NCT #NCT03648827

CLINICAL PROTOCOL

PHASE 2 CLINICAL PHARMACOLOGY STUDY TO ASSESS DYSTROPHIN LEVELS IN SUBJECTS WITH nmDMD BEFORE AND AFTER TREATMENT WITH ATALUREN

PTC124-GD-045-DMD

06 MAY 2019

VERSION 3.0

PTC THERAPEUTICS, INC.

100 CORPORATE COURT

SOUTH PLAINFIELD, NJ 07080 USA.

PROTOCOL SYNOPSIS

Name of Sponsor/Company: PTC Therapeutics
Name of Investigational Product: Ataluren
Title of Study: Phase 2 clinical pharmacology study to assess dystrophin levels in subjects with nonsense mutation Duchenne muscular dystrophy (nmDMD) before and after treatment with ataluren.
Study center(s): Approximately 10 sites
Objectives: ***Primary*:** • To assess the change in levels of dystrophin in ambulatory nmDMD subjects after treatment with ataluren for 40 weeks using a quantitative dystrophin assay, such as electrochemiluminescence (ECL) ***Secondary*:** • To assess dystrophin levels/intensity and protein localization by immunohistochemistry after 40 weeks of ataluren treatment ***Exploratory*:** • To assess the change from baseline in revised North Star Ambulatory Assessment (rNSAA) and timed function tests (TFTs) after 40 weeks of ataluren treatment • To assess changes from baseline in creatine kinase (CK) levels after 40 weeks of ataluren treatment • To evaluate the pharmacokinetics (PK) of ataluren after 40 weeks of treatment
Primary Endpoint Assessment: • The percent change from baseline in dystrophin levels following 40 weeks of ataluren therapy, as measured by ECL **Secondary Endpoint:** • The percent change in dystrophin levels/intensity from baseline after 40 weeks of ataluren therapy as determined by a validated immunohistochemistry assay **Exploratory Endpoints:** • Change from baseline in rNSAA after 40 weeks of ataluren treatment • Change from baseline in TFTs after 40 weeks of ataluren treatment • Change from baseline in CK levels after 40 weeks of ataluren treatment • PK assessments at Week 1 and Week 40
Number of patients (planned): Total of approximately 15 to 20 ataluren-naïve subjects age ≥2 and <8 years

Methodology:
This study is designed to generate additional data on the effect of ataluren on producing dystrophin protein in nmDMD subjects. This study will evaluate dystrophin levels from ataluren-naïve subjects before and after treatment with ataluren for 40 weeks.
Due to some of the challenges of dystrophin quantification, the study has been designed to mitigate these challenges as best possible.

- Biopsies will be collected from subjects ≥2 and <8 years of age who are symptomatic to increase the chance of collecting less heterogenous muscle tissue.
- Up to approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be collected from the [redacted] and [redacted] muscles before initiation of ataluren therapy and after 40 weeks of treatment. If the [redacted] muscle is considered by the investigator to be too small for a biopsy sample (or the obtained sample is considered not evaluable for analysis), the [redacted] muscle may be used.
- The same muscles will be sampled pre- and post-40 weeks of ataluren therapy.
- Two validated, highly sensitive assays will be used to measure ataluren-associated increases in dystrophin levels and to evaluate whether the protein localizes correctly to the muscle membrane.

Dystrophin will be quantified using ECL and immunohistochemistry.

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. Age ≥2 and <8 years.
4. 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 CK. Medical documentation of phenotypic evidence of DMD needs to be provided upon request by the PTC Therapeutics medical monitor.
5. Documentation of the presence of a nonsense point mutation in the dystrophin gene as determined by gene sequencing. *Note: Review and approval of documentation by sponsor or designee is required prior to enrollment.*
6. Willing to undergo muscle biopsy

Exclusion Criteria:

1. Ongoing intravenous (IV) aminoglycoside or IV vancomycin therapy.
2. Known contra-indication to muscle biopsy (ie, such as bleeding or clotting disorders).
3. Prior or ongoing therapy with ataluren.
4. Known hypersensitivity to any of the ingredients or excipients of the study drug (eg, refined polydextrose, polyethylene glycol 3350, poloxamer 407, mannitol 25C, crospovidone XL10, hydroxyethyl cellulose, colloidal silica, magnesium stearate).
5. Exposure to another investigational drug within 2 months prior to start of study treatment, or ongoing participation in any interventional clinical trial.
6. 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.*
7. Elevated serum creatinine or cystatin C levels at screening. *Note: If the initial test result is abnormal, it is permissible to re-test serum creatinine or cystatin C.*

8. 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 course of treatment or follow-up would be completed, or could impair the assessment of study results.
Reference therapy, dosage, and mode of administration: Ataluren will be dosed at 10, 10, 20 mg/kg per day
Duration of Treatment 40 weeks

Table 1. Schedule of Assessments

Study Procedure	Screening[1]	Visit 1[2,3]	Visit 2	Visit 3 End of Study[4]	Unscheduled Visit	Early Termination[4]	Follow-up[5]	Notes
Week (visit window)	**-45 to -1 days**	**Baseline Week 1**	**Week 20 (± 14 days)**	**Week 40 (± 14 days)**				
Eligibility								
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							
Dystrophin genotyping	X							Documentation of the presence of a nonsense point mutation in the dystrophin gene as determined by gene sequencing must be reviewed and approved by sponsor prior to enrollment. Blood will be drawn for sequencing of the dystrophin gene to confirm the presence of a nonsense mutation.
Safety Assessments								
Physical Exam	X	X		X	X		X	Full physical-exam (including evaluation of cardiovascular system, chest and lungs, thyroid, abdomen, nervous system, skin and mucosae, musculoskeletal system, eyes, ears, nose, mouth, throat, spine, lymph nodes, extremities, and genitourinary) will be performed at screening. A targeted physical exam will be conducted at Visits 1 and Visit 3, and follow-up visit. A targeted exam will include nervous and musculoskeletal systems At an unscheduled visit, physical exam will be done as necessary. Physical exam should be performed prior to rNSAA and TFTs assessments and biopsy.

