEFINITION OF TERMS 5
1 INTRODUCTION 6
2 STUDY DESIGN 6
3 STUDY OBJECTIVES AND ENDPOINTS 7
3.1 Study Objectives 7
3.1.1 Primary Objective 7
3.1.2 Secondary Objective 7
3.1.3 Pharmacokinetic 7
3.2 Study Endpoints 7
3.2.1 Primary Endpoint 7
3.2.2 Secondary Endpoint 7
3.2.3 Pharmacokinetic 7
3.3 Sample Size 7
4 STUDY POPULATIONS 7
4.1 Screened Population 7
4.2 Enrolled Population 7
4.3 Intent to Treat (ITT) Population 7
4.4 Safety Population 8
5 GENERAL CONSIDERATIONS 8
5.1 Tables, Figures, and Listings 8
5.2 Missing Data Handling 8
6 SUBJECT DATA 8
6.1 Subject Disposition and Study Populations 8
6.2 Treatment Exposure 8
6.3 Study Drug Compliance 8
6.4 Demographic and Baseline Characteristics 8
6.5 Concomitant Medications and Non-Drug Treatments 8
6.6 Medical History 9
7 EFFICACY EVALUATION 9
7.1 Muscle Biopsy 9
7.1.1 Electrochemiluminescence (ECL) 9
7.1.2 Immunohistochemistry (IHC) 9
7.2 Timed Function Test 9
8 SAFETY EVALUATION 10
8.1 Adverse Events 10
8.2 Clinical Laboratory 10
8.3 Vital Signs 10
8.4 Other Safety Assessments 10

9 PHARMACOKINETIC BLOOD SAMPLE.....10
10 CHANGES FROM THE PROTOCOL.....10
11 BIBLIOGRAPHY.....10

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Term	Definition
AE	adverse event
CRF	Case Report Form
DMD	Duchenne muscular dystrophy
ECL	Electrochemiluminescence
FDA	Food and Drug Administration
IHC	immunohistochemistry
ITT	Intent to Treat
MedDRA	Medical Dictionary for Regulatory Activities
nmDMD	nonsense mutation Duchenne muscular dystrophy
PK	pharmacokinetics
PT	preferred term
SAE	serious adverse event
SAP	statistical analysis plan
SOC	system organ class
TFT	Timed Function Test
WHODRUG	World Health Organization Drug Dictionary

1 INTRODUCTION

Under the current standard of care, nonsense mutation Duchenne muscular dystrophy (nmDMD) remains a disease with devastating consequences and bleak prognosis. The progressive and irreversible effects of nmDMD underscore the importance of early intervention with treatments that have the potential to slow physical deterioration and delay the natural course of this fatal disease. While treatment with corticosteroids target the inflammatory component of the disease, additional treatments are needed to address the loss of dystrophin, the underlying cause of the disease.

The mechanism by which ataluren restores dystrophin has been established in comprehensive preclinical studies and supported in clinical evaluations. Moreover, the clinical benefit of ataluren has been demonstrated in two large randomized controlled trials PTC124-GD-007-DMD (hereafter referred to as Study 007) and PTC124-GD-020-DMD (hereafter referred to as Study 020).

The purpose of this statistical analysis plan (SAP) is to describe the procedures and the statistical methods that will be used in analyzing and reporting results for study PTC124-GD-046-DMD (hereafter referred to as Study 046). This SAP is prepared based on the study protocol V2.0 (dated 08Mar2019).

2 STUDY DESIGN

This is a single-site, non-interventional study designed to generate data on the effect of ataluren for producing dystrophin protein in nmDMD subjects. This study will evaluate dystrophin levels from approximately 6 ambulatory nmDMD male subjects who have been receiving ataluren, dosed daily 10 mg/kg in the morning, 10 mg/kg at midday, and 20 mg/kg in the evening (10, 10, 20 mg/kg), for ≥9 months from ongoing PTC-sponsored nmDMD clinical trials.

The study will have a single visit (Visit 1) which will occur that the site of [redacted]. At this visit, inclusion/exclusion criteria, demographics and medical history will be assessed. In addition, blood will be drawn for clinical labs and pharmacokinetics (PK) analysis pre-morning ataluren dose. A second blood sample for PK will also be taken 2 hours after morning ataluren dose. The muscle biopsy will also be performed at this visit. Adverse events will be monitored at Visit 1 and by a follow-up phone call approximately 1-week post Visit 1.

Muscle biopsy of the [redacted] and [redacted] muscles will be performed using an established core muscle biopsy procedure. If the [redacted] muscle is considered by the investigator to be too small for a muscle biopsy sample, the [redacted] muscle may be used. A total of no more than approximately 450mg of muscle tissue (up to [redacted] cores per muscle) from each muscle will be obtained. Vacuum needle muscle biopsy will be performed using local anesthesia or mild sedation, if needed. All biopsies will be preserved/frozen using standard protocol for electrochemiluminescence (ECL) and immunohistochemistry (IHC).

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objective

To assess the levels of dystrophin in ambulatory nmDMD subjects currently being treated with ataluren for ≥9 months using a quantitative electrochemiluminescence (ECL) assay.

3.1.2 Secondary Objective

To assess dystrophin levels/intensity and protein localization by immunohistochemistry.

3.1.3 Pharmacokinetic

To evaluate the steady state pharmacokinetics (PK) of ataluren.

3.2 Study Endpoints

3.2.1 Primary Endpoint

Mean dystrophin levels as measured by ECL.

3.2.2 Secondary Endpoint

Levels and localization of dystrophin protein levels as determined by immunohistochemistry.

