NCT #NCT03796637

CLINICAL PROTOCOL

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.

PROTOCOL NUMBER: PTC124-GD-046-DMD

20 MARCH 2019

VERSION 2.0

PTC THERAPEUTICS, INC.
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PROTOCOL SYNOPSIS

Name of Sponsor/Company: PTC Therapeutics
Name of Investigational Product: Ataluren
Title of Study: 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.
Study Centers: The study will be performed at a single site at [redacted]
Phase of Development: Phase 2
Objectives: ***Primary*:** • To assess the levels of dystrophin in ambulatory nonsense mutation Duchenne muscular dystrophy (nmDMD) subjects currently being treated with ataluren for ≥9 months using quantitative electrochemiluminescence (ECL) assay ***Secondary*:** • To assess dystrophin levels/intensity and protein localization by immunohistochemistry ***Pharmacokinetic:*** • To evaluate the steady state pharmacokinetics (PK) of ataluren
Endpoints: ***Primary:*** • Mean dystrophin levels as measured by ECL ***Secondary:*** • Levels and localization of dystrophin protein as determined by immunohistochemistry ***Pharmacokinetic***: • PK assessments using sparse sampling and population PK modeling in a separate report. The parameters include Cl/F, V/F, AUC_{0-24}, C_{max}, and C_{ave}.
Study Description and Methodology Single-site, non-interventional study to obtain additional data on the effect of ataluren on dystrophin protein production in nmDMD subjects who currently have been taking ataluren for ≥9 months. Quantifying dystrophin levels from nmDMD subject muscle biopsies is challenging due to the difficulty of obtaining muscle samples that contain sufficient amounts of intact muscle cells for analysis. This study has been designed to mitigate these challenges, to the extent possible, by adherence to the following practice parameters: • Biopsies will be collected from ambulatory nmDMD subjects; a subject population that are more likely to have greater amounts of intact muscle cells compared with older, non-ambulatory patients with nmDMD. • A total of no more than approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be collected from the [redacted] and [redacted]. If the [redacted] muscle is considered by the investigator to be too small for muscle biopsy sampling, the [redacted] muscle may be used. • Two validated, highly sensitive assays, ie, ECL and immunohistochemistry, will be used to measure dystrophin protein levels and to evaluate whether the protein localizes correctly to the muscle membrane.
Study Population: Approximately 6 ataluren-treated subjects with nmDMD.

Main inclusion and exclusion criteria

***Inclusion criteria*:**

1. Evidence of signed and dated informed consent/assent document(s) indicating that the patient (and/or his parent/legal guardian) has been informed of all pertinent aspects of the trial.
2. Male sex.
3. Ambulatory (ie, 10 m walk/run in <30seconds) and functional grade on the Brooke Upper Extremity Scale of a 1 or a 2.
4. Currently being treated with ataluren 10, 10, 20 mg/kg for ≥9 months, with no gap in treatment of >1 month prior to study entry.
5. Phenotypic evidence of DMD based on the onset of characteristic clinical symptoms or signs (eg, proximal muscle weakness, waddling gait, and Gowers' maneuver) and an elevated serum creatine kinase (CK). Medical documentation of phenotypic evidence of DMD needs to be provided upon request by the PTC Therapeutics medical monitor.
6. Willing to undergo muscle biopsy.

Exclusion Criteria:

1. Known contra-indication to muscle biopsy (ie, such as bleeding or clotting disorders).
2. Exposure to another investigational drug within 2 months prior to enrollment in the study or ongoing participation in any non-ataluren interventional clinical trial.
3. Requirement for daytime ventilator assistance or any use of invasive mechanical ventilation via tracheostomy. *Note: Evening non-invasive mechanical ventilation such as use of bilevel positive airway pressure (Bi-PAP) therapy is allowed.*
4. Prior or ongoing medical condition (eg, concomitant illness, psychiatric condition, behavioral disorder, alcoholism, drug abuse), medical history, physical findings or laboratory abnormality that, in the Investigator's opinion, could adversely affect the safety of the subject, makes it unlikely that the muscle biopsy or follow-up would be completed, or could impair the assessment of study results.

Statistical analyses:

Dystrophin levels will be summarized using descriptive statistics.

SCHEDULE OF ASSESSMENTS

Study Procedure	Visit 1[1]	Follow-up (phone call)	Notes
Week (visit window)		**Week 1 (± 3days)**	
Informed Consent	X		A signed and dated informed consent must be obtained before conducting any study procedures.
Inclusion/Exclusion	X		
Demographics	X		
Medical History	X		
Physical Exam	X		Full physical exam will be performed.
Clinical Labs	X		Biochemistry, hematology, and urinalysis laboratory assessments.
Height/Weight/BMI	X		
Vitals (HR & BP)	X		Vital signs will include systolic and diastolic blood pressure, pulse rate, and body temperature. The pulse rate and blood pressure determinations will be performed with the subject in a sitting position after a 5-minute rest. Blood pressure will be measured in triplicate and the average will be recorded.
Timed function tests	X		Tests include time to climb 4 stairs, time to descend 4 stairs, time to rise from supine, and time to run/walk 10 meters.
PK blood sampling	X		PK samples will be drawn pre-morning dose and 2 hours post-morning dose. Ataluren plasma concentrations will be analyzed in a population PK model for the estimation of key pharmacokinetic parameters including Cl/F, V/F, AUC_{0-24}, C_{max}, and $C_{ave.}$
Muscle biopsy	X		Muscle biopsy will be taken from the [redacted] and [redacted]. If the [redacted] muscle is considered by the investigator to be too small for a muscle biopsy sample, the [redacted] muscle may be used
AE/SAE Monitoring	X	X	
Concomitant medications	X		Treatments, including ataluren, taken one month prior to enrollment and during Visit 1 will be recorded.

Abbreviations: AE, adverse event; AUC_{0-24}, area under the concentration curve from time 0 to 24 hours; BMI, body mass index; BP, blood pressure; Cl/F, clearance; C_{ave}, average plasma concentration; Cmax, maximal plasma concentration; HR, heart rate; PK, pharmacokinetics; SAE, serious adverse event; V/F, volume of distribution

[1] Subjects may have to return to the clinic for up to 3 days for completion of assessments and muscle biopsy.

