Official Title: A Randomized, Double Blind, Placebo-Controlled, Study to Assess the Efficacy, Safety, and Tolerability of RO7239361 in Ambulatory Boys With Duchenne Muscular Dystrophy

NCT Number: NCT03039686

Document Date: Protocol Version 4: 16-August-2018

PROTOCOL

TITLE:	**A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED, STUDY TO ASSESS THE EFFICACY, SAFETY, AND TOLERABILITY OF RO7239361 IN AMBULATORY BOYS WITH DUCHENNE MUSCULAR DYSTROPHY**
PROTOCOL NUMBER:	WN40227
VERSION NUMBER:	4
EUDRACT NUMBER:	2016-001654-18
IND NUMBER:	120,702
TEST PRODUCT:	Anti-Myostatin Adnectin (RO7239361)

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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	16-Aug-2018 07:13:59

1. DOCUMENT HISTORY

Document	Date of Issue	Summary of Changes
Revised Protocol 4 (Version 4)	*See electronic date stamp.*	
Amendment 6	*See electronic date stamp.*	• *Deleted references to the previous Bristol-Myers Squibb protocol and product numbers throughout the text, except in the Introduction, Schedule of Assessments, and Treatment sections* • *Change the primary endpoint from the 4 Stair Climb velocity to the North Star Ambulatory Assessment total score* • *Added a new inclusion criterion requiring a minimum NSAA score of 15 points at screening* • *Changed the 4SCV from the primary endpoint to a secondary endpoint* • *Added 95th percentile stride velocity, as recorded using the ActiMyo as a secondary endpoint* • *Added the caregiver video assessment at selected sites as a new exploratory endpoint* • *Added the Caregiver Global Impression of Change at selected sites as a new exploratory endpoint* • *Added the Pediatric Quality of Life Inventory Multidimensional Fatigue Scale at selected sites as an exploratory endpoint* • *Shortened the Study Rationale section to include a high-level summary of available clinical data and to refer to the Investigator's Brochure for specific details* • *Updated the duration of the open-label (OL) extension phase and the frequency of visits during the OL extension phase* • *Added the specific sites indicated for subcutaneous injection* • *Deleted the proposed interim analysis* • *Updated the statistical analysis section to establish hierarchical testing of the doses* • *Added definitions for different situations of incorrect administration of study drug* • *Deleted urine biomarkers and miRNA biomarkers as exploratory endpoints* • *Added assessment of serum creatine kinase level in muscle to the exploratory pharmacokinetic, pharmacodynamic, and immunogenicity endpoints*

Document	Date of Issue	Summary of Changes
Revised Protocol 03	29 Jan 2018	Incorporates Amendment 05
Amendment 05	29 Jan 2018.	– Added assessment of CGI-C. – Reduced pulmonary function tests – Reduced anthropometry assessments – Reduced myometry assessments – Reduced TFTs and 6MWT in the open-label phases – Clarified signature of informed consent prior to Day –42 screening period – Clarified FVC in the exclusion criteria – Clarified contraception methods – Clarified GDF-11 sample timepoint – Clarified ActiMyo assessments – Clarified use of videos – Updated requirement for safety reporting of overdose – Clarified monitoring of ADAs during 24-week safety follow-up phase – Updated Medical Monitor information – Updated for consistency with IB, version 6 – Corrected typographical errors
Revised Protocol 02	21-Aug-2017.	Incorporates Amendment 04
Amendment 04	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 01	20-Apr-2017	Incorporates Amendment 03
Amendment 03	20-Apr-2017	– Added text defining significant change in dosage for prednisone and deflazacort. – Added measurement of ulna length to baseline and on treatment time points. – Timing of on treatment videotaping of functional assessments has been clarified. – Timing of Health Care Resource Utilization assessments has been clarified. – Added clarification that DXA scanning is not required at early termination. – History of hypersensitivity of the components of the study drug added as an exclusion. – Threshold for adjusting dosing weight tier increased from 1 kg to 2 kg. – Text clarifying that malfunctions of pre-filled syringes should be reported to the sponsor in accordance with local regulations has been added. – Guidance regarding skin biopsy added. – Text describing pharmacogenomics removed. – Corrections of typographical errors.
Original Protocol	05-Dec-2016	Not Applicable

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PROTOCOL *AMENDMENT* ACCEPTANCE FORM

TITLE:	**A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED, STUDY TO ASSESS THE EFFICACY, SAFETY, AND TOLERABILITY OF RO7239361 IN AMBULATORY BOYS WITH DUCHENNE MUSCULAR DYSTROPHY**
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MEDICAL MONITOR*S*:	[redacted], M.D. [redacted], M.D., Ph.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.

1 SYNOPSIS

Protocol Title: A Randomized, Double Blind, Placebo-Controlled, Study to Assess the Efficacy, Safety, and Tolerability of RO7239361 in Ambulatory Boys with Duchenne Muscular Dystrophy

Study Phase 2/3 Rationale

The purpose of this study is to evaluate the efficacy, safety and tolerability of weekly subcutaneous (SC) doses of RO7239361 compared to placebo in ambulatory boys with Duchenne muscular dystrophy (DMD).

Study Population:

Key Inclusion Criteria:

- Males, ≥ 6 to < 12 years of age at time of randomization
- Diagnosis of DMD, confirmed by medical history (e.g., onset of clinical signs or symptoms before 5 years of age together with an elevated serum creatine kinase level observed before or after initial diagnosis) and by genotyping
- Participants ≥ 15 kg
- Ambulatory without assistance
- Participants must be receiving corticosteroids (*CS;* 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 prednisone or > 0.24 mg/kg deflazacort) or dosing regimen for at least 12 weeks 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
- *North Star Ambulatory Assessment (NSAA) score ≥ 15 points at screening*
- 4SC ≤ 8 seconds at screening
- Participants must agree to avoid major changes in their physical or respiratory therapy regimen during the double-blind phase, to the extent possible

Key Exclusion Criteria:

- Participants with cognitive impairment or behavioral issues that, in the judgement of the investigator, will compromise their ability to comply with study procedures
- Participants on intermittent *CS* regimens with off periods of 20 days or longer (e.g.: 10 days on, 20 days off)
- 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 12 weeks 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 12 weeks prior to start of study treatment
- Treatment with exon skipping therapies within 6 months prior to the start of study drug administration.
- Treatment with ataluren *currently or within 12 weeks prior to the start of study drug administration*