Study Procedure	Screening[1]	Visit 1[2,3]	Visit 2	Visit 3 End of Study[4]	Unscheduled Visit	Early Termination[4]	Follow-up[5]	Notes
Week (visit window)	**-45 to -1 days**	**Baseline Week 1**	**Week 20 (± 14 days)**	**Week 40 (± 14 days)**				
Clinical Labs	X	X		X	X	X		For Visit 1, Visit 3, and unscheduled and early termination visits, subjects should fast approximately 8 hours prior to assessment. Biochemistry and other laboratory assessments will be analyzed by the central laboratory. Biochemistry, hematology, and urinalysis laboratory assessments. Creatine kinase will be assessed via the UV method or an equivalent method. At an unscheduled visit, clinical laboratory assessments will be done as necessary. At Visit 1 and Visit 3, clinical laboratory assessments should be performed prior to biopsy.
Height/Weight/BMI		X	X	X		X		Height (in cm) and weight (in kg) will be measured at Visit 1, Visit 2, and Visit 3-Early Termination. If the subject is unable to stand, sitting arm span and ulna length should be assessed as surrogate measurements for height.
Vitals (HR & BP)		X		X	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. Vitals should be taken prior to rNSAA and TFTs assessments and biopsy.
AE/SAE Monitoring	X	X	X	X	X	X	X	AEs must be assessed and documented at each clinic visit. Subjects, parents/caregiver or legal guardian will be encouraged to report AEs of concern at any time in the intervals between visits.
Concomitant medications	X	X	X	X	X	X		Concomitant medications will need to be collected starting 30 days prior to first dose of study drug.
Efficacy Assessments								
Muscle biopsy		X		X				If a subject received ≥6 months of therapy but early terminated, a biopsy may be performed if subject, parents or legal guardian agrees.
rNSAA		X		X				
TFTs		X		X				

Study Procedure	Screening[1]	Visit 1[2,3]	Visit 2	Visit 3 End of Study[4]	Unscheduled Visit	Early Termination[4]	Follow-up[5]	Notes
Week (visit window)	**-45 to -1 days**	**Baseline Week 1**	**Week 20 (± 14 days)**	**Week 40 (± 14 days)**				
Pharmacokinetic Assessments								
PK blood sampling		X		X				PK samples will be drawn on Visit 1, samples will be drawn pre-first study dose and 2 hours postdose, and on Visit 3 will be drawn pre-morning dose and 2 hours postdose.
Study Drug Administration								
Dispense Drug		X	X					First dose of study drug should be administered following completion of all Baseline (Visit 1) assessments. Because of potential changes in subject body weight over time, a dose adjustment may be made based on the subject's bodyweight at Visit 2 (Week 20). Central pharmacy to dispense drug direct to subject upon completion of Visit 2.
Unused Drug Return/ Compliance			X	X		X		All used and unused study drug kits and unused sachets will be returned as directed.

Abbreviations: AE, adverse event; BP, blood pressure; BMI, body mass index; HR, heart rate; PK, pharmacokinetic; rNSAA, Revised North Star Ambulatory Assessment; SAE, serious adverse event; TFT, timed-function tests; [redacted]; UV, urine volume

1 Screening procedures must take place within 45 days of baseline visit (Visit 1).
2 Any screening procedure completed within and including 7 days of Visit 1 can serve as baseline and does not need to be repeated at Visit 1.
3 Visit 1 will be performed at [redacted]. Subjects may have to return to the clinic for up to 3 days for completion of assessments and biopsy.
4 Visit 3 will be performed at [redacted].
5 Early termination assessments should be performed for subjects who discontinue prematurely from the study.
6 The follow-up visit is for subjects who terminated early/discontinued from the study. Visit to occur ~30 days after termination/discontinuation or until recovery from or stabilization of the AE, whichever comes last. The follow-up visit will also occur for subjects who decided to discontinue ataluren therapy even if they completed assessments at Visit 3.

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
NCT Number	NCT03648827
Protocol Number	PTC124-GD-045-DMD
Protocol Version	Version 3.0
Protocol Version Date	06 May 2019
Protocol Phase	Phase 2
Protocol Title	Phase 2 Clinical Pharmacology Study to Assess Dystrophin Levels in Subjects with nmDMD Before and After Treatment with Ataluren
PTC Clinical Lead/Medical Monitor	[redacted] PTC Therapeutics, Inc. 100 Corporate Court South Plainfield, NJ 07080 USA Telephone (office): [redacted] Facsimile: [redacted] E-mail: [redacted]
PTC Biostatistician	[redacted] PTC Therapeutics, Inc. 100 Corporate Court South Plainfield, NJ 07080 USA Telephone (office): [redacted] Facsimile: [redacted] E-mail: [redacted]
PTC Study Manager	[redacted] PTC Therapeutics, Inc. 100 Corporate Court South Plainfield, NJ 07080 USA Telephone (office): [redacted] Facsimile: [redacted] E-mail: [redacted]

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: _____

Address: _____

City: _____

State/Province: _____

Country: _____

Phone: _____

Fax: _____

Email: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Term	Definition
ACE	Angiotensin-converting enzyme
AEs	Adverse events
ARBs	Angiotensin receptor blockers
BMD	Becker muscular dystrophy
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
cGMP	Current good manufacturing practice
CK	Creatine kinase
CRF	Case report form
CRO	Contract research organization
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficients of variability
DMD	Duchenne muscular dystrophy
ECL	Electrochemiluminescence
EDC	Electronic Data Capture
eCRF	Electronic case report form
EMA	European Medicines Agency
GCP	Good clinical practices
HR	Heart rate
IB	Investigator brochure
IRB	Institutional Review Board
ITT	Intention-to-treat
IV	Intravenous
LOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	messenger ribonucleic acid
nmCF	Nonsense mutation Cystic Fibrosis
nmDMD	Nonsense mutation Duchenne muscular dystrophy
RSI	Reference Safety Information
rNSAA	Revised North Star Ambulatory Assessment
SAEs	Serious adverse events
TFTs	Timed-function tests
[redacted]	

1 INTRODUCTION

1.1 Disease Background

Duchenne muscular dystrophy (DMD) is a rare, debilitating, progressive, and ultimately fatal childhood genetic disease (Bushby 2010a, Bushby 2010b). It is an X-linked genetic muscle disorder that results from a mutation in the DMD gene, which encodes for the dystrophin protein. 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 being 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, the ability to climb stairs, and the ability to walk. Complete loss of ambulation typically occurs in the early teenage years. Loss of the 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 at 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).

Currently, there are no medications that 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); 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 base 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.

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 11.0% (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.

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 the increase in dystrophin protein in boys with nmDMD. The biopsy technique to be used is less invasive than the standard biopsy and is of relative low-risk to the patient. The use of two highly sensitive validated assays to evaluate dystrophin protein levels will increase the likelihood that each biopsy sample will yield interpretable results. Hence, the study design and the importance of establishing the ability of ataluren to increase dystrophin production results in a positive risk/benefit.

2 STUDY OBJECTIVE AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

- To assess the change in levels of dystrophin in ambulatory nmDMD subjects after treatment with ataluren for 40 weeks using quantitative assay, such as electrochemiluminescence (ECL)

2.1.2 Secondary Objectives

- To assess dystrophin levels/intensity and protein localization by immunohistochemistry after 40 weeks of ataluren treatment

2.1.3 Exploratory Objectives

- To assess the change from baseline in revised North Star Ambulatory Assessment (rNSAA) and timed-function tests (TFTs) after 40 weeks of ataluren treatment
- To assess changes from baseline in creatine kinase (CK) levels after 40 weeks for ataluren treatment
- To evaluate the steady state pharmacokinetics (PK) of ataluren after 40 weeks of treatment

2.2 Endpoints

2.2.1 Primary Endpoint

The primary endpoint is:

- The percent change from baseline in dystrophin levels after 40 weeks of ataluren therapy, as measured by ECL

2.2.2 Secondary Endpoints

The secondary endpoint is:

- The percent change in dystrophin levels/intensity from baseline after 40 weeks of ataluren therapy as determined by a validated immunohistochemistry assay