PROTOCOL IDENTIFIERS AND STUDY PERSONNEL

Project Code	PTC124-GD
Therapeutic Area	Genetic Disorders – Duchenne Muscular Dystrophy
PTC Therapeutics Substance Identifier	Ataluren (PTC124)
IND Number	068431
Protocol Number	PTC124-GD-046-DMD
Protocol Version	Version 2.0
Protocol Version Date	20 March 2019
Protocol Phase	Phase 2
Protocol Title	Phase 2, non-interventional, clinical study to assess dystrophin levels in subjects with nmDMD who have been treated with ataluren for ≥9 months

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PTC THERAPEUTICS PROTOCOL APPROVAL SIGNATURES

[redacted]

Date

PTC Therapeutics, Inc.

[redacted]

Date

PTC Therapeutics, Inc.

[redacted]

Date

PTC Therapeutics, Inc.

[redacted]

Date

PTC Therapeutics, Inc.

PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

I have read the protocol document and, on behalf of my institution, agree to comply with the protocol and all applicable regulations.

Principal Investigator	**Date**

Institution:	
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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Term	Definition
AE	Adverse event
Bi-PAP	Bilevel positive airway pressure
BMD	Becker muscular dystrophy
BMI	Body mass index
BP	Blood pressure
CK	Creatine kinase
CRF	Case report form
CRO	Contract research organization
CTCAE	Common Terminology Criteria for Adverse Events
DMD	Duchenne muscular dystrophy
EC	Ethics Committee
ECL	Electrochemiluminescence
eCRF	Electronic case report form
FVC	Forced vital capacity
GCP	Good Clinical Practice
HR	Heart rate
ICF	Informed consent form
ICH	International Councilfor Harmonisation
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger ribonucleic acid
nmDMD	Nonsense mutation Duchenne muscular dystrophy
PK	Pharmacokinetic
RSI	Reference Safety Information
SAE	Serious adverse event
[redacted]	

1 INTRODUCTION

1.1 Disease Background

Duchenne muscular dystrophy (DMD) is a rare, debilitating, progressive, and ultimately fatal childhood disease (Bushby 2010a, Bushby 2010b). It is an X-linked genetic muscle disorder that results from a mutation in the dystrophin gene. Dystrophin is a 427 kDa structural protein present at the muscle sarcolemma that provides stability to the muscle and is expressed in skeletal, respiratory, and cardiac muscle (Bushby 2010a, Mah 2016, Rae 2016). It is also expressed in neurons in specific parts of the brain, such as the hippocampus (Aartsma-Rus 2016).

Dystrophin acts as a shock absorber during muscle contraction by linking the actin of the contractile apparatus to the layer of connective tissue that surrounds each fiber (Aartsma-Rus 2016). Consequently, dystrophin bears the mechanical stresses that occur during muscle contraction, stabilizing muscle cell membranes, and protecting muscles from injury (Petrof 1993). Dystrophin mutations disrupt the connection with the actin cytoskeleton and connective tissue resulting in chronic muscle damage, inflammation, and eventually replacement of muscle fibers by fat and fibrotic tissue resulting in progressive and irreversible loss of muscle function (Muntoni 2003). The culmination of muscle function loss leads to early death; the average life expectancy of DMD patients is about 25 years of age.

Duchenne muscular dystrophy is noticeable in young children, who typically display underdeveloped or delayed development of motor skills relative to healthy boys (Bushby 2010a). Muscle function continues to deteriorate in pre-adolescence resulting in the loss of gross motor functions, including the ability to rise from the floor, to climb stairs, and to walk. Complete loss of ambulation typically occurs in the early teenage years. Loss of functional abilities occur sequentially, with the loss of one function preceding and predicting the loss of subsequent functions. Importantly, the age at loss of ambulation is predictive of the age of onset of severe respiratory insufficiency (forced vital capacity [FVC] <1 liter) requiring the need for ventilation assistance (Humbertclaude 2012), which in turn is predictive of death within 3 years (Phillips 2001).

To date, no medications cure or reverse the effects of DMD. The goals of current interventions are to help slow or stabilize disease progression, prolong patients' ability to manage activities of daily living, and delay the onset of subsequent deterioration. Recent clinical guidelines recommend treatment with glucocorticoids, which address the inflammatory component of the disease, and have beneficial effects on prolonging ambulation and muscle and respiratory function (Bushby 2010a, Bushby 2010b). Additional treatments are necessary, particularly those that treat the underlying cause of the disease. Because of the role of the dystrophin protein, dystrophin restoration therapy would be expected to stabilize or slow disease progression in patients with DMD.

Approximately 10% to 15% of boys with DMD have the disease due to a nonsense mutation in the dystrophin gene (Aartsma-Rus 2006, Bladen 2015), resulting in a premature stop codon in the dystrophin mRNA (messenger ribonucleic acid). Consequently, ribosomal

translation of nonsense codon containing mRNA results in premature termination of translation before a full-length, functional protein is generated.

1.2 Ataluren

Ataluren is a small molecule being developed for the treatment of nonsense mutation Duchenne muscular dystrophy (nmDMD). Ataluren promotes ribosomal readthrough of premature stop codons enabling the formation of full-length, functional dystrophin protein (Welch 2007).

The efficacy and safety of ataluren for the treatment of nmDMD were assessed in 2 randomized, double-blind, placebo--controlled, 48-week trials (PTC124-GD-007-DMD [NCT00592553] [Study 007] and PTC124-GD-020-DMD [NCT01826487] [Study 020]). Data from these studies supported conditional marketing authorization of ataluren for the treatment of nmDMD in ambulatory patients aged ≥5 years in Europe and subsequently extended to ≥2 years of age based on pharmacokinetic extrapolation from study PTC124-GD 030-DMD.

A detailed description of the chemistry, pharmacology, efficacy, and safety of ataluren is provided in the Investigator's Brochure (IB).

1.2.1 Ataluren-associated increased ribosomal readthrough of premature stop codons

The ability of ataluren to promote ribosomal readthrough of premature stop codons has been evaluated in a wide range of in vivo and in vitro preclinical experiments, including different organ and animal model systems, as well as cell-free and cell-based systems. In particular, preclinical studies in the mdx mouse and the *sapje* zebrafish nmDMD models and human myotubes consistently demonstrate that ataluren increases dystrophin production across key tissues, including skeletal muscle, heart, and diaphragm.