- *Treatment with* any other investigational drug (excluding deflazacort *in CS dose-finding trials*) currently or within 12 weeks prior to the start of study drug administration
- Concomitant or previous participation at any time in a gene therapy study
- Participants with a FVC of < 50% of predicted value (in participants able to produce a valid FVC, as judged by the clinical evaluator or respiratory therapist)
- Cutaneous AEs sustained during participation in a prior clinical trial that resolved less than 12 weeks prior to the start of study drug administration
- Current or prior treatment within 12 weeks prior to the start of study drug administration with androgens or human growth hormone
- Prior treatment with RO7239361 or any other anti-myostatin agent
- History of lower limb fracture within 12 weeks prior to the start of study drug administration
- History of upper limb fracture within 8 weeks prior to the start of study drug administration.
- Any injury *that* may impact functional testing. Previous injuries must be fully healed prior to consenting.
- Expectation of major surgical procedure, such as scoliosis surgery, during the double-blind phase of this study
- Requirement of daytime ventilator assistance
- Initiation of nighttime ventilation less than 4 weeks prior to the start of study drug administration
- Expectation that daytime or nighttime ventilation may be initiated during the double-blind phase of this study
- Clinical signs or symptoms of *uncontrolled* congestive heart failure (*CHF*) (American College of Cardiology/American Heart Associated Stage C or Stage D)
- For participants participating in cMRI substudy: *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 cMRI).
- Unwilling or unable to administer study drug at home

Objectives and Endpoints:

Objectives	Endpoints
Primary • To compare the efficacy of RO7239361 to placebo in ambulatory boys with Duchenne muscular dystrophy	• The change from baseline in the *North Star Ambulatory Assessment (NSAA) total score* at Week 48 in RO723936-treated participants compared to placebo-treated participants.
Secondary • To compare the efficacy of RO7239361 to placebo using the following tests: – *4 stair climb velocity (4SCV)* – Stand from supine velocity – 10 M walk/run velocity – PODCI transfers and basic mobility subscale – Proximal lower extremity flexor (knee extension and knee flexion) strength, measured using manual myometry – 6 Minute Walk Distance (6MWD) – Clinical Global Impression of Change (CGI-C) – *Stride velocity recorded with ActiMyo* • To assess the safety and tolerability of RO7239361 in boys with DMD as reflected by new or worsening lab abnormalities (as defined by CTCAE criteria), serious adverse events (SAEs) and adverse events (AEs) leading to discontinuation.	• Change from baseline at Week 48 in RO7239361-treated participants compared to placebo- treated participants in the following – *4 stair climb velocity (4SCV)* – Stand from supine velocity – 10 M walk/run velocity – PODCI transfers and basic mobility subscale – Proximal lower extremity flexor (knee extension and knee flexion) strength, measured using manual myometry – 6-Minute Walk Distance (6MWD) – Clinical Global Impression of Change (CGI-C) – *95th percentile stride velocity, as recorded with ActiMyo in a subset of the overall study population* • Tabulations of the numbers of unique participants with new or worsening laboratory abnormalities, SAEs and AEs leading to discontinuation, in RO7239361 arms compared to the placebo arm.
Tertiary/Exploratory • To assess the safety and tolerability of RO7239361 in boys with DMD as reflected by vital signs, measures of cardiac function	• Summaries and listings of vital signs, ECG parameters, echocardiogram parameters. – Proportion of participants with on treatment adverse events in RO7239361 arms compared to the placebo arm – Proportion of participants with on treatment decrease in ejection fraction (EF), measured using echocardiography, in RO7239361 arms compared to the placebo arm

Objectives	Endpoints
• To compare the efficacy of RO7239361 to placebo using the following tests: - Performance of upper limb (PUL) total score	• Change from baseline at Week 48 in RO7239361 treated participants relative to placebo treated participants in: – Performance of upper limb (PUL) total score
• To evaluate other measures of efficacy, quality of life *and* health care utilization	• Change from baseline at Week 48 in RO7239361 treated participants compared to placebo treated participants: – Pulmonary function tests, including FVC, % predicted FVC FEV_1, MEP, *and* MIP – PODCI total score – *Caregiver Global Impression of Change (CaGI-C) in a subset of the overall study population* – *Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL MFS) in a subset of the overall study population* – Cardiac magnetic resonance imaging (cMRI) measures of ejection fraction, fractional strain, and volume of fibrosis in a subset of the overall study population • Strength of elbow flexors, elbow extensors, and pinch, measured by manual myometry • Health Utilities Index III and PedsQL Family Impact Module scores • *Assessments at selected timepoints during the study of:* – Dual x-ray absorptiometry (DXA) measures of lean body mass, fat mass and bone mineral density – *Activity levels in a subset of the overall study population, as recorded with ActiMyo* – *Scoring of functional assessments, as recorded by a subset of parents/caregivers participating in the caregiver video assessment* – *Number of falls during treatment in the RO7239361 arms compared with the placebo arm*

Objectives	Endpoints
• *To evaluate RO7239361 pharmacokinetics, pharmacodynamics, and immunogenicity*	• Assessments at select timepoints during the study of: – Serum RO7239361 concentrations – Serum free myostatin and myostatin drug complex concentrations – Serum ADA concentrations – *Serum creatine kinase level in muscle*
• To explore the effect of genetic variation on target engagement, pharmacodynamic (PD) effects or functional test endpoints	• Assess the impact on genetic variation in the *DYSTROPHIN* gene or in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1) in RO7239361 and placebo treated participants, on target engagement, pharmacodynamic (PD) and functional endpoints

Overall Design:

This is a multi-center, randomized, double-blind, placebo-controlled study to assess the efficacy, safety and tolerability of two different weekly SC doses of RO7239361 in ambulatory boys with Duchenne muscular dystrophy (DMD).

Eligible participants will be randomized to receive doses of RO7239361 or placebo (1:1:1 across the three treatment arms). The randomization will be stratified by:

- Age (6 through 7 and 8 through 11)
- CS regimen: daily (prednisone, prednisolone or deflazacort) vs. intermittent (any non-daily dosing of prednisone, prednisolone or deflazacort)

A separate randomization will be conducted for Japan (N~15) with stratification for age only (6 through 7 and 8 through 11) not CS regimen.

RO7239361 or placebo will be administered weekly. The first three doses (Days 1, 8 and 15) of study *drug* must be administered at the site. Parents/caregivers will be trained to administer SC injections during the first three dose administrations. Parents/caregivers can administer the injection at home starting at Day 22. *Site staff should ensure that the parent or caregiver is able to perform injections at home.* Parents/caregivers will be dispensed an approximately 6- *or 12*-week supply of drug in pre-filled syringes (RO7239361 or placebo), *during the double-blind phase and open-label (OL) extension phase, respectively,* for weekly study drug/placebo administration to the participant.

Study visits and endpoint measurements will occur as indicated in Table 2-1 though Table 2-4.

