Official Title:	A Multi-Site, Randomized, Placebo-Controlled, Double-Blind, Multiple Ascending Subcutaneous Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of RO7239361 (BMS-986089) in Ambulatory Boys with Duchenne Muscular Dystrophy
NCT Number:	NCT02515669
Document Dates:	Protocol Amendment/Version 6: 14-Oct-18

PROTOCOL

TITLE:	**A MULTI-SITE, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND, MULTIPLE ASCENDING SUBCUTANEOUS DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF RO7239361 IN AMBULATORY BOYS WITH DUCHENNE MUSCULAR DYSTROPHY**
PROTOCOL NUMBER:	WN40226
VERSION NUMBER:	6
EUDRACT NUMBER:	2015-005455-28
IND NUMBER:	120,702
TEST PRODUCT:	Anti-Myostatin Adnectin (RO7239361)
MEDICAL MONITOR:	[redacted] *M.D.*
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	See electronic date stamp below.

FINAL PROTOCOL *AMENDMENT* APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Company Signatory	14-Oct-2018 20:17:19

DOCUMENT HISTORY

Document	Date of Issue	Summary of Changes
Protocol 06 (Version 6)	See electronic date stamp.	Incorporates Amendment 06
Amendment 06	See electronic date stamp.	- Changed IMP dose in the open-label extension (OLE) phase - Changed IMP from vial to prefilled syringes (PFS) in the OLE - Changed the study assessments and procedures to add a new clinic visits, PK/PD and safety assessments after the switch to PFS - Updated Medical Monitor information - Product Development Background and Overall Risk/Benefit sections have been updated - Updated monitoring of anti-drug antibodies (ADAs) during 24-week follow-up period - Deleted references to BMS study and drug number
Revised Protocol 05 (Version 5)	21 Aug 2017	Incorporated Amendment 05
Amendment 05	21 Aug 2017	– Changed Sponsor from Bristol-Myers Squibb to F. Hoffmann-La Roche Ltd – Changed study drug name from BMS-986089 to RO7239361 – Updated Medical Monitor information
Revised Protocol 04	21-Feb-2017	Incorporated Amendment 04
Amendment 04	21-Feb-2017	- Added OLE phase to the study - Updated plan for clinical study reports - Incorporated administrative corrections which were included in Administrative Letters 03 and 04
Revised Protocol 03	21-Apr-2016	Incorporated Amendment 03
Amendment 03	21-Apr-2016	- Clarification added after the withdrawal of study drug into an appropriate-sized syringe, the product should be administered SC within 4 hours to align with our current investigational brochure. -Updated CN001-001 safety information -Removed the exclusion criteria: baseline 4SC 9 Day -1) more than a 20% or 0.5 second reduction, whichever is greater, from the valid screening 4SC 9 Day -45 to Day -7) used to determine eligibility. Revised - The wash out period of prior investigational drug exclusion criteria is clarified to 5 half-lives, removing the 3 month criteria.

		- The addition of vital signs in the open label phase at week 2 & 3. - The addition of the PODCI measurement in the open-label phase at Weeks 12, 24 and 36. - Administrative corrections which were included in the administrative letter 01 and 02 - Administrative changes and clarifications
Protocol 02	09-Dec-2015	Incorporates Amendment 02
Amendment 02	09-Dec-2015	- Removed ACTIVE assessment from study procedures - Clarified inclusion/exclusion criteria (4SC, use of bisphosphonates, subject travel distance to site, etc.) - Updated number of planned interim analyses - Administrative changes and clarifications

Document	Date of Issue	Summary of Change
Revised Protocol 01	18-Sep-2015	Incorporated Amendment 01
Amendment 01	18-Sep-2015	- Updated schedule and clarified data to be included in the interim analyses - Added additional laboratory assessments for additional safety monitoring and added storage of residual plasma sample remaining after GLDH analysis for up to five years after the last subject's last study visit - Clarified the secondary and exploratory endpoints associated with MRI and MRS procedures and clarified that MRS will be conducted at *selected* sites as training is completed - Added video recording of some of the exploratory functional assessments to ensure quality of assessments or identify potential issues requiring re-training of clinical evaluators - Administrative changes and clarifications
Original Protocol	08-Jul-2015	Not applicable

PROTOCOL *AMENDMENT* ACCEPTANCE FORM

TITLE:	**A MULTI-SITE, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND, MULTIPLE ASCENDING SUBCUTANEOUS DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF RO7239361 IN AMBULATORY BOYS WITH DUCHENNE MUSCULAR DYSTROPHY**
PROTOCOL NUMBER:	WN40226
VERSION NUMBER:	6
EUDRACT NUMBER:	2015-005455-28
IND NUMBER:	120,702
TEST PRODUCT:	Anti-Myostatin Adnectin (RO7239361)
MEDICAL MONITOR:	[redacted] *M.D.*
SPONSOR:	F. Hoffmann-La Roche Ltd

I agree to conduct the study in accordance with the current protocol.

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

Please retain the signed original of this form for your study files. Please return a copy of the signed form as instructed by your study monitor.

SYNOPSIS

Protocol Title: A Multi-Site, Randomized, Placebo-Controlled, Double-Blind, Multiple Ascending Subcutaneous Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetics of RO7239361 in Ambulatory Boys with Duchenne Muscular Dystrophy

Investigational Product(s), Dose and Mode of Administration, Duration of Treatment with Investigational Product(s): Each subject will be administered weekly subcutaneous (SC) doses of RO7239361 or placebo for 24 weeks. Subjects will be assigned to one of three dose panels. The initial 24-week double-blind phase will be followed by a 48-week open-label (OL) phase. During the OL phase, all subjects will receive weekly SC doses of RO7239361 for 48 weeks at the active dose corresponding to the dose in their originally assigned panel. In the OL extension *(OLE)* phase, all subjects will continue to receive weekly unblinded SC doses of RO7239361 for up to 228 weeks, or until RO7239361 is commercially available, whichever comes first. Doses may be adjusted based on emerging data.

Study Phase: Ib/II

Research Hypothesis: The purpose of this study is to evaluate the safety, tolerability, pharmacokinetics, free myostatin suppression, and immunogenicity of multiple SC doses of RO7239361 in boys with Duchenne muscular dystrophy (DMD).

Objectives:

Primary Objective:

- To assess the safety and tolerability of multiple SC doses of RO7239361 in boys with DMD

Secondary Objectives:

- To evaluate the *pharmacokinetics* of multiple SC doses of RO7239361 in boys with DMD
- To evaluate the immunogenicity of multiple SC doses of RO7239361 in boys with DMD
- To evaluate the effects of RO7239361 on free myostatin and myostatin-drug complex levels in boys with DMD
- To evaluate magnetic resonance imaging (MRI) measures of right thigh maximal cross-sectional area (CSA_{max}) and contractile *versus* non-contractile content

Exploratory Objectives:

- To explore the pharmacodynamic *(PD)* effects of multiple doses of RO7239361 in boys with DMD on:
  - Measures of function, including timed function tests (TFTs): 4-stair climb (4SC), 4-stair descend (4SD), 10-m walk/run, *standing* from supine), 6-minute walk distance (6MWD), and the North Star Ambulatory Assessment Scale (NSAA)
  - Serum analytes that include but are not limited to:
    - miR-206, miR-133a, miR-1, miR-6 (control miR), miR-21, miR-39, and other miRNAs
    - *Transforming growth factor–β* (TGF-β) concentrations, which are associated with fibrosis of muscle seen in DMD
    - Serum creatine kinase concentrations and creatine kinase-muscle (CK-MM), which are associated with muscle atrophy in DMD
  - Urine analytes that include but are not limited to:
    - Titin protein amino-terminal fragment concentrations that are associated with muscle damage
  - Manual myometry measures of upper and lower extremity strength
  - Ankle range of motion in degrees
  - Dual-energy X-ray absorptiometry (DXA) scanning measures of total lean body mass, fat mass, and bone mineral density
  - Magnetic resonance spectroscopy (MRS) measures of right thigh lipid fraction
  - Health-related quality of life as measured by the Pediatric Outcome Data Collection Instrument (PODCI)
  - Pulmonary function tests (PFTs), including forced vital capacity (FVC), forced expiratory volume in

1 second (FEV_1), maximal expiratory pressure (MEP), maximal inspiratory pressure (MIP), cough peak flow (CPF), and peak flow rate (PFR)

- Upper extremity motor ability measured using the Performance of Upper Limb Scale (PUL)

- To explore the effect of genetic variation in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1) on target engagement, PD, or function test endpoints [13, 14, 15]

Study Design: This is a multi-site, randomized, placebo-controlled, double-blind, multiple-ascending SC dose study to assess the safety and tolerability of RO7239361 in ambulatory boys with DMD. The initial 24-week double-blind phase will be followed by a 48-week OL phase. The initial phase will have two parts: Part A and Part B. Following the 48-week OL phase, subjects will have the option to continue treatment in an OLE phase.

Part A will include 3 dose panels of ≥ 5 to < 11 year old (the age of the subject is the age at randomization) ambulatory subjects with DMD and will consist of 4 weeks of double-blind dosing. Panels 1–3 will randomize approximately 8 subjects each (randomized 3:1 to RO7239361 or placebo). If, after all subjects in a Panel have been randomized, and it is determined that older or younger ages are underrepresented, the Panel may be opened to additional subjects of specific ages, even if 8 subjects have already been randomized. Opening Panels 1–3 to more than 8 subjects may provide more robust safety and tolerability data in certain age ranges. Roche will inform sites if and when dose panels are opened to additional subjects. All subjects will receive weekly (Day 1, Day 8, etc.) SC doses of RO7239361 or placebo.

In Panels 1 and 2, up to 4 subjects can be randomized on 1 day. Once the third subject randomized reaches Day 8, the remaining 4 subjects in that *dose panel* may be randomized. The rationale for this sentinel dosing is to observe safety and tolerability for 7 days post dose, in at least 1 subject randomized to RO7239361 before continuing to dose the remaining subjects within a *panel.* In addition, dose initiation in Panels 2 and 3 will begin only after Day 14 has been completed by at least 6 of 8 subjects participating in the preceding *panel* and all available safety and tolerability data have been reviewed and deemed safe to proceed by the study *investigators* (Principal Investigator or designee) and the Roche Medical Monitor. Prior to dose escalation the Roche Medical Monitor and the investigators must agree that it is safe to proceed with dose escalation to the next panel.

A body weight–tiered (for *Panels* 2 and 3), fixed-dose strategy targeting the approximate achievement of moderate (> 50% suppression), high (> 85%) and near complete (> 95%) suppression of serum free myostatin levels for Panels 1, 2, and 3, respectively, was used to select the three doses for the study. The body weight–tiered (for Panels 2 *and* 3) doses are presented in Tables 1, 2, and 3 below:

Table 1: Panel 1 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg	4.0 mg/0.08 mL

Table 2: Panel 2 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg to ≤ 45 kg	12.5 mg/0.25 mL
> 45 kg	20.0 mg/0.4 mL

Table 3: Panel 3 Dose	
Body Weight Tier	**Fixed Dose (mg)/Volume (mL)**
≥ 15 kg to ≤ 45 kg	35 mg/0.7 mL
> 45 kg	50.0 mg/1 mL

Table 4: Expansion Panel Dose	
Body Weight Tier	**Fixed Dose (mg)/Volume (mL)**
≥ 15 kg to ≤ 45 kg	35 mg/0.7 mL
> 45 kg	50.0 mg/1 mL

Following the PK and PD assessment in this study, if the PD targets were not achieved (e.g., myostatin inhibition was much higher than 40% in all dose groups OR if none of the dose groups achieved >90% inhibition), dose modification may be performed and additional dose cohorts may be enrolled to confirm exposures and myostatin inhibition at the modified dose. Modified doses will be selected so that the estimated exposures in pediatric subjects will not exceed those observed in adults.

The dose selected for the Expansion Panel will be a safe and well tolerated dose that is less than or equal to the highest dose tested in the panels. The Expansion Panel will provide additional safety, tolerability, PD, and functional data at a dose anticipated to be efficacious. As such, a dose that produces > 90% suppression in serum levels of free myostatin is expected to be efficacious. A review of safety data from Panels 1–3 supports the selection of the Panel 3 dose (35 mg for subjects ≥ 15 kg to ≤ 45 kg; 50 mg for subjects > 45 kg).

Part B will include 20 weeks of continued double-blind dosing in Panels 1–3 and an Expansion Panel of 24-week double-blind dosing. The Expansion Panel will randomize approximately 16 subjects (randomized 3:1 to RO7239361 or placebo). The Expansion Panel will be initiated only after at least 2 weeks of dosing has been completed by at least 6 subjects participating in Panel 3 and all available safety and tolerability data, including but not limited to, injection-site reactions, reported adverse events (AEs), clinical laboratory results and vital signs have been reviewed and deemed safe to proceed by the study investigators and the Roche Medical Monitor. The Expansion Panel may be opened to additional subjects to either explore another dose that may be safe and efficacious or to provide additional safety and tolerability data.

Upon completion of 24 weeks of double-blind dosing, all subjects will be eligible to enter the OL phase. In this phase, all subjects will receive unblinded RO7239361 SC doses, weekly for 48 weeks. Subjects in the OL phase will receive the active dose corresponding to the dose in their originally assigned Panel. Dosing in the OL phase may be adjusted based upon emerging safety and tolerability data and/or upon the results of the interim analysis (IA) of the double-blind data. The schedule of events in the OL phase is described in Table 5.1-3.

Upon completion of the 48-week OL phase, all subjects will have the option to continue treatment in the OLE phase. Dosing in the OLE phase may be adjusted based on emerging safety and tolerability data and/or upon the results of IAs. *During the OLE phase, all subjects will switch from the vial formulation to a prefilled syringe (PFS) dosage formulation corresponding to the same dose from Panel 3 (i.e., the 35-mg dose if a subject's body weight is ≥15 kg to ≤45 kg and the 50-mg dose if the subject's body weight is >45 kg).* The schedule of events in the OLE phase is described in Table 5.1-4.

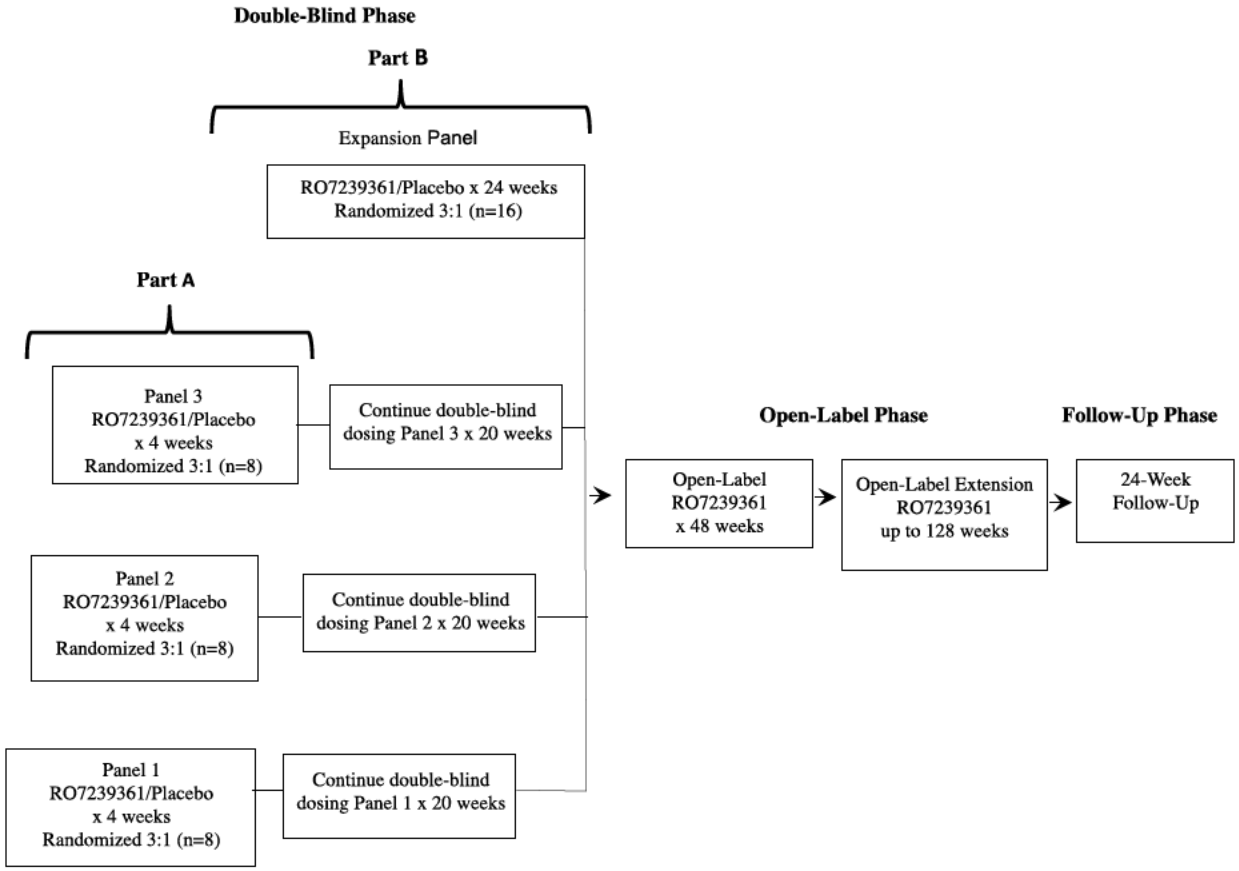
Any subject who discontinues *from* the study early or who does not enroll in a rollover protocol should enter the follow-up phase of the study. The follow-up phase of the study is up to 24 weeks *in length*. *Subjects* should provide immunogenicity samples at all follow-up visits.

Up to five IAs are planned. *The* content and timing of the IAs are described in Section 8.5 of the protocol.

Measures of function, including TFTs (4SC, 4SD, 10-*m* walk/run, stand from supine), 6MWD, the NSAA, manual myometry measures of upper and lower extremity strength, ankle range of motion, PFTs, and PUL measures of upper extremity motor ability, echocardiograms, laboratory assessments, MRI and MRS scanning of the right thigh

will be collected during the double-blind phase, OL phase, and follow-up phase at the intervals specified in Table 5.1-2 to Table 5.1-4 (MRS procedures will be completed at selected sites, based on completed training). Study visits will occur as indicated in Table 5.1-1 though Table 5.1-4.

Figure 1: Study Design



Study Population:

Key Inclusion Criteria:

- Males, ≥ 5 to < 11 years of age at time of randomization
- Diagnosis of Duchenne muscular dystrophy, confirmed by medical history (e.g., onset of clinical signs or symptoms before 6 years of age together with an elevated serum creatine kinase level) and by genotyping
- Subjects ≥ 15 kg
- Ambulatory without assistance
- Subjects must be receiving corticosteroids (prednisone, prednisolone, or deflazacort) for at least 6 months prior to the start of study drug, with no significant change in dosage (> 0.2 mg/kg) or dosing regimen for at least 3 months prior to the start of study drug, with the expectation that dosage and dosing regimen will not change significantly for the duration of the study.
- 4SC ≤ 8 seconds at screening (Day -45 to Day -7)

Key Exclusion Criteria:

- Ejection fraction < 55% on echocardiogram, based on central read
- Subjects with known cognitive impairment or behavioral issues that will compromise their ability to comply with study procedures, in the judgment of the investigator.

- Any change (initiation, change in drug class, dose modification unrelated to change in body weight, interruption or re-initiation) in prophylaxis/treatment for congestive heart failure (CHF) within 3 months prior to start of study treatment.
- Any change (initiation, change in drug class, dose modification unrelated to change in body weight, interruption or re-initiation) in prophylaxis/treatment for bone density within 3 months prior to start of study treatment.
- Treatment with ataluren, or any investigational drug (excluding deflazacort) currently or within 5 half-lives (whichever is longer) prior to the start of study drug administration. If the half-life of the prior treatment is unknown, the investigator must consult with the Roche Medical Monitor to determine washout duration.
- Treatment with exon skipping therapies within 6 months prior to the start of study drug administration
- History of major surgical procedure within 6 weeks prior to the start of study drug administration.
- Any injury, which may impact functional testing. Previous injuries, including fractures, must be fully healed prior to consenting.
- Expectation of major surgical procedure, such as scoliosis surgery, during the treatment period of this study.
- Implanted ferromagnetic metal (implanted metal that is not ferromagnetic, such as surgical steel or titanium implants may be allowed if the implants will not compromise the quality of the MRI, MRS or DXA scans) or unable to complete right thigh MRI scan or DXA scan
- Ongoing immunosuppressive therapy (other than corticosteroids)
- Current or prior treatment within 3 months prior to the start of study drug administration with androgens, human growth hormone
- Requirement of daytime ventilator assistance
- Uncontrolled clinical signs and symptoms of *CHF* (American College of Cardiology/American Heart–associated Stage C or Stage D).

Study Drug: Includes both investigational (medicinal) product (IP) and non-investigational (medicinal) product (non-IP) as listed:

Study Drug

Medication	Potency	IP/Non-IP
RO7239361 vial	50 mg/mL	IP
RO7239361 matching placebo vial	0 mg/mL	IP
RO7239361 35-mg prefilled syringe	*50 mg/mL*	*IP*
RO7239361 50-mg prefilled syringe	*71.4 mg/mL*	*IP*

Study drug preparation and administration instructions will be provided in a separate manual.

Study Assessments:

- **Safety Outcome Measures**: All subjects who received treatment (RO7239361 or placebo) will be evaluated for safety. Safety assessments will be based on medical review of adverse event reports (including injection-site reactions) and the results of vital sign measurements, ECGs, echocardiograms, physical examinations, immunogenicity, and clinical laboratory tests. The incidence of observed adverse events will be tabulated and reviewed for potential significance and clinical importance. Cardiac safety assessments (e.g., echocardiogram) will be used to evaluate baseline ejection fraction and to assess for any impact of RO7239361 on this endpoint.
- **PK Measures**: The pharmacokinetics of RO7239361 following multiple doses will be assessed by measuring serum concentrations of RO7239361 at selected timepoints.
- **Target Engagement Measures**: Effects of multiple doses of RO7239361 on free myostatin and drug myostatin complex will be assessed by measuring these markers in serum at selected timepoints.
- **Immunogenicity Measures**: Detection of anti-RO7239361 antibodies (anti-drug *antibodies* [ADAs]) will be assessed at selected timepoints. Additional characterization of immunogenicity samples for any detected antibodies may also be performed.