Production of dystrophin from ataluren treatment was also investigated in nmDMD patients in the Phase 2a PTC124-GD-004-DMD study (Study 004) (Finkel 2013). In this trial, 38 nmDMD patients received treatment with ataluren for 28 days. Results from the proof of concept study indicate that ataluren treatment leads to production of full-length dystrophin protein; post-treatment increases in dystrophin were quantified in 61% (23/38) of the patients. The mean change in muscle biopsy dystrophin expression from baseline to Day 28 was 11% (p=0.008). In addition, immunohistochemistry experiments indicated the protein was correctly localized to membranes of muscle cells, suggesting functional activity. Myotubes derived from pre-treatment muscle biopsies were cultured in vitro in the presence of ataluren and 100% of the samples showed increases in dystrophin expression. Dystrophin expression was also evaluated in Study 007 in which nmDMD patients were treated with ataluren or placebo. Results from Study 007 were also supportive of an increase in dystrophin levels, consistent with Study 004 results. The increase in dystrophin levels in the two prior clinical studies are limited by the quality of muscle biopsy samples and the use of a non-validated immunofluorescence method of detecting dystrophin protein.

1.3 Risk/Benefit Assessment

Under the current standard of care, 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 Study 007 and Study 020.

This study is designed to quantitatively evaluate dystrophin protein levels in muscle biopsies from boys with nmDMD who currently have been receiving ataluren for ≥9 months. The muscle biopsy technique to be used is less invasive than the standard muscle biopsy and is of relative low-risk to the subject. The use of two highly sensitive validated assays to evaluate dystrophin protein levels will increase the likelihood that each muscle biopsy sample will yield interpretable results. Hence, the study design and the importance of establishing the association of ataluren treatment with dystrophin expression results in a positive risk/benefit.

2 STUDY OBJECTIVE AND ENDPOINTS

2.1 Objectives

2.1.1 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

2.1.2 Secondary Objectives

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

2.1.3 Pharmacokinetic:

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

2.2 Endpoints

2.2.1 Primary Endpoint

The primary endpoint is:

- Mean dystrophin levels as measured by ECL

2.2.2 Secondary Endpoints

The secondary endpoint is:

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

2.2.3 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 STUDY DESIGN

3.1 Overall Design

This is a single-site, non-interventional study designed to generate additional data on the effect of ataluren for producing dystrophin protein in nonsense mutation nmDMD subjects. This study will evaluate dystrophin levels from subjects with nmDMD who currently have been receiving ataluren for ≥9 months.

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.

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 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.

3.1.1 Muscle biopsy samples

Muscle biopsies will be performed by appropriately trained staff at [redacted] All subjects will have a physical exam and a history taken and recorded prior to the procedure to assess muscle biopsy locations and determine if there is any intercurrent illness that places a subject at higher risk.

Muscle biopsy of the [redacted] and right gastrocnemius [redacted] will be performed using an established core muscle biopsy procedure (Gallo 2018). 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 450 mg of muscle tissue (up to [redacted] cores per 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 ECL and immunohistochemistry (see Section 3.1.2).

The need for conscious sedation will be evaluated on a case by case basis and will be provided by physicians who are credentialed to monitor and provide sedation in an outpatient setting. Following sedation, vital signs will be monitored until the subject returns to pre-sedation baseline values. These techniques are standard of care at [redacted] for minor procedures on young patients.

3.1.2 Evaluation of dystrophin levels

Two validated assays, ie, ECL and immunohistochemistry, will be used to assess dystrophin levels. The assays provide slightly different information, and together give a robust analysis.

3.1.2.1 Electrochemiluminescence assay

[redacted]
Electrochemiluminescence technology provides high sensitivity with a low background. The assay will be optimized and validated by an external contract research organization (CRO). Subject muscle biopsy samples will be shipped to and analyzed for dystrophin levels by a CRO.

3.1.2.2 Immunohistochemistry assay

Immunohistochemistry will semi-quantitatively assess dystrophin protein levels and evaluate whether the dystrophin protein is correctly localized to the membrane of the muscle cell, consistent with a functional protein. Dystrophin measured using this assay will be the secondary endpoint. [redacted] Subject muscle biopsy samples will be shipped to and analyzed for dystrophin levels by a CRO.

3.2 Scientific Rationale for Study Design

The study is designed to generate additional data on the effect of ataluren on promoting the production of dystrophin protein in nmDMD subjects. It is known that even small increases in dystrophin levels can result in milder disease phenotype in nmDMD patients. The levels of dystrophin in patients with Becker muscular dystrophy (BMD) have been reported to be higher than that observed in patients with Duchenne muscular dystrophy (DMD) (Anthony 2014). Consequently, patients with BMD generally have less severe disease compared with those with DMD; BMD patients often show a later onset of disease and slower disease progression (Muntoni 2003, Aartsma-Rus 2006).

As described in Section 1.2.1, the ability of ataluren to promote ribosomal readthrough of premature stop codons has been demonstrated in a range of in vivo and in vitro preclinical assays. In addition, ataluren-associated increases in dystrophin protein levels was assessed in a prior clinical study. Of patients treated with ataluren 10, 10, 20 mg/kg, 55% (8/20) had an increase in dystrophin expression post-ataluren treatment (Finkel 2013). The findings from this study were consistent with ataluren treatment increasing dystrophin levels and indicated that the protein correctly localized to the muscle cell membranes, suggesting functional activity (Finkel 2013).

Assessing dystrophin levels in patients with nmDMD can be challenging due to inherent difficulty in obtaining muscle samples that contain sufficient amounts of intact muscle cells for analysis, as nmDMD muscle, particularly in older patients, are often heterogenous with respect to fibrofatty replacement of muscle.

From prior clinical trial experience, it is anticipated that although levels or dystrophin may increase in nmDMD muscle from baseline following ataluren therapy, the amount of dystrophin will still be low; hence, sensitive assays with good specificity are necessary to evaluate change in dystrophin levels with treatment.

Method of muscle biopsy sampling can also impact results. For example, variability can be introduced by assessing a bilateral muscle sampled from one side of the body before treatment and from the other side after treatment.

This current study is designed to mitigate some of these difficulties.

- Biopsies will be collected from ambulatory nmDMD subjects who have grade 1 or 2 of the Brook Upper Extremity Scale (Brooke 1989, Connolly 2015) to increase the chance of collecting less heterogenous muscle tissue.
- A total of no more than approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be collected from the [redacted] and [redacted]. If the [redacted] muscle is considered by the investigator to be too small for muscle biopsy sampling, the [redacted] muscle may be used.
- Two validated, highly sensitive assays will be employed to measure ataluren-associated increases in dystrophin protein levels and to evaluate whether the protein localizes correctly to the muscle membrane.