After completion of this 48-week double-blind phase, participants may enter the *OL* phase in which all participants will receive active RO7239361 study drug. *Participants* who discontinue

the study *drug at any time (i.e.,* early *discontinuation for subjects who* do not enter the *OL extension* phase *or at completion of the OL extension phase)* will enter *a 24-week safety* follow-up phase of the study. Participants will be monitored for safety at three visits *during* the follow-up phase. The participant *will be requested to* provide immunogenicity samples at all follow-up visits during the 24-week follow-up phase.

Number of Participants:

Statistical Considerations:

Sample Size:

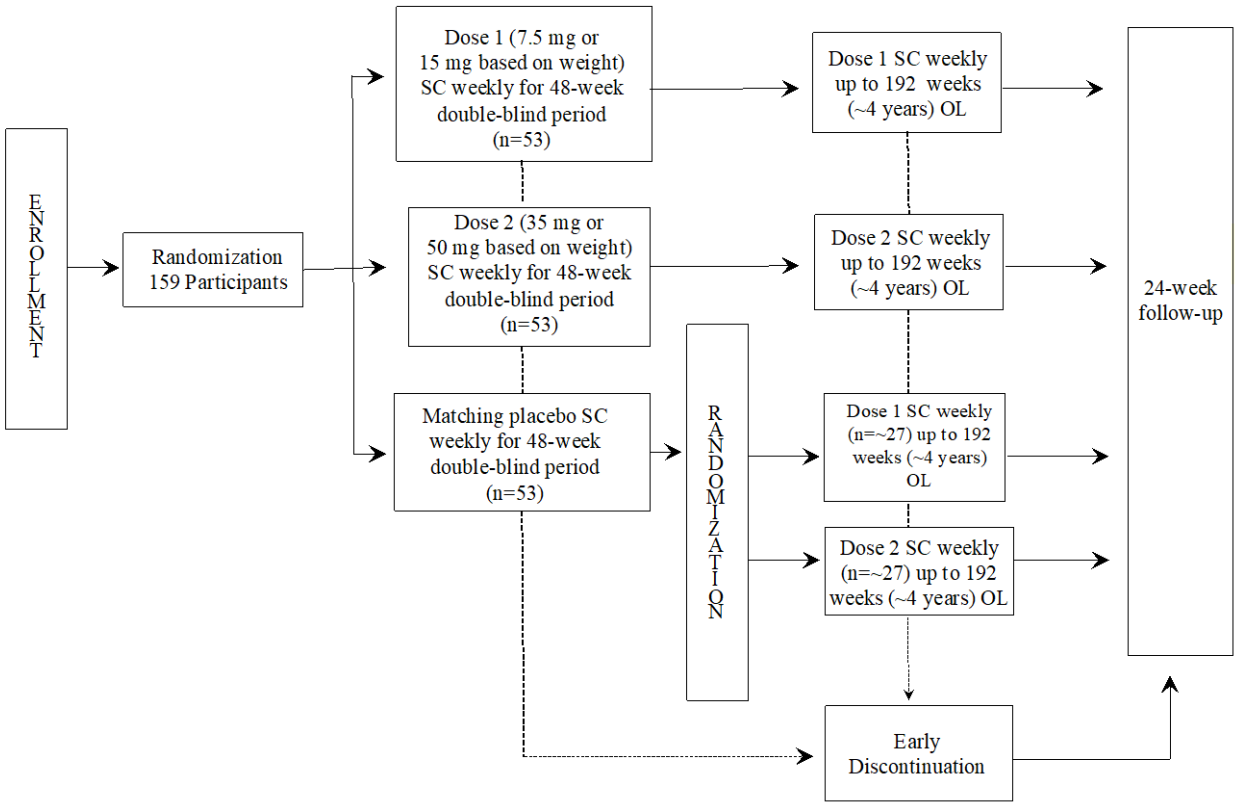
Assuming the standard deviation of the *NSAA is 4.4 points*, with approximately 150 evaluable subjects, the final analysis will have 80% power to detect a between group difference of *2.5 points*. The number randomized will be increased to allow for an approximate dropout rate on the order of 5% at the primary analysis, so *approximately* 159 participants will be randomized. Approximately 15 of these participants will be randomized in Japan, and the remaining participants will be randomized outside of Japan.

The final analysis will test for differences *between each RO7239361 treatment and placebo* in either direction. *The doses will be tested against placebo in a hierarchical manner, with the high dose (Dose 2) tested versus placebo in the first step of the hierarchy. If the null hypothesis of no difference between the high dose and placebo is rejected in favor of the RO7239361 dose at a two-sided α level of 0.05, then the low dose (Dose 1) will be tested against placebo.*

The primary analysis, on the change from baseline in *NSAA total score*, will be conducted using a mixed model repeated measures (MMRM) analysis with treatment, visit, and the treatment-by-visit interaction entered into the model as fixed effects, and participant entered as a random effect. The baseline value of the *NSAA total score* will be entered into the model as a covariate. The stratification factors will be entered into the model as blocks.

Sensitivity analyses will include the following: an analysis of covariance (ANCOVA) using the *modified intent-to-treat (*mITT*)* data set; an ANCOVA on the mITT data set with missing data imputed using multiple imputations; and an MMRM analysis using the per-protocol data.

Treatment Arms and Duration:



OL=open label; SC=subcutaneous.

Study Treatment:

Each participant will be administered weekly, SC doses of RO7239361 *(previously, BMS-986089)* or placebo for 48 weeks in a randomized, double- blind design. Participants who complete the *48-week* double- blind phase are eligible to receive *OL* RO7239361 (active treatment) as part of *an OL extension* phase of this study. *The OL extension phase will last up to 192 weeks or until RO7239361 is commercially available in the subject's country, per local regulations, or the study is terminated by the Sponsor, whichever comes first. Please refer to Section 7.8 for details regarding treatment after the end of study.* Doses *in the OL extension phase* may be adjusted based on emerging data.

Study Drug		
Medication	**Potency**	**IP/Non-IP**
RO7239361 7.5 mg dose	10.7 mg/mL (0.7mL/syringe)	IP
RO7239361 15 mg dose	21.4 mg/mL (0.7mL/syringe)	IP
RO7239361 35 mg dose	50 mg/mL (0.7mL/syringe)	IP
RO7239361 50 mg dose	71.4 mg/mL (0.7mL/syringe)	IP
RO7239361 matching placebo	0 mg/mL (0.7mL/syringe)	IP

Other medications used as support medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, may be considered as non-investigational products. In this study, corticosteroids are considered *a* non-investigational product and will not be provided by the sponsor.

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

2 SCHEDULE OF ACTIVITIES

Study procedures and assessments are present in Table 2-1, Table 2-2, Table 2-3, and Table 2-4.