3.2.3 Pharmacokinetic

PK assessments using sparse sampling and population PK modeling in a separate report. The parameters include Cl/F, V/F, $AUC_{0\text{-}24}$, C_{max}, and C_{ave}.

3.3 Sample Size

No formal sample size calculation is performed for this study. The sample size was determined empirically based on feasibility and subject availability.

4 STUDY POPULATIONS

4.1 Screened Population

Screened population is defined as all patients who has signed informed consent.

4.2 Enrolled Population

Enrolled population is defined as the subset of screen population who are not screen failures.

4.3 Intent to Treat (ITT) Population

This analysis set will include all enrolled subjects with a valid assessment of dystrophin level, as measured by ECL.

4.4 Safety Population

This analysis set will include all subjects who received at least one dose of ataluren and will be used for all summaries of safety.

5 GENERAL CONSIDERATIONS

5.1 Tables, Figures, and Listings

In general, continuous variables will be summarized using descriptive statistics including n (number of subjects), mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using frequency count as number and percentage of subjects.

Listings will be created for selected electronic case report form (CRF) domain sorted by subject and associated dates.

5.2 Missing Data Handling

Observed data will be used for all analyses. Missing data will not be imputed.

6 SUBJECT DATA

6.1 Subject Disposition and Study Populations

The number of subjects in each analysis population will be summarized using frequency count.

The number of subjects who completed or discontinued from the study and the reasons of discontinuation will be summarized based on the ITT populations.

6.2 Treatment Exposure

Since this is a non-interventional study, treatment exposure summary is not applicable to this study. The use of ataluren will be presented in the subject listings only.

6.3 Study Drug Compliance

Since this is a non-interventional study, study drug compliance is not applicable to this study.

6.4 Demographic and Baseline Characteristics

Demographic and baseline characteristics including age at biopsy date, ethnicity group, race, weight, height, and BMI at biopsy date will be summarized based on the ITT populations.

6.5 Concomitant Medications and Non-Drug Treatments

Concomitant Medications will be coded using the World Health Organization Drug Dictionary (WHODRUG), version March 1, 2019. Non-drug treatments will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 22.1. Prior medications and non-drug treatments are defined as those taken any time prior to biopsy date. Concomitant medications and non-drug treatments are defined as those taken any time on or after biopsy date.

The use of prior and concomitant medications and non-drug treatments will be presented in the subject listings only.

6.6 Medical History

Medical history will be coded using MedDRA, version 22.1. Medical history will be listed in the listings only.

7 EFFICACY EVALUATION

All efficacy summaries will be generated based on the ITT population. In addition to actual reported values, in case any measured dystrophin levels are below the lower limit of quantification (LLOQ), the levels will be set at 0.5*LLOQ for the statistical analysis.

7.1 Muscle Biopsy

Muscle biopsies will be taken from several locations. Multiple cores of muscles will be taken from each location and multiple samples will be prepared from each muscle cores. Dystrophin levels will be measured from each sample by both ECL and IHC. The dystrophin levels measured by ECL will be considered as the primary efficacy endpoint. The dystrophin levels measured by IHC will be considered as the secondary efficacy endpoint.

7.1.1 Electrochemiluminescence (ECL)

Dystrophin levels will be summarized by muscle locations and across muscle locations. Within each muscle location, the best, the average and the median of dystrophin levels from each muscle cores for each subject will be summarized using descriptive statistics. The same summary statistics will be presented across muscle locations. In addition to dystrophin levels, the percent normal (defined as dystrophin level normalized to healthy subjects) measured by ECL will also be summarized using descriptive statistics.

7.1.2 Immunohistochemistry (IHC)

The dystrophin levels measured by IHC will be summarized using descriptive statistics in the similar fashion as for ECL. In addition to dystrophin levels (the mean staining intensity), the percentage of positive fibers will also be summarized using descriptive statistics. Within each muscle location, the best, the average and the median of dystrophin levels (the mean staining intensity) and the percentage of positive fibers from each muscle cores for each subject will be summarized using descriptive statistics. The same summary statistics will be presented across muscle locations.

7.2 Timed Function Test

Timed function tests (TFT) include the time taken to run/walk 10 meters, climb 4 stairs, descend 4 stairs, and stand from supine. Since subjects only have one assessment in timed function test results. The TFT assessments will be presented in the subject listings only.

8 SAFETY EVALUATION

All safety summaries will be generated based on the safety population.

8.1 Adverse Events

Adverse events (AE) will be coded using the MedDRA version 22.1. A treatment-emergent adverse event (TEAE) is defined as an adverse event that occurs on or after the biopsy date. Any AE with and end date prior to the biopsy date will not be considered as an TEAE.

The following summaries will be provided for TEAEs:

1. TEAE overview
2. TEAEs by system organ class (SOC) and preferred term (PT)

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 reported on the CRFs ("life-threatening", "severe", "moderate", "mild", "unknown", and "not reported") will be presented in the summaries.

In addition, subjects experienced serious adverse events (SAE) will be presented in the subject listings only.

8.2 Clinical Laboratory

Hematological, biochemistry, and urine evaluations (normal, abnormal - not clinically significant, and abnormal – clinically significant) collected at visit 1 will be presented in the subject listings only.

8.3 Vital Signs

Vital signs including systolic blood pressure, diastolic blood pressure, pulse rate and oral temperature will be presented in the subject listings only.

8.4 Other Safety Assessments

Other safety assessments including physician assessments will be presented in the subject listings only.

9 PHARMACOKINETIC BLOOD SAMPLE

PK assessments using sparse sampling and population PK modeling will be presented in a separate report. Plasma concentration will be presented in the subject listings only.

10 CHANGES FROM THE PROTOCOL

There are no major changes to the planned analyses from the protocol.

11 BIBLIOGRAPHY

None