3.3 Justification of Dose

Not applicable

3.4 End of Study Definition

The end of the study is defined as completion of the follow-up phone call to assess adverse events (AEs) which occurs approximately 1 week (±3 days) following Visit 1.

4 STUDY POPULATION

4.1 Overview

4.2 Inclusion Criteria

1. Evidence of signed and dated informed consent/assent document(s) indicating that the subject (and/or his parent/legal guardian) has been informed of all pertinent aspects of the trial.
2. Male sex.
3. Ambulatory (ie, 10m walk/run in <30seconds) and functional grade on the Brooke Upper Extremity Scale of a 1 or a 2.
4. Currently being treated with ataluren 10, 10, 20 mg/kg for ≥9 months, with no gap in treatment of >1 month, prior to study entry.
5. Phenotypic evidence of DMD based on the onset of characteristic clinical symptoms or signs (eg, proximal muscle weakness, waddling gait, and Gowers' maneuver) by 6 years of age and an elevated serum creatine kinase (CK). Medical documentation of phenotypic evidence of DMD needs to be provided upon request by the PTC Therapeutics medical monitor.
6. Willing to undergo muscle biopsy.

4.3 Exclusion Criteria

1. Known contra-indication to muscle biopsy (ie. such as bleeding or clotting disorders).
2. Exposure to another investigational drug within 2 months prior to study enrollment or ongoing participation in any non-ataluren interventional clinical trial.
3. Requirement for daytime ventilator assistance or any use of invasive mechanical ventilation via tracheostomy. *Note: Evening non-invasive mechanical ventilation such as use of bilevel positive airway pressure (Bi-PAP) therapy is allowed*.
4. Prior or ongoing medical condition (eg, concomitant illness, psychiatric condition, behavioral disorder), medical history, physical findings or laboratory abnormality that, in the investigator's opinion, could adversely affect the safety of the subject, makes it unlikely that the course of treatment or follow-up would be completed, or could impair the assessment of study results.

5 ENROLLMENT PROCEDURES

5.1 Source and Number of Subjects

Approximately 6 subjects will be enrolled. Subjects who currently have been receiving ataluren therapy for ≥9 months will be recruited.

5.2 Screening

The Investigator must inform each subject and/or parent/legal guardian of the nature of the study, explain the potential risks, and obtain written informed consent/assent from the subject and/or parent(s)/legal guardian (as required by local regulations) prior to performing any study-related screening procedures.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

Not applicable as not an interventional study.

6.2 Concomitant Therapy

Including ataluren, any treatments (ie, prescription and non-prescription drugs, health foods, herbal remedies, self-prescribed drugs, street drug, tobacco products, or alcohol) that are taken by a subject for one month prior to study enrollment and during Visit 1 are considered concomitant medications. Information regarding any concomitant medications will be collected and documented in the electronic case report form (eCRF) and in the source documents by the clinic staff.

To the extent possible, administration of any prescription or over-the-counter drug products should be minimized during the study period. Subjects should be discouraged from use of "health supplements" (eg, creatine, glutamine, coenzyme Q), herbal remedies, growth hormone, self-prescribed drugs, at any time during clinical studies of ataluren.

If considered necessary for the subject's well-being, drugs for concomitant medical conditions or for symptom management may be given at the discretion of the investigator. The decision to authorize the use of any other drug(s) should consider the subject's safety, the medical need, the potential for drug interactions, the possibility for masking symptoms of a more relevant underlying event, and whether use of a concomitant medication will compromise the outcome or integrity of the study.

Subjects and parents/caregivers or legal guardian should be instructed about the importance of the need to inform the clinic staff of the use of any drugs or remedies (whether prescribed, over-the-counter, or illicit) before and during the course of the study.

The investigator is encouraged to consult the PTC medical monitor or designee with questions relating to specific drugs.

6.3 Discontinuation of Study Intervention

Not applicable

6.4 Participant Discontinuation/Withdrawal from the Study

The parent/caregiver or legal guardian has the right to withdraw consent and discontinue the study at any time.

This study may be discontinued by the relevant regulatory authority, IRB/EC (Institutional Review Board/Ethics Committee), and/or PTC Therapeutics at any time.

7 STUDY ASSESSMENT AND PROCEDURES

7.1 Schedule of Assessments and Study Parameters

Table 1. Schedule of Assessments

Study Procedure	Visit 1[1]	Follow-up (phone call)	Notes
Week (visit window)		**Week 1 (± 3days)**	
Informed Consent	X		A signed and dated informed consent must be obtained before conducting any study procedures.
Inclusion/Exclusion	X		
Demographics	X		
Medical History	X		
Physical Exam	X		Full physical exam will be performed.
Clinical Labs	X		Biochemistry, hematology, and urinalysis laboratory assessments.
Height/Weight/BMI	X		
Vitals (HR & BP)	X		Vital signs will include systolic and diastolic blood pressure (BP), pulse rate, and body temperature. The pulse rate and BP determinations will be performed with the subject in a sitting position after a 5-minute rest. Blood pressure will be measured in triplicate and the average will be recorded.
Timed function tests	X		Tests include time to climb 4 stairs, time to descend 4 stairs, time to rise from supine, and time to run/walk 10 meters.
PK blood sampling	X		PK samples will be drawn pre-morning dose and 2 hours post-morning dose. Ataluren plasma concentrations will be analyzed in a population PK model for the estimation of key pharmacokinetic parameters including Cl/F, V/F, AUC_{0-24}, C_{max}, and C_{ave}.
Muscle biopsy	X		Muscle biopsy will be taken from the [redacted] and [redacted]. If the [redacted] muscle is considered by the investigator to be too small for a muscle biopsy sample, the [redacted] muscle may be used.
AE/SAE Monitoring	X	X	
Concomitant medications	X		Treatments, including ataluren, taken one month prior to enrollment and during Visit 1 will be recorded.

Abbreviations: AE, adverse event; AUC_{0-24}, area under the concentration curve from time 0 to 24 hours; BMI, body mass index; BP, blood pressure; Cl/F, clearance; C_{ave}, average plasma concentration; Cmax, maximal plasma concentration; HR, heart rate; PK, pharmacokinetics; SAE, serious adverse event; V/F, volume of distribution

[1] Subjects may have to return to the clinic for up to 3 days for completion of assessments and muscle biopsy.