- **Imaging Measures:** MRI measures of right thigh maximal cross-sectional area (CSA_{max}), and contractile versus non-contractile content and MRS measures of right thigh lipid fraction (MRS procedures will be completed at selected sites, based on completed training).
- **Exploratory *PD* Measures**: Effects of multiple doses of RO7239361 on *the following*:
  - Measures of function, including TFTs, 6MWD, and the NSAA scale
  - Serum analytes that may include but are not limited to:
    - miR-206, miR-133a, miR-1, miR-6 (control miR), miR-21, and miR-39
    - TGF-β concentrations, which are associated with fibrosis of muscle seen in DMD
    - Follistatin
    - Serum creatine kinase concentrations and creatine kinase-muscle (CK-MM) which are associated with muscle atrophy in DMD
  - Urine analytes that include but are not limited to:
    - Titin protein amino-terminal fragment concentrations that are associated with muscle damage
  - Upper and lower extremity strength obtained via hand held myometry assessments of strength
  - Measures of ankle range of motion
  - DXA scanning measures of total lean body mass, fat mass, and bone mineral density
  - MRS measure of right thigh lipid fraction
  - Pulmonary function tests, including FVC, FEV_1, MEP, MIP, CPF, and PFR
  - Health-related quality of life (will be obtained using the PODCI)
  - Upper extremity motor ability as measured using the PUL
- **Genetic variation**: Explore the effect of genetic variation in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1) on target engagement, PD or function test endpoints[13,14,15]

Statistical Considerations:

Sample Size: Overall, approximately 40 subjects will be randomized in this study. Panels 1–3 will each have approximately 8 subjects and the Expansion Panel will have approximately 16 subjects. In all panels, the age range is between ≥ 5 and < 11. The panels may be opened to additional subjects if certain ages are underrepresented in the panels. Further details are included in Section 8.1.

Endpoints:

Primary Endpoint:

The primary endpoints of the study are safety and tolerability endpoints, including incidence of AEs, serious AEs, AEs leading to discontinuation and death, as well as marked treatment emergent abnormalities in clinical laboratory tests, vital sign measurements, ECGs, echocardiograms, and physical examinations across treatment conditions during 24 weeks of double-blind treatment.

Secondary Endpoints:

The secondary endpoints include C_{max} and C_{trough} of serum RO7239361 concentrations, serum concentration of free myostatin and drug-myostatin complex, percent inhibition of free myostatin at trough, anti-RO7239361 antibodies, and MRI measurements of changes from baseline in right thigh CSAmax and fold number changes in contractile versus non-contractile content during the 24-week double-blind treatment period, compared to placebo. Additional characterization of immunogenicity samples for any detected antibodies will also be performed. All timepoints are specified in Table 5.1-1 through Table 5.1-3.

Exploratory Endpoints:

- Measures of function, including TFTs, 6MWD, and the NSAA
- Serum analytes that may include but are not limited to:
  - miR-206, miR-133a, miR-1, miR-6 (control miR), miR-21, miR-39, and other miRNAs
  - TGF-β concentrations which are associated with fibrosis of muscle seen in DMD
  - Follistatin

- Serum creatine kinase concentrations and creatine kinase-muscle (CK-MM) which are associated with muscle atrophy in DMD

- Urine analytes that include but are not limited to:
  - Titin protein amino-terminal fragment concentrations that are associated with muscle damage
- Upper and lower extremity strength obtained by hand held myometry
- Ankle range of motion
- DXA measures of total lean body mass, fat mass and bone mineral density
- MRS measures of right thigh lipid fraction
- Measures of pulmonary function, including FVC, FEV_1, MEP, MIP, CPF and PFR
- Health related quality of life as measured by the PODCI
- Upper extremity motor ability as measured using the PUL
- Genetic variation status of in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1)

Analyses:

Primary Endpoint:

Safety: All recorded *AEs* will be listed and tabulated by system organ class, preferred term and treatment. Any physical examination findings will be listed. ECG, echocardiogram, vital signs and clinical laboratory test results and corresponding change from baseline values will be listed and summarized by treatment panel. Values for ECG, vital signs and clinical laboratory test results outside the pre-specified criteria will also be listed and summarized. The placebo data will be pooled as one group.

Summary statistics will be presented for each echocardiogram, ECG, vital sign parameters, and the corresponding changes from baseline by treatment panel and timepoint. Plots of the mean change from baseline value for each parameters versus time since dosing will be presented by treatment panel. The last pre-dose value is defined as baseline value.

Secondary Endpoints:

Pharmacokinetics: RO7239361 serum concentrations will be summarized by treatment group, study day and times postdose. To assess the attainment of steady state, geometric mean C_{trough} values will be plotted versus study day. C_{max} and C_{trough} will be summarized by treatment group, study day and times postdose.

Dose Proportionality: Dose proportionality of RO7239361 will be assessed by estimating the slope of the linear regression of the natural log of the PK parameter (C_{max} and C_{trough}) on the natural log of dose (by tiered weight) using the power model described by Gough et al.[27]

Target Engagement: Free myostatin and myostatin-drug complex will be tabulated by dose and study day, and the corresponding changes from baseline and percent change from baseline will be calculated and summarized. Profile plots may be provided.

A dose-response (exposure-response) analysis may be applied to characterize the relationship between dose levels (PK parameters) of RO7239361 and free myostatin and/or myostatin drug complex

Immunogenicity: Immunogenicity test results will be listed and summarized by treatment. If ADAs are observed, subgroup analyses may be conducted to compare AEs, labs, biomarker data, and PK results across treated subjects with and without ADAs, if sizes of the subgroups warrant such.

MRI measures: A dose-response (exposure-response) analysis may be applied to characterize the relationship between dose levels (PK parameters) of RO7239361 and MRI measures of right thigh maximal cross-sectional area (CSA_{max}) and contractile versus non-contractile content.

Exploratory Analysis:

PK and target engagement data collected in this study may be used to develop a population PK/PD model to estimate model-derived population and individual PK/PD parameters (e.g., CL/F, Vc/F, Ka, etc) and may be reported separately from the clinical study report.

All the exploratory endpoints such as miRNA 206, urine titin fragment concentration, exploratory blood biomarkers, measures of function, DXA measures of total lean body mass, fat mass, and bone mineral density, MRS measures of right thigh lipid fraction, and PFTs will be tabulated by dose and study day; and the corresponding changes from baseline and percent change from baseline will be calculated and summarized. Profile plots may be provided.

Key primary, secondary or exploratory endpoints may be characterized for *subject* subgroups with specific genetic variations (including. but not limited to, LTBP4, SPP1). When sample size permit, dose/exposure response model will be used to quantify dose-dependent treatment effects.

Interim Analyses:

Up to five IAs are planned *until all subjects have completed Week 72 of the study, after which additional IAs may be conducted*. The first IA may be conducted when 4-week data of Panels 1 *and* 2 are available. The timing of the second and third IAs may be based on when the 4-week data of Panel 3 are available and when *the* 24-week data of Panels 1 and 2 are available, the timing to be based on the milestone. A fourth IA will be conducted when all subjects have completed the (24-week) double-blind phase. A fifth IA will be conducted to support regulatory submissions for marketing approval. The first three IAs will include available safety and tolerability data but not PD or functional data from the Expansion Panel.

TABLE OF CONTENTS

1 INTRODUCTION AND STUDY RATIONALE

Duchenne muscular dystrophy (DMD) is an X-linked human disease that affects 1 in 3600–6000 live male births, with an estimated 15,000 patients with the disease in the *United States*. DMD results from mutations in the *Dystrophin* gene (locus Xp21.2) that lead to absent or defective dystrophin production.[1] The clinical manifestation of this molecular lesion is progressive weakness and limitation in motor capacity, with proximal muscles affected initially. Current standard of care is limited to symptomatic treatment, including medical and physical therapies to improve cardiac and respiratory function as well as corticosteroids to improve skeletal muscle strength and function. However, corticosteroids are associated with significant adverse side effects, including obesity, diabetes, short stature, osteopenia, and fracture.[2] Translarna (ataluren) recently received a conditional marketing authorization in the European Union *(EU)* with a restricted indication for the treatment of DMD specifically resulting from a nonsense mutation in the *Dystrophin* gene in ambulatory patients aged 5 years and older. Since Translarna is restricted to the 13% of DMD patients with genetically identified nonsense mutation in the *Dystrophin* gene[3], and is the only approved therapy (in the EU only) to treat DMD, there remains a significant unmet medical need for novel agents to treat DMD.

Myostatin, also known as growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor–β (TGF-β) superfamily of secreted growth factors. It is a negative regulator of skeletal muscle growth and development that is expressed predominantly in skeletal muscle beginning at embryogenesis and persisting through adulthood. Nonclinical models support the anti-myostatin mechanism as potentially efficacious in DMD. Deletion of the *Myostatin* gene from *mdx* mice, *Dystrophin* gene null, resulted in attenuation of the dystrophic phenotype relative to *mdx* mice with intact *Myostatin* genes.[4] Similarly, weekly administration of anti-myostatin monoclonal antibodies to *mdx* mice resulted in increases in muscle size and strength.[5]

RO7239361 is a bivalent anti-human myostatin antagonist adnectin, which has been formatted with a human IgG1 Fc tail to prolong its half-life in circulation. RO7239361 is being developed as a *once per week (QW)*, subcutaneously administered therapeutic to increase muscle mass and strength. RO7239361 has a unique mechanism of action (MOA) that involves the competitive inhibition of Alk4/5 (signaling receptor) recruitment and binding to free myostatin or myostatin-ActRIIb complex. This MOA leads to the inhibition of downstream pSMAD2/3 intracellular signaling with physiological consequences of increased muscle volume and body weight. RO7239361 is being developed as a potential symptomatic therapy to improve muscle function in DMD. As most DMD patients are treated with corticosteroids, in this study RO7239361 will be given to subjects on stable corticosteroid therapy.

1.1 Study Rationale

This is a multi-site, randomized, placebo-controlled, double-blind, multiple-ascending SC dose study to assess the safety and tolerability of RO7239361 in ambulatory boys with DMD. The

initial 24-week double-blind phase will be followed by a 48-week OL phase and then by an OL extension (OLE) phase. The initial double-blind phase will have two parts: Part A and Part B. Part A will include 4 weeks of double-blind, multiple-ascending SC dosing of RO7239361 in 3 panels of ambulatory boys with DMD. Each panel will include approximately 8 subjects (randomized 3:1 to RO7239361 or placebo) and is aimed at understanding the safety, tolerability, *pharmacokinetic* (PK), and target engagement (free myostatin lowering) profile of RO7239361 after 4 weeks of dosing. In Part B, subjects in Panels 1–3 will continue to receive their originally assigned weekly dose of RO7239361 or placebo. In addition, Part B will include an Expansion Panel of approximately 16 subjects (randomized 3:1 to RO7239361 or placebo). The aim of Part B is to collect additional safety, tolerability, and exploratory data and measures of function over a 24-week period of dosing of RO7239361 or placebo at doses of RO7239361 that are anticipated to be efficacious. Upon completion of 24 weeks of double-blind dosing, all subjects will be eligible to enter the OL phase. In the OL phase, all subjects will receive weekly unblinded SC doses of RO7239361 for 48 weeks. The OL phase is included to provide subjects an opportunity to initiate or to continue treatment with RO7239361 following completion of the double-blind phase. Subjects in the OL phase will receive the active dose corresponding to their originally assigned panel. In the OLE phase, all subjects will continue to receive weekly unblinded SC doses of RO7239361 for up to 228 weeks, or until RO7239361 is commercially available, whichever comes first.

This study is designed as a dose-ranging study to characterize the safety, PK, target engagement (i.e., myostatin suppression), and the relationship between PK and target engagement over at least 3 doses in children with DMD. Doses proposed for this study are based upon achieving and maintaining a moderate to high degree of suppression in serum free myostatin levels at steady-state trough across 3 dose levels. A body weight–tiered (for Panels 2 *and* 3), fixed-dose strategy targeting the achievement of moderate (>50% suppression), high (>85%) and near complete (>95%) suppression of serum free myostatin levels for Panels 1, 2, and 3, respectively, was used to select the three doses for the study. The body weight–tiered doses for Panels 2 and 3, based on two tiers of body weight, namely ≥ 15 kg to ≤ 45 kg and > 45 kg are presented in Table 3.1-1 to Table 3.1-3.

A pediatric PK–*pharmacodynamic* (PD) extrapolation model, developed using preliminary PK-PD data from the single-ascending dose *(SAD)* study in normal healthy volunteers was used to determine doses based on different degrees of target (i.e., free myostatin) suppression. The starting dose in children with DMD was selected as one that is expected to achieve and maintain a moderate level of myostatin suppression (i.e., >50%) at steady-state trough. Prior studies in Cynomolgus monkeys have shown that significant increases in thigh muscle volume were observed at doses that generated 80% suppression of free myostatin at trough. The proposed starting dose, targeting achievement of >50% suppression of free myostatin at trough, represents not only a safe starting dose in children with DMD but can also help identify if a moderate level of suppression in free myostatin is associated with PD activity or other measures of efficacy in children with DMD. The starting dose range of 4.0 mg QW in children with DMD is lower than the starting dose of 15 mg QW that was used in the multiple-ascending dose *(MAD)* study in adult healthy volunteers and produced greater than 80% suppression of

free myostatin following repeated *QW* dosing.

The middle dose was selected as a dose that is approximately a half log increment higher than the starting dose. This dose is expected to produce at least 85% suppression in free myostatin levels at trough. Myostatin suppressions at trough of >80% were associated with significant increases in muscle volume in a non-human primate study. The highest proposed dose is a <3-fold increment over the mid dose, and targets the achievement and maintenance of at least 95% suppression in levels of free myostatin at trough. Robust suppression of free myostatin (>90%) was observed for the 45-mg QW dose in the MAD study. Similarly, high levels of suppression are expected for the 90-mg QW dose as well as the highest planned dose of 180 mg QW in the MAD study.

An interim analysis (IA) revealed that at steady state, suppression of free serum myostatin from baseline of ~77%, 92%, and 97% over the dosing interval was observed at the 4-, 12.5- and 20-, and 35- and 50-mg doses, respectively. PD steady state was reached after ~6 weeks. Model-predicted steady-state weekly exposure of RO7239361 in children with DMD and the associated safety margins based on comparison to (1) weekly exposure at 12 weeks from the juvenile rat toxicity study, and (2) weekly no-observed-adverse-event level (NOAEL) exposure from the pivotal toxicity study, are presented in Table 1.1-1.

Table 1.1-1: Exposure Margins for Dose Panels 1–3[a] and Expansion Panel Dose

Dose Panel	Model Predicted Steady-State Exposure AUC (µg•h/mL)	Exposure Margins				
		Rat 6-Week Pivotal Toxicity Study (NOAEL)	Rat 6-Month Pivotal Toxicity Study (LOAEL)	Juvenile Male Rat 12-Week Pivotal Toxicity Study (HD)	Monkey 6-Week Pivotal Toxicity Study (NOAEL)	Monkey 6-Month Pivotal Toxicity Study (HD)
Panel 1 4.0 kg	~366 *450 [b]*	46x	23x	239x	308x	402x
Panel 2 ≥ 15 kg to ≤ 45 kg: 12.5 mg > 45 kg: 20 mg	1883 *1240 [b]*	9x	5x	46x	60x	78x
Panel 3 ***and Expansion Panel dose (vials)*** ≥ 15 kg to ≤ 45 kg: 35.0 mg > 45 kg: 50 mg	5480 *3620 [b]*	3x	2x	16x	21x	27x
Open-label extension dose (prefilled syringe)	*5780 [c]*	*3x*	*1.5x*	*15x*	*20x*	*25x*

[a] Exposure margins were calculated as the ratio of the highest predicted weekly AUC at steady state in children within a given panel to observed weekly steady state exposure of 1) 16,900 µg•h/mL from the 6-week rat pivotal toxicity study, 2) 8,540 µg•h/mL from the 6-month rat pivotal toxicity study, 3) 87,500 µg•h/mL from the 12-week juvenile rat toxicity study, 4) 113,000 µg•h/mL from the 6-week monkey pivotal toxicity study and 5) 147,000 µg•h/mL from the 6-month monkey pivotal toxicity study.

[b] *Model-predicted steady-state exposure before the interim analysis and based on the interim analysis, respectively.*

[c] *Model-predicted steady-state exposure based on the interim analysis and assuming 1.6-fold higher exposure with the prefilled syringe compared with the vial formulation.*

In healthy volunteers who participated in Study WN40225 (a study designed to compare the bioavailability of RO7239361 at different injection sites), a single dose (50 mg) of RO7239361 following SC administration with a prefilled syringe (PFS) in the arm, thigh, or abdomen was safe and generally well tolerated in healthy adult participants. The PFS achieved approximately 1.6-fold higher exposure than that of the vial formulation (see Investigator's Brochure for further details on Study WN40225 results). If this would also be the case in patients with DMD, the exposure achieved in this study during the OLE phase with the PFS dosage formulation (corresponding to the same dose of 35–50 mg from Panel 3) would still be 1.5 times lower than the exposure achieved with the 180-mg vials in healthy subjects during MAD phase of Study

CN001001. The drug was well tolerated up to doses of 180 mg QW in healthy volunteers.

1.2 Rationale for Change to Prefilled Syringe

A more convenient PFS presentation was developed to support the start of the pivotal trial (WN40227) in DMD patients, which may support submission for marketing authorization of RO7239361 PFS. The higher of the two doses currently being tested in Study WN40227 is being proposed as the OLE dose following switch of presentation, which corresponds to the Panel 3 dose being evaluated in Study WN40226 (35 and 50 mg, depending on subject body weight). The selected dose offers the highest suppression of free serum myostatin (97%) from the doses tested. In addition to free serum myostatin, the higher exposure reached with 35- and 50-mg PFS could potentially suppress higher levels of tissue myostatin. The increased myostatin suppresion could provide greater benefit to patients.

The exposure (area under the concentration–time curve [AUC]) predicted for the 35- and 50-mg PFS in Study WN40227 would be approximately 5780 µg•h/mL; therefore, the initially calculated safety margins (relative to the projected AUC in DMD patients at the high dose; see Table 1.1-1) remain approximately the same. RO7239361 was generally safe and well tolerated in healthy adult subjects receiving multiple SC doses of up to 180 mg (the observed exposure approximately 1.5-fold higher than in DMD patients receiving 35 and 50 mg QW by PFS in Study WN40227). Ongoing safety monitoring in Study WN40227 has not identified any new safety signals to date.

Therefore, the change from vial formulation to high-dose PFS would align both ongoing studies (WN40226 and WN40227) in DMD patients, allowing easier administration for patients and potentially to increase the chances of a beneficial effect from higher myostatin suppression.

1.3 Rationale for Specific Inclusion/Exclusion Criteria

Age Range:

A minimum age of inclusion of 5 years is planned, as children younger than 5 years are less likely to consistently understand and be able to cooperate with performing the functional endpoints, such as *the 4-stair climb* (4SC), reliably. An upper age limit for inclusion of < 11 years is planned to enable a focus on children who are able to perform the 4SC and whose lower extremities have some intact muscle tissue. RO7239361 is expected to preserve and improve muscle strength primarily by increasing the size of existing muscle fibers. Therefore, it is anticipated that the optimal effect of RO7239361 will be before muscle fibers have been replaced by fat and fibrosis. A further rationale for limiting the age range in this study is to minimize variability with respect to the timing and extent of functional and physical deficits, and due to the patient's age, growth, maturity, and concomitant medications. Both natural history and clinical trial data have highlighted the significant variability in functional endpoints.[6,7,8] Key approaches to minimizing this variability include restricting the age range for inclusion, thereby reducing variability stemming from growth. Although functional endpoints (the 4SC *and the 6-minute walk distance* [6MWD]) are exploratory in this study, safety, tolerability, PK, and TE data from this study will inform dose selection for future studies, for which function will be the primary endpoint.

Use of Corticosteroids:

As corticosteroids have demonstrated benefit on motor function in boys with DMD[9], boys included in this study will be required to be on a corticosteroid (e.g., prednisone/prednisolone or deflazacort) for at least 6 months prior to the start of study treatment, with no significant change in dosage (> 0.2 mg/kg) or dosing regimen for at least 3 months prior to the start of study treatment. The goal of this inclusion criterion is to control variability in motor function and strength stemming from corticosteroid use.

Both prednisone/prednisolone and deflazacort have been shown to be effective in increasing and preserving muscle strength in DMD[9,10] supporting this pharmacotherapy as an important standard of care. Following initiation of corticosteroids, a period of improvement in muscle strength is often observed. To control this potential source of variance, inclusion in this initial study will be restricted to *subjects* who have been taking corticosteroids for at least 6 months, plan to continue corticosteroids for the duration of the double-blind phase of the clinical trial, and have not had a significant change in dose in the 3 months preceding initiation of study treatment.

Use of Translarna (Ataluren) or Investigational Agents:

Subjects receiving Translarna (ataluren) will not be eligible for this study as ataluren was only recently conditionally approved in the *EU* for a small subset of DMD subjects (i.e., those with nonsense mutations). Because of this recent approval, the magnitude and time course of treatment response of this agent in DMD *are* not well understood. Similarly, limited data are available regarding the safety and tolerability of this drug. Data regarding safety, tolerability, and the time course for efficacy are also limited for exon-skipping therapies and other investigational agents. These information gaps render controlling for potential effects of ataluren or investigational agents challenging and thus could complicate interpretation of the data emerging from this study. To avoid a potentially confounding impact of poorly understood effects of ataluren and investigational drugs (with the exception of deflazacort), *subjects* receiving these treatments currently or *within* 5 half-lives prior to the start of study treatment will be excluded from participation. Consult with the Roche Medical Monitor to determine the washout duration if unknown.

Use of Growth Hormone or Androgens:

Growth hormone may exert effects on cardiac morphology and function, while androgens may alter skeletal muscle function in boys with DMD.[11,12] To avoid potential confounding effects of growth hormone and androgens on the endpoints being measured in this study, *subjects* receiving these treatments currently or within 3 months or 5 half-lives (whichever is longer) prior to the start of study treatment will be excluded from participation.

1.4 Research Hypothesis

The purpose of this study is to evaluate the safety, tolerability, pharmacokinetics, free myostatin suppression, and immunogenicity of multiple SC doses of RO7239361 in boys with *DMD*.

1.5 Objectives(s)

1.5.1 Primary Objectives

To assess the safety and tolerability of multiple SC doses of RO7239361 in boys with DMD

1.5.2 Secondary Objectives

- To evaluate the PK of multiple SC doses of RO7239361 in boys with DMD
- To evaluate the immunogenicity of multiple SC doses of RO7239361 in boys with DMD
- To evaluate the effects of RO7239361 on free myostatin and myostatin-drug complex levels in boys with DMD
- To evaluate magnetic resonance imaging (MRI) measures of right thigh maximal cross-sectional area (CSA_{max}), and contractile versus non-contractile content

1.5.3 Exploratory Objectives

- To explore the pharmacodynamic effects of the multiple doses of RO7239361 in boys with DMD on:
  - Measures of function, including timed function tests (TFTs): 4SC, 4-stair descend (4SD), 10-m walk/run, *standing* from supine, 6MWD, and the North Star Ambulatory Assessment Scale (NSAA)
  - Serum analytes that may include but are not limited to:
    - miR-206, miR-133a, miR-1, miR-6 (control miR), miR-21, and miR-39
    - TGF-β concentrations, which are associated with fibrosis of muscle seen in DMD
    - Follistatin
    - Creatine kinase concentrations and creatine kinase-muscle (CK-MM), which are associated with muscle atrophy in DMD
  - Urine analytes that include but are not limited to:
    - Titin protein amino-terminal fragment concentrations that are associated with muscle damage
  - Manual myometry measures of upper and lower extremity strength
  - Ankle range of motion in degrees
  - DXA measure of total lean body mass, fat mass and bone mineral density
  - Magnetic resonance spectroscopy (MRS) measures of right thigh lipid fraction
  - Health related quality of life as measured by the Pediatric Outcome Data Collection Instrument (PODCI)
  - Pulmonary function tests (PFTs), including forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), maximal expiratory pressure (MEP), maximal inspiratory pressure (MIP), cough peak flow (CPF), and peak flow rate (PFR)
  - Upper extremity motor ability measured using the Performance of Upper Limb Scale (PUL)

- Explore the effect of genetic variation in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1) on target engagement, PD, or function test endpoints.[13, 14, 15]

1.6 Product Development Background

1.6.1 Pharmacology

RO7239361 is a potent inhibitor of myostatin signaling activity with subnanomolar affinity for myostatin. RO7239361 also inhibits GDF11 with subnanomolar affinity, but does not bind to other members of the TGF-β family of ligands, including activin A, TGF-β3, GDF9, and GDF10. A more complete summary of the nonclinical pharmacology studies is provided in the Investigator's Brochure.