2.2.3 Exploratory Endpoints

Exploratory endpoints include:

- Change from baseline in rNSAA after 40 weeks of treatment
- Change from baseline in TFTs after 40 weeks of treatment
- Change from baseline in CK levels after 40 weeks of treatment
- PK assessments at Week 1 and Week 40

3 STUDY DESIGN

3.1 Overall Design

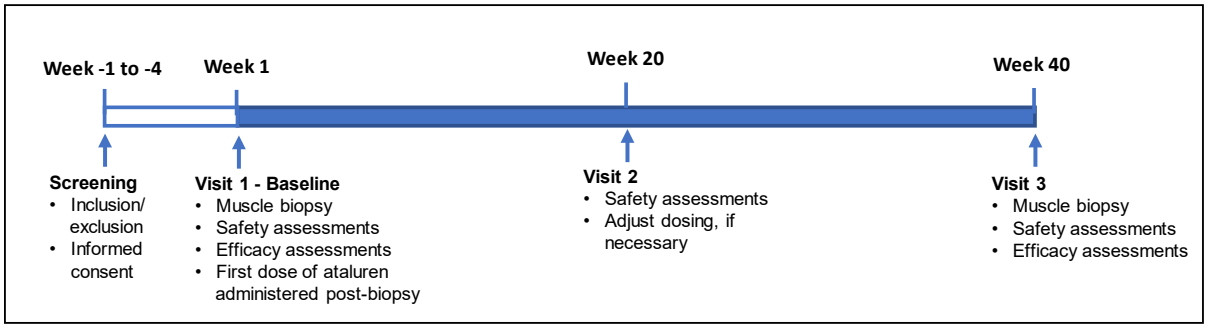
This is an open-label, single-arm, Phase 2 study designed to evaluate the ability of ataluren treatment to increase dystrophin protein levels in muscle cells of patients with nmDMD. The study will evaluate the levels of dystrophin before and after 40 weeks of ataluren therapy using muscle biopsies and two validated assay methods.

Approximately, 15 to 20 ataluren-naïve male subjects, aged ≥2 and <8 years of age will be administered ataluren.

Ataluren will be 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).

The study will have a screening visit and 3 additional visits (Figure 1). At the screening visit, which will be performed at a patient's local site, inclusion/exclusion criteria, demographics and medical history will be assessed. In addition, blood will be drawn for genotyping to confirm that a subject carries a nonsense mutation in the dystrophin gene. For all subjects, Visit 1 and Visit 3 will occur at the same site, that of [redacted]. During Visit 1 and Visit 3 biopsies will be performed and used for assessing dystrophin protein levels. At Visit 1, the first dose of ataluren will be administered following the biopsy at [redacted] and subjects, parent/caregivers or legal guardian will be dispensed a supply of ataluren which will be administered at home. Visit 2 will be in the clinic at the subjects' local site and weight will be measured to determine if the dose of ataluren requires adjustment. Adverse events, (SAEs), and compliance will also be captured, and an additional supply of ataluren will be dispensed.

Figure 1. Study Schematic



3.1.1 Muscle biopsy samples

Biopsies will be performed before initiation of ataluren therapy and after 40 weeks of treatment by appropriately trained staff at [redacted]. All subjects will have a physical exam and a history taken and recorded within 7 days of the procedure, usually on the day before the procedure to assess biopsy locations and determine if there is any intercurrent illness that places a subject at higher risk.

Biopsy of the [redacted] and [redacted] muscles will be performed using an established core biopsy procedure (Gallo 2018). If the [redacted] muscle is considered by the investigator to be too small for a biopsy sample (or the obtained sample is considered not evaluable for analysis), the [redacted] muscle may be used. Up to approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be taken from each muscle at both Visit 1 and Visit 3. Conscious sedation will be offered with propofol and fentanyl to provide sedation and analgesia by physicians 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 measure changes from baseline in dystrophin levels following ataluren therapy. The assays provide slightly different information, and together give a robust analysis.

[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 biopsy samples will be shipped to and analyzed for dystrophin levels by a CRO.

3.1.2.1 Immunohistochemistry

Immunohistochemistry will semi-quantitatively assess dystrophin protein levels [redacted] Dystrophin measured using this assay will be the secondary endpoint. [redacted] Subject biopsy samples will be shipped to and analyzed for dystrophin levels by a CRO.

3.1.3 Assessment of rNSAA and TFTs

As exploratory outcomes, the study will also assess the efficacy of ataluren using the rNSAA and TFTs using standardized procedures pre- and post-40 weeks of ataluren therapy. The rNSAA evaluates physical function and TFTs measure peak physical capacity.

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 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 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 level 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 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 subjects ≥2 and <8 years of age who are symptomatic to increase the chance of collecting less heterogenous muscle tissue.
- Up to approximately 450 mg of muscle tissue (up to [redacted] cores per muscle) will be collected from the [redacted] muscles located on the [redacted] side of the body before initiation of ataluren therapy and after 40 weeks of treatment. If the [redacted] muscle is considered by the investigator to be too small for biopsy sampling (or the obtained sample is considered not evaluable for analysis), the [redacted] muscle may be used.
- The same muscles will be sampled pre- and post-40 weeks of ataluren therapy.

- Two validated, highly sensitive assays will be used to measure ataluren-associated increases in dystrophin levels and to evaluate whether the protein localizes correctly to the muscle membrane.

3.3 Justification of Dose

The proposed ataluren dosing regimen of 10, 10, 20 mg/kg has been demonstrated to be safe and effective in delaying disease progression in nmDMD patients in the Phase 2 clinical Study 007 and the Phase 3 Study 020. It is also the dose that received conditional approval by the EMA (European Medicines Agency).

3.4 End of Study Definition

The end of the study is defined as completion of assessment on Visit 3, or after follow-up for those subjects with early termination or who decided to stop receiving ataluren. PTC will ensure that patients who complete the study and want to remain on ataluren will have access to the drug.

4 STUDY POPULATION

4.1 Overview

The study population is pre-defined to be homogeneous. The inclusion/exclusion criteria select for subjects with severe phenotype only (nmDMD) and against patients with BMD. The inclusion criteria require phenotypic evidence of DMD based on signs and symptoms onset by 6 years of age. This includes proximal muscle weakness, waddling gait, and Gowers' maneuver and elevated serum CK levels.

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. Age ≥2 and <8 years.

4. 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 CK. Medical documentation of phenotypic evidence of DMD needs to be provided upon request by the PTC Therapeutics medical monitor.

5. Documentation of the presence of a nonsense point mutation in the dystrophin gene as determined by gene sequencing. *Note: Review and approval of documentation by sponsor or designee is required prior to enrollment.*

6. Willing to undergo muscle biopsy

4.3 Exclusion Criteria

1. Ongoing intravenous (IV) aminoglycoside or IV vancomycin therapy.

2. Known contra-indication to muscle biopsy (ie. such as bleeding or clotting disorders)

3. Prior or ongoing therapy with ataluren.

4. Known hypersensitivity to any of the ingredients or excipients of the study drug (eg, refined polydextrose, polyethylene glycol 3350, poloxamer 407, mannitol 25C, crospovidone XL10, hydroxyethyl cellulose, colloidal silica, magnesium stearate).