Table 2-1: Screening Procedural Outline

Procedure	Screening Visit (Day -42 to Day -1)	Notes
Eligibility Assessments		
Informed Consent	X	A participant is considered enrolled only when a protocol specific informed consent is signed Informed consent may be signed prior to Day-42.
Register / enroll participant in IWRS	X	
Inclusion/Exclusion Criteria	X	
Medical History	X	Include disease history and any toxicities or allergies related to previous treatments.
Concomitant Medications	X	Include complete history of corticosteroids.
Physical Examination (PE)	X	
Physical Measurements	X	Includes height, weight, hand circumference and ulna length; see Clinical Evaluator (CE) manual.
Vital Signs	X	Includes body temperature (recommend using tympanic thermometer), respiratory rate, and seated blood pressure and heart rate. Blood pressure and heart rate should be measured after the participant has been resting quietly for at least 5 minutes.
Electrocardiogram (ECG)	X	ECG should be recorded after the participant has been supine for at least 5 minutes. See Eli 150c/250c User Guide
Echocardiogram	X	See ECHO Manual
Laboratory Assessments	X	Section 9.3.4
Urinalysis	X	

Table 2-1: Screening Procedural Outline

Procedure	Screening Visit (Day -42 to Day -1)	Notes
Cardiac MRI (At select U.S. centers only)	X	See Section 9.1.3.2 and the imaging procedure manual. Baseline cMRI should be obtained between Day -40 and Day -14. The adequacy of cMRI scan should be confirmed by the imaging vendor prior to randomization. Subjects enrolled at select U.S. centers will be required to attempt to complete the cMRI.
DXA	X	See Section 9.1.3.1 and the imaging procedure manual; Baseline DXA should be obtained between Day -42 and Day -14. Adequacy of DXA scan should be confirmed by the imaging vendor prior to randomization. DXA is not required for subjects enrolled at sites in Germany.
NSAA	*X*	*See the Clinical Evaluator Manual. The NSAA assessment must be performed to determine subject eligibility (NSAA ≥15 total score inclusion criterion), videotaped for ATOM review and confirmed to be adequate prior to randomization*
TFTs	X	Includes 4SC, 10 m walk/run, stand from supine At screening visit: 4SC must be ≤ 8 seconds. See CE Manual; A videotape of screening TFTs must be reviewed by ATOM and confirmed adequate prior to randomization; see Bracket Videographer Manual
6MWD	X	A videotape of screening 6MWD must be reviewed by ATOM and confirmed adequate prior to randomization.
PFTs	X	To include FVC, % predicted FVC, FEV_1, MEP, *and* MIP. See PFT Site Manual.
ActiMyo	X	Subjects at *selected* sites *who agree to participate* will wear the ActiMyo *device from screening until Day 1.* See *the* ActiMyo manual.
Adverse Event Reporting		
Monitor for Serious Adverse Events	X	All SAEs must be collected from the date of participant's written consent until 50 days post discontinuation of dosing in the present study. If the participant enters a separate rollover treatment study of RO7239361, the participant's SAEs will be reported in that study.

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

Procedure	D1[a]	D8 ±1 day (Wk 1)	D15 ±1 day (Wk 2)	D29 ±1 day (Wk 4)	Wk 6 ±3 days	Wk 12 ±3 days	Wk 18 ±3 days	Wk 24 ±3 days	Wk 30 ±3 days	Wk 36 ±3 days	Wk 42 ±3 days	Wk 48 or early D/C ±3 days	Notes
Physical Examination (PE)						X		X				X	
Targeted PE	X	X	X	X	X		X		X	X	X		See Section 9.3.1
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 photographs of injection site reactions without personally identifying information be obtained for submission to the sponsor or designee Some subjects with injection site reaction or rash may be asked to participate in additional procedures to determine type of immune reaction. See Section 9.2.8.1.
Weight	X					X		X		X		X	See Study Procedure Manual
Height	X					X		X		X		X	See CE Manual
Ulna Length	X												See CE Manual Only to be measured instead of standing height if height cannot be measured
Hand Circumference	X							X				X	See CE Manual
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	See note in screening procedures.
Con med assessment	X	X	X	X	X	X	X	X	X	X	X	X	Include corticosteroids
Physical therapy and respiratory therapy assessment	X					X		X		X		X	Assess current physical and respiratory therapy regimen
Echocardiogram						X		X		X		X	See ECHO Manual

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

Procedure	D1[a]	D8 ±1 day (Wk 1)	D15 ±1 day (Wk 2)	D29 ±1 day (Wk 4)	Wk 6 ±3 days	Wk 12 ±3 days	Wk 18 ±3 days	Wk 24 ±3 days	Wk 30 ±3 days	Wk 36 ±3 days	Wk 42 ±3 days	Wk 48 or early D/C ±3 days	Notes
Electrocardiogram (ECGs)	X							X				X	ECGs should be recorded after the participant has been supine for at least 5 minutes. See Eli 150c/250c User Guide
Laboratory Assessments	X	X		X		X		X		X		X	Section 9.3.4 Placement of an intravenous lock is allowed for onsite visits when multiple blood draws are required.
Urinalysis	X			X		X		X		X		X	
Blood PK Sampling	X	X	X	X		X		X		X		X	See section 9.4 for the sample collection schedule. Must be collected prior to administration of study drug.
Immunogenicity Sampling	X		X	X		X		X		X		X	Must be collected prior to administration of study drug. In the event of a positive immunogenicity response, additional neutralizing antibody (NAB) testing will be conducted.
Serum Free Myostatin and Myostatin-Drug Complex	X	X	X	X		X		X		X		X	Myostatin-drug complex will not be analyzed at baseline Must be collected prior to administration of study drug.
Serum Biomarkers	X					X		X		X		X	
Additional Research Sample	X							X				X	
Exploratory genotyping sample	X												
TFTs	X					X		X		X		X	See Clinical Evaluator Manual; TFT assessment must be videotaped for ATOM review at D1, Wk 24 and Wk 48 visits

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

Procedure	D1[a]	D8 ±1 day (Wk 1)	D15 ±1 day (Wk 2)	D29 ±1 day (Wk 4)	Wk 6 ±3 days	Wk 12 ±3 days	Wk 18 ±3 days	Wk 24 ±3 days	Wk 30 ±3 days	Wk 36 ±3 days	Wk 42 ±3 days	Wk 48 or early D/C ±3 days	Notes
6MWD	X					X		X		X		X	See Clinical Evaluator Manual; 6MWD assessment must be videotaped for ATOM review at D1, Wk 24 and Wk 48 visits
ActiMyo	X (*Scr* to Day 1)				X (Wk 6 to Wk 12)		*X (Wk 18 to Wk 24)*		X (Wk 30 to Wk 36)		X (Wk 42 to Wk 48)		Subjects at *selected* sites who opt in will wear ActiMyo *device* at home and during visits. See ActiMyo manual.
NSAA	X					X		X		X		X	See Clinical Evaluator Manual; NSAA assessment must be videotaped for ATOM review at D1, Wk 24 and Wk 48 visits
PFTs	X							X				X	To include FVC, % predicted FVC, FEV1, MEP, *and* MIP. See PFT Site Manual
Upper & Lower Extremity Myometry	X							X				X	See Clinical Evaluator Manual; myometry assessment must be videotaped for ATOM review at D1, Wk 24 and Wk 48 visits
Grip & Pinch Strength	X							X				X	See Clinical Evaluator Manual
PUL	X							X				X	See Clinical Evaluator Manual; PUL assessment must be videotaped for ATOM review at D1, Wk 24 and Wk 48 visits
Right Ankle Range of Motion	X							X				X	See Clinical Evaluator Manual
Caregiver video assessment	*X*					*X*		*X*		*X*		*X*	*Subjects at selected sites who opt in will record videos at selected timepoints of the study (as specified), or at any applicable visits after start date. The visit window is +2 weeks. See Section 9.1.6 and the parent/caregiver manual.*