1.6.2 Toxicity

In the pivotal 6-month intermittent (every 2 weeks [Q2W]) SC toxicity studies in rats and monkeys, RO7239361 was well tolerated at all doses (0, 5, 20, or 60 mg/kg), with no effects on central nervous system (CNS), respiratory, or cardiovascular (CV) endpoints. PD-related effects observed in both species at all doses included increased body weight and lean mass and increased skeletal muscle weight, with no evidence of skeletal muscle toxicity and minimal skeletal muscle hypertrophy. In rats and monkeys, RO7239361 treatment-induced anti-drug antibody (ADA) responses resulted in decreased serum RO7239361 concentrations and attenuation of associated pharmacological changes in some animals.

The primary RO7239361-related toxicology findings in rats in the above-mentioned study were as follows: 1) increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (not associated with adverse histopathological changes in the liver); 2) mild inhibition of primary IgG T cell–dependent antibody response (TDAR); and 3) increased incidence of slight biliary hyperplasia in the liver of females treated with 60 mg/kg of RO7239361. The latter represented an exacerbation of an age-related background finding (Hailey et al. 2014). Because of the magnitude of the effects in some rats, the increases in serum AST and ALT were considered adverse; therefore, a NOAEL was not achieved in this study. All other findings were considered non-adverse.

The primary RO7239361-related non-adverse toxicology findings in monkeys were: 1) increased adrenal gland weight in monkeys treated with 60 mg/kg; and 2) slightly increased mononuclear cell infiltration in choroid plexus at doses ≥5 mg/kg and minimal follicular lymphocytic infiltration in bone marrow at 60 mg/kg. The latter findings were both exacerbations of background findings in monkeys. RO7239361-related findings were partially to fully reversible following a 3-month postinjection period. The high dose of 60 mg/kg (AUC from Time 0 to 168 hours [AUC_{0-168h}] =147,000 μg•h/mL) was considered the NOAEL in monkeys. The exposure multiple at the NOAEL in monkeys relative to the projected AUC at the maximum clinical dose (50 mg; AUC =5,480 μg •h/mL) is approximately 27-fold.

Juvenile toxicity was investigated in the 12-week SC toxicity studies in juvenile albino rats. RO7239361 administration at doses up to 180 mg/kg Q2W, starting on postnatal Day 7 was safe and well tolerated, with no adverse findings. RO7239361-related findings included: 1) non-adverse increases in bone mineral content and density (at all doses); 2) a slight delay in balanopreputial separation, without effects on mating or fertility (at ≥60 mg/kg) and delayed vaginal opening with no impact on function (at ≥20 mg/kg); 3), mild inhibition of primary IgG TDAR (males, treated with ≥60 mg/kg); and 4) minimally decreased testis, epididymis, and seminal vesicle weights with no microscopic correlate (at all doses). An increase in liver enzymes as observed in the chronic study in adult animals was not seen. The exposure multiple at the clinically tolerated dose of 180 mg/kg ($AUC_{0\text{-}168h}$ 85,600 and 127000 μg•h/mL) in juvenile rats, relative to the projected AUC at the maximum clinical dose of 50 mg is approximately 16x and 23x compared with exposures in male and female rats, respectively.

Overall, RO7239361 was generally tolerated in adult and juvenile rats and adult monkeys at all doses tested. Intended pharmacology, including myostatin TE and increased muscle mass, was demonstrated in both species.

In preliminary reproductive toxicity studies conducted with RO7239361, maternal mortality and pronounced liver toxicity (marked hepatocellular necrosis correlated with elevated ALT, AST, and total bilirubin) were observed in pregnant rats beginning on gestation Day 17 (1 day after the last dose) at doses ≥100 mg/kg ($AUC_{0\text{-}168h}$ ≥57,500 μg•h/mL; 10x relative to the maximum clinical dose of 50 mg). The maternal NOAEL in this study was considered to be the low dose of 25 mg/kg/Q2W ($AUC_{0\text{-}168h}$ = 24,400 μg•h/mL; 4x relative to the maximum clinical dose of 50 mg) based on the absence of adverse maternal findings. The maternal findings appear to be specific to pregnant rats given that there were no signs of hepatocellular necrosis or mortality at any dose tested in any other toxicity study, including a dose range–finding study in pregnant rabbits (maternal NOAEL $AUC_{0\text{–}168h}$ = 234,000 μg•h/mL) at RO7239361 exposures comparable or higher than those in the pregnant rat study. In addition to the maternal effects in rats, the presence of the fetal malformation of protruding tongue was noted in both pregnant rats and rabbits at all RO7239361 doses tested. This finding is considered pharmacologically mediated as it is also observed in myostatin-null animals.[34,35] However, the impact of this finding on postnatal development, particularly with respect to nursing, is not known at this time.

Overall, data from nonclinical toxicology studies in adult rats and monkeys and juvenile male rats indicates RO7239361 was well tolerated at all doses tested, demonstrated muscle-related pharmacology, including myostatin TE and increased muscle mass in both species, and provided an acceptable safety profile for Phase Ib/II clinical investigations in pediatric DMD subjects.

1.6.3 Preclinical Metabolism and Pharmacokinetics

RO7239361 exhibits PK properties consistent with Fc-fused proteins. Following intravenous (IV) administration, the steady-state volume of distribution (V_{ss}) of RO7239361 ranged from 0.034 to 0.069 in nonclinical species, suggesting RO7239361 largely resides in the extracellular

space. Total body clearance (CLTp) of RO7239361 was 0.68 mL/h/kg (mouse), 0.38 mL/h/kg (rat), and 0.21 mL/h/kg (monkey). The terminal half-life ($T_{1/2}$) was 65 (mouse), 117 (rat), and 129 hours (monkey). RO7239361 was slowly absorbed following SC administration with a T_{max} of 24 and 56 hours in the mouse and rat, respectively. RO7239361 demonstrated good SC bioavailability in mice and rats (approximately 84% in both species) and modest bioavailability in monkeys (~ 30%).

1.6.4 Clinical Pharmacology and Safety

The first-in-human, combined SAD and MAD study (CN001001) *has been completed. Overall, 43 subjects received study drug/placebo during the SAD phase, and 97 subjects received study drug/placebo during the MAD phase. A single dose or multiple doses (up to 180 mg SC QW) of RO7239361 were generally safe and well tolerated.*

There were no deaths, serious adverse events (SAEs) or AEs leading to discontinuation during the study.

The most frequently occurring AEs during the SAD phase were injection-site hemorrhage (n=3; 7.0%) and upper respiratory tract infection (n =3; 7.0%). The most frequently occurring AE during the MAD phase was injection-site erythema (n =12; 12.4%), followed by upper respiratory tract infection (n =11; 11.3%), and rash (n =9; 9.3%). All events were mild or moderate.

Injection-site and other skin reactions observed in the MAD phase were not associated with systemic symptoms, did not require cessation of study drug administration, and, in most cases, did not require treatment. ADAs to RO7239361 were detected in 45.2% and 27.8% of subjects receiving RO7239361 in the SAD phase and in the MAD phase, respectively. The presence of ADAs did not appear to affect exposure to RO7239361. Injection-site and other skin reactions were slightly more frequent in ADA-negative subjects (18.1%) than in ADA-positive subjects (6.9%).

No clinically significant dose-related changes in laboratory values occurred during the study. The most common marked laboratory abnormalities were transient elevations in CK and reductions in leukocytes. These laboratory abnormalities were not associated with AEs and were observed in both RO7239361- and placebo-treated subjects. There were no treatment-related changes in ECG parameters or vital sign measurements.

In Study WP40225, which was designed to compare the bioavailability of RO7239361 given at different injection sites, a total 76 healthy volunteers received single-dose SC injections of RO7239361 from a PFS in the abdomen, arm, and thigh.

There were no deaths, SAEs, or AEs leading to discontinuation during the study.

Overall, 25 of 76 subjects (32.9%) experienced AEs during the study. The incidence and severity of AEs were generally comparable across the groups with administration of RO7239361 in abdomen, arm, or thigh.

All AEs were mild or moderate in intensity. The most common AE was injection-site hemorrhage (n =3), followed by diarrhea, headache, and injection-site erythema (n=2 for each). All injection-site AEs were mild and resolved without treatment.

The most common marked laboratory abnormalities were transient reductions in leukocytes and elevation in ALT. These laboratory abnormalities were not associated with clinical symptoms and did not require pharmacological intervention. There were no treatment-related changes in ECG parameters or vital sign measurements during the study. Overall, 13 subjects had a positive ADA titer (≤2). The earliest detection of ADAs was on Day 64. The presence of ADAs did not appear to affect the exposure, safety, and tolerability of RO7239361.

Additional safety information on RO7239361 is available in the most recent version of the Investigator Brochure.

1.6.4.1 Pharmacokinetics of RO7239361

RO7239361 achieves peak concentrations *in serum between 3 and 5 days post-injection* and has a terminal half-life of approximately 7 to 10 days *across all doses following single SC dose administration (5, 15, 45, 90, and 180 mg). The increase in exposure with dose of RO7239361 appeared to be generally proportional up to 90 mg but was greater than proportional at the highest dose of 180 mg (Study CN001001).*

Following multiple RO7239361 administration, T_{max} of RO7239361 was achieved approximately 2–3 days after dosing. The half-life estimates following the last dose were from 10 to 13 days. PK parameters increased in a slightly greater than proportional manner over the explored dose range (15 mg Q1W to 180 mg Q1W) (Study CN001001).

In the current 226 study, the pharmacokinetics of RO7239361 in ambulatory boys with DMD were analyzed using a population-PK approach. The pharmacokinetics of RO7239361 were well characterized using a PD/PK model based on the target-mediated drug disposition concept, and body weight was found to be the only covariate. Steady state was reached after ~12 weeks of Q1W administration. The pharmacokinetics of RO7239361 appear to be dose proportional, considering the between-subject variability. T_{max} of RO7239361 was achieved within 24–30 hours after dosing.

1.7 Overall Risk–Benefit Assessment

More detailed information about the known and expected benefits and risks and reasonably anticipated AEs of RO7239361 are presented in the Investigator's Brochure.

The potential benefits of treatment with RO7239361 have not been established.

There are no identified risks to date related to RO7239361 administration. Potential risks include immunogenicity, hypersensitivity reactions, injection-site reactions, and adverse findings from nonclinical studies or observations with the use of other anti-myostatin agents. A more complete summary of potential risks is provided in the RO7239361 Investigator's Brochure.

Immunogenicity/Hypersensitivity: *ADAs* to RO7239361 were detected in *subjects receiving RO7239361 in SAD and MAD phases of Study* CN001001. *Most subjects with injection-site or other skin reactions were ADA-titer negative, suggesting a lack of relationship between the presence of ADAs and risk for cutaneous AEs. In Study WP40225, 17.1% of the healthy subjects who received a SC single dose of RO7239361 in the abdomen, arm, or thigh had a positive ADA titer (low, ≤2). The presence of ADAs appeared to have no effect on exposure in both studies, and no subject developed an acute hypersensitivity reaction.*

Subject safety will be closely monitored in this study. All subjects who are administered study drug will be closely monitored for possible immunogenicity-related AEs. If present, antibodies will be assessed for neutralizing activity by assessment of serum free myostatin levels and muscle volume endpoints. Subjects who do not enter a separate rollover treatment study of RO7239361 will enter the follow-up phase of the study. Subjects *will be requested to provide immunogenicity samples at each visit during the 24-week follow-up phase* (Table 5.1-5).

Potential Acute Hypersensitivity Reactions to RO7239361: No participants in Study CN001001 or WP40225 receiving RO7239361 developed an acute hypersensitivity reaction. The risk of a subject developing an acute hypersensitivity reaction to RO7239361 is considered low. However investigators and sites should be prepared to manage possible acute hypersensitivity reactions to initial doses of RO7239361. Management of acute hypersensitivity reactions is further described in Section 3.4.2.2.

Injection-Site Reactions may occur when a drug is injected subcutaneously. In the MAD phase of CN001001, mild injection-site erythema was the most common *AE* observed, occurring in 12.4% of subjects treated with RO7239361. *AE*s of injection-site erythema did not require treatment or cessation of study drug and were not accompanied by systemic symptoms. Injection sites will be rotated and inspected regularly. Injection sites will be monitored from Day 1 though the end of the study.

Teratogenicity (Fetal Malformation of Protruding Tongue): *The presence of the fetal malformation of protruding tongue was noted in both pregnant rats and rabbits at all RO7239361 doses tested, which is considered a pharmacologically mediated effect of unknown consequence on postnatal development. Subjects who are sexually active should be advised to use appropriate methods of contraception (see Section 6.4).*

In addition, mortality and marked liver toxicity, including hepatocellular necrosis, were observed in pregnant rats (Section 1.4.2), which may reflect a species-specific effect and are not considered relevant to the use of RO7239361 in DMD patients since: 1) there was no mortality or evidence of liver toxicity at comparable or higher exposures in monkeys, juvenile male rats, and pregnant rabbits; and 2) there is no evidence for treatment-related clinical liver toxicity to date in humans given RO7239361. Liver function will continue to be closely monitored in studies of RO7239361 by following liver enzymes and bilirubin levels as well as clinical signs.

Liver Enzymes Elevation: *Increased serum levels of AST and ALT observed in rats given RO7239361 for 6 months (see Section 1.4.2) were reversible with continued dosing and were not associated with histopathologic evidence of hepatocellular or skeletal muscle injury or inflammation. No ALT and AST elevations were observed at higher RO7239361 exposures in monkeys dosed for 6 months or juvenile rats dosed for 12 weeks. Monkey and juvenile rat exposures 17x and 10x, respectively, the lowest-observed-adverse-effect level (LOAEL) for the transaminase elevation in the 6-month rat study.*

In the MAD phase of Study CN001001 in healthy subjects, 9 subjects (12.5%) receiving RO7239361 had ALT elevations >1.25 x upper limit of normal (ULN) (and <3x ULN, except 1 subject with a single value of ALT elevation [4.6x ULN] on Day 99, with concurrent CK elevations). Most of the ALT elevations occurred only at single timepoint. None of the ALT elevations was accompanied with bilirubin elevations.

Taken together, the serum transaminase increases seen in rats in the 6-month study suggest a time-dependent finding that is likely rat specific. Regardless, potential hepatotoxicity will be assessed by monitoring of aminotransferases, bilirubin in serum, and evaluations of clinical signs and symptoms in subjects. The hepatocyte-specific markers gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) will continue to be used for liver monitoring.

Immunoglobulin G Suppression after Primary Immunization: *A minimal to mild decrease in the IgG response to a primary immunization was observed in some animals treated with RO7239361. Given the small magnitude of the decrease in the IgG response to primary immunization and lack of effect of RO7239361 treatment on IgM response or IgG response to booster, potential effect of RO7239361 treatment on efficacy of immunization in humans is expected to be minimal. Caregivers should be advised to ensure that their child's immunizations are up to date prior to receiving study drug.*

Potential Cardiac Effects of RO7239361: RO7239361 also recognizes GDF11, which has been implicated in heart failure associated with aging in mice[20]. While the mechanism underlying the development of aging related re-modeling of the heart in mice likely differs from that underlying the development of heart failure in DMD, potential cardiac effects of RO7239361 will be monitored in this trial using electrocardiography and echocardiography. Notably, there have been no cardiac findings in animals who received RO7239361 in the toxicology studies. Further, no significant treatment- related changes in ECG parameters or vital signs have been observed in healthy adult subjects receiving single or multiple doses of RO7239361 in *Study* CN001001.

The most up to date safety information on RO7239361 is available in the most recent version of the Investigator's Brochure.

2 ETHICAL CONSIDERATIONS

2.1 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the

International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).

The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion prior to initiation of the study.

All potential serious breaches must be reported to Roche immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks.

This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment).

2.2 Institutional Review Board/Independent Ethics Committee

Before study initiation, the investigator must have written and dated approval/favorable opinion from the IRB/IEC for the protocol, Informed Consent Form *or Assent Form*, subject recruitment materials (e.g., advertisements), and any other written information to be provided to subjects. The investigator or Roche should also provide the IRB/IEC with a copy of the Investigator Brochure's or product labeling information to be provided to subjects and any updates.

The investigator or Roche should provide the IRB/IEC with reports, updates and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

2.3 Informed Consent

Investigators must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate.

For subjects unable to give their written consent, in accordance with local regulations, one or both parents, a guardian, or a legally acceptable representative (LAR) must be informed of the study procedures and must document permission by signing the informed consent form approved for the study prior to clinical study participation.

Each subject must be informed about the nature of the study to the extent compatible with his or her understanding. Should a subject become capable or reach the age of majority, his or her consent should be obtained as soon as possible. The explicit wish of a subject who is a minor or unable to give his or her written consent, but who is capable of forming an opinion and assessing information to refuse participation in, or to be withdrawn from, the clinical study at any time should be considered by the investigator.

Minors who are judged to be of an age of reason as determined by local requirements should also give their assent. The assent should be documented based on local requirements. Continued

assent should be documented when important new information becomes available that is relevant to the subject's assent.

Roche will provide the investigator with an appropriate (i.e., global or local) sample Informed Consent Form, which will include all elements required by ICH, GCP and applicable regulatory requirements. The sample informed consent form will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Investigators must:

1) Provide a copy of the consent form, assent form if applicable and written information about the study in the language in which the subject is most proficient prior to clinical study participation. The language must be non-technical and easily understood.
2) Allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study.
3) Obtain an informed consent signed and personally dated by the subject or the subject's legally acceptable representative and by the person who conducted the informed consent discussion.
4) Obtain the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects, prior to the beginning of the study, and after any revisions are completed for new information.
5) If informed consent is initially given by a subject's legally acceptable representative or legal guardian, and the subject subsequently reaches majority, becomes emancipated, and/or capable of making and communicating his or her informed consent during the study, consent must additionally be obtained from the subject.
6) Revise the informed consent (and Assent Form if applicable) whenever important new information becomes available that is relevant to the subject's consent. The investigator, or a person designated by the investigator, should fully inform the subject or the subject's legally acceptable representative or legal guardian, of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules applicable to regulatory requirements, the subjects' signed ICF and, in the *United States*, the subjects' signed *Health Insurance Portability and Accountability Act* (HIPAA) authorization.

The consent form must also include a statement that Roche and regulatory authorities have direct access to subject records.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

3 INVESTIGATIONAL PLAN

3.1 Study Design and Duration

This is a Phase Ib/II, multi-center, randomized, placebo-controlled, double-blind, multiple-ascending SC dose study to assess the safety and tolerability of RO7239361 in

ambulatory boys with DMD. The study design schematic is presented in Figure 3.1-1. Subjects will undergo screening evaluations to determine eligibility and baseline evaluations within 45 days prior to first administration of study drug/placebo. The initial 24-week double-blind phase will be followed by a 48-week OL phase and an OLE phase. The double-blind phase will have two parts: Part A and Part B.

Part A will include 3 dose panels of ≥ 5 to < 11 year old (the age of the subject is the age at randomization) ambulatory subjects with DMD and will consist of 4 weeks of double-blind dosing. Panels 1–3 will randomize approximately 8 subjects each (randomized 3:1 to RO7239361 or placebo). If, after all subjects in a panel have been randomized, and it is determined that older or younger ages are underrepresented, the panel may be opened to additional subjects of specific ages, even if 8 subjects have already been randomized. Opening Panels 1–3 to more than 8 subjects may provide more robust safety and tolerability data in certain age ranges. Roche will inform sites if and when dose panels are opened to additional subjects. All subjects will receive weekly (Day 1, Day 8, etc.) SC doses of RO7239361 or placebo.

In Panels 1 and 2, up to 4 subjects can be randomized on 1 day. Once the third subject reaches Day 8, the remaining 4 subjects in that dose panel may be randomized. The rationale for this sentinel dosing is to observe safety and tolerability for 7 days post-dose in at least 1 subject randomized to RO7239361 before the remaining subjects within that panel are randomized. In addition, dose initiation in Panels 2 and 3 will begin only after Day 14 has been completed by at least 6 of 8 subjects participating in the preceding panel and all available safety and tolerability data have been reviewed and deemed safe to proceed by the study investigators (Principal Investigator or designee) and the Roche Medical Monitor. Prior to dose escalation the Roche Medical Monitor and the investigators must agree that it is safe to proceed with dose escalation to the next panel. In addition, available safety, PK, and target engagement (suppression of free myostatin) from preceding panels may be evaluated before the start of the next panel for timely decisions about dose adjustment or early termination. No formal inferences requiring any adjustment to statistical significance level will be performed.

Doses for Panels 1–3 are based upon safety, tolerability, PK, and PD data observed in Study CN001001 ("A Randomized, Placebo-Controlled, Single and Multiple Ascending Subcutaneous Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of RO7239361 in Healthy Adult Subjects"). A body weight–tiered (for Panels 2 and 3), fixed-dose strategy targeting the achievement of moderate (> 50% suppression), high (> 85%), and near complete (> 95%) suppression of serum free myostatin levels for Panels 1, 2, and 3, respectively, was used to select the three doses for the study. The body weight–tiered doses (for Panels 2 *and* 3) are presented in the Table 3.1-1 to Table 3.1-3 below.

Following the PK and PD assessment in this study, if the PD targets are not achieved (e.g., myostatin inhibition was much higher than 40% in all dose groups OR if none of the dose groups achieved > 90% inhibition), dose modification may be performed and additional dose cohorts may be enrolled to confirm exposures and myostatin inhibition at the modified dose. Modified doses will be selected such that the estimated exposures in pediatric subjects will not exceed those observed at the highest tolerated and safe dose in adults.

Table 3.1-1: Panel 1 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg	4.0 mg/0.08 mL

Table 3.1-2: Panel 2 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg to ≤ 45 kg	12.5 mg/0.25 mL
> 45 kg	20.0 mg / 0.4 mL

Table 3.1-3: Panel 3 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg to ≤ 45 kg	35.0 mg/0.7 mL
> 45 kg	50.0 mg/1 mL

Table 3.1-4: Expansion Panel Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg to ≤ 45 kg	35.0 mg/0.7 mL
> 45 kg	50.0 mg/1 mL

The dose selected for the Expansion Panel will be a safe and well-tolerated dose that is less than or equal to the highest dose tested in the escalation panels. The Expansion Panel will provide additional safety, tolerability, *PD,* and functional data at a dose anticipated to be efficacious. As such, a dose that produces > 90% suppression in serum levels of free myostatin is expected to be efficacious. A review of safety data from Panels 1–3 supports the selection of the Panel 3 dose (35 mg for subjects ≥ 15 kg to ≤ 45 kg *and* 50 mg for subjects > 45 kg).