5. Exposure to another investigational drug within 2 months prior to start of study treatment, or ongoing participation in any interventional clinical trial.

6. 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.*

7. Elevated serum creatinine or cystatin C levels at screening. *Note: If the initial test result is abnormal, it is permissible to re-test serum creatinine or cystatin C.*

8. 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 15 to 20 subjects will be enrolled. Subjects will be recruited from dystrophinopathy populations who receive care or referred for evaluation at the investigational site. Investigators will discuss the possibility of participation directly with parent(s)/legal guardian in the clinic.

5.2 Screening

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

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study intervention description

Open-label ataluren will be provided as white to off-white granules for oral suspension. The drug substance and drug product are manufactured under current good manufacturing practice (cGMP) conditions. The formulation includes matrix and suspending agents, surfactants, and various excipients that aid in the manufacturing process. The granules for oral suspension are packaged in aluminum foil, child resistant sachets (packets) and supplied in dose strengths containing 125, 250, or 1000 mg of the active drug substance.

Ataluren will be supplied free of charge by PTC Therapeutics for appropriate distribution to the subjects, parent/caregivers or legal guardian.

6.1.2 Dosing and administration

During the 40 week treatment period, dosing of ataluren will be based on milligrams of drug per kilogram of subject body weight and will be adjusted to allow for dosing with up to 2 of the available sachet dose strengths (125 mg, 250 mg, and/or 1000 mg). The sachet dose strengths and number of sachets to be taken per dose will be calculated (see appendix for dosing table).

The clinic staff (eg, pharmacist or other qualified person) will be responsible for dispensing study drug according to instructions at the Baseline Visit (Visit 1). A central pharmacy will ship study drug directly to the subject upon Visit 2 completion. Multiple kits may be dispensed at a single visit (maximum of two different strengths) at Visits 1 and 2 (Week 1 and Week 20). At Visit 1 and Visit 2 drug will be dispensed.

6.1.3 Return of study drug

Subjects and/or parents or legal guardian should return all used and unused kits and unused sachets as instructed. Study drug dispensing to the subjects and the return of any unused study drug for compliance assessments will be documented.

For any subject experiencing an overdose, observation for any symptomatic side effects should be instituted, and vital signs and biochemical and hematological parameters should be followed closely (consistent with the protocol or more frequently, as needed). Appropriate supportive management to mitigate adverse effects should be initiated. Pending the acquisition of sufficient human experience with the drug, use of gastric lavage or induction of emesis is not specifically recommended or contraindicated.

The PTC Therapeutics medical monitor must be contacted if an overdose occurs. Under applicable regulations, overdosing may be considered a SAE and should be reported accordingly (see Section 7.3).

6.1.4 Inadvertent exposure and spill precautions

Based on available data from nonclinical studies, ataluren does not appear to be acutely toxic or genotoxic at levels that are likely to result from inadvertent exposure to the contents of the packet, if opened. Based on general experience with the drug during manufacturing, it does not appear that exposure to formulated ataluren is likely to be irritating to skin or eyes. However, personnel handling the drug should use reasonable precautions to avoid eye contact, skin contact, inhalation, or ingestion of the material in the packets. Refer to the ataluren Investigator Brochure (IB) for current information on inadvertent exposures and spill precautions.

6.2 Preparation/Handling/Storage/Accountability

Study drug sachets should be stored at room temperature, away from the reach of children until time of reconstitution and should only be opened at the time of dose preparation. The available stability data from representative samples support the use of the drug product for 48 months when stored at room temperature. The full contents of the sachets should be mixed with at least 30 mL (1 ounce) of liquid (water, milk, fruit juice, fruit punch), or 3 tablespoons of semi-solid food (yogurt, pudding, or applesauce) and administered with food. The prepared dose should be mixed well and stirred for approximately 30 to 60 seconds before administration. The amount of the liquid can be increased based on subject preference.

Each prepared dose is best administered immediately after preparation. The prepared dose should be discarded if not consumed within 24 hours of preparation (if kept refrigerated), or within 3 hours of preparation (if kept at room temperature).

The clinic staff will instruct each subject or parent/caregiver or legal guardian on the specific number of sachets to be taken from each kit for each dose and will provide detailed oral directions regarding drug preparation. In addition, detailed written drug mixing and dosing instructions will be provided to the subject or parent/caregiver or legal guardian when drug supplies are dispensed.

It is anticipated that palatability of ataluren will not be a concern with this young population of nmDMD subjects; and consequently, will not impact ingestion of the drug. Palatability was assessed in study PTC124-GD-030-DMD (Study 030), a Phase 2, randomized, open-label study that evaluated the safety, PK, and pharmacodynamics of ataluren in nmDMD patients ≥2 and <5 years of age (N=14). Palatability of ataluren was investigated using a palatability questionnaire that assessed characteristics of the drug such as smell and taste. A parent/caregiver could respond on behalf of the subject. Of the 14 study subjects, 10 (71.5%) agreed or strongly agreed that the medication was pleasant based on the child's reaction. Only 2 (14.3%) respondents reported having problems giving the medication to the child.

6.2.1 Accountability

Study personnel must ensure that all study drug supplies are kept in a secure locked area with access limited to authorized personnel. Study drug must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study drug to other investigators, clinics, or allow the study drug supplies to be used other than as directed by this protocol.

The investigator/ site personnel and/or central pharmacy must maintain accurate records of the receipt of all study drug shipped by PTC Therapeutics or its designee, including, but not limited to, the date received, lot number, amount received, amount returned, and the disposition of all study drug product. Drug accountability records must also be maintained by the site and central pharmacy that include the subject's assigned study number, date and amount of study drug dispensed, and relevant lot and sachet numbers.

Study drug must be returned to PTC Therapeutics or its designee, except where sites are required to destroy study drug per their local standard operating procedures.

6.2.2 Formulation, appearance, packaging, and labeling

Drug kits will be provided, each of which contains sachets of one of the dose strengths (125, 250, or 1000 mg). Sachets and cartons will be color-coded to indicate dosage strength (125 mg - yellow, 250 mg -pink, 1000 mg - blue). Each kit will have a unique kit identification number. The content of the labeling will be in accordance with local regulatory specifications and requirements.

6.3 Measures to Minimize Bias: Randomization and Blinding

Bias due to sample evaluation will be abrogated by the fact that all biopsy samples will be frozen and the pre-therapy and post-treatment biopsy samples will be analyzed at the same time for dystrophin levels by ECL and immunohistochemistry assays. In addition, the persons performing and evaluating the findings of the two assays will be blinded to the timing of sample collection.

6.4 Concomitant Therapy

Other than the study drug, any treatments (including prescription and non-prescription drugs, health foods, herbal remedies, self-prescribed drugs, street drug, tobacco products, or alcohol) that are taken by a subject during the screening period, during study drug administration, and for 4 weeks after discontinuation of study drug are considered concomitant medications. Information regarding any concomitant medications will be collected and documented in the case report form (CRF) and in the source documents by the clinic staff. Study intervention discontinuation and participant discontinuation/withdrawal.