7.2 Safety Assessments and Other Assessments

Subjects will be monitored closely for AEs and laboratory abnormalities during the study.

For AEs and laboratory abnormalities, the investigator should use his/her judgment in determining whether the event or abnormality is clinically significant, whether diagnostic evaluation is warranted, and whether potential interruption of study drug therapy is appropriate. In general, life-threatening (Grade 4) or severe (Grade 3) AEs or laboratory abnormalities should be considered clinically significant, although recurrent or persistent moderate events (Grade 2) may also be considered clinically significant in certain circumstances. Reference should be made to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 for grading the severity of adverse events and laboratory abnormalities.

While specific monitoring, diagnostic testing, and supportive care measures must be instituted based on the clinical judgment of the investigator, investigators should contact the PTC Therapeutics medical monitor to obtain guidance and to ascertain whether similar events are being seen at other sites. The PTC Therapeutics medical monitor should be notified of any AE or laboratory abnormality that leads to dose interruption and should be apprised of ancillary laboratory or other diagnostic findings and the evolving data from any work-up of the initial abnormality. The PTC Therapeutics medical monitor may suggest review of the case with gastroenterology, endocrinology, nephrology consultants or with other experts (either at the site or retained by PTC Therapeutics).

7.3 Adverse Events and Serious Adverse Events

7.3.1 Definition of adverse events

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered related to the drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease in a study subject who is enrolled in this study.

For this protocol, untoward medical occurrences that should be reported as AEs include the following:

- All AEs relating to the muscle biopsy
- Apparently unrelated illnesses, including worsening of a preexisting illness
- Injury or accidents. *Note that if a medical condition is known to have caused the injury or accident (a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as 2 separate AEs. The outcome of the accident (hip fracture secondary to the fall) should be recorded in source documents*.

- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test)
- Laboratory that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities associated with a clinical event should be captured in the source documents. Laboratory abnormalities not requiring clinical intervention or further investigation will be captured as part of overall laboratory monitoring and should not be reported as AEs.
- A preexisting condition (eg, allergic rhinitis) must be noted on the appropriate eCRF for Visit 1; however, it should not be reported as an AE unless the condition worsens or episodes increase in frequency during the AE reporting period. Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that occurs during the AE reporting period should be reported as the AE and the resulting appendectomy should be recorded in the source documents and eCRF. If a surgical procedure was planned prior to entry into the study, and the surgery is not performed because of a worsening of a baseline condition, this should not be reported as an AE. *Note that, as described in Section 7.3.2 any hospitalization occurring as the consequence of an AE during the study period should be reported as a serious adverse event (SAE)*.

Each AE is to be classified as serious or non-serious by the investigator using medical and scientific judgment.

7.3.2 Definition of serious adverse events

An SAE is an untoward medical occurrence or effect associated with the use of a study drug at any dose, regardless of whether it is considered to be related to the study drug, which results in one of the following:

- Results in death. This includes all deaths that occurred within 30 days of Visit 1, including deaths due to disease progression. Any death occurring later than 30 days following Visit 1need not be reported as an SAE unless it is a result of an event that started within the period covered by the on-study definition. The reported AE should be the event that caused the death. In addition, any AE resulting in death that occurs subsequent to the AE reporting period and that the investigator assesses as possibly related to the muscle biopsy procedure should also be reported as serious.
- Is life-threatening. This refers to an event in which the subject was at risk of death at the time of the event. It does not include an event that, had it occurred in a more severe form, hypothetically might have caused death.

- Requires hospitalization or prolongation of existing hospitalization (excluding hospitalizations for administration of the study drug, procedures required by the study protocol, or treatment-related diagnostic procedures; other planned hospitalizations; or hospitalizations related only to progression of disease). Treatments in the emergency room for procedures such as hydration that do not require admitting the subject to the hospital and observational durations in the emergency room for less than 24 hours do not fall into this category.
- Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions, not related to cancer.
- Any other medically important event that the investigator or the sponsor judges to be serious or which is defined as serious by the regulatory agency in the local country. These are AEs that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Medical judgment should be exercised in deciding whether an AE is serious based on above definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

7.3.3 Eliciting adverse event information

The investigator is to report all directly observed AEs and all AEs spontaneously reported by the study subject/parent(s)/legal guardian/legally acceptable representative. In addition, each study subject/parent(s)/legal guardian/legally acceptable representative will be questioned about AEs at each scheduled clinic visit after study drug administration or during any telephone contact with the subject/parent(s)/legal guardian/legally acceptable representative. The type of question asked should be open-ended, for example, "*How have you been feeling*?" or a similar type of query.

7.3.4 Recording Non-serious AEs and SAEs

All AEs (both serious and non-serious) which are not related to the muscle biopsy that occur in subjects during the AE reporting period must be recorded in the eCRF of the ataluren nmDMD trial in which the subject was recruited from. Those events considered related to the muscle biopsy should be reported to the Study 046. In addition, any known untoward event that occurs subsequent to the AE reporting period that the investigator assesses as related to the muscle biopsy procedure should also be recorded as an AE in Study 046.

All AEs are to be recorded in the source documents and on the eCRF using concise medical terminology; whenever possible, terms contained in the Medical Dictionary for Regulatory Activities (MedDRA) should be employed. In addition, the following information should be recorded:

- Indication of whether the event is serious or non-serious (see Section 7.3.2)
- Relationship to study procedure (see Section 7.3.5)

- Severity of the event (see Section 7.3.6)
- Onset date
- Resolution date, or date of death
- Action taken
- Outcome of the event

Classification of the event as serious or non-serious determines the reporting procedures to be followed.

7.3.5 Describing adverse event relationship to muscle biopsy procedure

The investigator should provide an assessment of the relationship of the AE to the study muscle biopsy, ie, whether there is a reasonable possibility that the muscle biopsy procedure caused the AE, using the considerations outlined in Table 2.