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

Procedure	D1[a]	D8 ±1 day (Wk 1)	D15 ±1 day (Wk 2)	D29 ±1 day (Wk 4)	Wk 6 ±3 days	Wk 12 ±3 days	Wk 18 ±3 days	Wk 24 ±3 days	Wk 30 ±3 days	Wk 36 ±3 days	Wk 42 ±3 days	Wk 48 or early D/C ±3 days	Notes
PODCI	X					X		X		X		X	Completed by Parent/Caregiver (should be the same parent/caregiver at each visit)
HUI-3	X							X				X	Completed by Parent/Caregiver (should be the same parent/caregiver at each visit)
Health Care Resource Utilization	X			X		X	X	X	X	X	X	X	Protocol mandated procedures, test & encounters are excluded
PedsQL Family Impact Module	X					X		X		X		X	Completed by Parent/Caregiver (should be the same parent/caregiver at each visit)
CaGI-C												*X*	*Completed by parent/caregiver (who has been involved in the study from baseline) who opt in at selected sites*
CGI-C												X	Completed by Clinician (e.g., Principal Investigator or other clinician who has interacted with the individual from baseline)
CaGI-C												*X*	*Completed by parent/caregiver (who has been involved in the study from baseline) who opt in at selected sites*
Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL MFS)	*X*					*X*		*X*		*X*		*X*	*Completed by parent/caregiver who opt in at selected sites (should be the same parent/caregiver at each visit)*
Cardiac MRI												X	Visit window +/- 1 week. Conducted at early termination if ≥ 24 weeks from previous cMRI. See Section 9.1.3.2 and the imaging procedure manual. cMRI will be completed at select U.S. sites only. Subjects enrolled at select U.S. centers

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

Procedure	D1[a]	D8 ±1 day (Wk 1)	D15 ±1 day (Wk 2)	D29 ±1 day (Wk 4)	Wk 6 ±3 days	Wk 12 ±3 days	Wk 18 ±3 days	Wk 24 ±3 days	Wk 30 ±3 days	Wk 36 ±3 days	Wk 42 ±3 days	Wk 48 or early D/C ±3 days	Notes
													will be required to attempt to complete the cMRI.
DXA								X				X	Visit window +/- 1 week. Not required at early termination visit. See Section 9.1.3.1 and the DXA Scanning Guide. DXA is not required for subjects enrolled at sites in Germany or for early termination visits.
Monitor for Non-Serious Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	All participants 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). Falls should be assessed at each study visit [b].
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 participant's written consent until 50 days post discontinuation of dosing. Falls should be assessed at each study visit [b].
Randomize	X												
Access IWRS to obtain study drug kit assignment	X	X	X	X	X	X	X	X	X	X	X	X	Access IWRS as needed to obtain sufficient drug. Access IWRS as needed to replace *kits*.

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

Procedure	D1[a]	D8 ±1 day (Wk 1)	D15 ±1 day (Wk 2)	D29 ±1 day (Wk 4)	Wk 6 ±3 days	Wk 12 ±3 days	Wk 18 ±3 days	Wk 24 ±3 days	Wk 30 ±3 days	Wk 36 ±3 days	Wk 42 ±3 days	Wk 48 or early D/C ±3 days	Notes
Weekly Study Drug Administration	X	X	X	→	→	→	→	→	→	→	→	→	RO7239361 (*BMS-986089*) or placebo will be administered every week (every 7 ± 2 days, unless otherwise stated). Parents/caregivers will be trained to administer SC injections. Parents/caregivers can administer the injection starting at Day 22. Parents/caregivers will be dispensed approximately 6 week supply of prefilled syringes (RO7239361 or placebo) for weekly study drug administration to the participant. On site study visit days, participants should be dosed in clinic, after PK, myostatin and immunogenicity sampling.

[a] Day 1 must occur within 42 days of the screening visit. Some Day 1 procedures (ECG, TFTs, 6MWD, NSAA, PFTs, Upper and Lower Extremity Myometry, Grip and Pinch Strength, right ankle range of motion, PUL, PODCI, HUI-3 and PedsQL Family Impact Module) may be completed on Day -1 to accommodate subject's schedule.

[b] *Falls will be reported as an adverse event if worsening occurs (e.g., more frequent falls) compared with pre-treatment.*

Table 2-3: Open-Label Phase Procedural Outline

Procedure	Wk 1[a]	Wk 2 ±1 day	Wk 3 ±1 day	Wk 6 ±3 days	Wk 12 ±3days	Wk 18 ±3days	Wk 24 ±3days	Wk 36 ±3days	Wk 48/ or early D/C ±3 days	*Wk 60* ±5 days	*Wk 72 to Wk 192 ±5 days*	**Notes** **Week 1 of the open-label phase occurs 1 week after the end of the Week 48 double-blind visit** *After Week 24 visits to take place every 12 weeks*
Safety Assessments												
Physical Examination (PE)									X			
Targeted PE	X				X		X	X			*X*	See Section 9.3.1; *to be assessed every 24 weeks after Wk 48 of the OL phase*
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 photographs of injection site reactions without personally identifying information be obtained for submission to the sponsor or designee. Some subjects with injection site reaction or rash may be asked to participate in additional procedures to determine type of immune reaction. See Section 9.2.8.1
Vital Signs	X			X	X	X	X	X	X	*X*	*X*	See note in screening procedures
Con med assessment	X	X	X	X	X	X	X	X	X	*X*	*X*	Include corticosteroid assessment
Physical therapy and respiratory therapy assessment	X				X		X	X	X	*X*	*X*	Assess current physical therapy and respiratory therapy
Weight	X				X		X	X	X	*X*	*X*	See Study Procedure Manual
Height	X				X		X		X	*X*	*X*	See CE Manual
Ulna Length	X										*X*	See CE Manual Only to be measured instead of standing height if height cannot be measured.
Hand Circumference							X		X		*X*	See CE Manual; *to be assessed every 24 weeks after Wk 48 of the OL phase*
Echocardiogram							X		X		*X*	See ECHO Manual; *to be assessed every 24 weeks after Wk 48 of the OL phase*