To the extent possible, administration of any prescription or over-the-counter drug products other than study medication 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 and their potential for interactions with ataluren.

6.4.1 Corticosteroids

Corticosteroid therapy in DMD improves muscle strength and function in the short term (Matthews 2016). To minimize potential confounding effects of changes in corticosteroid therapy on the interpretation of study results, the use of these medications must be standardized as much as possible during the study.

A stable and standardized corticosteroid regimen should be maintained for 3 months prior to Visit 1. Adjustments in corticosteroid dosage for increases in body weight are permitted during study treatment but are not mandatory.

6.4.2 Aminoglycosides and Vancomycin

Ataluren should not be co-administered with IV aminoglycosides (eg, tobramycin, gentamicin, amikacin, kanamycin and/or IV vancomycin), based on cases of decreased renal function observed in a clinical trial of ataluren in patients with nonsense mutation cystic fibrosis (nmCF). Elevations of serum creatinine occurred in several nmCF patients treated with ataluren and IV aminoglycosides/vancomycin together with other antibiotics for cystic fibrosis exacerbations. The serum creatinine elevations resolved in all cases, with discontinuation of the IV aminoglycoside/vancomycin, and either continuation or interruption of ataluren. These findings suggested that co-administration of ataluren and IV aminoglycosides/vancomycin may potentiate the nephrotoxic effect of these antibiotics. For additional details, refer to the IB.

In subjects, who require treatment for serious infections, investigators should consider substituting with alternate antibiotics for systemic aminoglycosides/vancomycin when clinically appropriate. For subjects requiring systemic antibiotic therapy, IV aminoglycosides/vancomycin may be used when medically necessary. If IV aminoglycosides/vancomycin are administered, study drug must be interrupted during the course of these antibiotic treatments. Subjects requiring IV aminoglycoside or IV vancomycin therapy should be closely monitored in an appropriate setting, such as a hospital. In subjects receiving potentially nephrotoxic agents such as IV aminoglycosides or IV vancomycin, antibiotic drug levels (if appropriate) and serum creatinine and blood urea nitrogen (BUN) should be followed closely. The antibiotic trough level (if appropriate) and creatinine and BUN should be measured within 24 to 48 hours of administration of the first antibiotic dose, and further antibiotic dosing should be based on these results. Creatinine and BUN should be measured prior to initiating IV aminoglycoside or vancomycin therapy and at least weekly during the course of antibiotic treatment.

6.4.3 Hydration

Because of the potential risk of renal dysfunction during periods of dehydration in subjects receiving study drug, it is important to encourage study subjects to maintain adequate hydration throughout the study. Subjects should be adequately hydrated prior to receiving potentially nephrotoxic agents such as IV aminoglycosides or IV vancomycin, and hydration status should be carefully monitored throughout the administration of these agents. Investigators should be particularly vigilant with subjects who are experiencing nausea, vomiting, diarrhea, or fever, or who have laboratory evidence of dehydration.

6.4.4 Cardiac Drugs for Cardiomyopathy Prophylaxis/Treatment

The use of angiotensin-converting enzyme (ACE) inhibitors for the management of DMD-associated cardiomyopathy has become widespread in the DMD population (El-Aloul 2017). In subjects who are unable to tolerate ACE inhibitors, angiotensin receptor blockers (ARBs) are sometimes used. Beta-blocker therapy is sometimes initiated after ACE inhibitor/ARB therapy for progressive cardiac decline. Finally, the use of an aldosterone antagonist has recently demonstrated favorable effects on cardiac function in DMD (Raman 2015). Because changes in the use of cardiac drugs could introduce confounding influences on the interpretation of results, such changes should be minimized during study drug therapy.

For subjects who are not on cardiac drugs for the prophylaxis/treatment of cardiomyopathy, initiation of such drugs during the 40 weeks of study drug treatment is discouraged unless there is a strong medical need.

For subjects who are on cardiac drugs, they should be on a stable dose for at least 1 month prior to Visit 1 and a stable regimen should be maintained during the 40 weeks of study drug treatment. Adjustments in dosage or type of drug are permitted to avoid symptoms (eg, cough associated with ACE inhibitors) but attempts should be made to avoid entirely removing subjects from cardiomyopathy prophylaxis/treatment. Subjects who require initiation,

interruption, dose modification, or reinstitution of cardiomyopathy prophylaxis/ treatment may remain on study drug therapy.

6.4.5 Drugs Metabolized by Cytochrome P450 Enzymes

As the primary route of ataluren metabolism is via glucuronidation by UDPglucuronosyl transferase 1-9 (UGT1A9), clinically significant interactions between ataluren and coadministered drugs metabolized by cytochrome P450 enzymes (CYPs) are unlikely. In particular, ataluren is not an inhibitor of CYP1A2, CYP2B6, CYP2C19, CYP2D6, and CYP3A4/5, and does not have induction potential on the major CYP enzymes.

In vitro, ataluren is a weak inhibitor of CYP2C8 and CYP2C9, but in vivo drug-drug interactions mediated by these enzymes are not expected according to the criteria described in the EMA guideline on the investigation of drug interactions (EMA 2012). As an added measure of safety, however, investigators should pay specific attention to use of drugs that are known substrates of these enzymes, particularly when such drugs may have a low therapeutic index.

6.4.6 Other Potential Drug Interactions

Based on in vitro studies, ataluren is a substrate of UGT1A9. Coadministration with rifampin, a strong inducer of metabolic enzymes, including UGT1A9, decreased ataluren exposure by 30%. The significance of these findings is unknown. Caution should be exercised when ataluren is coadministered with medicinal products that are inducers of UGT1A9 (eg, rifampicin).

In vitro data indicate that ataluren is an inhibitor of organic anion transporter 1 (OAT1), organic anion transporter 3 (OAT3) and organic anion transporting polypeptide 1B3 (OATP1B3). Caution should be exercised when ataluren is co-administered with e.g. OAT1, OAT3, or OATP1B3 (eg, oseltamivir, acyclovir, ciprofloxacin, captopril, furosemide, bumetanide, valsartan, pravastatin, rosuvastatin, atorvastatin, pitavastatin) because of the risk of increased plasma concentration of these drugs.

6.5 Discontinuation of Study Intervention

If after appropriate consideration of study drug interruption/modification and consultation with the PTC Therapeutics medical monitor, it is not appropriate for a subject to continue with study treatment, then study drug should be permanently discontinued. After permanent discontinuation of study drug for a safety concern, and if the initial event was reported as a SAE, then a follow-up SAE report form should be completed. In the case of a treatment discontinuation due to an event that is not an SAE, the PTC Therapeutics medical monitor should be notified (see Section 7.3). In addition, details regarding the reasons for discontinuation and the adverse events (AEs) leading to the discontinuation should be recorded in the source documents and in the appropriate CRF. The Early Termination Visit CRF should be completed and appropriate follow-up, ie, at ~4 weeks as per protocol or until recovery from or stabilization of the AE, whichever comes last.