Table 2. Relationship of Study Muscle Biopsy Procedure to Adverse Event

	Description
Probable	A clinical event in which a relationship to the study muscle biopsy procedure seems probable because of such factors as consistency with known effects of the muscle biopsy procedure, a clear temporal association with the muscle biopsy procedure; or lack of alternative explanations for the event.
Possible	A clinical event occurring coincident with the muscle biopsy procedure and which may or may not be explained by concurrent disease or concomitant drugs or chemicals.
Unlikely	A clinical event with a temporal relationship to the study muscle biopsy procedure that does not preclude causality but for which there is a clear alternate cause that is more likely to have caused the adverse event than the procedure. Such alternatives include a concomitantly administered drug, the subject's disease state, other medical conditions, or environmental factors.
Unrelated	A clinical event, for which a relationship to the study muscle biopsy procedure seems improbable because of factors such as inconsistency with known effects of the muscle biopsy procedure, lack of a temporal association with the muscle biopsy procedure, and/or presence of alternative explanations for the event. Alternative explanations might include a known relationship of the adverse event to a concomitant drug, medical history of a similar event, the subject's disease state, other medical conditions, or environmental factors.

7.3.6 Grading of severity of adverse event related to muscle biopsy procedure

The severity of AE will be graded using the CTCAE Version 5.0. For each episode, the highest severity grade attained should be reported.

If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the AE. For purposes of consistency with the CTCAE, these severity grades are defined in Table 3.

Table 3. Grading of Adverse Event Severity Grade

	Adjective	Description
Grade 1	Mild	Sign or symptom is present, but it is easily tolerated, is not expected to have a clinically significant effect on the subject's overall health and well-being, does not interfere with the subject's usual function, and is not likely to require medical attention
Grade 2	Moderate	Sign or symptom causes interference with usual activity or affects clinical status, and may require medical intervention
Grade 3	Severe	Sign or symptom is incapacitating or significantly affects clinical status and likely requires medical intervention and/or close follow-up
Grade 4	Life-threatening	Sign or symptom results in a potential threat to life
Grade 5	Fatal	Sign or symptom results in death

7.3.7 Adverse Event Reporting

Investigator site reporting requirements for AEs are summarized in Table 4 and Table 5.

Table 4. Investigator Site Requirements for Reporting Adverse Events Not Related to Muscle Biopsy

Event	Recorded on the Parent Study eCRF	Reported on the SAE Report Form to PTC Pharmacovigilance Within 24 Hours of Awareness
Serious AE	All	All
Non-Serious AE	All	None

Abbreviations: AE, adverse event; eCRF, electronic case report form; SAE, serious adverse event

Table 5. Investigator Site Requirements for Reporting Muscle Biopsy-Related Adverse Events

Event	Recorded on the Study 046 eCRF	Reported on the SAE Report Form to PTC Pharmacovigilance Within 24 Hours of Awareness
Serious AE	All	All
Non-Serious AE	All	None

Abbreviations: AE, adverse event; eCRF, electronic case report form; SAE, serious adverse event

All AEs should be followed up by the investigator until they are resolved, or the investigator assesses them as chronic or stable. The investigator should consider protocol guidelines and use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. In the event of additional investigations, the PTC Therapeutics Pharmacovigilance Department or designee should be informed via e-mail or fax. A subject withdrawn from the study because of an AE must be followed by the investigator until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. Follow up may need to continue after the subject has discontinued from the study, and additional investigations may be requested by the PTC Therapeutics medical monitoring team.

The first day of AE reporting will coincide with the date of signing of Informed Consent and including a minimum of 30 calendar days after Visit 1.

7.3.8 Serious adverse event reporting

All SAEs should be reported via the SAE report form to PTC Therapeutics within 24 hours of becoming aware of the event(s). In addition, the AE portion of the eCRF must also be completed.

The SAE report form should be signed by the investigator; however, if the investigator is unable to sign at the time of the event or within 24 hours, the form should be signed by the clinical staff member reporting the SAE (eg, the study coordinator). The SAE report form must be faxed or e-mailed to the PTC Therapeutics Pharmacovigilance Department or designee and to the site IRB/EC (if required by local regulations) within 24 hours.

Follow-up information to the SAE should be clearly documented as "follow-up" in the SAE report form and must also be faxed or e-mailed to the same party. All follow-up SAE report forms for the event must be signed by the investigator. Any source documents (eg, progress notes, nurses' notes, laboratory and diagnostic test results, discharge summaries) provided to the sponsor should be redacted so that the subject's name, address, and other personal identity information are obscured. Only the subject's study number and initials are to be provided (in regions where the provision of such information is permitted). The information in the AE portion of the eCRF and the SAE report form(s) must match or be reconciled. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should be used on both forms.

In the rare event that the investigator does not become aware of the occurrence of an SAE immediately (for example, if a subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and to document his/her first awareness of the AE.

The PTC Therapeutics Pharmacovigilance Department contact information for reporting SAEs is provided below. This information is also provided in the Study Manual and in the SAE report form.

PTC Therapeutics Safety Department
Attention: Pharmacovigilance
E-mail: [redacted]
Facsimile: [redacted]

7.3.9 Reporting Pregnancy

Subjects enrolled in this trial will have not reached sexual maturity; therefore, there are no requirements for pregnancy avoidance for study participants.

7.3.10 PTC Therapeutics Adverse Event Reporting Requirement

As the sponsor of the study, PTC Therapeutics is responsible for reporting certain safety information, particularly SAEs and subject deaths related to participation in the study, to each investigator in an expedited manner. If notification of an AE requiring expedited reporting to investigators is received, PTC Therapeutics or its designated representative will contact each investigational site participating in this study by e-mail, fax, and/or overnight mail such that the investigator can promptly notify the site IRB/EC per their local requirements. The initial expedited safety report will be provided as required according to local regulations (eg, within 15 days) after the earliest date PTC Therapeutics or an agent of PTC Therapeutics (eg, a site monitor) becomes aware of an AE. This awareness date is the date the regulatory reporting clock begins, and the date is considered Day 0.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical Hypotheses

Dystrophin levels will be summarized using descriptive statistics. No statistical hypothesis testing will be performed.

8.1.1 Sample Size Determination

No formal sample size calculation is performed for this study.

8.2 Population for Analyses

8.2.1 Intention-to-treat analysis set

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

8.3 Safety Analysis Set

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

8.4 Statistical Analyses

8.4.1 General approach

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

8.4.2 Analysis of primary efficacy endpoints

The dystrophin level will be summarized by muscle biopsy locations using descriptive statistics based on ITT analysis set.