Table 2-3: Open-Label Phase Procedural Outline

Procedure	Wk 1[a]	Wk 2 ±1 day	Wk 3 ±1 day	Wk 6 ±3 days	Wk 12 ±3days	Wk 18 ±3days	Wk 24 ±3days	Wk 36 ±3days	Wk 48/ or early D/C ±3 days	*Wk 60* ±5 days	*Wk 72 to Wk 192 ±5 days*	**Notes** **Week 1 of the open-label phase occurs 1 week after the end of the Week 48 double-blind visit** *After Week 24 visits to take place every 12 weeks*
Safety Assessments												
Electrocardiogram (ECGs)									X		*X*	ECGs should be recorded after the participant has been supine for at least 5 minutes. See Eli 150c/250c User Guide; *to be assessed every 24 weeks after Wk 48 of OL phase.*
Laboratory Tests	X				X		X	X	X	*X*	*X*	Placement of an intravenous lock is allowed for onsite visits when multiple blood draws are required.
Urinalysis					X		X	X	X	*X*	*X*	
Monitor for Non-Serious Adverse Events	X	X	X	X	X	X	X	X	X	*X*	*X*	See notes above. Falls should be assessed at each study visit [b].
Monitor for Serious Adverse Events	X	X	X	X	X	X	X	X	X	*X*	*X*	See notes above. All SAEs must be collected from the date of participant's written consent until 50 days post discontinuation of dosing in the present study. If the participant enters a separate rollover treatment study of RO7239361, the participant's SAEs will be reported in that study. Falls should be assessed at each study visit [b].
Blood PK Sampling	X	X	X		X		X	X	X			Must be collected prior to administration of study drug. See section 9.4 for the sample collection schedule.
TFTs	X						X		X		*X*	See Clinical Evaluator Manual Functional measures at Open Label Week 1 Visit must be obtained prior to dosing; TFT assessment must be videotaped for ATOM review at Wk 24 and Wk 48 visits. *To be assessed every 24 weeks after Wk 48 of the OL phase.*

Table 2-3: Open-Label Phase Procedural Outline

Procedure	Wk 1[a]	Wk 2 ±1 day	Wk 3 ±1 day	Wk 6 ±3 days	Wk 12 ±3days	Wk 18 ±3days	Wk 24 ±3days	Wk 36 ±3days	Wk 48/ or early D/C ±3 days	*Wk 60* ±5 days	*Wk 72 to Wk 192 ±5 days*	**Notes** **Week 1 of the open-label phase occurs 1 week after the end of the Week 48 double-blind visit** *After Week 24 visits to take place every 12 weeks*
Safety Assessments												
6MWD	X						X		X		X	See Clinical Evaluator Manual Functional measures at *OL* Week 1 visit must be obtained prior to dosing; 6MWD assessment must be videotaped for ATOM review at Wk 24 and Wk 48 visits. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
NSAA	X						X		X		X	See Clinical Evaluator Manual Functional measures at *OL* Week 1 visit must be obtained prior to dosing; NSAA assessment must be videotaped for ATOM review at Wk 24 and Wk 48 visits. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
ActiMyo					*X (Wk 6 to Wk 12)*		*X (Wk 18 to Wk 24)*	*X (Wk 30 to Wk 36)*	*X (Wk 42 to Wk 48)*			*Subjects at selected sites who opt in during the double-blind phase of the study will wear the ActiMyo device at home and during visits of the OL phase. See the ActiMyo manual.*
PUL	X						X		X		X	See Clinical Evaluator Manual Functional measures at Open Label Week 1 Visit must be obtained prior to dosing; PUL assessment must be videotaped for ATOM review at Wk 24 and Wk 48 visits. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
PODCI							X		X			Completed by parent/caregiver (should be the same parent/caregiver at each visit)
CGI-C									*X*			Completed by clinician (e.g., Principal Investigator or other clinician who has interacted with the individual from baseline) *Should be the same clinician at both visits.*

Table 2-3: Open-Label Phase Procedural Outline

Procedure	Wk 1[a]	Wk 2 ±1 day	Wk 3 ±1 day	Wk 6 ±3 days	Wk 12 ±3days	Wk 18 ±3days	Wk 24 ±3days	Wk 36 ±3days	Wk 48/ or early D/C ±3 days	*Wk 60* ±5 days	*Wk 72 to Wk 192 ±5 days*	Notes **Week 1 of the open-label phase occurs 1 week after the end of the Week 48 double-blind visit** *After Week 24 visits to take place every 12 weeks*
Safety Assessments												
Immunogenicity Sampling	X	X	X		X		X		X		*X*	Must be collected prior to administration of study drug. In the event of a positive immunogenicity response, additional NAB testing will be conducted. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
Serum Free Myostatin and Myostatin-Drug Complex	X	X	X		X		X	X	X			Must be collected prior to administration of study drug.
Serum Biomarkers					X		X	X	X			
PFTs	X						X		X		*X*	To include FVC, % predicted FVC, FEV_1, MEP, *and* MIP See Clinical Evaluator Manual Functional measures at *OL* Week 1 visit must be obtained prior to dosing. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
Upper & Lower Extremity Myometry	X						X		X		*X*	See Clinical Evaluator Manual Functional measures at *OL* Week 1 visit must be obtained prior to dosing; myometry assessment must be videotaped for ATOM review at Wk 24 and Wk 48 visits. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
Grip & Pinch Strength	X						X		X			See Clinical Evaluator Manual Functional measures at *OL* Week 1 visit must be obtained prior to dosing.
Right Ankle Range of Motion	X						X		X		*X*	See Clinical Evaluator Manual Functional measures at *OL* Week 1 visit must be obtained prior to dosing. *To be assessed every 24 weeks after Wk 48 of the OL phase.*