6.6 Participant Discontinuation/Withdrawal from the Study

All subjects who receive ataluren should remain in the study whenever possible. However:

- The parent/caregiver or legal guardian has the right to withdraw consent and discontinue ataluren at any time.
- If the subject's condition substantially worsens after initiating ataluren, the subject will be carefully evaluated by the Investigator. The subject will be withdrawn from treatment if continuing would place them at risk.
- The Investigator may withdraw the subject from ataluren, if, in the Investigator's clinical judgment, it is not in the subject's best interest to continue.
- Compliance will be evaluated at Visit 2, Visit 3, and at an early termination visit. Compliance is defined as a subject taking 80% to 120% of doses. In the event a subject becomes significantly noncompliant with ataluren administration, study procedures, or study requirements, the subject should be withdrawn from ataluren when the circumstances surrounding noncompliance increase risk to the subject or are anticipated to substantially compromise the interpretation of study results.
- If genotyping of the dystrophin gene indicates that patient does not carry a nonsense mutation in the gene.
- This study may be discontinued by the relevant regulatory authority, IRB/EC (Institutional Review Board/Ethics Committee), and/or PTC Therapeutics at any time.

The date ataluren is discontinued and the reason for discontinuation will be recorded in the source documents and in the electronic case report form (eCRF). The PTC medical monitor (or designee) should be informed via e-mail of when a subject discontinues study drug.

When ataluren is discontinued (regardless of the reason), the Investigator is expected to capture all the evaluations required at the end of study or follow-up and any additional evaluations should be completed that may be necessary to ensure that the subject is free of untoward effects. The subject should be encouraged to seek appropriate follow-up for any continuing health problems.

7 STUDY ASSESSMENT AND PROCEDURES

7.1 Schedule of Assessments and Study Parameters

Table 1. Schedule of Assessments

Study Procedure	Screening[1]	Visit 1[2,3]	Visit 2	Visit 3 End of Study[3]	Unscheduled Visit	Early Termination[4]	Follow-up[5]	Notes
Week (visit window)	**-45 to -1 days**	**Baseline Week 1**	**Week 20 (± 14 days)**	**Week 40 (± 14 days)**				
Eligibility								
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							
Dystrophin genotyping	X							Documentation of the presence of a nonsense point mutation in the dystrophin gene as determined by gene sequencing must be reviewed and approved by sponsor prior to enrollment. Blood will be drawn for sequencing of the dystrophin gene to confirm the presence of a nonsense mutation.
Safety Assessments								
Physical Exam	X	X		X	X		X	Full physical-exam (including evaluation of cardiovascular system, chest and lungs, thyroid, abdomen, nervous system, skin and mucosae, musculoskeletal system, eyes, ears, nose, mouth, throat, spine, lymph nodes, extremities, and genitourinary) will be performed at screening. A Full physical-exam will be performed at screening and targeted physical exams at Visits 1 and Visit 3, and follow-up visit. A targeted exam will include nervous and musculoskeletal systems. At an unscheduled visit, physical exam will be done as necessaryPhysical exam should be performed prior to rNSAA and TFTs assessments and biopsy.

Study Procedure	Screening[1]	Visit 1[2,3]	Visit 2	Visit 3 End of Study[3]	Unscheduled Visit	Early Termination[4]	Follow-up[5]	Notes
Week (visit window)	**-45 to -1 days**	**Baseline Week 1**	**Week 20 (± 14 days)**	**Week 40 (± 14 days)**				
Clinical Labs	X	X		X	X	X		For Visit 1, Visit 3, and unscheduled and early termination visits, subjects should fast approximately 8 hours prior to assessment. Biochemistry and other laboratory assessments will be analyzed by the central laboratory. Biochemistry, hematology, and urinalysis laboratory assessments. Creatine kinase will be assessed via the UV method or an equivalent method. At an unscheduled visit, clinical laboratory assessments will be done as necessary. At Visit 1 and Visit 3, clinical laboratory assessments should be performed prior to biopsy.
Height/Weight/BMI		X	X	X		X		Height (in cm) and weight (in kg) will be measured at Visit 1, Visit 2, and Visit 3-Early Termination. If the subject is unable to stand, sitting arm span and ulna length should be assessed as surrogate measurements for height.
Vitals (HR & BP)		X		X	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. Vitals should be taken prior to rNSAA and TFTs assessments and biopsy.
AE/SAE Monitoring	X	X	X	X	X	X	X	AEs must be assessed and documented at each clinic visit. Subjects, parents/caregiver or legal guardian will be encouraged to report AEs of concern at any time in the intervals between visits.
Concomitant medications	X	X	X	X	X	X		Concomitant medications will need to be collected starting 30 days prior to first dose of study drug.
Efficacy Assessments								
Muscle biopsy		X		X				If a subject received ≥6 months of therapy but early terminated, a biopsy may be performed if subject, parents or legal guardian agrees.
rNSAA		X		X				
TFTs		X		X				

Study Procedure	Screening[1]	Visit 1[2,3]	Visit 2	Visit 3 End of Study[3]	Unscheduled Visit	Early Termination[4]	Follow-up[5]	Notes
Week (visit window)	**-45 to -1 days**	**Baseline Week 1**	**Week 20 (± 14 days)**	**Week 40 (± 14 days)**				
Pharmacokinetic Assessments								
PK blood sampling		X		X				PK samples will be drawn on Visit 1, samples will be drawn pre-first study dose and 2 hours postdose, and on Visit 3 will be drawn pre-morning dose and 2 hours postdose.
Study Drug Administration								
Dispense Drug		X	X					First dose of study drug should be administered following completion of all Baseline (Visit 1) assessments. Because of potential changes in subject body weight over time, a dose adjustment may be made based on the subject's bodyweight at Visit 2 (Week 20). Central pharmacy to dispense drug direct to subject upon completion of Visit 2.
Unused Drug Return/ Compliance			X	X		X		All used and unused study drug kits and unused sachets will be returned as directed.

Abbreviations: AE, adverse event; BP, blood pressure; BMI, body mass index; HR, heart rate; PK, pharmacokinetic; rNSAA, Revised North Star Ambulatory Assessment; SAE, serious adverse event; TFT, timed-function tests; [redacted], [redacted] UV, urine volume

1 Screening procedures must take place within 45 days of baseline visit (Visit 1).
2 Any screening procedure completed within and including 7 days of Visit 1 can serve as baseline and does not need to be repeated at Visit 1.
3 Visit 1 and Visit 3 will be performed at [redacted]. Subjects may have to return to the clinic for up to 3 days for completion of assessments and biopsy.
4 Early termination assessments should be performed for subjects who discontinue prematurely from the study.
5 The follow-up visit is for subjects who terminated early/discontinued from the study. Visit to occur~30 days after termination/discontinuation or until recovery from or stabilization of the AE, whichever comes last. The follow-up visit will also occur for subjects who decided to discontinue ataluren therapy even if they completed assessments at Visit 3.

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) AE 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.CTCAE

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).

Cases of decreased renal function have been observed in patients with nmCF receiving ataluren and IV aminoglycosides together with other antibiotics for cystic fibrosis exacerbations (Section 6.4.2). As a precaution, Table 2provides information on actions to be taken in the event that abnormalities are noted in specified renal laboratory parameters. Thresholds are provided for interrupting study drug immediately or for interrupting study drug after confirmation of a value beyond the threshold. For AEs or laboratory abnormalities not listed in Table 2, 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 is appropriate.