8.4.3 Analysis of secondary efficacy endpoints

The secondary efficacy endpoints will be summarized in the same way as the primary efficacy endpoint.

8.4.4 Safety analyses

Evaluations of safety will be performed using the safety analysis set. The incidence of AEs and SAEs will be tabulated using frequency count.

8.4.5 Planned interim analyses

There are no planned interim analyses for this study.

8.4.6 Sub-group analyses

Not Applicable

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 Regulatory, Ethical, and Study Oversight Considerations

9.1.1 Informed consent process

By signing the protocol, the investigator assures that informed consent/assent will be obtained from each parent/legal guardian prior to study entry and that the informed consent/assent will be obtained in accordance with current regulations.

The investigator or sub-investigator will give each parent/legal guardian full and adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. An informed consent/assent document will be provided to each parent/legal guardian in a language in which the parent/legal guardian is fluent. This information must be provided to the parent/legal guardian prior to undertaking any study related procedure. Adequate time should be provided for the parent/legal guardian to read the informed consent, to understand the risks and benefits of participating in the study, and to ask any questions that the parent/legal guardian may have about the study. The parent/legal guardian should be able to ask additional questions as and when needed during the conduct of the study. Where applicable, the subject will sign an age-appropriate assent form.

Each parent/legal guardian will be given a copy of the signed consent/assent form. The original signed informed consent forms will be retained by the investigator with the study records.

The written subject information must not be changed without prior approval by PTC Therapeutics and the IRB/EC.

9.1.2 Study discontinuation and closure

PTC Therapeutics reserves the right to discontinue the study prior to inclusion of the intended number of subjects. The investigator, after consultation with the PTC Therapeutics medical monitor, reserves the right to discontinue the study at the investigator site for safety reasons at any time.

After a decision to terminate the study, investigators must contact all subjects who are continuing their participation in the study and must do so within a time period set by PTC Therapeutics. As directed by PTC Therapeutics, all study materials must be collected, and all electronic data entry forms completed to the greatest extent possible.

9.1.3 Confidentiality and privacy

Research records will be collected and stored in a manner that protects the confidentiality of subject information. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs, paper CRFs, or other records provided to or

retained by PTC Therapeutics (or its authorized designee). The names and identities of the subjects need not be divulged; however, the records must nevertheless be inspected. This will be accomplished by blanking out the subject's name and replacing the name with the subject's study identification number on any record provided to or retained by PTC Therapeutics. The informed consent form must include appropriate statements explaining these requirements.

By signing this protocol, the investigator affirms to PTC Therapeutics that the investigator will maintain, in confidence, information furnished by PTC Therapeutics and will divulge such information to the IRB/IEC under an appropriate understanding of confidentiality with such board.

9.1.4 Future use of stored specimens and data

As part of the current study, muscle tissue via biopsies will be collected to evaluate the effect of ataluren treatment on the expression of dystrophin protein as measured by ECL and immunohistochemistry. Sample processing will be performed by laboratories under the direction of the sponsor. Processing, analysis, and storage will be performed at a secure laboratory facility to protect the validity of the data and maintain subject privacy.

The samples will be stored for 10-years. Only PTC Therapeutics and its representatives and agents will have access to muscle biopsy/extracts and they will not be shared with secondary researchers.

These same samples will potentially be used to look at biochemical biomarkers that may be altered due to increased dystrophin levels and to generate new hypotheses (eg, identify factors involved in disease progression and identify possible new drug targets). No additional genetic testing will be performed or cell lines created. The results will be communicated to PTC Therapeutics.

The subject/parent/caregiver will provide confirmation to allow any remaining specimens to be used for exploratory research work by PTC Therapeutics within the Informed Consent Form (ICF). The ICF will contain a separate authorization for the use of remaining tissue for exploratory research. Subjects/parents/caregivers will be told that participation in exploratory research is optional and that they can decline participation in this research. Subjects/parents/caregivers may withdraw their consent and request disposal of their stored samples at any time and for any reason during the storage period.

No subject-identifying information (eg, initials, date of birth, government identifying number) will be associated with any of the biologic samples. All samples will be single coded and the sponsor will take steps to ensure that data are protected accordingly, and confidentiality is maintained to the extent possible.

Given the research nature of the analysis, it will not be possible to return individual data to subjects. The results that may be generated are not currently anticipated to have clinical relevance to the patients or their family members. Therefore, these results will not be disclosed to the patients or their physicians.

9.1.5 Clinical monitoring

In accordance with 21 Code of Federal Regulations Part 312.56 and/or relevant ICH International Council for Harmonisation) guidelines, PTC Therapeutics or a designee will periodically inspect all eCRFs, study documents, research facilities, and clinical laboratory facilities associated with this study at mutually convenient times, before, during, and after completion of the study. As required by applicable regulations (Responsibilities of Sponsors and Investigators), the monitoring visits provide PTC Therapeutics with the opportunity to evaluate the progress of the study; verify the accuracy and completeness of data in the eCRFs; ensure that all protocol requirements, relevant regulations, and investigator's obligations are being fulfilled; and resolve any inconsistencies in the study records. This includes inspection of all documents and records required to be maintained by the Investigator, including but not limited to medical records (office, clinic, or hospital) for the subjects in this study. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs or other records provided to or retained by PTC Therapeutics. The investigator/institution guarantees direct access to source documents by PTC Therapeutics and appropriate regulatory authorities.

It is important that the investigator and relevant institutional personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

9.1.6 Quality assurance and quality control

To ensure compliance with GCP (Good Clinical Practice) and all applicable regulatory requirements, PTC, PTC's representatives, a regulatory authority or and Institutional Review board may conduct a quality assurance audit. Reasons for quality assurance audit may include but are not limited to: random selection, geographic proximity, suspected GCP violation, high enrolling site, recurring protocol deviations, etc. The purpose of a Sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Council for Harmonisation, and any applicable regulatory requirements. The Investigator should contact the Sponsor immediately if contacted by a regulatory agency about an inspection.

9.1.7 Data handling and record keeping

To enable evaluations and/or audits from regulatory authorities or PTC Therapeutics, the investigator agrees to keep accurate and complete records, including the identity of all participating subjects (sufficient information to link eCRFs and clinic records/source documents), all original signed informed consent forms, electronic copies (ie, CD-ROM, USB, etc.) or paper copies of the data that have been captured in the EDC for each subject (eCRFs), and detailed records of study drug disposition. All records and documents pertaining to the study will be maintained by the investigator until notification is received from PTC Therapeutics that the records no longer need to be retained.