Part B will include 20 weeks of continued double-blind dosing in Panels 1–3 and an Expansion Panel of 24 weeks *of* double- blind dosing. The Expansion Panel will randomize approximately 16 subjects (randomized 3:1 to RO7239361 or placebo). The Expansion Panel will be initiated after at least 2 weeks of dosing has been completed by at least 6 subjects participating in Panel 3 and all available safety and tolerability data, including, but not limited to, injection-site reactions, reported AEs, clinical laboratory results, and vital sign *measurements* have been reviewed and deemed safe to proceed by the study investigators and the Roche Medical Monitor. The Expansion Panel may be opened to additional subjects to either explore another dose that may be safe and efficacious or randomize subjects of under-represented ages.

Upon completion of 24 weeks of double-blind dosing, all subjects will be eligible to enter the OL phase. In this phase, all subjects will receive open-label RO7239361 SC doses, weekly for 48 weeks. Subjects in the OL phase will receive the active dose corresponding to the dose in their originally assigned panel. Dosing in the OL phase may be adjusted based upon emerging safety and tolerability data and/or upon the results of the interim analyses (IAs) of the double-blind data. The schedule of events in the OL phase is described in Table 5.1-3. Subjects who complete the 48-week OL phase, may continue in the OLE phase. Subjects in the OLE phase will continue to receive weekly doses of RO7239361 corresponding to the dose in their originally assigned panel for up to 228 weeks, or until RO7239361 is commercially available, whichever comes first. The study may be stopped earlier if the study is terminated by the Sponsor. In the OLE phase, all subjects will have visits every 12 weeks. The first visit in the OLE phase will occur 12 weeks after OL Week 48 and every 12 weeks thereafter for up to 228 weeks, or until RO7239361 is commercially available.

After approval of Version 6 of Protocol WN40226, and subjects have signed the corresponding updated Informed Consent Form, all participants will switch from the vial formulation to the PFS formulation of RO7239361. Subjects will continue to receive injections from a vial until the PFS becomes available at the site, based on availability of supplies and local approvals.

During the OLE phase, all subjects will switch from the vial formulation to PFS dosage formulation for weekly doses of study drug, corresponding to the same dose in Panel 3 (35 mg dose if a subject's body weight is ≥15 kg to ≤45 kg and the 50-mg dose if the subject's body weight is >45 kg). Subjeccts will receive their first dose of RO7239361 from a PFS during a scheduled clinic visit and will return to the clinic for the second PFS dose 1 week later. In addition, subjects will come to the clinic on Day 45 after the switch to the PFS (approximately 2 days after dosing at home) for PK blood sampling. Visits will take place every 12 weeks thereafter, as originally scheduled for the OLE phase.

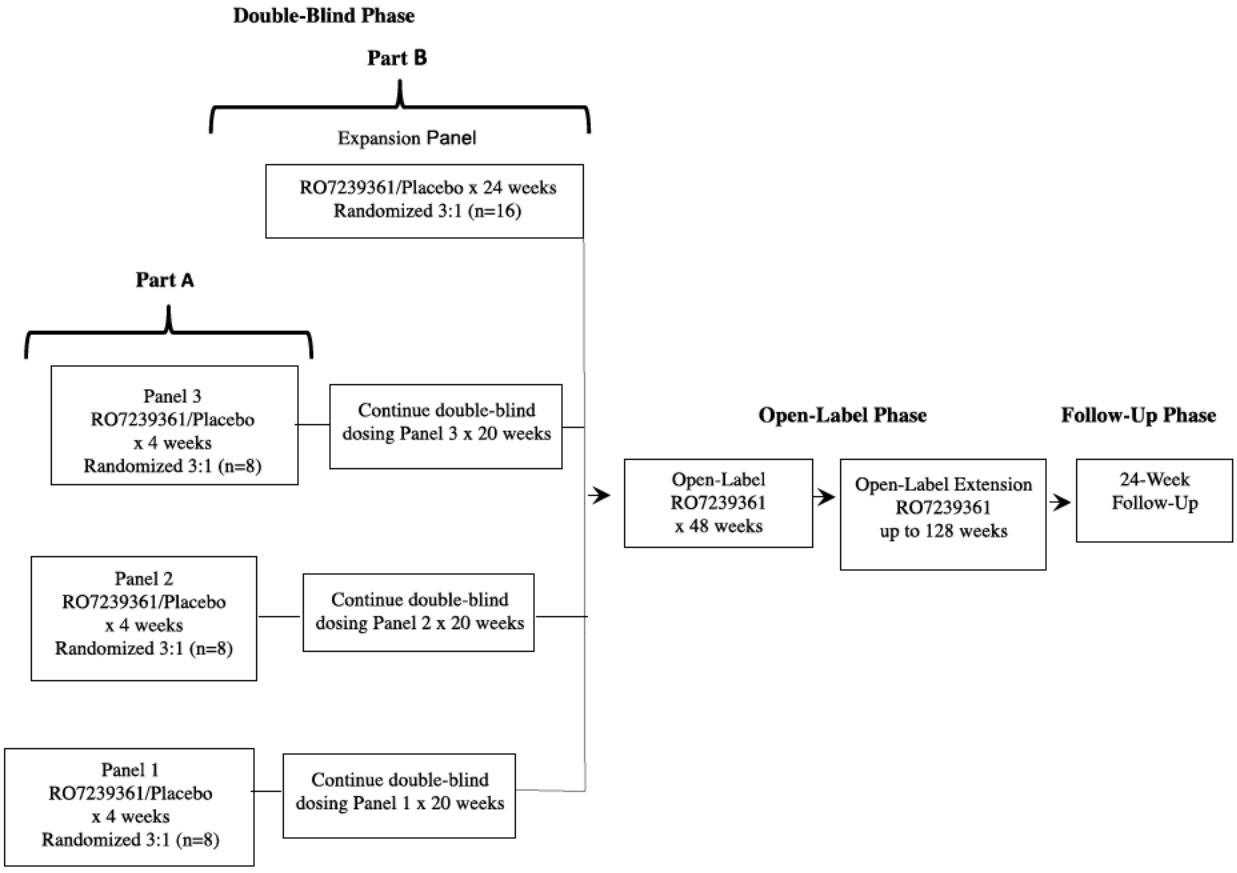
Any subject who discontinues *from* the study early or who does not enroll in a rollover protocol should enter the follow-up phase of the study. The follow-up phase of the study is up to 24 weeks *in length* after *the* last dose.

Up to five IAs are planned *until all subjects have completed Week 72 of the study, after which additional IAs may be conducted. The* content and timing of the IAs are described in Section 8.5.

Measures of function, including TFTs (4SC, 4SD, 10-m walk/run, *standing* from supine), 6MWD, the NSAA, PFTs, manual myometry measures of upper and lower extremity strength, ankle range of motion, and PUL measures of upper extremity motor ability, as well as safety assessments (e.g., ECG, echocardiogram, laboratory tests, and physical exams) and other exploratory assessments (e.g., DXA scan, serum and urine biomarkers) will be collected at selected timepoints as specified in Table 5.1-1 through Table 5.1-2.

Subjects may have ECGs, echocardiograms and laboratory assessments during the follow-up phase, as needed, to follow up on *AEs*. Subjects in the follow-up phase will be followed for up to 24 weeks after last dose for measurement of anti-RO7239361 antibodies (ADAs) and immunological *AE* assessment (Table 5.1-5).

Figure 3.1-1: Study Design Schematic



Subject safety will be closely monitored in this study.

Approximately *265* mL of blood will be drawn from each subject during the study.

The duration of the study is *up to approximately 6* years (a 45-day [approximately 6-week] screening phase+24-week double-blind phase+48-week OL phase+*228 weeks* of OLE phase+24-week follow-up phase). The end of the study is the last visit in the follow-up phase in the study, up to 24 weeks after last injection.

3.2 Post-Study Access to Therapy

At the end of the OLE period, Roche will not continue to provide Roche-supplied study drug to subjects/investigators unless Roche chooses to extend the study. The investigator should ensure that the subject receives appropriate standard of care to treat the condition under study.

3.3 Study Population

For entry into the study, the following criteria MUST be met prior to dosing on Day 1. No exceptions will be granted.

3.3.1 Inclusion Criteria

Signed Written Informed Consent

a) Prior to study participation, written informed consent from subjects, or in the case of

minors, written permission (informed consent) from parents, guardians, or legally acceptable representatives must be obtained according to local laws and regulations.

b) Assent from minor subjects should be obtained per local laws and regulations and should be documented in accordance with local requirements.

Target Population

c) Males, ambulatory without assistance

d) Subjects ≥ 15 kg

e) Diagnosis of Duchenne muscular dystrophy confirmed by medical history (e.g., onset of clinical signs or symptoms before 6 years of age with an elevated serum creatine kinase level) and by genotyping

f) Subjects must be receiving corticosteroids (e.g., prednisone, prednisolone, or deflazacort) for at least 6 months prior to the start of study drug administration, with no significant change in dosage (> 0.2 mg/kg) or dosing regimen for at least 3 months prior to the start of study treatment, with the expectation that dosage and dosing regimen will not change significantly for the duration of the double-blind phase of the study.

g) 4 stair climb ≤ 8 seconds at screening (Day -45 to Day -7); subjects who perform the 4SC in > 8 seconds may be retested within the screening window

h) Subject re-enrollment: This study permits the re-enrollment of a subject *who* has discontinued from the study as a pre-treatment failure (i.e., the subject has not been randomized and has not been treated). If re-enrolled, the subject must be re-consented.

Age and Reproductive Status

i) Males, ≥ 5 to < 11 years on day of randomization

3.3.2 Exclusion Criteria

Medical History and Concurrent Diseases

a) History of or current renal disease

b) Ejection fraction < 55% on screening echocardiogram, according to central read

c) Any major surgery within 6 weeks prior to the start of study drug administration

d) Expectation of major surgical procedure, such as scoliosis surgery, during the study drug administration phases of this study (approximately 72 weeks)

e) Any injury, which may impact functional testing. Previous injuries, including fractures, must be fully healed prior to consenting.

f) Any known cognitive impairment or behavioral issues that will compromise ability to comply with study procedures, in the judgment of the investigator.

g) Any change (initiation, change in drug class, dose modification unrelated to change in body weight, interruption or re-initiation) in prophylaxis/treatment for congestive heart failure (CHF) within 3 months prior to start of study drug administration

h) Treatment with ataluren, or any investigational drug (excluding deflazacort) currently, or

within 5 half-lives prior to the start of study drug administration. If the half-life of the prior treatment is unknown, the investigator must consult with the Roche Medical Monitor to determine the washout duration.

i) Treatment with exon-skipping therapies within 6 months prior to the start of study drug administration

j) Current or prior treatment within 3 months of study drug administration with androgens or human growth hormone

k) Ongoing immunosuppressive therapy (other than corticosteroids)

l) Requirement of daytime ventilator assistance

m) Uncontrolled clinical signs and symptoms of *CHF* (American College of Cardiology/American Heart–associated Stage C or Stage D)

n) Inability to be venipunctured, tolerate venous access, and tolerate SC injection

o) Any other sound medical, psychiatric, and/or social reason as determined by the investigator

p) Any change (initiation, change in drug class, dose modification unrelated to change in body weight, interruption or re-initiation) in prophylaxis/treatment for reduced bone density within 3 months prior to start of study drug administration

Physical and Laboratory Test Findings

q) Evidence of organ dysfunction or any clinically significant deviation from normal in physical examination, vital signs, ECG, or clinical laboratory determinations beyond what is consistent with the target population

Allergies and Adverse Drug Reaction

r) History of any significant drug allergy (such as anaphylaxis or hepatotoxicity)

Other Exclusion Criteria

s) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (e.g., infectious disease) illness

t) Inability to comply with restrictions and prohibited activities/treatments as listed in Section 3.4

u) Subjects and caregivers who reside a significant distance from the investigative site that would negatively impact the ability to complete study procedures and/or complete the study, in the judgment of the investigator

v) Subjects with implanted ferromagnetic metal (implanted metal that is not ferromagnetic, such as surgical steel or titanium implants may be allowed if the implants will not compromise the quality of the MIR, MRS, or DXA scans) or who are unable to complete right thigh MRI scan or DXA scan without sedation. See Section 3.4.2.1.

Subjects participating in non-interventional, natural history studies are eligible to participate in Study WN40226. Subjects participating in the FOR-DMD study (NCT01603407) are eligible to participate in Study WN40226 if the FOR-DMD study treatment is unblinded to the investigator to permit documentation of the corticosteroid regimen on the WN40226 CRF and the

corticosteroid dose or regimen of the subject is not likely to significantly change during the course of Study WN40226.

Eligibility criteria for this study have been carefully considered to ensure the safety of the study subjects and that the results of the study can be used. It is imperative that subjects fully meet all eligibility criteria.

3.3.3 Women of Childbearing Potential

Not applicable.

3.4 Concomitant Treatments

3.4.1 Prohibited and/or Restricted Treatments

Prohibited and/or restricted medications taken prior to study drug administration in the study are described below. Medications taken within 4 weeks prior to study drug administration must be recorded on the CRF. Prior therapies to treat DMD should be reported on the CRF, regardless of when the prior therapy was administered. Routine standard of care immunizations are recommended on study.

1) Prior exposure to RO7239361
2) Treatment with ataluren, or any investigational drug (excluding deflazacort) currently or within 5 half-lives prior to the start of study drug administration. If the half-life of the prior treatment is unknown, the investigator must consult with the Roche medical monitor to determine the washout duration.
3) Treatment with exon- skipping therapies within 6 months prior to the start of study drug administration
4) Current or prior treatment within 3 months of study drug administration with androgens or human growth hormone
5) Use of any other drugs, including over-the-counter medications and herbal preparations, within 1 week prior to start of study drug administration or during study participation except those medications cleared by the Roche Medical Monitor

3.4.2 Other Restrictions and Precautions

3.4.2.1 Imaging Contraindications (MRI/MRS/DXA)

The Principal Investigator and/or the imaging specialist at the study site imaging facility is responsible for determining if a subject is contraindicated from having the imaging procedures specified in the time and events schedule (Table 5.1-1, Table 5.1-2, and Table 5.1-3). Imaging contraindications and risks should be considered.

The ultimate decision to perform the imaging should rest with the site radiologist, the investigator, and the standard set by the local Ethics Committee.

3.4.2.2 Management of Possible Acute Hypersensitivity Reactions to RO7239361

Hypersensitivity or acute allergic reactions may occur as a result of the biologic nature of RO7239361. Qualified personnel should administer the first three weekly SC doses of RO7239361 during both the double-blind phase and during the *OL* phase. I*n addition, qualified personnel should administer or supervise the administration by the caregiver of the first two doses of the PFS formulation at the clinic.* Appropriate emergency equipment should be available in the event of a serious anaphylactic reaction. For the first three weekly doses of both the double-blind and *OL* phases, *and for the first two PFS doses in the OLE phase,* subjects should remain on site for 3 hours after drug administration for observation. Subjects and the parents/caregivers will be provided with emergency contact information by the site. Subjects and the parents/caregivers will be instructed to *recognize any signs and symptoms of hypersensitivity reaction and for the need to seek emergency medical care* in case of an extreme reaction.

The following information is provided to assist in the recognition of hypersensitivity reactions and in the management of those reactions should they occur during or after the administration of RO7239361. Care should be taken to treat any acute toxicity expeditiously, should it occur. When dosing RO7239361, equipment such as a portable tank or wall-source of oxygen, endotracheal intubation set, oral airway, mask, ambu-bag, syringes, injectable epinephrine, injectable anti-histamine, and injectable glucocorticosteriods should be kept in the vicinity where the subject is dosed (for the first three weekly doses).

Signs of potential acute hypersensitivity reactions include symptomatic hypotension; dyspnea; acute pain the chest, back or extremities/or chills, fever, urticaria; or generalized erythema.

Clinically relevant hypersensitivity reactions will be reported as AEs and treated according to current standard of care medical practice. Management is as follows:

Symptomatic Hypotension: Place subject in the Trendelenburg position and administer intravenous fluid. Additional medical intervention may also include the use of epinephrine, glucocorticosteroids, antihistamines and pressor agents.

Dyspnea: Observe the subject for worsening of the event and for the appearance of additional signs and symptoms of anaphylaxis. Antihistamines, epinephrine and glucocorticosteroids may be administered as indicated.

Acute Pain in the Chest, Back or Extremities: Observe subject for worsening of the event and for the appearance of additional signs and symptoms of anaphylaxis, Antihistamines, epinephrine and glucocorticosteroids may be administered as indicated.

Chills, Fever, Urticaria or Generalized Erythema: Treat these possible signs and symptoms of an allergic reaction to biologic products with acetaminophen and antihistamines.

The decision whether to continue the subject in the study will be made by the investigator, in consultation with the Roche Medical Monitor.

3.5 Discontinuation of Subjects following any Treatment with Study Drug

Subjects MUST discontinue investigational product (and non-investigational product at the discretion of the investigator) for any of the following reasons:

- Subject's request to stop study treatment and/or participation in the study
- Any clinical AE, laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Termination of the study by Roche
- Loss of the ability of the parent/legal guardian to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness
- Unblinding a subject for any reason (emergency or non-emergency)
- Inability to comply with protocol
- Discretion of the investigator

Clinical Criteria for Stopping Dose Escalation or Dose Discontinuation:

- Dose escalation will be halted and dosing within a dose panel may be stopped until safety information can be reviewed in the event that:
  - Two (2) or more subjects experience the same severe or very severe adverse event that is considered related to RO7239361, or;
  - Two (2) or more subjects show a decrease in ejection fraction to less than 50% (confirmed by repeat echocardiogram) that is considered related to RO7239361
- Dosing will be paused within a subject until safety information is reviewed and drug-induced liver injury (DILI) is ruled out in the event that:
  - ALT > 5 x baseline AND no corresponding increase in CPK to indicate muscle origin as the reason for the increase in ALT

  OR
  - ALT > 10 x ULN AND ALT > 2 x baseline AND no corresponding increase in CPK to indicate muscle origin as the reason for the increase in ALT

  OR
  - Total bilirubin > 2 x ULN

  OR
  - Symptoms or signs of hepatic inflammation such as nausea, vomiting, right upper quadrant pain, or tenderness with no other immediately apparent possible cause of these symptoms or signs, such as viral gastroenteritis or constipation.

In addition to the above stopping rules, dosing may be paused or halted for a subject or a panel if a review of safety and tolerability data, or the clinical judgment of the investigator, suggest the emergence of a new potentially serious safety signal, including DILI.

The following categories and definitions of intensity as determined by a physician should be

used *assessing the severity of* AEs:

- Mild (Grade 1) - Awareness of event but easily tolerated
- Moderate (Grade 2) - Discomfort enough to cause some interference with usual activity
- Severe (Grade 3) - Inability to carry out usual activity
- Very Severe (Grade 4) - Debilitating, significantly incapacitates subject despite symptomatic therapy

Dosing may not be resumed until a thorough review of safety and tolerability data has been completed and the Roche medical monitor, Roche pharmacovigilance representative, and the investigators agree that it is safe to proceed.

Any subject meeting the above stopping criteria or otherwise removed from the study for abnormal laboratory, or echocardiogram will be followed until their abnormal laboratory result and/or echocardiogram values return to baseline. Subjects who discontinue early should enter the post-treatment follow-up phase of the study for this follow-up.

All subjects who discontinue investigational product should comply with protocol-specified follow-up procedures as outlined in Section 5. The only exception to this requirement is when a subject's parent/legal guardian withdraws consent for all study procedures, including those in the study follow-up phase or loses the ability to consent freely (i.e., is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness).

If study drug is discontinued prior to the subject's completion of the study, the reason for the discontinuation must be documented in the subject's medical records and entered on the appropriate CRF page.

3.6 Post-Study Drug Follow-Up

In this study, overall safety and tolerability is a key endpoint of the study. Post-study follow-up is of critical importance and is essential to preserving subject safety and the integrity of the study. Subjects who discontinue study drug must continue to be followed for collection of outcome and/or survival follow-up data as required and in line with Section 5 until death or the conclusion of the study. There will be follow-up visits up to 24 weeks after last dose of study drug (Table 5.1-5).

3.6.1 Withdrawal of Consent

Subjects whose parent/legal guardian requests to discontinue study drug will remain in the study and must continue to be followed for protocol specified follow-up procedures. The only exception to this is when a subject's parent/legal guardian specifically withdraws consent for any further contact with him/her or persons previously authorized by the parent/legal guardian to provide this information. Subject's parent/legal guardian should notify the investigator of the decision to withdraw consent from future follow-up **in writing**, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is from further treatment with study drug only or also from study procedures and/or posttreatment study follow-up, and entered on the appropriate CRF page. In

the event that vital status (whether the subject is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

Withdrawal of consent may be requested by subjects, parents, guardians, or legally acceptable representatives, in accordance with local regulations. The wishes of minor subjects to withdraw their assent should also be respected.

3.6.2 Lost to Follow-Up

All reasonable efforts must be made to locate subjects to determine and report their ongoing status. This includes follow-up with persons authorized by the subject as noted above. Lost to follow-up is defined by the inability to reach the subject after a minimum of three documented phone calls, faxes, texts, or emails as well as lack of response by subject to one registered mail letter. All attempts should be documented in the subject's medical records. If it is determined that the subject has died, the site will use permissible local methods to obtain the date and cause of death.

If investigator's use of third-party representative to assist in the follow-up portion of the study has been included in the subject's informed consent, then the investigator may use a sponsor-retained third-party representative to assist site staff with obtaining subject's contact information or other public vital status data necessary to complete the follow-up portion of the study. The site staff and representative will consult publicly available sources, such as public health registries and databases, in order to obtain updated contact information. If after all attempts, the subject remains lost to follow-up, then the last known alive date as determined by the investigator should be reported and documented in the subject's medical records.

4 STUDY DRUG

Study drug includes both investigational (medicinal) product (IP/IMP) and non-investigational (medicinal) product (non-IP/non-IMP) and can consist of the following:

- All products, active or placebo, being tested or used as a comparator in a clinical trial
- Study required premedication
- Other drugs administered as part of the study that are critical to claims of efficacy (e.g., background therapy, rescue medications)
- Diagnostic agents (e.g., glucose for glucose challenge) given as part of the protocol requirements must also be included in the dosing data collection

RO7239361 or placebo will be administered as a SC injection. The table below indicates the solution strength for each panel. After the withdrawal of study drug into an appropriate-sized syringe, the product should be administered SC within 4 hours.

PFS dose administration: RO7239361 will be administered as a SC injection with a PFS in the abdomen, thigh, or the back of the upper arm. Injection sites should be rotated according to the "instruction for use" (IFU). Refer to the current version of the Investigator's Brochure for PK and safety data following single-dose administration across these injection sites in healthy subjects

(Study WP40225). The following table indicates the solution strength for each PFS.