Table 2. Renal Monitoring Parameters and Actions To Be Taken

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
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

Abbreviations: BUN = blood urea nitrogen; ULN = upper limit of normal
a Laboratory abnormalities may be confirmed immediately or at the next scheduled clinic visit based on investigator judgment.

7.3 Adverse Events and Serious Adverse Events

7.3.1 Definition of adverse events (AE)

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 administered study drug in this study.

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

- All AEs during the course of treatment with study drug administration
- All AEs resulting from medication misuse, abuse, withdrawal, or overdose, of study drug
- All AEs resulting from medication errors such as dispensing or administration error outside of what is described in the protocol
- 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 or electrocardiogram abnormalities 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 CRF 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 treatment with study

drug 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 an 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 (SAE)

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 on treatment or within 30 days after last study drug administration, including deaths due to disease progression. Any death occurring later than 30 days following the last dose need 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 study drug 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 Unexpected Adverse Events

The IB contains the Reference Safety Information (RSI) which will be used for assessing expectedness. If an event is not listed in the RSI, it should be considered unexpected or if the AE occurs at a greater severity, specificity or frequency, it should be considered unexpected.

7.3.4 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.

7.3.5 Recording Non-serious AEs and SAEs

All AEs (both serious and non-serious) that occur in subjects during the AE reporting period must be recorded, whether or not the event is considered drug related. In addition, any known untoward event that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational drug/product should also be recorded as an AE.

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 drug (see Section 7.3.6)
- Severity of the event (see Section 7.3.7)
- 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.

7.3.6 Describing adverse event relationship to study drug

The investigator should provide an assessment of the relationship of the AE to the study drug, ie, whether there is a reasonable possibility that the study drug caused the AE, using the considerations outlined in Table 3.

Table 3. Relationship of Study Drug to Adverse Event Relationship

	Description
Probable	A clinical event in which a relationship to the study drug seems probable because of such factors as consistency with known effects of the drug; a clear temporal association with the use of the drug; improvement upon withdrawal of the drug; recurrence upon rechallenge with the drug; lack of alternative explanations for the event.
Possible	A clinical event occurring coincident with administration of the study drug and which may or may not be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal or rechallenge may be lacking.
Unlikely	A clinical event with a temporal relationship to the study drug exposure 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 study drug. 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 drug seems improbable because of factors such as inconsistency with known effects of the study drug, lack of a temporal association with study drug administration, lack of association of the event with study drug withdrawal or rechallenge, 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.

7.3.7 Grading of severity of adverse event

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 intensity grades are defined in Table 4.

Table 4. 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

7.3.8 Adverse Event Reporting

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

Table 5. Investigator Site Requirements for Reporting Adverse Events

Event	Recorded on the eCRF	Reported on the SAE Report Form to PTC Pharmacovigilance Within 24 Hours of Awareness
Serious AE	All	All
Non-Serious AE	All	None
Occupational exposure	All (regardless of whether associated with an AE), except occupational exposure	Occupational exposure (regardless of whether associated with an AE)

Abbreviations: 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 the last administration of study drug.

7.3.9 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.

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

7.3.10 Reporting Pregnancy

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

7.3.11 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

The null hypothesis of this study is that the change in dystrophin levels following 40 weeks of ataluren therapy, as measured by an immunoassay such as ECL is zero with the alternative hypothesis being that the change is greater than zero. To evaluate this hypothesis a one-sided test at the 5% alpha level will be employed.

8.2 Sample Size Determination

Approximately, 15 to 20 ataluren-naïve male subjects, aged ≥2 and <8 years of age is planned to be enrolled in this study. Limited information is available about the magnitude and intra-subject variability of the change in dystrophin levels, as measured by an immunoassay such as ECL following 40 weeks ataluren therapy in this patient population. Assuming a baseline dystrophin level of 0.2% (as per previous dystrophin replacement therapy [Center for Drug Evaluation and research Application number: 206488Orig1s000 Eteplirsen Summary Review]), Table 6 shows the number of subjects needed to achieve a 85% power to detect an increase from baseline in dystrophin of 0.04%, 0.06% and 0.08%, for intra-subject coefficients of variability (CV) ranging from 20% to 35%, using a one-sided test at alpha 5%. As an example, if the increase from baseline is 0.08% and the intra-subject coefficient is 30%, 13 evaluable subjects would be required to have a power of 85% to detect this increase.

Table 6. Summary of number of subjects required to achieve 85% power to detect increase in dystrophin levels from baseline

Increase of dystrophin from baseline	Intra Subject CV			
	20%	25%	30%	35%
0.04%	19	29	41	55
0.06%	10	15	21	28
0.08%	7	10	13	18

Abbreviations: CV, coefficients of variability

The sample size will be re-assessed after the 10th subject has completed the Week 40 assessments.

8.3 Population for Analyses

8.3.1 Intention-to-treat (ITT) analysis set

This analysis set will include all enrolled subjects with treatment assignments. In addition, subjects in this analysis set must have a valid assessment of dystrophin levels at baseline, as measured by ECL. This analysis set will be used for the primary endpoint analyses and summaries of other efficacy endpoints.

8.3.2 Per Protocol Analysis Set

This analysis set will include all subjects in the ITT set who meet the following additional criteria: received study treatment, completed the 40-week treatment period without major protocol violations and dose interruptions and have a valid assessment of dystrophin levels at Week 40. This analysis set will be used for supportive efficacy analyses

8.3.3 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

8.4.2 Analysis of primary efficacy endpoint

The primary endpoint will be log transformed and analyzed in a mixed model of repeated measures with week/treatment as a fixed effect and subject as a random effect. Two-sided 90% CI for the ratio (%) of dystrophin level at Week 40 versus baseline will be constructed. The dystrophin level will be summarized by muscle biopsy locations using descriptive statistics.

Baseline dystrophin levels in this patient population is expected to be close to zero, in case any measured dystrophin levels are below the lower limit of quantification (LOQ), the levels will be set at 0.5*LOQ for the statistical analysis. Subjects with baseline dystrophin levels [redacted] of the predicted levels will be excluded from the primary analysis to minimize the potential bias in the assessment of change in dystrophin levels.

8.4.3 Analysis of secondary efficacy endpoint

The secondary endpoint will be analyzed in the same way as the primary endpoint.

8.4.4 Pharmacokinetic analyses

Plasma concentration will be summarized with descriptive statistics.

A separate analysis to evaluate the PK data observed in the study will be conducted in parallel if the plasma concentrations are within in the established therapeutic range.

8.4.5 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. Change from baseline results will be presented for clinical laboratory values and vital signs.

8.4.6 Planned interim analyses

There are no planned interim analyses for this study.

8.4.7 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. The parent/legal guardian's signature on the informed consent form should be obtained at the investigator site in the presence of the investigator or a qualified representative (eg, sub investigator). 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/IEC.