The investigator must obtain written permission from PTC Therapeutics before disposing of any records. The investigator will promptly notify PTC Therapeutics in the event of accidental loss or destruction of any study records. If the investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to PTC Therapeutics as applicable.

9.1.8 Protocol deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, investigator, or site staff. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. – either tests not done, incorrect tests done, or not done within the time frame specified in the protocol
- Procedural deviations, failure to update the ICF when new risks become known, or failure to obtain IRB approvals for the protocol and ICF revisions

Significant deviations are any deviations that impact subject eligibility (ie, protocol inclusion/exclusion violations), subject safety or a subject's ability to continue in the clinical trial.

At the outset of the study, a process for defining and handling protocol deviations will be established with the CRO. This will include determining which deviations will be designated significant; thus, requiring immediate notification to the PTC Therapeutics medical monitor and the sponsor.

Prospective deviations (eg, protocol waivers) are prohibited per PTC policy.

The investigator is responsible for seeing that any known protocol deviations are recorded handled as agreed.

9.1.9 Publication and data sharing policy

The information developed during the conduct of this clinical study is considered confidential by PTC Therapeutics. This information may be disclosed as deemed necessary by PTC Therapeutics.

PTC Therapeutics intends that the data from this study will be presented and published. The PTC Therapeutics staff under the direction of the PTC Therapeutics Chief Medical Officer or designee in collaboration with the investigator will be responsible for writing presentations and manuscripts for publication. Investigators will not be allowed to publish or present the data from this study without prior agreement with PTC Therapeutics.

The investigator is obliged to provide the sponsor with complete test results and all data derived by the investigator from the study. During the study, only the sponsor may make study information available to other study Investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the Sponsor.

The sponsor may publish any data and information from the study (including data and information generated by the investigator) without the consent of the Investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

Data from all sites participating in the study will be pooled and analyzed by the sponsor or the sponsor's designee. The first publication of the study results shall be made in conjunction with the results from other study sites as a multicenter publication. If a multicenter publication is not forthcoming within 24 months of completion of the study at all sites, the investigator may publish or present the results generated at his or her site.

The investigator will provide the sponsor with a copy of any proposed publication or presentation for review and comment at least 60 days prior to such presentation or submission for publication. The sponsor shall inform the investigator in writing of any changes or deletions in such presentation or publication required to protect the sponsor's confidential and proprietary technical information and to address inaccurate data or inappropriate interpretations in the context of any pooled multicenter results. At the expiration of such 60-day period, the investigator may proceed with the presentation or submission for publication unless the sponsor has notified the institution or the investigator in writing that such proposed publication or presentation discloses the sponsor's confidential and proprietary technical information. Further, upon the request of the sponsor, the investigator will delay the publication or presentation for an additional 90 days to permit the sponsor to take necessary actions to protect its intellectual property interests.

9.2 Additional Considerations

Not applicable

9.3 Protocol Amendment History

Original Protocol Issued: 12 Nov 2018

Amendment 1 (20 Mar 2019)

The overall reason for the amendment: The overall reason for the amendment was to ensure that sufficient muscle tissue for analysis was obtained from the biopsy procedure.

Item No.	Protocol Section	Amendment/Update	Reason/Rationale
1	Synopsis	Changed from, "[redacted] muscle biopsy samples will be collected from the [redacted] and [redacted]." to "A total of no more than approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be obtained from the [redacted] and [redacted]"	To ensure sufficient muscle tissue for analysis
2	Synopsis	Inclusion criteria #4 changed from, "Currently being treated with ataluren 10, 10, 20 mg/kg for ≥9 months, with no gap in treatment of >1 month, in an ongoing PTC-sponsored nmDMD clinical trial prior to study entry." to "Currently being treated with ataluren 10, 10, 20 mg/kg for ≥9 months, with no gap in treatment of >1 month prior to study entry."	To allow IND subjects to be included
3	List of Abbreviations	Changed from, "International Conferences on Harmonisation" to "International Council for Harmonisation"	Correction
4	Section 3.1	Changed 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."to "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."	To allow IND subjects to be included
5	Section 3.1.1	Changed from, "A total of [redacted] cores from each muscle will be obtained." to "A total of no more than approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be obtained."	To ensure sufficient muscle tissue for analysis
6	Section 3.2	Changed from, "Three muscle biopsy samples will be collected from the [redacted] and [redacted]". to "A total of no more than approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be obtained from the [redacted] and [redacted]."	To ensure sufficient muscle tissue for analysis
7	Section 4.2	Inclusion criteria #4 changed from, "Currently being treated with ataluren 10, 10, 20 mg/kg for ≥9 months, with no gap in treatment of >1 month, in an ongoing PTC-sponsored nmDMD clinical trial prior to study entry." to "Currently being treated with ataluren 10, 10, 20 mg/kg for ≥9 months, with no gap in treatment of >1 month prior to study entry."	To allow IND subjects to be included

Item No.	Protocol Section	Amendment/Update	Reason/Rationale
8	Section 5.1	Changed from, "Subjects will be recruited from ongoing PTC sponsored nmDMD clinical trials who currently have been receiving ataluren therapy for ≥9 months." to "Subjects who currently have been receiving ataluren therapy for ≥9 months will be recruited."	To allow IND subjects to be included
9	Section 7.3.1	Changed from, • "All AEs during the study visit and follow-up, including AEs related to the muscle biopsy" to • "All AEs relating to the muscle biopsy"	Correction
10	Section 7.3.3	Section deleted	Reference Safety Information (RSI) in the ataluren IB does not apply to muscle biopsies
11	Section 8.4.2	Changed from, "The dystrophin level will be summarized by muscle biopsy locations [redacted] using descriptive statistics based on ITT analysis set." to "The dystrophin level will be summarized by muscle biopsy locations using descriptive statistics based on ITT analysis set."	To allow biopsy from the [redacted] muscle if [redacted] muscle is considered by the investigator to be too small for muscle biopsy sampling.
12	Section 9.1.5	Changed from, "International Conferences on Harmonisation" to International Council for Harmonisation"	Correction
13	Section 9.1.6	Changed from, "International Council on Harmonisation" to "International Council for Harmonisation"	Correction
14	Synopsis and Protocol	Document date/version and TOC were updated.	Update

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