Table 4-1: Study Drug in Double-Blind Phase

Panel / Dose	RO7239361 or Placebo Solution Strength
Panel 1: 4.0 mg	50 mg/mL or placebo
Panel 2: 12.5 mg or 20 mg	50 mg/mL or placebo
Panel 3: 35 mg or 50 mg	50 mg/mL or placebo
Expansion Panel: 35 mg or 50 mg	50 mg/mL or placebo

Table 4-2: Study Drug in Open-Label Phase

Panel / Dose	RO7239361 Solution Strength
Panel 1: 4.0-mg *vial*	50 mg/mL
Panel 2: 12. 5-mg or 20-mg *vial*	50 mg/mL
Panel 3: 35-mg or 50-mg *vial*	50 mg/mL
Expansion Panel: 35-mg or 50-mg *vial*	50 mg/mL
Pre-filled syringe: 35 mg	*50 mg/mL*
Pre-filled syringe: 50 mg	*71.4 mg/mL*

On Days 1, 8, and 15, each subject will receive SC doses of RO7239361 or placebo at the study site. For subsequent weekly SC doses (starting at Day 22), subjects may receive the weekly dose at home (by the subject's parent/caregiver) or at the study site. The parent/caregiver cannot administer the study drug to the subject until the parent/caregiver has been trained by study site staff and the parent/caregiver is comfortable administering study drug to the subject. After Day 15 and once trained, the parent/caregiver will receive RO7239361/placebo (during double-blind phase) or RO7239361 (during the OL phase) in vial and sterile syringes *or PFS (during the OLE phase)* for weekly administration at home to the subject. The first three weekly doses in the OL phase must be administered at the study site*, as well as the first two PFS doses during the OLE phase*.

Doses within each panel may be adjusted based upon emerging safety, tolerability, PK, and target engagement (free myostatin suppression) data from the present study.

Product description and storage information is described in Table 4-3.

Table 4-3: Study Drugs

Product Description Class and Dosage	Potency	IP/ Non-	Blinded or Open Label	Packaging/Appearance	Storage Conditions (per label)
RO7239361 solution for injection	50 mg/vial (1 mL vial)	IP	Blinded (during 24-week blinded phase) and open label (during 48 week OL phase and OL Extension phase)	Colorless to slightly yellow, clear to opalescent solution, essentially free of particulate matter packaged in a 5 -ccc type I glass vial with a 20-mm stopper and 20-mm flip-off seal. Secondary packaging is a one vial carton	2-8□□C (36-46□□F), protect from light, protect from freezing
Placebo for RO7239361 solution for injection	0 mg/vial (1 mL vial)	IP	Blinded	Colorless to slightly yellow solution, clear to opalescent, essentially free of particulate matter packaged in a 5-cc type I glass vial with a 20-mm stopper and 20-mm flip-off seal. Secondary packaging is a one vial carton.	2-8° C (36-46° F), protect from light, protect from freezing
RO7239361 prefilled syringe for injection of 35 mg per syringe	*50 mg/mL*	*IP*	*Open label (during OL Extension phase)*	*Solution packaged in a 1-cc glass syringe equipped with a safety syringe device. Secondary packaging is a one syringe carton.*	*2-8° C (36-46° F), protect from light, protect from freezing*
RO7239361 prefilled syringe for injection of 50 mg per syringe	*71.4 mg/mL*	*IP*	*Open label (during OL Extension phase)*	*Solution packaged in a 1-cc glass syringe equipped with a safety syringe device Secondary packaging is a one syringe carton.*	*2-8° C (36-46° F), protect from light, protect from freezing*

4.1 Investigational Product

An *IP*, also known as *IMP* in some regions, is defined as a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) differently than the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form.

The *IP* should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that *IP* is only dispensed to study subjects. The *IP* must be dispensed only from official study sites by authorized personnel according to local regulations.

In this protocol, the *IP* is RO7239361 solution for injection *(vial and PFS)* and matching placebo solution for injection *(for vial)*.

4.2 Non-investigational Product

Other medications used as support or escape medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, may be considered as non-*IPs*. In this study, corticosteriods are considered non-*IP* and will not be provided by the Sponsor.

4.3 Storage and Dispensing

The product storage manager should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by Roche. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact Roche immediately.

Study drug not supplied by Roche will be stored in accordance with the package insert.

IP documentation (whether supplied by Roche or not) must be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (e.g., required diluents, administration sets).

Parents/caregivers must be instructed by the site staff to bring used containers of study drug to each study visit; this will allow site staff to assess study drug accountability. Study drug must be transported to and from the site in a cooler with an ice pack to maintain the appropriate storage temperature. If approved by the local IRB/EC, the cooler and ice pack for study drug transport will be provided by the Sponsor.

Parents/caregivers must be instructed by the site staff on Sharps handling procedures. Parents/caregivers should be instructed to return the Sharps container to the study site for proper disposal.

4.4 Method of Assigning Subject Identification

Within each panel, subjects will be randomized to receive either RO7239361 or placebo according to a computer-generated randomization scheme.

During the screening visit, the investigative site will enter into the enrollment option of the interactive web response system (IWRS) designated by Roche for assignment of a 5-digit subject number that will be unique across all sites. Enrolled subjects, including those not dosed, will be assigned sequential subject numbers starting with 00001 (e.g., 00001, 00002, 00003.... 00010). The patient identification number (PID) will ultimately be comprised of the site number and subject number. For example, the first subject screened (i.e., enrolled) at site number 1, will have a PID of 0001 00001. Once it is determined that the subject meets the eligibility criteria following the screening visit, the investigative site will enter into the IWRS to randomize the subject into the open-dose panel.

Subjects will not be replaced if they are discontinued from the study secondary to an *AE* unless the *AE* can be determined to be unrelated to treatment. Subjects may be replaced if corticosteroid use is discontinued while on treatment. If a subject is replaced after dosing, then the replacement subject will be assigned the original subject's number plus 100. The replacement subject will receive the same treatment as the subject being replaced but a new randomization number will be assigned to him. For example, Subject 00004 would be replaced by Subject 00104.

4.5 Selection and Timing of Dose for Each Subject

The levels of free myostatin at Week 5 in Study CN001001 of normal healthy volunteers were used to determine the dose ranges for the present study. The lowest dose in this study (Panel 1 dose; see Table 4.5-1) is a dose that targets achievement of >50% suppression in levels of free myostatin at trough after 5 weeks of weekly dosing and is lower than the starting weekly dose of that study of normal healthy volunteers that produced approximately 83% reduction in free myostatin at trough at Week 5. The dose for Panel 1 has a safety margin in children in the weight range anticipated for subjects in this study[21] of 15 kg to 70 kg of about 232x relative to the Week 12 exposures in the juvenile rat toxicity study.

Table 4.5-1: Panel 1 Dose

Body Weight Tier	Fixed Dose (mg) /Volume (mL)
≥ 15 kg	4.0 mg /0.08 mL

The middle dose in this study (Panel 2 dose; Table 4.5-2) is a dose that targets achievement of > 85% suppression in levels of free myostatin at trough after 5 weeks of weekly dosing. The dose for Panel 2 has a safety margin in children in the weight range of 15 kg to 70 kg of about 45x, relative to the Week 12 exposures in the juvenile rat toxicity study.

Table 4.5-2: Panel 2 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg to ≤ 45 kg	12.5 mg/0.25 mL
> 45 kg	20.0 mg/0.4 mL

The highest dose in this study (Panel 3; Table 4.5-3) is a dose that targets achievement of > 95% suppression in levels of free myostatin at trough after 5 weeks of weekly dosing. The dose for Panel 3 has a safety margin in children in the weight range of 15 kg to 70 kg of about 16x relative to the Week 12 exposures in the juvenile rat toxicity study. A review of safety data from Panels 1–3 supports the selection of the Panel 3 dose (35 mg for subjects ≥ 15 kg to ≥ 45 kg; 50 mg for subjects > 45 kg) for the Expansion panel.

Table 4.5-3: Panel 3 Dose

Body Weight Tier	Fixed Dose (mg)/Volume (mL)
≥ 15 kg to ≤ 45 kg	35.0 mg/0.7 mL
> 45 kg	50.0 mg/1 mL

Table 4.5-4: Expansion Panel Dose

Body Weight Tier	Fixed Dose (mg) /Volume (mL)
≥ 15 kg to ≤ 45 kg	35.0 mg/0.7 mL
> 45 kg	50.0 mg/1 mL

Subjects will receive SC doses of RO7239361/placebo weekly. During Part A (first four weeks of Panels 1–3), study drug should be administered every 7 days. During Part B of the double-blind phase (Weeks 5–24 of Panels 1–3 and Expansion Panel) and during the 48-week OL phase, the study drug should be administered every 7 days within a ± 1-day window.

Subjects may receive the weekly dose at home, starting on Day 22. If the parent/caregiver has been trained by study site staff and is comfortable administering study drug to the subject, the parent/caregiver will receive an adequate supply of study drug in vial (either RO7239361 or placebo during the double-blind phase and RO7239361 in the OL and OLE phases) and sterile syringes for weekly administration at home to their child in between study visits. The first three weekly doses in the OL phase must be administered at the study site*, as well as the first two PFS doses in the OLE phase.*

In Panels 2 and 3, during the first four weeks of dosing (Days 1–29) the dose of RO7239361 should not be adjusted based on weight. Starting at Week 5, subjects whose weight exceeds or drops below the dosing weight tier to which they were assigned by > 1 kg should be assigned to the new weight-based dose in the subject's assigned dose panel.

4.6 Blinding/Unblinding

Blinding of treatment assignment is critical to the integrity of this clinical study. However, in the event of a medical emergency in an individual subject in which knowledge of the investigational product is critical to the subject's management, the blind for that subject may be broken by the investigator. The subject's safety takes priority over any other considerations in determining if a treatment assignment should be unblinded.

Before breaking the blind of an individual subject's treatment, the investigator should determine that the unblinded information is necessary (i.e., that it will alter the subject's immediate management). In many cases, particularly when the emergency is clearly not related to the investigational product, the problem may be properly managed by assuming that the subject is receiving active product. It is highly desirable that the decision to unblind treatment assignment be discussed with the medical monitor, but the investigator always has ultimate authority for the decision to unblind. The Principal Investigator should only call in for emergency unblinding AFTER the decision to discontinue the subject has been made.

In cases of accidental unblinding, contact the medical monitor and ensure every attempt is made to preserve the blind.

Any request to unblind a subject for non-emergency purposes should be discussed with the Medical Monitor.

In case of an emergency, the investigator(s) has unrestricted access to randomization information via the interactive voice response system (IVRS) and is capable of breaking the blind through the IVRS without prior approval from the Sponsor. Following the unblinding, the investigator shall notify the Medical Monitor and/or study director.

Further considerations about unblinding are discussed below. Because data emerging from each panel of Part A might be needed for timely decisions about adjustments to procedures in subsequent panels, including early termination of the study, data from these panels can be unblinded after documented completion and review of the corresponding *CRFs*, prior to the formal locking of the study database. Also, designated staff of Roche can be unblinded at any time. The Bioanalytical Sciences section or its designate will be unblinded to the randomized treatment assignments in order to minimize unnecessary analysis of samples from control group subjects. A pharmacokineticist(s) or designate in Clinical Pharmacology and Pharmacometrics, a biostatistician(s) and programmer(s) in Global Biometric Sciences may be unblinded in order to prepare preliminary summaries of PK, *PD,* and safety data, as needed before data is more generally unblinded. Additionally, data from subjects can be unblinded for *IAs* that are discussed in Section 8.5. Except for the *IA*, including all data from the double-blind period, summaries for these analyses will not reveal individual subjects' treatment assignments. Except as noted above, other members of Roche will remain blinded. Target

engagement and immunogenicity results from subjects will be masked to site, subject and Roche personnel, excluding the pharmacokineticist or designate, biostatistician and programmer as referenced above.

The randomization assignments will be released to the study sites by the final study report generated for the study.

4.7 Treatment Compliance

Study drug will be administered in the clinical facility or off-site by the parent/caregiver. The parent/caregiver may administer study drug on or after the Day 22 dose and once trained and comfortable with SC injections of study drug/placebo. For any dose administered at the clinical facility, the dose and location of each SC injection must be recorded in the source record.

For any dose administered off-site (off-site dosing is allowed starting on Day 22) of double-blind phase and at Week 4 of the open label phase), the parents/caregivers must be instructed on proper administration of study drug. The site staff must also instruct the parents/caregivers to bring used and/or empty vials to each study visit. The subjects and parents/caregivers will be provided with a dosing diary to record administration of study drug and be instructed to record the location and dose of each SC injection. Parents/caregivers must be instructed to bring the used and/or empty vials and the dosing diary to each study visit; this will allow site staff to assess treatment compliance and study drug accountability. A dosing diary will also be provided to the parent/caregiver to record the subject's corticosteroid therapy. Study drug must be transported to and from the site in a cooler with an ice pack to maintain the appropriate storage temperature. If approved by the local IRB/EC, the cooler and ice pack for study drug transport will be provided by the Sponsor.

Parents/caregivers must be instructed by the site staff on Sharps handling procedures. Parents/caregivers should be instructed to return the sharps container to the study site for proper disposal.

4.8 Destruction of Study Drug

For this study, study drugs (those supplied by Roche or sourced by the investigator) such as partially used study drug containers, vials and syringes may be destroyed on site.

Any unused study drugs can only be destroyed after being inspected and reconciled by the responsible Roche study monitor unless study drug containers must be immediately destroyed as required for safety, or to meet local regulations (e.g., cytotoxics or biologics).

On-site destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the site's SOPs and a copy provided to Roche upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The

method of disposal (i.e., incinerator, licensed sanitary landfill, or licensed waste disposal vendor) must be documented.

- Accountability and disposal records are complete, up-to-date, and available for the monitor to review throughout the clinical trial period.

If conditions for destruction cannot be met the responsible Roche study monitor will make arrangements for return of study drug.

It is the investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

4.9 Return of Study Drug

If study drug will not be destroyed upon completion or termination of the study, all unused and/or partially used study drug that was supplied by Roche must be returned to Roche. The return of study drug will be arranged by the responsible study monitor.

It is the investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

4.10 Retained Samples for Bioavailability/Bioequivalence

Not applicable.

5 STUDY ASSESSMENTS AND PROCEDURES

5.1 Flow Charts/Time and Events Schedules

Study assessments and procedures are presented in Table 5.1-1, Table 5.1-2, Table 5.1-3, and Table 5.1-5.

Table 5.1-1: Screening and Baseline Procedural Outline

Procedure	Screening Visit (Day -45 to Day -7)	Baseline Day -2 Visit*	Baseline Day -1 Visit*	Notes * Day -2 and Day -1 assessments and laboratory draws may be combined and performed on either Day -2 or Day -1
Eligibility Assessments				
Informed Consent	X			A subject is considered enrolled only when a protocol specific informed consent is signed.
Register / enroll subject in IWRS	X			
Inclusion/Exclusion Criteria	X			
Medical History	X			Include any toxicities or allergy related to previous treatments.
Concomitant Medications	X	X	X	
Safety Assessments				
Physical Examination (PE)	X		X	
Physical Measurements	X			Includes height, weight, and BMI.
Vital Signs	X			Includes body temperature, respiratory rate, and seated blood pressure and heart rate. Blood pressure and heart rate should be measured after the subject has been resting quietly for at least 5 minutes.
Electrocardiogram (ECG)	X			ECG should be recorded after the subject has been supine for at least 5 minutes.
Echocardiogram	X		X	If the screening echocardiogram was done between Day -15 and Day -7, the echocardiogram does not need to be repeated at Day -1 visit
Laboratory Assessments	X	X		
Glutamate dehydrogenase assay (GLDH)		X		
Urinalysis		X		

Table 5.1-1: Screening and Baseline Procedural Outline

Procedure	Screening Visit (Day -45 to Day -7)	Baseline Day -2 Visit*	Baseline Day -1 Visit*	Notes * Day -2 and Day -1 assessments and laboratory draws may be combined and performed on either Day -2 or Day -1
Secondary endpoints				
Immunogenicity		X		
Serum Free and Total Myostatin			X	
Right Thigh MRI	X			See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual; Baseline right thigh MRI scans should be obtained between Day -35 *and* Day -14. Adequacy of right thigh MRI should be confirmed by the imaging vendor prior to randomization.
Exploratory Endpoints				
Exploratory Serum Biomarkers		X		See Section 5.6.3
Backup chemistry samples (serum ferritin and transferrin)		X		To be analyzed if needed.
Blood Sample for Exploratory Genotyping		X		
Exploratory Urine Biomarkers		X		See Section 5.6.2
TFTs	X		X	Includes 4SC, 4SD, 10-m walk/run, *standing* from supine At screening visit: 4SC must be ≤ 8 seconds.
6MWD	X		X	See Study Procedure Manual.
NSAA			X	See Study Procedure Manual.
PFTs			X	See Study Procedure Manual.
Upper and Lower Extremity Myometry			X	See Study Procedure Manual.

Table 5.1-1: Screening and Baseline Procedural Outline

Procedure	Screening Visit (Day -45 to Day -7)	Baseline Day -2 Visit*	Baseline Day -1 Visit*	Notes * Day -2 and Day -1 assessments and laboratory draws may be combined and performed on either Day -2 or Day -1
Ankle Range of Motion			X	See Study Procedure Manual.
PODCI			X	Completed by the parent/caregiver of the subject.
PUL			X	See Study Procedure Manual.
DXA	X			See Sections 3.4.2.1 and 5.6.1.2 and the imaging procedure manual. Baseline DXA should be obtained between Day -35 and Day -14. Adequacy of DXA scan should be confirmed by the imaging vendor prior to randomization.
MRS	X			See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual. Baseline right thigh MRS scans should be obtained between Day -35 *and* Day -14. Adequacy of right thigh MRS should be confirmed by the imaging vendor prior to randomization. MRS will be completed at *selected* sites, based on completion of training.
Adverse Event Reporting				
Monitor for Serious Adverse Events	X	X	X	All SAEs must be collected from the date of subject's written consent until 50 days (5 half-lives) post discontinuation of dosing in the present study. If the subject enters a separate rollover treatment study of RO7239361, the subject's SAEs will be reported in that study.

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ±3 days	Study Week 24 / or early D/C ± 3 days	Notes
Safety Assessments													
Physical Examination (PE)										X		X	
Targeted PE				X	X		X		X		X		Guide exam by review of systems
Monitor Injection Sites	X	X	X	X	X	X	X	X	X	X	X	X	Report findings as AEs, as appropriate. It is strongly recommended that site staff take non-subject identifying photographs of moderate to severe injection-site reactions for submission to the Sponsor.
Weight	X						X			X		X	
Height												X	
Vital Signs	X		X	X	X	X	X	X	X	X	X	X	See note in screening procedures.
Echocardiogram							X			X		X	See Study Procedure Manual.

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
Electro-cardiogram (ECG)												X	ECGs should be recorded after the subject has been supine for at least 5 minutes.
Laboratory Tests				X	X		X			X		X	See note in screening procedures and Section 5.3.3. Placement of an intravenous lock is allowed for on-site visits when multiple blood draws are required.
Urinalysis					X		X		X	X	X	X	
GLDH			X		X		X			X		X	See Section 5.6.4 Residual sample retained for potential exploratory plasma biomarker

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
Adverse Event Reporting													
Monitor for Non-Serious Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	All subjects administered study drug will be closely monitored for AEs, including possible immunogenicity-related AEs, such as rash, fever. Injection sites will be evaluated at each visit (see above).
Monitor for Serious Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	All SAEs must be collected from the date of subject's written consent until 50 days (5 half-lives) post discontinuation of dosing in the present study. If the subject enters a separate rollover treatment study of RO7239361, the subject's SAEs will be reported in that study.

Table 5.1-2: On Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
Pharmacokinetic (PK) Assessments													
Blood PK Sampling	X	X	X	X	X	X	X	X		X		X	See Section 5.5 of protocol.
Secondary Endpoints													
Immunogenicity Sampling	X				X		X			X		X	The Immunogenicity sample must be collected before administration of study drug. In the event of a positive immunogenicity response, additional neutralizing antibody (NAB) testing may be conducted.
Free Myostatin and Myostatin-Drug Complex		X	X	X	X	X	X	X		X		X	Must be collected prior to administration of study drug.

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
Right thigh MRI										X		X	Visit window ±1 week. Conducted at early termination if ≥ 8 weeks from previous MRI. See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual.
Exploratory Endpoints													
Serum Biomarkers					X		X		X	X	X	X	See Section 5.6.3
Urine Biomarkers					X		X		X	X	X	X	See Section 5.6.2
Backup chemistry samples (serum ferritin and transferrin)					X		X		X	X	X	X	To be analyzed if needed.
TFTs							X		X	X	X	X	See Study Procedure Manual.
6MWD							X		X	X	X	X	See Study Procedure Manual

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
NSAA							X		X	X	X	X	See Study Procedure Manual.
PFTs							X		X	X	X	X	See Study Procedure Manual
Upper & Lower Extremity Myometry							X			X		X	See Study Procedure Manual
PUL							X			X		X	See Study Procedure Manual
Ankle Range of Motion							X			X		X	See Study Procedure Manual
PODCI										X		X	See Study Procedure Manual
DXA												X	Visit window ±1 week. Not required at early termination visit. See Sections 3.4.2.1 and 5.6.1.2 and the imaging procedure manual

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
Right thigh MRS										X		X	Visit window ± 1 week. Conducted at early termination if ≥ 8 weeks from previous MRS. See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual. MRS will be completed at *selected* sites, based on completion of training.
Clinical Drug Supplies													
Randomize	X												
Access IWRS to obtain study drug container assignment	X			X	X	X	X	X	X	X	X	X	Access IVRS as needed to obtain either a weekly supply (for on-site dosing) or 6-week supply (for at home dosing). Access IVRS as needed for to replace containers.

Table 5.1-2: On-Treatment Procedural Outline, Double-Blind Phase: All Panels

Procedure	D1[a]	D4[b]	D5[b]	D8 ± 1 day (Week 1)	D15 ± 1 day (Week 2)	D22[b] ± 1 day (Week 3)	D29 ± 1 day (Week 4)	D33[b] ± 1 day	Study Week 6 ± 3 days	Study Week 12 ± 3 days	Study Week 18 ± 3 days	Study Week 24 / or early D/C ± 3 days	Notes
Weekly Study Drug Administration	X			X	X	X	X						RO7239361 will be administered every week (every 7 days, unless otherwise stated). Parents and caregivers will be trained to administer SC injections. Parents/caregivers who are comfortable with SC administration can administer the injection starting at Day 22. Parents/caregivers will be dispensed an approximately 6 week supply of drug in vial (RO7239361 or placebo) and sterile syringes for weekly study drug/placebo administration to the subject.

[a] Day 1 must occur within 45 days of the screening visit.

[b] May be a home visit conducted by visiting nurse if approved by IRB and investigator. If visit is a home visit, the subject's signs and symptoms will be assessed.