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. All biopsy samples/extracts will be deidentified. The samples will be stored for 10-years. Only PTC Therapeutics and [redacted] will have access to 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. No additional genetic testing will be done 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. Participants who decline to participate in this optional research may do so.

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The investigator or authorized designee will explain to each subject the objectives of the exploratory research. Participants will be told that it is optional to participate in exploratory research and may withdraw their consent at any time and for any reason during the storage period.

9.1.5 Clinical monitoring

In accordance with 21 Code of Federal Regulations Part 312.56 and/or relevant ICH 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 good clinical practices (GCP) 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 on 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 Electronic Data Capture (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
- Failure to comply with dispensing or dosing requirements
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- 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 such as incorrect storage of study drug, 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

Amendment 1: 22 Oct 2018 (Version 2.0)

Amendment 2: 06 May 2019 (Version 3.0)

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, to extend the screening window, to add information on concomitant use of corticosteroids and cardiac drugs for cardiomyopathy prophylaxis/treatment, to update the exploratory PK assessments and analyses, and to update the ataluren dosing table.

Item No.	Protocol Section	Amendment/Update	Reason/Rationale
1	Synopsis and Sec 2.2.3	Exploratory endpoints: deleted specific PK parameters	Allow for analysis of other parameters
2	Synopsis, Sec 3.1.1, and Sec 3.2 Bullet #2	Revised from [redacted] muscle biopsy samples to up to approximately 450 mg of muscle tissue (up to [redacted] cores per muscle); allowed sample from the [redacted] muscle if obtained sample is not evaluable for analysis	To ensure sufficient muscle tissue for analysis
3	Synopsis Table 1 and Sec 7 Table 1	Extended screening window from 30 to 45 days; added details for assessing height/weight and physical exams, deleted details of PK analyses	Increase visit window; clarification
4	~~Section 3.1.4~~	Information on PK analyses moved to Section 8.4.4	Clarification
5	Section 4.1	Added text on homogeneity of study population	Clarification
6	Section 6.2	Added that drug will be administered with food.	Clarification
7	Section 6.4.1	Added information on Corticosteroids	Clarification
8	Section 6.4.4	Added information on Cardiac Drugs for Cardiomyopathy Prophylaxis/Treatment	Concomitant use of cardiac drugs for cardiomyopathy prophylaxis/treatment
9	Section 7.2	Added text and table on renal functioning and monitoring parameters	Safety precautions

Item No.	Protocol Section	Amendment/Update	Reason/Rationale
10	Sec 7.3.8, Table 5	Deleted: Exposure during pregnancy or breastfeeding"	Clarification
11	Section 7.3.9	Deleted: "This information is also provided in the Study Manual and in the SAE report form." Deleted telephone number for SAE reporting.	Clarification
12	Section 8.4.2	Added: "The dystrophin level will be summarized by muscle biopsy locations using descriptive statistics."	Clarification
13	Section 8.4.4	Added information on PK analyses	Clarification
14	Section 8.4.5	Added information on safety evaluations	Clarification
15	Section 9.3	Added Protocol Amendment History	Update
16	Appendix	Added dosing table	Update
17	Synopsis and Protocol	Document date/version, abbreviations and references were updated; small grammatical changes were made throughout.	Update

10 REFERENCES

Aartsma-Rus, A, Ginjaar, IB and Bushby, K. The importance of genetic diagnosis for Duchenne muscular dystrophy. J Med Genet 2016;53(3):145-151.

Aartsma-Rus, A, Van Deutekom, JC, Fokkema, IF, Van Ommen, GJ and Den Dunnen, JT. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle Nerve 2006;34(2):135-144.

Anthony, K, Arechavala-Gomeza, V, Ricotti, V, Torelli, S, Feng, L, Janghra, N, et al. Biochemical characterization of patients with in-frame or out-of-frame DMD deletions pertinent to exon 44 or 45 skipping. JAMA Neurol 2014;71(1):32-40.

Bladen, CL, Salgado, D, Monges, S, Foncuberta, ME, Kekou, K, Kosma, K, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat 2015;36(4):395-402.

Bushby, K, Finkel, R, Birnkrant, DJ, Case, LE, Clemens, PR, Cripe, L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010a;9(1):77-93.

Bushby, K, Finkel, R, Birnkrant, DJ, Case, LE, Clemens, PR, Cripe, L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol 2010b;9(2):177-189.

El-Aloul, B, Altamirano-Diaz, L, Zapata-Aldana, E, Rodrigues, R, Malvankar-Mehta, MS, Nguyen, CT, et al. Pharmacological therapy for the prevention and management of cardiomyopathy in Duchenne muscular dystrophy: A systematic review. Neuromuscul Disord 2017;27(1):4-14.

EMA. Guideline on the investigation of drug interactions. 2012;(CPMP/EWP/560/95/Rev. 1 Corr.*).

Finkel, RS, Flanigan, KM, Wong, B, Bonnemann, C, Sampson, J, Sweeney, HL, et al. Phase 2a study of ataluren-mediated dystrophin production in patients with nonsense mutation Duchenne muscular dystrophy. PLoS One 2013;8(12):e81302.

Gallo, A, Abraham, A, Katzberg, HD, Ilaalagan, S, Bril, V and Breiner, A. Muscle biopsy technical safety and quality using a self-contained, vacuum-assisted biopsy technique. Neuromuscul Disord 2018;28(5):450-453.

Humbertclaude, V, Hamroun, D, Bezzou, K, Berard, C, Boespflug-Tanguy, O, Bommelaer, C, et al. Motor and respiratory heterogeneity in Duchenne patients: implication for clinical trials. Eur J Paediatr Neurol 2012;16(2):149-160.

Mah, JK. Current and emerging treatment strategies for Duchenne muscular dystrophy. Neuropsychiatr Dis Treat 2016;12:1795-1807.

Matthews, E, Brassington, R, Kuntzer, T, Jichi, F and Manzur, AY. Corticosteroids for the treatment of Duchenne muscular dystrophy. Cochrane Database Syst Rev 2016;(5):Cd003725.

Muntoni, F, Torelli, S and Ferlini, A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol 2003;2(12):731-740.

Petrof, BJ, Shrager, JB, Stedman, HH, Kelly, AM and Sweeney, HL. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. Proc Natl Acad Sci U S A 1993;90(8):3710-3714.

Phillips, MF, Quinlivan, RC, Edwards, RH and Calverley, PM. Changes in spirometry over time as a prognostic marker in patients with Duchenne muscular dystrophy. Am J Respir Crit Care Med 2001;164(12):2191-2194.

Rae, MG and O'Malley, D. Cognitive dysfunction in Duchenne muscular dystrophy: a possible role for neuromodulatory immune molecules. J Neurophysiol 2016;116(3):1304-1315.

Raman, SV, Hor, KN, Mazur, W, Halnon, NJ, Kissel, JT, He, X, et al. Eplerenone for early cardiomyopathy in Duchenne muscular dystrophy: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2015;14(2):153-161.

Welch, EM, Barton, ER, Zhuo, J, Tomizawa, Y, Friesen, WJ, Trifillis, P, et al. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447(7140):87-91.

11 APPENDIX

PTC124-GD-045-DMD Dosing Table