Table 2-3: Open-Label Phase Procedural Outline

Procedure	Wk 1[a]	Wk 2 ±1 day	Wk 3 ±1 day	Wk 6 ±3 days	Wk 12 ±3days	Wk 18 ±3days	Wk 24 ±3days	Wk 36 ±3days	Wk 48/ or early D/C ±3 days	*Wk 60* ±5 days	*Wk 72 to Wk 192 ±5 days*	**Notes** **Week 1 of the open-label phase occurs 1 week after the end of the Week 48 double-blind visit** *After Week 24 visits to take place every 12 weeks*
Safety Assessments												
Caregiver video assessment							*X*		*X*			*Subjects at selected sites who opt in to participate in the caregiver video assessment during the double-blind phase will record videos at the specified timepoints. Visit window: + 2 weeks. See Section 9.1.6 and the Parent/Caregiver manual.*
CaGI-C									*X*			*Completed by parent/caregiver (who has been involved in the study from baseline) who opt in at selected sites.* Should be the same parent/caregiver at each visit.
HUI-3							X		X			Completed by parent/caregiver (should be the same parent/caregiver at each visit)
Health Care Resource Utilization	X						X		X			Protocol mandated procedures, tests, and encounters are to be excluded.
PedsQL Family Impact Module							X		X			Completed by parent/caregiver (should be the same parent/caregiver at each visit)
Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL MFS)							*X*		*X*			*Completed by parent/caregiver who opt in at selected sites (should be the same parent/caregiver at each visit).*
Cardiac MRI (At *selected* sites)									X		X*	Visit window +/- 1 week. Conducted at early termination if ≥ 24 weeks from previous cMRI. See the imaging procedure manual. **To be assessed every 48 weeks during the OL phase.*

Table 2-3: Open-Label Phase Procedural Outline

Procedure	Wk 1[a]	Wk 2 ±1 day	Wk 3 ±1 day	Wk 6 ±3 days	Wk 12 ±3days	Wk 18 ±3days	Wk 24 ±3days	Wk 36 ±3days	Wk 48/ or early D/C ±3 days	*Wk 60* ±5 days	*Wk 72 to Wk 192 ±5 days*	**Notes** **Week 1 of the open-label phase occurs 1 week after the end of the Week 48 double-blind visit** *After Week 24 visits to take place every 12 weeks*
Safety Assessments												
DXA							X		X		X	Visit window +/- 1 week. Not required at early termination visit. See Section 9.1.3.1 and the imaging procedure manual. DXA is not required for subjects enrolled at sites in Germany. *To be assessed every 24 weeks after Wk 48 of the OL phase.*
Randomize	X											Participants originally randomized to active drug will continue to receive the dose to which they were originally randomized. Participants originally randomized to placebo will be randomized to the high or low dose of RO7239361 on Day 1 of the OL phase.
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 can start administering at home starting at OL Week 4. Study drug, RO7239361, in pre-filled syringes for weekly study drug administration, will be dispensed as needed. Parents/caregivers will be dispensed approximately 12-week supply of prefilled syringes RO7239361 for weekly study drug administration to the participant. On site study visit days, participants should be dosed in clinic, after PK, immunogenicity and myostatin sampling.
Access IWRS to obtain study drug *kit* assignment	X	X	X	X	X	X	X	X	X	X	X	Supplied by Roche in pre-filled syringes

Table 2-3: Open-Label Phase Procedural Outline

[a] Week 1 of the *open-label (OL) phase* occurs one week after the end of the Week 48 double-blind visit. *After Week 24 of the OL phase, visits will take place every 12 weeks, with some assessments performed every 12, 24, or every 48 weeks as specified on the schedule of activities. The OL phase will last up to 192 weeks or until RO7239361 is commercially available in the subject's country, per local regulations, or the study is terminated by the Sponsor, whichever occurs first. Please refer to Section 7.8 for details regarding treatment after the end of study.*

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, echocardiogram)

2) Safety (clinical labs)

3) Pharmacokinetic Sampling

4) Biomarker Sampling

[b] *Falls will be reported only as an adverse event if worsening (e.g., more frequent falls) compared with pre-treatment.*

Table 2-4: 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
NSAA	X	X	X	For participants who discontinue study *drug* before Week 48 of the double-blind Phase, a follow-up visit will be schedule to coincide with the date at which the Week 48 visit of the double-blind phase would have occurred, when possible.
Adverse Event Reporting				
Monitor injection site	X	X	X	Report findings as AEs, as appropriate. It is strongly recommended that photographs of injection site reactions without personally identifying information be obtained for submission to the sponsor or designee. Some subjects with injection site reaction or rash may be asked to participate in additional procedures to determine type of immune reaction. See Section 9.2.8.1
Monitor for previously reported or new onset of non-serious adverse events	X	X	X	All non-serious adverse events must be collected until 50 days post discontinuation of dosing in the present study. If the participant enters a separate rollover treatment study of RO7239361, the participant's non-serious adverse events will be reported in that study
Monitor for previously reported or new onset of serious adverse events (SAEs)	X	X	X	All SAEs must be collected from the date of participant's written consent until 50 days post discontinuation of dosing in the present study. If the participant enters a separate rollover treatment study of RO7239361, the participant's SAEs will be reported in that study.
Safety Assessments				
ECG			X	Only required for participants who do not participate in a separate rollover study and who discontinue study drug due to cardiovascular concerns.
Echocardiogram			X	Only required for participants who do not participate in a separate rollover study and who discontinued study drug due to cardiovascular concerns.

Table 2-4: 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
Laboratory Assessments	X	X	X	Only required for participants who do not participate in a separate rollover study and who discontinued study drug due to an abnormal laboratory result. Lab *result*s should be followed until the participant's lab values return to baseline or are considered stable by the investigator.
Immunogenicity	X	X	X	Required for all patients who do not participate in a separate rollover study. In the event of a positive immunogenicity response, additional NAB testing will be conducted.

3 INTRODUCTION

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 The clinical manifestation of this molecular lesion is progressive weakness and limitation in motor capacity, with proximal muscles affected initially followed by distal muscles. Cardiac and respiratory failure typically begin to develop in adolescence and lead to death in the second or third decade of life. 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) received a conditional marketing authorization in the European Union with a restricted indication for the treatment of DMD specifically resulting from a nonsense mutation in the *DYSTROPHIN* gene. Exondys51 (eteplirsen) *received accelerated approval* by the U.S. FDA for treatment of DMD resulting from mutations amenable to skipping exon 51. As both Translarna and Exondys51 target restricted populations with specific mutations, *the* unmet need for safe and effective treatments in DMD remains significant.

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, the most commonly used mouse model of DMD, which carries a premature stop codon in exon 23 of *DYSTROPHIN*, resulted in attenuation of the dystrophic phenotype relative to mdx mice with intact *Myostatin* genes.[3,4] Similarly, weekly administration of anti-myostatin monoclonal antibodies to *mdx* mice resulted in increases in muscle size and strength.[5]

RO7239361 *(previously, BMS-986089)* is a bivalent human *anti*-myostatin 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 weekly, subcutaneously administered therapeutic to increase muscle mass and strength. RO7239361 has a unique mechanism of action (MOA) that includes the competitive inhibition of Alk4/5 (signaling receptor) recruitment and binding to free myostatin or the 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. [54, 55] RO7239361 is being developed as a potential therapy to improve muscle function in DMD. As most ambulatory DMD patients are treated with corticosteroids (CS), in this study of ambulatory patients with DMD, RO7239361 will be given to subjects on stable *CS* therapy.