Abbreviations: D = Day

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Safety Assessments												
Physical Examination (PE)											X	
Targeted PE	X			X	X	X	X		X			Guide exam by review of systems and include abdominal exam
Monitor Injection Sites	X	X	X	X	X	X	X	X	X	X	X	Report findings as AEs, as appropriate. It is strongly recommended that site staff take non-subject identifying photographs of moderate to severe injection-site reactions for submission to the Sponsor.
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	
Weight	X				X		X		X		X	
Height											X	

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Echocardiogram							X				X	See Study Procedure Manual
Electrocardiogram (ECG)											X	ECGs should be recorded after the subject has been supine for at least 5 minutes.
Laboratory Tests	X				X		X		X		X	Placement of an intravenous lock is allowed for on-site visits when multiple blood draws are required.
Urinalysis					X		X		X		X	
GLDH	X				X		X		X		X	See Section 5.6.4 Residual sample retained for potential exploratory plasma biomarker
Adverse Event Reporting												
Monitor for Non-Serious Adverse Events	X	X	X	X	X	X	X	X	X	X	X	

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Monitor for Serious Adverse Events	X	X	X	X	X	X	X	X	X	X	X	All SAEs must be collected from the date of subject's written consent until 50 days (5 half lives) post discontinuation of dosing in the present study. If the subject enters a separate rollover treatment study of RO7239361, the subject's SAEs will be reported in that study.
Pharmaco-kinetic (PK) Assessments												
Blood PK Sampling	X				X		X		X		X	See Section 5.5 of protocol.

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Secondary Endpoints												
Immunogenicity Sampling	X				X		X				X	Immunogenicity sample must be collected before administration of study drug. In the event of a positive immunogenicity response, additional NAB testing may be conducted.
Free Myostatin and Myostatin-Drug Complex	X				X		X		X		X	

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Right thigh MRI											X	Visit window ±1 week. Conducted at early termination if ≥ 8 weeks from previous MRI See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual.
Exploratory Endpoints												
Serum Biomarkers					X		X		X		X	See Section 5.6.3
Urine Biomarkers					X		X		X		X	See Section 5.6.2
Backup chemistry samples (Serum ferritin and transferrin)					X		X		X		X	To be analyzed if needed.

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
TFTs					X		X		X		X	See Study Procedure Manual
6MWD					X		X		X		X	See Study Procedure Manual
NSAA					X		X		X		X	See Study Procedure Manual
PFTs					X		X		X		X	See Study Procedure Manual
Upper and Lower Extremity					X		X		X		X	See Study Procedure Manual
PUL					X		X		X		X	See Study Procedure Manual
Ankle Range of Motion					X		X		X		X	See Study Procedure Manual
PODCI					X		X		X		X	See Study Procedure Manual
DXA											X	Visit window ± 1 week. Not required at early termination visit. See Sections 3.4.2.1 and 5.6.1.2 and the imaging procedure manual

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Right thigh MRS											X	Visit window ± 1 week. Conducted at early termination if ≥ 8 weeks from previous /MRS. See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual. MRS will be completed at *selected* sites, based on completion of training.

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1 day	Week 3 ± 1 day	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Clinical Drug Supplies												
Weekly study drug administration	X	X	X	→	→	→	→	→	→	→	→	RO7239361 will be administered every week (every 7 days, unless otherwise stated). The first 3 doses of RO7239361 (Week 1, Week 2 and Week 3) must be administered at the clinic. Parents/caregivers who are willing to administer study drug can start administering at home starting at Open Label Week 4. Study drug (RO7239361) and sterile syringes for weekly study drug administration, will be dispensed as needed.

Table 5.1-3: Open-Label Phase Procedural Outline

Procedure	Week 1	Week 2 ± 1	Week 3 ± 1	Week 6 ± 3 days	Week 12 ± 3 days	Week 18 ± 3 days	Week 24± 3 days	Week 30 ± 3 days	Week 36 ± 3 days	Week 42 ± 3 days	Week 48 or early D/C ± 3 days	Notes
Access IWRS to obtain study drug container assignment	X	X	X	X	X	X	X	X	X	X		Supplied by Roche with sterile syringes

In the event of multiple procedures are required at a single timepoint, the following is a list of procedures from highest priority to low:

1) Safety (ECG)

2) Safety (clinical labs)

3) Pharmacokinetic Sampling

4) Biomarker Sampling

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	*After Switch to PFS during OL Extension [b] Day 1, Day 8, Day 45, and Week 12 (All visits ±1 day)*	**Week 60, Week 108, Week 156, Week 204 (All visits ±3 days)**	**Week 72, Week 120, Week 168, Week 216 (All visits ± 3 days)**	**Week 84, Week 132, Week 180 (All visits ±3 days)**	**Weeks 96, Week 144, Week 192 (All visits ±3 days)**	**Week 228 or End of Treatment or Early D/C**	**Notes**
Safety							
Physical Examination (PE)	X				X	X	
Targeted PE	X	X	X	X			Guide exam by review of systems and include abdominal exam
Monitor Injection Sites	X	X	X	X	X	X	Report findings as AEs, as appropriate. It is strongly recommended that site staff take non-subject identifying photographs of moderate to severe injection-site reactions for submission to the Sponsor.
Vital Signs	X	X	X	X	X	X	
Weight	X [c]	X	X	X	X	X	
Height					X		

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	*After Switch to PFS during OL Extension [b] Day 1, Day 8, Day 45, and Week 12 (All visits ±1 day)*	**Week 60, Week 108, Week 156, Week 204 (All visits ±3 days)**	**Week 72, Week 120, Week 168, Week 216 (All visits ± 3 days)**	**Week 84, Week 132, Week 180 (All visits ±3 days)**	**Weeks 96, Week 144, Week 192 (All visits ±3 days)**	**Week 228 or End of Treatment or Early D/C**	**Notes**
Echocardiogram		X		X		X	See Study Procedure Manual
Electrocardiogram (ECGs)					X	X	ECGs should be recorded after the subject has been supine for at least 5 minutes.
Laboratory Tests	X[d]	X		X		X	Placement of an intravenous lock is allowed for on-site visits when multiple blood draws are required.
Urinalysis	X[d]	X		X		X	
GLDH	X[d]	X		X		X	See Section 5.6.4 Residual sample retained for potential exploratory plasma biomarker

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	*After switch to PFS during OL Extension [b] Day 1 Day 8 Day 45 Week 12 (all visits ± 1 day)*	**Week 60, Week 108, Week 156, Week 204 (all visits ± 3 days)**	**Week 72, Week 120, Week 168, Week 216 (all visits ± 3 days)**	**Week 84, Week 132, Week 180 (all visits ± 3 days)**	**Weeks 96, Week 144, Week 192 (all visits ± 3 days)**	**Week 228 or End of Treatment or Early D/C**	**Notes**
Adverse Event							
Monitor for Non-Serious Adverse Events	X	X	X	X	X	X	
Monitor for Serious Adverse Events	X	X	X	X	X	X	All SAEs must be collected from the date of subject's written consent until 50 days (5 half lives) post discontinuation of dosing in the present study.
Pharmacokinetic (PK) Assessments							
Blood PK Sampling	X	X		X		X	See Section 5.5 of protocol.

Table 5.1-4: Open Label Extension Phase Procedural Outline [a]

Procedure	*After switch to PFS during OL Extension [b] Day 1 Day 8 Day 45 Week 12 (all visits ± 1 day)*	**Week 60, Week 108, Week 156, Week 204 (all visits ± 3 days)**	**Week 72, Week 120, Week 168, Week 216 (all visits ± 3 days)**	**Week 84, Week 132, Week 180 (all visits ± 3 days)**	**Weeks 96, Week 144, Week 192 (all visits ± 3 days)**	**Week 228 or End of Treatment or Early D/C**	**Notes**
Secondary							
Immunogenicity Sampling	X[e]	X		X		X	Immunogenicity sample must be collected before administration of study drug. In the event of a positive immunogenicity response, additional NAB testing may be conducted.
Free Myostatin and Myostatin-Drug Complex	**X**	X		X		X	

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	After switch to PFS during OL Extension [b] Day 1 Day 8 Day 45 Week 12 (all visits ± 1 day)	Week 60, Week 108, Week 156, Week 204 (all visits ± 3 days)	Week 72, Week 120, Week 168, Week 216 (all visits ± 3 days)	Week 84, Week 132, Week 180 (all visits ± 3 days)	Weeks 96, Week 144, Week 192 (all visits ± 3 days)	Week 228 or End of Treatment or Early D/C	Notes
Right thigh MRI					X	X	Visit window ±1 week. Conducted at end of treatment or early termination if ≥ 24 weeks from previous MRI See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual.
Exploratory							
Serum Biomarkers	X	X		X		X	See Section 5.6.3
Urine Biomarkers		X		X		X	See Section 5.6.2
TFTs					X	X	See Study Procedure Manual
6MWD					X	X	See Study Procedure Manual
NSAA					X	X	See Study Procedure Manual

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	*After switch to PFS during OL Extension [b]* *Day 1* *Day 8* *Day 45* *Week 12* *(all visits ± 1 day)*	**Week 60, Week 108, Week 156, Week 204 (all visits ± 3 days)**	**Week 72, Week 120, Week 168, Week 216 (all visits ± 3 days)**	**Week 84, Week 132, Week 180 (all visits ± 3 days)**	**Weeks 96, Week 144, Week 192 (all visits ± 3 days)**	**Week 228 or End of Treatment or Early D/C**	**Notes**
PFTs					X	X	See Study Procedure Manual
Upper and Lower Extremity Myometry					X	X	See Study Procedure Manual
PUL					X	X	See Study Procedure Manual
Ankle Range of Motion					X	X	See Study Procedure Manual
PODCI					X	X	See Study Procedure Manual
DXA					X	X	Visit window ± 1 week. Conducted at end of treatment or early termination if ≥ 24 weeks from previous DXA. See Sections 3.4.2.1 and 5.6.1.2 and the imaging procedure manual

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	After switch to PFS during OL Extension [b] Day 1 Day 8 Day 45 Week 12 (all visits ± 1 day)	Week 60, Week 108, Week 156, Week 204 (all visits ± 3 days)	Week 72, Week 120, Week 168, Week 216 (all visits ± 3 days)	Week 84, Week 132, Week 180 (all visits ± 3 days)	Weeks 96, Week 144, Week 192 (all visits ± 3 days)	Week 228 or End of Treatment or Early D/C	Notes
Right thigh MRS					X	X	Visit window ±1 week. Conducted at end of treatment or early termination if ≥ 24 weeks from previous /MRS. See Sections 3.4.2.1 and 5.6.1.1 and the imaging procedure manual. MRS will be completed at *selected* sites, based on completion of training.

Table 5.1-4: Open-Label Extension Phase Procedural Outline [a]

Procedure	*After switch to PFS during OL Extension [b] Day 1 Day 8 Day 45 Week 12 (all visits ± 1 day)*	**Week 60, Week 108, Week 156, Week 204 (all visits ± 3 days)**	**Week 72, Week 120, Week 168, Week 216 (all visits ± 3 days)**	**Week 84, Week 132, Week 180 (all visits ± 3 days)**	**Weeks 96, Week 144, Week 192 (all visits ± 3 days)**	**Week 228 or End of Treatment or Early D/C**	**Notes**
Clinical Drug Supplies							
Weekly study drug adminis-tration	X [f]	X	X	X	X	X	RO7239361 will be administered every week (every 7 days, unless otherwise stated). Study drug (RO7239361) and sterile syringes or PFS for weekly study drug administration, will be dispensed as needed.
Access IWRS to obtain study drug container assignment	X	X	X	X	X	X	Supplied by Roche with sterile syringes

In the event of multiple procedures are required at a single timepoint, the following is a list of procedures from highest priority to low:
1) Safety (ECG)
2) Safety (clinical labs)
3) Pharmacokinetic Sampling
4) Biomarker Sampling

[a] Study visits will occur every 12 weeks in the *OLE* phase, for up to 228 weeks or until RO7239361 is commercially available, whichever comes first.

[b] The switch to PFS will take place at one of the scheduled clinic visits during the *OLE* phase. After this visit, *subjects* will be required to come to the clinic 1 week later for an additional visit and drug administration and at Day 45 for PK sampling (approximately 2 days after PFS dosing at home). Afterward, visits will take place every 12 weeks as scheduled.

[c] Body weight will be measured at Day 1 and 12 weeks after the first PFS dose.

[d] Laboratory tests, GLDH and urine analysis samples will be collected at 12 weeks after the first PFS dose, if not originally scheduled for the regular *OLE* visit.

[e] Immunogenicity samples will be collected at Day 1 (*prior to the* first PFS dose), Day 45, and 12 weeks after the first PFS dose, if not originally scheduled for regular *OLE* visit).

[f] Study drug will be administered at Day 1, Day 8, and Week 12.

Table 5.1-5: Post-Study Drug/Placebo Administration Follow-up Procedural Outline

Procedure	Follow-up Week 8 ±1 week	Follow-up Week 16 ±1 week	Follow up Week 24 ±1 week	Notes
Adverse Event Reporting				
Monitor injection site	X	X	X	Report findings as AEs, as appropriate. It is strongly recommended that site staff take non-subject identifying photographs of moderate to severe injection-site reactions for submission to the Sponsor.
Monitor for previously reported or new onset of non-serious adverse events	X			Only required if subject discontinued study early due to adverse event or does not participate in a separate rollover study. All non-serious adverse events must be collected until 50 days (5 half-lives) post discontinuation of dosing in the present study. If the subject enters a separate rollover treatment study of RO7239361, the subject's non-serious adverse events will be reported in that study
Monitor for previously reported or new onset of serious adverse events (SAEs)	X			All SAEs must be collected from the date of subject's written consent until 50 days (5 half-lives) post discontinuation of dosing in the present study. If the subject enters a separate rollover treatment study of RO7239361, the subject's SAEs will be reported in that study.
Safety Assessments				
ECG			X	Only required for subjects who discontinue study drug due to cardiovascular concerns.
Echocardiogram			X	Only required for subjects who discontinued study drug due to cardiovascular concerns.
Laboratory Assessments	X	X	X	Only required for subjects who discontinued study drug due to an abnormal laboratory result. Labs should be followed until the subject's lab values return to baseline or are considered stable by the Investigator

Table 5.1-5: Post Study Drug/Placebo Administration Follow-up Procedural Outline

Procedure	Follow-up Week 8 ±1 week	Follow-up Week 16 ±1 week	Follow up Week 24 ±1 week	Notes
Immunogenicity	X	X	X	Required for *all* subjects who *do not participate in a separate rollover study.* In the event of a positive immunogenicity response, additional NAB testing may be conducted.

5.1.1 Retesting during Screening or Lead-in Period

Retesting of laboratory parameters and/or other assessments within any single screening or lead-in period will be permitted (in addition to any parameters that require a confirmatory value).

Any new result will override the previous result (i.e., the most current result prior to randomization) and is the value by which study inclusion will be assessed, as it represents the subject's most current, clinical state.

Laboratory parameters and/or assessments that are included in Table 5.1-1, Screening Procedural Outline, may be repeated in an effort to find all possible well-qualified subjects. Consultation with the medical monitor may be needed to identify whether repeat testing of any particular parameter is clinically relevant.

5.2 Study Materials

The site will provide all required materials for the tests performed locally. The site will have available a well-calibrated scale for recording body weight, and a calibrated sphygmomanometer and thermometer for vital signs assessments. A current and fully stocked (advanced cardiac life support [ACLS] or basic cardiac life support [BCLS]) cart will be immediately available on the premises. The site will have urine collection containers, a monitored and alarmed refrigerator, and freezer (-20°C or below), as well as containers and dry ice for shipment and storage of blood and urine samples. A refrigerated centrifuge is recommended.

Roche will provide a Roche-approved protocol and any amendments or administrative letters (if required), and the *Investigator's Brochure*. *CRFs* (electronic or hard copy) will be provided by Roche. The central laboratory will provide labels and tubes for the collection of all required materials for the clinical laboratory tests performed by the central laboratory. Roche will also provide the relevant study manuals, a copy of *National Cancer Institute* Common Terminology Criteria for Adverse Events (*NCI* CTCAE), Version 4.0 and PODCI. Dosing diaries will be provided by Roche and should be used by the parent/caregiver to capture the dose administered, the time and date administered, and location of *SC* injection of RO7239361/placebo. The dosing diary will be used to document at home administration of study drug and should be completed by the parent/caregiver after each dose. A dosing diary will also be provided to record corticosteroid dosing. The dosing diaries should be reviewed by the site staff at the applicable study visits. If approved by the local IRB/EC, Roche will provide cooler bags, ice packs and Sharps containers for transport of study drug from the site to the subject's home.

<u>Supplied by the Imaging Core Lab</u>

The imaging core lab will supply the Imaging manual(s) for the DXA scan and for MRI/MRS scan, and if applicable, phantoms.

<u>Supplied by Cardiac Core Lab</u>

The cardiac core lab may supply 12-lead ECG machines and manuals for ECG and echocardiogram collection.

5.3 Safety Assessments

The data from the safety assessments required in this protocol (laboratory tests, ECGs, echocardiograms) will be transferred to Roche from the relevant vendor. The site staff are not required to report these procedures on the CRF. Any findings of potential clinical relevance should be evaluated and managed by the investigator per standard medical/clinical judgment in consultation with the Roche medical monitor. Site staff are required to report to the sponsor on the CRF any AE that is identified during safety assessments.

5.3.1 Echocardiogram

Guidelines for collection of echocardiograms will be provided in the Study Procedures Manual.

A central core lab will perform all imaging analyses. Sites will be informed of quality issues or needs for repeat scanning via queries from the core lab.

Any findings of potential clinical relevance that are not directly associated with the objectives of the protocol should be evaluated and handled by the study investigator as per standard medical/clinical judgment.

Reports (echocardiogram) from the central core lab will be provided to sites for inclusion in the subject's medical record and to provide to subject caregivers. Availability of reports will be determined by the central core lab.

Echocardiogram should be repeated in subjects with new cardiac symptoms.

5.3.2 Electrocardiograms

Guidelines for collection of ECGs will be provided in the Study Procedures Manual.

A central core lab will perform all imaging analyses. Sites will be informed of quality issues or needs for repeat scanning via queries from the core lab.

Any findings of potential clinical relevance that are not directly associated with the objectives of the protocol should be evaluated and handled by the study investigator as per standard medical/clinical judgment.

ECG reports from the central core lab will be provided to sites for inclusion in the subject's medical record and to provide to subject caregivers. Availability of reports will be determined by the central core lab.

ECG should be repeated in subjects with new cardiac symptoms.

5.3.3 Laboratory Test Assessments

A central laboratory will perform the analyses and will provide reference ranges for these tests.

The following clinical laboratory tests will be performed:

Table 5.3.3-1: Laboratory Assessments

	Screening	On Treatment	Follow up
Hematology			
Hemoglobin	X	X	X
Hematocrit	X	X	X
Total leukocyte count, including differential	X	X	X
Platelet count	X	X	X
Serum Chemistry			
Alanine aminotransferase (ALT)	X	X	X
Aspartate aminotransferase (AST)	X	X	X
Gamma-Glutamyl transferase (GGT)	X	X	X
Total bilirubin	X	X	X
Direct bilirubin	X	X	X
Alkaline phosphatase	X	X	X
Lactate dehydrogenase (LDH)	X	X	X
BUN	X	X	X
Creatinine	X	X	X
Creatine kinase	X	X	X
Total Protein	X	X	X
Albumin	X	X	X
Sodium	X	X	X
Potassium	X	X	X
Chloride	X	X	X
Calcium	X	X	X
Phosphorus	X	X	X
Magnesium	X	X	X
Iron	X	X	X
Plasma Chemistry			
Glutamate Dehydrogenase (GLDH)	X (Day -2 or Day -1)	X	X
Metabolic			
Thyroid stimulating hormone	X (screening)		

Table 5.3.3-1: Laboratory Assessments

	Screening	On-Treatment	Follow-up
Urine test			
Urinalysis (including protein, glucose, blood, leukocyte esterase, specific gravity)	X (Day -2 or day -1)	X (only required at visits specified in Table 5.1-2 to Table 5.1-5)	
Other sample collections			
Serum Biomarkers	X	X (see Table 5.1-2, Table 5.1-3, and Table 5.1-4)	
Urine biomarkers	X	X (see Table 5.1-2, Table 5.1-3, and Table 5.1-4)	
Immunogencity	X	X (see Table 5.1-2, Table 5.1-3, and Table 5.1-4)	X (see Table 5.1-5)
Free and total myostatin	X	X (see Table 5.1-2, Table 5.1-3, and Table 5.1-4)	
Exploratory genotyping	X		
Creatine kinase-muscle (CK-MM)	X (Day -2 or day -1)	X (Day 29, Weeks 6, 12, 18, and 24 in double-blind phase)	

All protocol-specified laboratory tests must be analyzed and reported by the central lab. In exceptional cases when local laboratory tests are performed, central lab samples should be submitted at the same time, if possible. A laboratory test may be repeated if it is determined to be inadequate for analysis. All local laboratory results should be reported on the appropriate supplemental lab CRF page. Any abnormal laboratory test result considered clinically significant by the investigator must be recorded on the appropriate AE page of the CRF (see Section 6.3).

Backup chemistry samples will be collected on the same schedule as the serum biomarker sample collection to test for serum ferritin and transferrin, if determined necessary.

Placement of an intravenous lock is allowed for on-site visits when multiple blood draws in a single day are required.

Further details of laboratory sample collection, processing and shipping will be provided to the site in a laboratory manual.

5.4 Efficacy Assessments

Not applicable.

5.4.1 Primary Efficacy Assessment

Not applicable.

5.4.2 Secondary Efficacy Assessments

Not applicable.

5.5 Pharmacokinetic Assessments

Pharmacokinetics of RO7239361 following multiple doses will be assessed by measuring serum concentration of RO7239361 at selected timepoints.

5.5.1 Pharmacokinetics: Collection and Processing

Table 5.5.1-1 lists the sampling schedule to be followed for the assessment of pharmacokinetics. Further details of blood collection and processing will be provided to the site in the procedure manual and laboratory manual. A PK test may be repeated if it is determined to be inadequate for analysis.

Table 5.5.1-1: Pharmacokinetic Sampling Schedule for RO7239361

Study Day of Sample Collection	Event	Time of Sample Collection, Relative to Time of Dose Administration	RO7239361 Serum Samples
Day 1, double-blind phase	Predose	00:00	X
Day 1, double- phase	Postdose	3:00 ±15 min	X
Day 1, double blind phase	Postdose	6:00 ±15 min	X
Day 4, double-blind phase	Post–Day 1 dose	72:00 ±8 hours	X
Day 5, double-blind phase	Post–Day 1 dose	96:00 ±8 hours	X
Day 8, double-blind phase	Predose	00:00	X
Day 15, double-blind phase	Predose	00:00	X
Day 22, double-blind phase	Predose	00:00	X
Day 29, double-blind phase	Predose	00:00	X
Day 33, double-blind phase	Post–Day 29 dose	96:00 ±8 hours	X
Week 12, double-blind phase	Predose	00:00	X
Week 24, double-blind phase	Predose	00:00	X
Week 1, open-label phase	Predose	00:00	X
Week 12, open-label phase	Predose	00:00	X
Week 24, open-label phase	Predose	00:00	X
Week 36, open-label phase	Predose	00:00	X
Week 48, open-label phase	Predose	00:00	X
**Day 1 after switch to PFS in open-label extension phase*	*Predose*	*00:00*	X
**Day 8 after switch to PFS in open-label extension phase*	*Predose*	*00:00*	X
**Day 45 after switch to PFS in open-label extension phase*	*Postdose*	*48:00*	X

Study Day of Sample Collection	Event	Time of Sample Collection, Relative to Time of Dose Administration	RO7239361 Serum Samples
**Week 12 after switch to PFS in open-label extension phase*	*Predose*	*00:00*	X
Week 60, open-label extension phase	Predose	00:00	X
Week 84, open-label extension phase	Predose	00:00	X
Week 108, open-label extension phase	Predose	00:00	X
Week 132, open-label extension phase	Predose	00:00	X

Table 5.5.1-1: Pharmacokinetic Sampling Schedule for RO7239361

Study Day of Sample Collection	Event	Time of Sample Collection, Relative to Time of Dose Administration	RO7239361 Serum Samples
Week 156, open-label extension phase	Predose	00:00	X
Week 180, open-label extension phase	Predose	00:00	X
Week 204, open-label extension phase	Predose	00:00	X
Week 228, open-label extension phase	Predose	00:00	X

5.5.2 Pharmacokinetic Sample Analyses

Serum samples will be analyzed for RO7239361 by a validated immunoassay. Pharmacokinetic samples collected from a subject who received placebo will not be analyzed.

5.5.3 Target Engagement Sample Analyses

Serum samples will be analyzed for free and total myostatin using a qualified commercial immunoassay kit. Serum will also be analyzed for drug-myostatin complex using a qualified immunoassay. The sample collection timepoints are in Table 5.1-1 through Table 5.1-4. A free and total myostatin test may be repeated if it is determined to be inadequate for analysis.

5.5.4 Labeling and Shipping of Biological Samples

Detailed instructions for the blood and serum sample collection, labeling, processing, storage, and shipping will be provided to the site in the procedure manual.

5.6 Biomarker Assessments

Biomarkers will be collected as specified in the Time and Events Schedules, Table 5.1-1, Table 5.1-2, Table 5.1-3, and Table 5.1-4.

5.6.1 Biomarker Imaging Assessments

Clinical sites will be trained in imaging procedures prior to scanning the first study subject and will be informed of quality issues or needs for repeat scanning via queries from the core lab.

Any findings of potential clinical relevance that are not directly associated with the objectives of the protocol should be evaluated and handled by the study investigator as per standard medical/clinical judgment.

Results/reports from the DXA, right thigh MRI, and MRS will not be provided to sites as this is a research measure.

5.6.1.1 Thigh MRI and MRS

Thigh MRI to collect measures of right thigh maximal cross-sectional area (CSA_{max}) and contractile versus non contractile content and MRS measures of right thigh lipid fraction will be performed at timepoints indicated in Table 5.1-1, Table 5.1-2, Table 5.1-3, and Table 5.1-4. MRS will be completed at *selected* sites, based on completion of training.

Right thigh MRI and MRS acquisition guidelines and submission processes will be in the imaging manual provided by the imaging lab that will perform the imaging analyses. Adequacy of MRI/MRS scans should be confirmed by the central imaging vendor prior to randomization.

Screening right thigh MRI/MRS should be obtained between Day -35 and Day -14 to allow for central imaging vendor review.

Early Termination or End of Treatment: *During the OL* phase, MRI/MRS will only be conducted if ≥ 8 weeks from previous MRI/MRS. In the *OLE* phase, MRI/MRS will only be conducted if greater than or equal to 24 weeks from previous MRI/MRS.

Re-enrollment: Repeat of MRI/MRS is not required if previously obtained within 6 weeks of Day 1/randomization.

5.6.1.2 DXA

DXA to collect measures of total lean body mass, fat mass and bone mineral density will be performed at timepoints indicated in Table 5.1-1, Table 5.1-2, and Table 5.1-3.

DXA acquisition guidelines and submission processes will be outlined in the imaging manual provided by the imaging lab that will perform the imaging analyses. Adequacy of DXA scans should be confirmed by the central imaging vendor prior to randomization.

Screening DXA should be obtained between Day -35 and Day -14 to allow for central imaging vendor review.

DXA is not required at the *OL* early termination visit.

In the *OLE* phase, DXA is required at early termination or end of treatment if at least 24 weeks since last scan.

Re-enrollment: Repeat of DXA is not required if previously obtained within 3 months of Day 1/randomization.

Effective doses for whole-body DXA examinations were found to be 0.0052, 0.0048, 0.0042 and 0.0042 mSv for a 5-, 10-, 15-year old child and adult respectively for an examination performed on the Hologic Discovery A device. Corresponding values for the Hologic Discovery W were 0.0105, 0.0096, 0.0084 and 0.0084 mSv.[22]

5.6.2 Exploratory Urine Biomarker Assessments

Urine samples will be collected at the times indicated in Table 5.1-1 through Table 5.1-4 for the measurement of exploratory biomarkers. The urine samples will be used to evaluate the levels of exploratory biomarkers that have been observed to be altered in patients with DMD and in preclinical models of DMD and muscle atrophy, such as fragments of the protein titin.[23,24]

Reports of the exploratory urine biomarkers will not be provided to sites as this is a research measure.

5.6.3 Exploratory Serum Biomarker Assessments

Blood will be drawn at the times indicated in Table 5.1-1 through Table 5.1-4 for the measurement of exploratory biomarkers. Serum samples will be used to evaluate the levels of exploratory biomarkers known to be involved in muscle growth and/or myostatin signaling (e.g., micro-RNAs such as mi-206 and others, and serum proteins such as TGF-β, IGF1, IGF2 and IGFBPs).

CK-MM will be analyzed during the double-blind phase of the study to examine concentrations associated with muscle atrophy in boys with DMD.

Reports of the exploratory serum biomarkers will not be provided to sites as this is a research measure.

5.6.4 Exploratory Plasma Biomarker Assessments

Residual samples remaining after GLDH analyses will be stored up to five years after the last subject randomized in the study reaches the last study visit. The samples may be used to support the biomarker assessments known to be involved in muscle growth and/or myostatin signaling (e.g., micro-RNAs such as mi-206 and others, and proteins such as TGF-β, IGF1, IGF2, and IGFBPs).

5.7 Exploratory Assessments

Some or all of the exploratory functional endpoint assessments including TFTs, 6MWT, NSAA, myometry, ankle range of motion, and PUL may be video recorded at some study visits while the subject is completing the assessments. The videos will be uploaded by the site to a secure vault, maintained by a vendor, and accessed by physical therapists for review to assure quality or identify potential issues requiring re-training of clinical evaluators at the site.

5.7.1 Functional Endpoints Assessments

Measurements of function, including TFTs (4SC, 4SD, 10-m walk/run, *standing* from supine), 6MWT, and the NSAA scale will be assessed at the timepoints specified in Table 5.1-1 through Table 5.1-4. Detailed instructions for performing each of these assessments will be provided in the Study Procedure Manual. Functional endpoints will be measured by a physiotherapist who has undergone study-specific training on the administration of these assessments.

TFTs include 4-stair climb, 4-stair descend, 10-m walk/run, and *standing* from supine. The 6MWT measures the distance a *subject* is able to traverse while walking for 6 minutes. Both the TFTs and the 6MWT are well validated and have been widely used in clinical trials.[9,25] The NSAA includes 17 items, ranging from standing to running. Each item is scored on a 3-point scale using the following criteria: 2 = normal; performs the task without assistance; 1 = modified, modified method but performs the task *independently* from physical assistance from another; 0 = unable to perform the task. The scale takes approximately 10–15 minutes to complete and is widely used in clinical trials[26].

5.7.2 Myometry and Ankle Range of Motion (ROM)

Muscle strength will be assessed using hand-held dynamometry at the timepoints specified in Table 5.1-1 through Table 5.1-4, following procedures outlined in the Study Procedure Manual. All measures will be obtained with a calibrated hand-held dynamometer.

Detailed instructions for performing manual myometry and ankle ROM assessments will be provided in the Study Procedure Manual. These assessments will be performed by a physiotherapist who has undergone study-specific training on the administration of these assessments.

5.7.3 Exploratory Genotyping

A 2-mL whole blood sample will be drawn on Day -2 or Day -1 (as indicated in Table 5.1-1) for potential analysis of genetic variation in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1). Human leukocyte antigen (HLA) genotyping may be conducted to better understand immunogenicity risk if ADAs are detected and it is determined that ADA impacts safety or efficacy. Further details of blood collection and processing will be provided to the site in the laboratory procedure manual. The exploratory genotyping samples will be used for up to 2 years after the study is closed and will be used for analyses between genotype and endpoints evaluated in this study.

5.7.4 Performance of Upper Limb Scale (PUL)

The PUL includes 22 items with an entry item to define the starting point of testing, with 21 items subdivided into shoulder level, middle level, and distal level dimension. Each dimension can be scored separately.[27] Subjects will complete PUL assessments at the timepoints specified in Table 5.1-2 through Table 5.1-4.

5.7.5 *Pulmonary Function Tests (PFTs)*

Pulmonary function will be assessed using the following tests: FVC, FEV_1, MEP, MIP, CPF, and PFR.[28] Guidelines for completion of the PFTs will be provided in the study procedure manual.

5.8 Outcomes Research Assessments

The PODCI will be completed at baseline, Weeks 12 and 24 of the double-blind phase, and at Weeks 12, 24, 36, and 48 of the OL phase. The instrument will be completed by the subject's parent/caregiver. The PODCI will generate eight scales: Upper Extremity and Physical Function Scale, Transfer and Basic Mobility Scale, Sports/Physical Functioning Scale, Pain/Comfort Scale, Treatment Expectations Scale, Happiness Scale, Satisfaction with Symptoms Scale and Global Functioning Scale.[29,30]

5.9 Other Assessments

5.9.1 *Injection-Site Assessments*

Subjects will be monitored for injection-site reactions from Day 1 through the last study visit. Report injection-site reactions as adverse events, as appropriate. It is strongly recommended that site staff take non-subject identifying photographs of moderate to severe injection-site reactions for submission to the Sponsor. The Draize Scale for erythema and edema will be used as a guide for reporting injection-site reaction AEs[31] (see Table 5.9.1-1).

Table 5.9.1-1: Grading Injection-Site Reactions

Erythema		Edema	
Description	**AE Grade**	**Description**	**AE Grade**
No erythema	-	No edema	-
Very slight erythema, barely perceptible	Mild	Very slight edema, barely perceptible	Mild
Well defined erythema	Moderate	Moderate edema, raised approximately 1 milimeter (mm)	Moderate
Severe erythema Beet redness to slight eschar formation	Severe	Sever edema, raised more than 1mm and beyond exposure area	Severe

5.10 Additional Research Collection

Not applicable.

6 ADVERSE EVENTS

An AE is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a clinical investigation subject administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease

temporally associated with the use of study drug, whether or not considered related to the study drug.

The causal relationship to study drug is determined by a physician and should be used to assess all AEs. The causal relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

AEs can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

6.1 Serious Adverse Events

A ***serious adverse event (SAE)*** is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.) Potential DILI is also considered an important medical event.

Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.

Although overdose, cancer, and potential DILI are not always serious by regulatory definition, these events must be handled as SAEs. (See Section 6.1.1 for reporting pregnancies.)

Any component of a study endpoint that is considered related to study therapy (e.g., death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported) should be reported as an SAE (see Section 6.1.1 for reporting details).

NOTE:

The following hospitalizations are not considered SAEs in Roche clinical studies:

- A visit to the emergency room or other hospital department <24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- Elective surgery, planned prior to signing consent
- Admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason)
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

6.1.1 Serious Adverse Event Collection and Reporting

Sections 5.6.1 and 5.6.2 of the Investigator's Brochure represent the Reference Safety Information to determine expectedness of serious adverse events for expedited reporting. Following the subject's written consent (or the parent/guardian/LAR's permission) to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur during the screening period and within 50 days of discontinuation of dosing.

The investigator must report any SAE that occurs after these time periods and that is believed to be related to study drug or protocol-specified procedure.

An SAE report must be completed for any event where doubt exists regarding its seriousness.

If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship must be specified in the narrative section of the SAE Report Form.

SAEs, whether related or not related to study drug, and pregnancies must be reported to Roche or designee within 24 hours of awareness of the event. SAEs must be recorded on the SAE Report Form. The preferred method for SAE data reporting collection is through the eCRF. The paper SAE forms are only intended as a back-up option when the eCRF system is not functioning. In this case, the paper forms are to be transmitted via email or confirmed facsimile (fax) transmission to:

SAE Email Address: Refer to Contact Information list.

SAE Facsimile Number: Refer to Contact Information list.

For studies capturing SAEs through electronic data capture (EDC), electronic submission is the

required method for reporting. In the event the electronic system is unavailable for transmission, paper forms must be used and submitted immediately. When paper forms are used, the original paper forms are to remain on site

SAE Telephone Contact (required for SAE reporting): Refer to Contact Information list.

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports must include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, the SAE report must be updated and submitted within 24 hours to Roche (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs must be followed to resolution or stabilization.

6.2 Non-serious Adverse Events

A ***non-serious adverse event*** is an AE not classified as an *SAE*.

6.2.1 Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin at initiation of study drug *until 50 days after discontinuation of dosing*. Non-serious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious (see Section 6.1.1). Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate. All identified non-serious AEs must be recorded and described on the Non-serious AE page of the CRF (paper or electronic).

Completion of supplemental CRFs may be requested for AEs and/or laboratory abnormalities that are reported/identified during the course of the study.

6.3 Laboratory Test Result Abnormalities

The following laboratory test result abnormalities should be captured on the non-serious AE CRF page or SAE Report Form (paper or electronic) as appropriate:

- Any laboratory test result that is clinically significant or meets the definition of an SAE
- Any laboratory test result abnormality that required the subject to have study drug discontinued or interrupted
- Any laboratory test result abnormality that required the subject to receive specific corrective therapy

Laboratory abnormalities that are reported as AEs or SAEs should be reported utilizing the *NCI* CTCAE, Version 4.0.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia *vs.* low hemoglobin value).

6.4 Pregnancy

Investigators shall counsel male subjects who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy. At a minimum, contraceptive counseling should be provided at the time of assent or consent. Investigators shall advise male subjects who are sexually active with WOCBP on the use of highly effective methods of contraception in non-pregnant partner, and on the use of condom in subjects with a pregnant partner, during treatment period and for 140 days following the last dose. Highly effective methods of contraception have a failure rate of <1% when used consistently and correctly. Pregnancy that occurs in a female partner of a male study participant should be reported to Roche.

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

- Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena® by the male subject's WOCBP partner. Female partners of male subjects participating in the study may use hormone-based contraceptives as one of the acceptable methods of contraception since they will not be receiving study drug.
- Non-hormonal IUDs, such as ParaGard®
- Complete abstinence

  Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

LESS EFFECTIVE METHODS OF CONTRACEPTION

- Diaphragm with spermicide
- Cervical cap with spermicide
- Vaginal sponge
- Male condom with or without spermicide
- Progestin only pills or male subject's WOCBP partner female condom*

* A male and female condom must not be used together.

6.5 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as SAEs (see Section 6.1.1 for reporting details).

6.6 Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiogram, x-ray filming, or any other potential safety assessment required or not

required by protocol should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

7 DATA MONITORING COMMITTEE AND OTHER EXTERNAL COMMITTEES

There will be a data monitoring committee (DMC) for this study. The DMC scope, frequency of review, membership, activities and other specifications are detailed in the DMC charter.

The DMC shall have access to partially unblinded data (coded treatment assignments), including but not limited to following: the frequency and spectrum of *SAEs*; Grades 3 *and* 4 laboratory abnormalities; occurrence of malignancies; and other select adverse events of interest. The DMC shall also review aggregate data results from the IAs. The DMC shall have the right to request full unblinding of the data (codes will be decoded to reveal actual treatment assignments). The DMC shall act as an advisor to the Sponsor and have responsibility for safeguarding the subjects' interests. The DMC shall bring any safety concerns to the attention of the Sponsor so that the Sponsor can review the data and prepare appropriate communications to the regulatory authorities.

8 STATISTICAL CONSIDERATIONS

8.1 Sample Size Determination

The total number of subjects of the study will be approximately 40, which consists of 3 dose panels of approximately 8 subjects each and an Expansion Panel of approximately 16 subjects. In each dose panel, subjects will be randomized 3:1 to either RO7239361 or placebo. Subjects who discontinue participation in the study prematurely may be replaced only if the reason for discontinuation is not related to study drug. For safety evaluation, the administration of RO7239361 to 6 subjects provides an 80% probability of observing at least one occurrence of any adverse event that would occur with 24% incidence in the population from which the sample is drawn.

8.2 Populations for Analyses

- Enrolled subjects: all consented subjects who entered screening
- Treated subjects: all subjects who received at least one dose of study drug or placebo

The *PK* data set includes all available concentration time data from the subjects who receive any RO7239361 medication. Additionally, all available derived PK parameter values will be included in the PK data set and reported, but only subjects with adequate PK profiles will be included in the summary statistics and statistical analysis.

The *PD* data set includes all available data from subjects for whom *PD* measurements are available at baseline and at least one other timepoint.

Subjects who receive placebo in any panel will be pooled into a single placebo group.

8.3 Endpoints

8.3.1 Primary Endpoint(s)

The primary objective is to assess safety and tolerability of multiple SC doses of RO7239361 in DMD boys. Primary endpoints are incidence of AEs, SAEs, AEs leading to discontinuation, and death, as well as marked treatment emergent abnormalities in clinical laboratory tests, vital sign measurements, ECGs, echocardiograms, and physical examinations across treatment conditions during the double-blind and *OL* phases of treatment.

8.3.2 Secondary Endpoint(s)

The first secondary objective to evaluate the PK of multiple SC doses of RO7239361 will be assessed using serum concentrations of RO7239361 at specified timepoints and will be measured by the following secondary endpoints:

- RO7239361 trough concentrations at Days 8, 15, 22, 29, Week 12, and Week 24
- Observed C_{max} of RO7239361 in the 1-week dosing interval following the doses on Day 1 and Day 29

The second secondary objective to evaluate the immunogenicity of multiple SC doses of RO7239361 will be measured by the assessment of immunogenicity samples for presence and titer of ADAs at the timepoints specified in Table 5.1-1 through Table 5.1-4. The following endpoints will be measured:

- Frequency of subjects with positive ADA assessment
- Frequency of subjects who develop positive ADA following a negative baseline
- Anti-RO7239361 antibodies on selected days

The third secondary objective to evaluate the effects of RO7239361 on free myostatin and myostatin-drug complex levels of RO7239361 will be measured by serum concentrations of free myostatin and myostatin-drug complex at the timepoints specified in Table 5.1-3. The following endpoints will be measured:

- Serum concentration of free myostatin and drug-myostatin complex, percent inhibition of free myostatin at trough

The fourth secondary objective is to evaluate right thigh muscle changes through MRI measurements. The secondary endpoints that will be measured are absolute changes and percentage changes from baseline of CSA_{max} and fold number changes in contractile versus non-contractile content for muscles in the right thigh during the 24-week double-blind treatment period, compared to placebo.

8.3.3 Exploratory Endpoint(s)

The *PD* effects of multiple doses of RO7239361 in DMD boys will be evaluated by the following exploratory endpoints:

- Measures of function, including TFTs (4SC, 4SD, 10-m walk/run, *standing* from supine), 6MWD, and the NSAA)
- Serum analytes that may include but are not limited to:
  - miR-206, miR-133a, miR-1, miR-6 (control miR), miR-21 and miR-39
  - TGF-β concentrations which are associated with fibrosis of muscle seen in DMD
  - Follistatin
  - Creatine kinase concentrations and CK-MM, which are associated with muscle atrophy in DMD
- Urine analytes that include but are not limited to:
  - Titin protein amino-terminal fragment concentrations that are associated with muscle damage
- Upper and lower extremity strength obtained by manual myometry
- Ankle range of motion in degrees
- DXA measures of total lean body mass, fat mass and bone mineral density
- MRS measures of right thigh lipid fraction
- Health-related quality of life as measured by the PODCI
- Measures of pulmonary function, including FVC, FEV_1, MEP, MIP, CPF, and PFR
- Genetic variation status of genes suspected to modify DMD disease expression (including but not limited to LTBP4, SPP1)
- PUL measure of upper extremity motor function

8.4 Analyses

8.4.1 Demographics and Baseline Characteristics

Frequency distributions of gender and race will be tabulated. Summary statistics for age, body weight, height, and body mass index (BMI) will be tabulated.

8.4.2 Efficacy Analyses

Exploratory efficacy endpoints including but not limited to TFT (4SC, 4SD, 10-m walk/run, *standing* from supine), 6MWD, NSAA and PFTs will be tabulated by dose and study day; and the corresponding changes from baseline and percent change from baseline will be calculated and summarized.

8.4.3 Safety Analyses

All recorded *AEs* will be listed and tabulated by system organ class, preferred term and treatment. Vital signs and clinical laboratory test results will be listed and summarized by treatment. Treatment emergent laboratory abnormalities will be listed by toxicity grade (according to the *NCI* CTCAE, Version 4.0) and summarized by treatment. Any significant

physical examination findings will be listed. Echocardiogram and ECG readings will be evaluated by the central cardiac site and abnormalities, if present, will be listed. Values for ECG, vital signs and clinical laboratory test results outside the pre-specified criteria will also be listed and summarized. ECG readings will be evaluated by the investigator and abnormalities, if present, will be listed. The placebo data will be pooled as one group.

Summary statistics will be presented for each echocardiogram/ECG/VS parameter and the corresponding changes from baseline by treatment and timepoint. Plots of mean change from baseline value for each parameter versus time since dosing will be presented by treatment. The last pre-dose value is defined as baseline value.

8.4.4 Pharmacokinetic Analyses

Serum RO7239361 concentrations will be summarized by treatment group, study day and times postdose. To assess the attainment of steady state, geometric mean Ctrough values will be plotted versus study day.

Dose proportionality of RO7239361 will be assessed by estimating the slope of the linear regression of the natural log of the PK parameter (C_{max} and C_{trough}) on the natural log of dose (by tiered body weight) using the power model described by Gough et al.[32] Descriptive summary statistics, including means, standard deviations, coefficients of variation, minima, medians, and maxima will be provided for serum RO7239361 concentrations at different timepoints.

PK and target engagement data collected in this study may be used to develop a population PK/PD model to estimate model-derived population and individual PK/PD parameter (e.g., CL/F, Vc/F, Ka, etc.) and may be reported separately from the clinical study report.

8.4.5 Target Engagement Analyses

Free myostatin and drug-myostatin complex will be tabulated by dose and study day; and the corresponding changes from baseline and percent change from baseline will be calculated and summarized. Profile plots may be provided. A dose-response (exposure-response) analysis may be applied to characterize the relationship between dose levels (PK parameters) of RO7239361 and free myostatin.

8.4.6 Biomarker Analyses

Right thigh CSA_{max}, contractile versus non-contractile content as a key secondary endpoint will be tabulated by dose and study day; and the corresponding changes from baseline and percent change from baseline will be calculated and summarized. Summary and individual profile plots will be provided as appropriate.

A model dose-response (exposure-response) analysis of non-contractile fraction content will be used to characterize the relationship between dose levels (PK parameters) of RO7239361 and changes from baseline in right thigh non-contractile fraction content among 4 muscles in the quadriceps femoris group. This analysis will combine data from Panels 1−3 and the expansion panel.

This model assumes that the change from baseline follows a normal distribution with a mean response that can be expressed in an E_{max} model. If the model results show evidence of heteroscedascity or the E_{max} model does not produce an acceptable fit, then either weighted analyses and/or alternate model specifications may be used. Alternative model specifications may include additional covariates that are relevant to the model.

Similar model-based analyses may be applied for other right thigh muscles/muscle groups CSA_{max} and lipid fraction content.

8.4.7 Exploratory Biomarker Analyses

All the exploratory endpoints such as miRNA 206, exploratory blood biomarkers, urinary titin fragments will be summarized.

Subgroup analyses of other primary, secondary, or exploratory endpoints may be conducted based on genetic variation in genes suspected to modify DMD disease expression (including, but not limited to, LTBP4, SPP1). The potential association between genetic variations with certain endpoints from safety, PD, or function test data may be analyzed. Depending on available data, all analyses related to genetic variation in genes may be conducted by pooling data from all RO7239361-treated *subjects* together or by dose/exposure-response modeling.

Summary and individual profile plots will be provided as appropriate.

8.4.8 Immunogenicity Analysis

Serum samples from all treated subjects, including placebo subjects, will be analyzed by a validated immunogenicity assay. Individual timepoints are considered positive if confirmed as specific against RO7239361. The subject will be called positive for immunogenicity as defined in the Statistical Analysis Plan. The number and percentage of subjects will be listed and summarized by treatment and by time, and the corresponding antibody titer values will be listed. If ADAs are observed, subgroup analyses may be conducted to compare AEs, labs, biomarker data, and PK results across treated subjects with and without ADAs, if sizes of the subgroups warrant such. In the event a subject has a positive immunogenicity response, additional NAB analyses may be conducted.

8.4.9 Outcomes Research Analyses

Parent-reported health-related quality of life using PODCI will be summarized by treatment and time for each domain or in combination.[29,30] The correlations between PODCI with clinical measurements may be analyzed.

8.4.10 Other Analyses

Not applicable.

8.5 Interim Analyses

Because data emerging from each panel of this exploratory study might be needed for timely decisions about adjustments to procedures in subsequent panels, including early termination of the study, data from panels can be unblinded after documented completion and review of the corresponding *CRFs*, prior to the formal locking of the study database. Analyses will only

consist of listings, summaries, and graphs of the available data. No formal inferences requiring any adjustment to statistical significance level will be performed.

Up to five IAs are planned. The first IA may be conducted when 4-week data of Panels 1 *and* 2 are available. The timing of the second and third IAs may be based on when the 4-week data of Panel 3 are available and when *the* 24-week data of Panels 1 *and* 2 are available, the timing to be based on the milestone. A fourth IA will be conducted when all subjects have completed the (24-week) double-blind phase. A fifth IA will be conducted to support regulatory submissions for marketing approval.

At the first IA, in addition to all available safety and tolerability, pharmacokinetics will be assessed following multiple-dose administration during the first 4 weeks. Additionally, dose/exposure-response analysis will be conducted to evaluate target engagement (myostatin inhibition). At the second and third IAs, all available safety, tolerability, PK, TE, biomarker data, strength, ankle range of motion, and function data may be analyzed. Depending on outcomes from IAs conducted earlier, the scope and timing of IAs scheduled later may change. The first three IAs will include all available blinded safety and tolerability data but not PD or functional data from the Expansion Panel, where applicable. The fourth IA will include all available data from the double-blind phase. The fifth IA will include all available data from the *OL* and *OLE* phases. A clinical study report will be generated based on these data for the purposes of marketing application submissions.

Clinical study report addendum will be generated at the end of the study.

9 STUDY MANAGEMENT

9.1 Compliance

9.1.1 Compliance with the Protocol and Protocol Revisions

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by, Roche. The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion
- Roche
- Regulatory authority(ies), if required by local regulations

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to Roche.

If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion; (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment.

If the revision is done via an administrative letter, investigators must inform their IRB(s)/IEC(s).

9.1.2 Monitoring

Representatives of Roche must be allowed to visit all study site locations periodically to assess the data quality and study integrity. On site they will review study records and directly compare them with source documents, discuss the conduct of the study with the investigator, and verify that the facilities remain acceptable.

In addition, the study may be evaluated by Roche internal auditors and government inspectors who must be allowed access to CRFs, source documents, other study files, and study facilities. Roche audit reports will be kept confidential.

The investigator must notify Roche promptly of any inspections scheduled by regulatory authorities and promptly forward copies of inspection reports to Roche.

9.1.2.1 Source Documentation

The investigator is responsible for ensuring that the source data are accurate, legible, contemporaneous, original and attributable, whether the data are hand-written on paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved, or transmitted electronically via computerized systems (and/or any other kind of electronic devices) as part of regulated clinical trial activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records (EMRs/EHRs), adverse event tracking/reporting, protocol required assessments, and/or drug accountability records).

When paper records from such systems are used in place of electronic format to perform regulated activities, such paper records should be certified copies. A certified copy consists of a copy of original information that has been verified, as indicated by a dated signature, as an exact copy having all of the same attributes and information as the original.

9.1.3 Investigational Site Training

Roche will provide quality investigational staff training prior to study initiation. Training topics will include but are not limited to: GCP, AE reporting, study details and procedure, electronic CRFs, study documentation, informed consent, and enrollment of WOCBP.

9.2 Records

9.2.1 Records Retention

The investigator must retain all study records and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period

specified by Roche, whichever is longer. The investigator must contact Roche prior to destroying any records associated with the study and Roche will notify the investigator when the study records are no longer needed.

If the investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, IRB). Notice of such transfer will be given in writing to Roche.

9.2.2 Study Drug Records

It is the responsibility of the investigator to ensure that a current disposition record of study drug (inventoried and dispensed) is maintained at the study site to include the following investigational product. Records or logs must comply with applicable regulations and guidelines and should include:

- Amount received and placed in storage area
- Amount currently in storage area
- Label identification number or batch number
- Amount dispensed to and returned by each subject, including unique subject identifiers
- Amount transferred to another area/site for dispensing or storage
- Non-study disposition (e.g., lost, wasted)
- Amount destroyed at study site, if applicable
- Amount returned to Roche
- Retained samples for bioavailability/bioequivalence, if applicable
- Dates and initials of person responsible for Investigational Product dispensing/accountability, as per the Delegation of Authority Form.

Roche will provide forms to facilitate inventory control if the investigational site does not have an established system that meets these requirements.

9.2.3 Case Report Forms

An investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated or entered as a control in the investigation. Data that are derived from source documents and reported on the CRF must be consistent with the source documents or the discrepancies must be explained. Additional clinical information may be collected and analyzed in an effort to enhance understanding of product safety. CRFs may be requested for AEs and/or laboratory abnormalities that are reported or identified during the course of the study.

For sites using the Roche electronic data capture tool, electronic CRFs will be prepared for all data collection fields except for fields specific to SAEs and pregnancy, which will be reported on the paper or electronic SAE form and Pregnancy Surveillance form, respectively. Spaces may be left blank only in those circumstances permitted by study-specific CRF completion guidelines provided by Roche.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

The investigator will maintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on CRFs.

The completed CRF, including any paper or electronic SAE/pregnancy CRFs, must be promptly reviewed, signed, and dated by the investigator or qualified physician who is a subinvestigator and who is delegated this task on the Delegation of Authority Form. For electronic CRFs, review and approval/signature is completed electronically through the Roche electronic data capture tool. The investigator must retain a copy of the CRFs, including records of the changes and corrections.

Each individual electronically signing electronic CRFs must meet Roche training requirements and must only access the Roche electronic data capture tool using the unique user account provided by Roche. User accounts are not to be shared or reassigned to other individuals.

9.3 Clinical Study Report and Publications

A Signatory Investigator must be selected to sign the clinical study report.

For this protocol, the signatory investigator will be selected as appropriate based on the following criteria:

- External principal investigator designated at protocol development
- Subject recruitment (e.g., among the top quartile of enrollers)
- Involvement in trial design
- Other criteria (as determined by the study team)

The data collected during this study are confidential and proprietary to Roche. Any publications or abstracts arising from this study require approval by Roche prior to publication or presentation and must adhere to Roche's publication requirements as set forth in the approved clinical trial agreement (CTA). All draft publications, including abstracts or detailed summaries of any proposed presentations, must be submitted to Roche at the earliest practicable time for review, but at any event not less than 30 days before submission or presentation unless otherwise set forth in the CTA. Roche shall have the right to delete any confidential or proprietary information contained in any proposed presentation or abstract and may delay publication for up to 60 days for purposes of filing a patent application.

10 GLOSSARY OF TERMS

Term	Definition
Complete Abstinence	If one form of contraception is required, complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence. If two forms of contraception is required, Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence. **Expanded definition** Complete abstinence as defined as complete avoidance of heterosexual intercourse is an acceptable form of contraception for all study drugs. This also means that abstinence is the preferred and usual lifestyle of the patient. This does not mean periodic abstinence (e.g., calendar, ovulation, symptothermal, profession of abstinence for entry into a clinical trial, post-ovulation methods) and withdrawal, which are not acceptable methods of contraception. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.
Additional Research	Those scientific activities which cannot be reasonably anticipated at the time of trial design, for which we would like to collect and/or retain samples from study participants. Examples of additional research include, but are not limited to, new assay development and validation, companion diagnostic development, new hypotheses in the interaction of drug and the human body, and exploration of emerging science in the understanding of disease.

10.1 List of Abbreviations

Term	Definition
4SC	4-stair climb
4SD	4-stair descend
6MWD	6-minute walk distance
ADA	Anti-RO7239361 antibodies
AE	adverse event
AI	accumulation index
AI_AUC	AUC Accumulation Index; ratio of AUC(TAU) at steady state to AUC(TAU) after the first dose
AI_Cmax	Cmax Accumulation Index; ratio of Cmax at steady state to Cmax after the first dose
AI_Ctau	Ctau Accumulation Index; ratio of Ctau at steady state to Ctau after the first dose
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AT	aminotransaminases
AUC	area under the concentration-time curve
AUC(INF)	area under the concentration–time curve from time zero extrapolated to infinite time
AUC(0-T)	area under the concentration–time curve from time zero to the time of the last quantifiable concentration
AUC(TAU)	area under the concentration–time curve in one dosing interval
A-V	atrioventricular
β-HCG	beta-human chorionic gonadotrophin
BA/BE	bioavailability/bioequivalence
%BE	percent biliary excretion
BID, bid	bis in die, twice daily
Bi-PAP	Bi-level positive airway pressure
BLQ	below limit of quantification

Term	Definition
BMI	body mass index
BP	blood pressure
BRt	total amount recovered in bile
%BRt	total percent of administered dose recovered in bile
BUN	blood urea nitrogen
C	Celsius
C12	concentration at 12 hours
C24	concentration at 24 hours
Ca^{++}	calcium
Cavg	average concentration
CBC	complete blood count
Cexpected-tau	expected concentration in a dosing interval
CFR	Code of Federal Regulations
CHF	congestive heart failure
CI	confidence interval
$C1^{-}$	chloride
CLcr	creatinine clearance
CLD	Dialysate clearance of drug from plasma/serum
CLNR	nonrenal clearance
CLR	renal clearance
CLT	total body clearance
CLT/F (or CLT)	apparent total body clearance
CLT/F/fu or CLT/fu	Apparent clearance of free drug or clearance of free if (if IV)
Cm	Centimeter
Cmax, CMAX	maximum observed concentration
Cmin, CMIN	trough observed concentration
CPF	Cough peak flow
CRC	Clinical Research Center

Term	Definition
CRF	Case Report Form, paper or electronic
CSAmax	Maximal cross-sectional area
Ct	Expected concentration at a certain time, usually at the end of an expected future dosing interval (e.g., concentration at 24 hours, concentration at 12 hours, etc.)
Ctau	Concentration in a dosing interval (e.g., concentration at 24 hours, concentration at 12 hours, etc.)
Ctrough	Trough observed plasma concentration
CV	coefficient of variation
CYP	cytochrome p-450
D/C	Discontinue
dL	deciliter
DMC	Data monitoring committee
DMD	Duchenne muscular dystophy
DRt	Total amount recovered in dialysate
%DRt	Total percent of administered dose recovered in dialysate
DSM IV	Diagnostic and Statistical Manual of Mental Disorders (4^{th} Edition)
DXA	Dual-energy X-ray absorptiometry
EA	extent of absorption
ECG	electrocardiogram
ECHO	echocardiogram
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
e.g.	exempli gratia (for example)
ESR	Expedited Safety Report
F	bioavailability
Fb	fraction of bound drug
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FI	fluctuation Index ([Cmax-Ctau)/Cavg])
%FRt	total percent of administered dose recovered in feces
FSH	follicle stimulating hormone

Term	Definition
fu	fraction of unbound drug
FVC	forced vital capacity
g	gram
GC	gas chromatography
GCP	Good Clinical Practice
G criteria	adjusted R^2 value of terminal elimination phase
GGT	gamma-glutamyl transferase
GFR	glomerular filtration rate
GDF-8	growth and differentiation factor-8
GLDH	glutamate dehydrogenase
h	hour
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HCO_3^-	bicarbonate
HIV	human immunodeficiency virus
HR	heart rate
HRT	hormone replacement therapy
IA	interim analysis
ICD	International Classification of Diseases
ICH	International Conference on Harmonisation
i.e.	id est (that is)
IEC	Independent Ethics Committee
IMP	investigational medicinal product
IND	Investigational New Drug Exemption
IP	investigational product
IRB	Institutional Review Board
IU	International Unit
IV	Intravenous
K	slope of the terminal phase of the log concentration-time curve

Term	Definition
K_3EDTA	potassium ethylenediaminetetraacetic acid
K^+	potassium
kg	kilogram
$\lambda\sigma z$	terminal disposition rate constant
L	liter
LC	liquid chromatography
LAR	legally acceptable representative
LDH	lactate dehydrogenase
MAD	multiple ascending dose
mg	milligram
MIP	maximal inspiratory pressure
MEP	maximal expiratory pressure
mL	nilliliter
mmHg	millimeters of mercury
MOA	mechanism of action
MR_AUC(0-T)	Ratio of metabolite AUC(0-T) to parent AUC(0-T), corrected for molecular weight
MR_AUC(INF)	Ratio of metabolite AUC(INF) to parent AUC(INF), corrected for molecular weight
MR_AUC(TAU)	Ratio of metabolite AUC(TAU) to parent AUC(TAU), corrected for molecular weight
MR_Cmax	Ratio of metabolite Cmax to parent Cmax, corrected for molecular weight
MR_Ctau	Ratio of metabolite Ctau to parent Ctau, corrected for molecular weight
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MRT	mean residence time
MS	mass spectrometry
MTD	maximum tolerated dose
μg	microgram
N	number of subjects or observations

Term	Definition
Na^+	sodium
N/A	not applicable
NAB	neutralizing antibodies
ng	nanogram
NIMP	non-investigational medicinal products
NOAEL	no observed adverse event level
Non-IP	non-investigational product
NSAA	North Star Ambulatory Assessment Scale
NSAID	non-steroidal anti-inflammatory drug
OL	open label
OLE	*open-label extension*
pAUCe	extrapolated partial AUC from last quantifiable concentration to infinity
Pb	percent bound drug
PD	pharmacodynamic
PFR	peak flow rate
PFS	prefilled syringe
PFTs	pulmonary function tests
PK	pharmacokinetic
PODCI	Pediatric Outcome Data Collection Instrument
PT	prothrombin time
PTT	partial thromboplastin time
QW	once per week
RBC	red blood cell
SAE	serious adverse event
SC	subcutaneous
SD	standard deviation
SOP	standard operating procedure
Sv	sieverts
TE	target engagement
TFTs	timed function tests
$T_{1/2}$	terminal half-life

Term	Definition
T-HALFeff_AUC	Effective elimination half-life that explains the degree of AUC accumulation observed
T-HALFeff_Cmax	Effective elimination half-life that explains the degree of Cmax accumulation observed)
TID, tid	ter in die, three times a day
Tmax, TMAX	time of maximum observed concentration
TR_AUC(0-T)	AUC(0-T) treatment ratio
TR_AUC(INF)	AUC(INF) treatment ratio
TR_Cmax	Cmax treatment ratio
UR	urinary recovery
%UR	percent urinary recovery
URt	total amount recovered in urine
%URt	total percent of administered dose recovered in urine
UV	ultraviolet
Vss/F (or Vss)	apparent volume of distribution at steady state
Vz	volume of distribution of terminal phase (if IV and if multi-exponential decline)
W	washout
WBC	white blood cell
WHO	World Health Organization
WOCBP	women of childbearing potential
x g	times gravity

11 REFERENCES

[1] Emery AE, Population frequencies of inherited neuromuscular disease—a world survey. Neuromuscular Disord 1991; 1:19-29.

[2] Manzur AY, Kuntzer T, Pike M, et al. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Systematic Review, 2008; 1:CD003725.

[3] European Medicines Agency http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Risk-management-plan_summary/human/002720/WC500167551.pdf Accessed 12-Oct-2014.

[4] Wagner KR, McPherron AC, Winik N, et al. Loss of myostatin attenuates severity of muscular dystrophy in mdx mice. Ann Neurol 2002;832-836.

[5] Bogdanovich S, Krag, TO, Barton ER, et al. Functional improvement of dystrophic muscle by myostatin blockade. Nature2002;420:418-421.

[6] Mcdonald CM, Henricson EK, Abresch RT, et al. The 6-minute walk test and other clinical endpoints in duchenne muscular dystrophy: Reliability, concurrent validity, and minimal clinically important differences from a multicenter study. Muscle Nerve 2013;48(3):357- 68

[7] Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. Muscle Nerve 2014;50:477-487.

[8] Pane M, Mazzone ES, Sivo S, et al. Long term natural history data in ambulant boys with duchenne muscular dystrophy: 36-month changes. PLoS One 2014;9:e108205.

[9] Griggs RC et al. Prednisone in Duchenne muscular dystrophy: a randomized, controlled trial defining the time course and dose response. Arch Neurol 1991; 48:383-388.

[10] Manzur AY, Kuntzer T, Pike M, et al. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database System Rev 2008, Issue 1. Art. No.: CD003725.

[11] Cittadini A, Comi LI, Longobardi S, et al. A preliminary randomized study of growth hormone administration in Becker and Duchenne muscular dystrophies. Eur Heart J 2003;24(7):664-72.

[12] Fenichel GM, Griggs RC, Kissel J, et al. A randomized efficacy and safety trial of oxandrolone in the treatment of Duchenne dystrophy. Neurology 2001;56(8):1075-9.

[13] Flannigan KM, Ceco E, Lamar K-M, et al. LTBP4 genotype predicts age of ambulatory loss in Duchenne muscular dystrophy. Ann Neurology 2013; 73: 481-488.

[14] Bello L, Piva L, Barp A, et al. Importance of SPP1 genotype as a covariate in clinical trials in Duchenne muscular dystrophy. Neurology 2012; 79: 159-162.

[15] Pegoraro E, Hoffman EP, Piva L, et al. SPP1 genotype is a determinant of disease severity in Duchenne muscular dstrophy. Neurology 2011;76:219-226.

[16] Wojcik E, Carrithers LM, Carrithers MD. Characterization of epithelial V-like antigen in human choroid plexus epithelial cells: potential role in CNS immune surveillance. Neurosci Lett 2011;495(2):115-20.

[17] Sato J, Doi T, Kanno T, et al. Histopathology of incidental findings in Cynomolgus monkeys (Macaca fascicularis) used in toxicity studies. J Toxicol Pathol 2012;25(1):63-101.

[18] Chamanza R, Marxfeld HA, Blanco AI, et al. Incidences and range of spontaneous findings in control cynomolgus monkeys (Macaca fascicularis) used in toxicity studies. Toxicol Pathol 2010;38(4):642-57.

[19] BMS-986089: In vitro antibody-dependent cell cytotoxicity assessment using human peripheral blood mononuclear cells (Study DN13164). Bristol-Myers Squibb Company; 2014. Document Control No. 930083084.

[20] Loffredo FS et al. Growth differentiation factor 11 is a circulating factor that reverses age- related cardiac hypertrophy. Cell 2013;153:828-839.

[21] Martigne L et al. Natural evolution of weight status in Duchenne muscular dystrophy: a retrospective audit. Brit J Nutr 2011;105:1486-1491.

[22] Damilakis J et al. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. Eur Radiol 2010; 20: 2707-2714.

[23] Rouillon J et al. Proteomics profiling of urine reveals specific titin fragments as biomarkers of Duchenne muscular dystrophy. Neuro Disord 2014;24:563-573.

[24] Sun S et al. Measurement of a MMP-2 degraded titin fragment in serum reflects changes in muscle turnover induced by atrophy. Exper Gerontol 2014;58:83-89.

[25] Henricson E et al. The Cooperative International Neuromuscular Research Group Duchenne Natural History Study: Glucocorticoid treatment preserves clinically meaningful functional milestones and reduces rate of disease progression as measured by manual muscle testing and other commonly used clinical trial outcome measures. Muscle Nerve; July 2013;55-67.

[26] Mazzone ES et al. Reliability of the North Star Ambulatory Assessment in a multicentric setting. Neuro Disord 2009;19:458-461.

[27] Pane M, et al. Reliability of the Performance of Upper Limb Assessment in Duchenne muscular dystrophy. Neuro Disord 2014; 24:201-206

[28] Sharma G. Pulmonary function testing in nuromuscular disorders. Pediatrics 2008; 123:S219-S221.

[29] Daltroy LH, Liang MH, Fossel AH, Goldberg MJ, and The Outcomes Instrument Development Work Group. The POSNA Pediatric Musculoskeletal Functional Health Questionnaire: report on reliability, validity, and sensitivity to change. J Pediatr Orthop 1998; 18: 561-71.

[30] McDonald CM, McDonald DA, Bagley A, et al. Relationship between clinical outcome measures and parent proxy reports of health-related quality of life in ambulatory children with Duchenne muscular dystrophy. J Child Neurology Sep 2010;25(9):1130-1144.

[31] Haschek W, Rousseaux C, Wallig M. 2010. Fundamentals of Toxicologic Pathology. 2nd edition. London: Elseviers, Inc.

[32] Gough K, Hutchison M, Keene O, *et al*. Assessment of dose proportionality: report from the statisticians in the Pharmaceutical Industry/Pharmacokinetics UK Joint Working Party. Drug Information J 1995;29:1039-48.

[33] *Wang K, Ouyang H, Xie Z, et al. Efficient generation of myostatin mutations in pigs using the CRISPR/Cas9 system. Sci Rep 2015;13:16623. doi: 10.1038/srep16623.*

[34] *Lv Q, Yuan L, Deng J, et al. Efficient generation of myostatin gene mutated rabbits by CRISPR/Cas9. Sci Rep 2016;6:25029.*