3.1 Study Rationale

Study WN40227 is a multi-center, randomized, double-blind, placebo-controlled study to assess the efficacy, safety, and tolerability of two different weekly subcutaneous (SC) doses of RO7239361 in ambulatory boys aged ≥ 6 to < 12 years with DMD.

DMD is caused by mutations within the *DYSTROPHIN* gene that result in loss of the mRNA reading frame, severely limiting dystrophin protein production.[6] The *DYSTROPHIN* gene lies on the X chromosome and thus DMD is primarily expressed in boys. The disease manifests as progressive weakness of skeletal, respiratory, and cardiac muscles due to the absence of dystrophin in the subsarcolemmal cytoskeleton.[7] Progressive weakness and contractures lead to loss of ambulation by age 13 and death from cardiorespiratory failure by the late 20s to early 30s.[8] In the absence of supportive treatment, patients rarely survive beyond their teens.[9] Advances in management, including CS use, physical therapy for contractures, ventilatory support, spinal surgery, and cardiac management have increased the life expectancy for patients with DMD.[9,10,11] With these advances, aggressively managed patients may ambulate beyond 14 years of age and survive into their early 30s.[9,10]

Although advances in the management of DMD have improved survival, chronic CS treatment is associated with adverse side effects that contribute to morbidity in DMD.[8,11] While Translarna (ataluren) has received a conditional marketing authorization in the EU for the treatment of DMD resulting from a nonsense mutation in the *DYSTROPHIN gene*,[12] this treatment is restricted to the *15*% of patients with DMD with *this type of mutation* [12,13] Similarly, Exondys51 (eteplirsen) has received U.S. FDA approval for treatment of DMD resulting from mutations amenable to exon 51 skipping, roughly only 13% of the DMD population. Thus, there remains a significant unmet medical need for novel agents to treat DMD.

Data from prior and ongoing clinical studies suggest that RO7239361 is generally safe and well tolerated and has expected effects on pharmacodynamic endpoints.

A first-in-human (FIH) assessment of RO7239361 (*Study CN001001*), a combined single-ascending dose (SAD)/multiple-ascending dose (MAD) study to investigate the safety, tolerability, pharmacokinetics, pharmacodynamics, and immunogenicity in healthy adult subjects, *showed that single and multiple doses (of up to 180 mg weekly) of RO7239361 were generally safe and well tolerated. The most common adverse events (AEs) considered by the investigator to be related to study drug were mild or moderate injection site reactions, and that, in most cases, did not require treatment. There were no acute hypersensitivity reactions during this study. The presence of anti-drug antibodies (ADAs) did not appear to affect the safety or exposure of RO7239361 in healthy subjects. Administration of both single and multiple doses of RO7239361 produced dose-dependent reductions in free serum myostatin and increases in myostatin-drug complex consistent with target engagement. Findings supporting the expected pharmacodynamic (PD) effects of RO7239361 include increases in whole right thigh muscle volume (measured by magnetic resonance imaging) and increases in the change from baseline in the percentage of lean body mass (as measured by dual X-ray absorptiometry [DXA]).*

Study WN40226 is a Phase 1b/2, multi-center, randomized, placebo-controlled, double-blind, multiple-ascending SC dose study in ambulatory boys ages ≥ 5 to < 11 years with DMD who are receiving CS. This study was designed as a dose-ranging study to characterize the safety, *pharmaockinetics*, target engagement (i.e., myostatin suppression) and the relationship between *pharmaockinetics* and target engagement over at least 3 doses in boys with DMD. *As of 8 February 2018*, *all 43 patients randomized had completed the 24-week double-blind phase of the study. In all, 42 patients completed the 48-week OL phase and 41 entered into the OL extension phase. Two patients withdrew from the study for non-safety reasons. The study met its primary objective of showing safety and tolerability of RO7239361. The most common AEs that were considered by the investigators to be related to study drug were mild to moderate injection-site reactions that resolved without change to study treatment; there were no acute hypersensitivity reactions associated with study drug reported. Immunogenicity data from 43 gs showed a single value of low positive ADA titer in 1 patient who had received RO7239361.*

*Pharmacokinetic (*PK*)* data from Panels 1 to 3 (*which included* all *three* doses tested) show dose-related increases in peak and overall exposure, which appear to be dose proportional. *Furthermore, an effect of body weight on the RO7239361 pharmacokinetics has been such that with increasing body weight, the clearance increases.* Preliminary target engagement data from Panels 1 to 3 show robust dose-related increases in free *serum* myostatin suppression. *As shown by the population PK/PD model, the three different dose levels achieved 77%, 92%, and 97% reduction in serum-free myostatin.*

Further information regarding the *available clinical data* of RO7239361 in *healthy volunteers and* patients with DMD can be found in the Investigator's Brochure (IB).

3.1.1 Rationale to Support Dose Selection

Two doses of RO7239361 *were selected for this* study *based on interim data from Study WN40226*; *doses* that *are* safe and *were predicted to* produce a moderate level of free myostatin suppression (≥ 70% relative to baseline) and a second dose that is safe and *was predicted to* produce a high*er* level of free myostatin suppression (≥ 90% relative to baseline).

Whereas statistically significant increases in muscle volume were observed at > 80% suppression of free myostatin levels relative to baseline in a dose ranging study in cynomolgus monkeys, numerical but non statistically significant increases were observed at approximately 54% suppression relative to baseline following administration of RO7239361 in that study. Similarly, in Study CN001001, the multiple dose study in normal healthy subjects, statistically significant increases in muscle volume were observed for RO7239361 versus placebo at doses that sustained free *serum* myostatin suppression above 68% at the time of MRI assessment (Day 57). In that same study, no significant increases in muscle volume were observed at doses or regimens that generated a suppression of approximately 42% or lower on Day 57. The increase in muscle volume observed in monkeys and humans following RO7239361 administration is expected to correlate with an increase in thigh muscle to non-contractile tissue fraction in children with DMD.

Given that increase in muscle mass and strength mediated by RO7239361 induced suppression of free myostatin is the underlying mechanism that is expected to ultimately drive the efficacy of RO7239361 in patients with DMD, dose selection for Study WN40227 is based on target engagement, i.e., free myostatin suppression.

All available PK and free myostatin data following administration of 4 mg QW and 12.5 mg QW doses from Study WN40226 were analyzed in an interim analysis (Interim Analysis 1). Data were modeled using a *population PK/PD model based on the concept of* target mediated drug disposition (TMDD). Using the estimates of the *PK/PD* model parameters and the associated variabilities, the PK and free myostatin suppression profile following various doses in an ambulatory DMD population were obtained. The population of subjects in the simulations reflected the age (6 to 12 years) and weight (≥15 kg) of ambulatory DMD subjects *in* Study WN40227. Simulations were performed for 1000 subjects in each 5 kg weight bin. Based on the results of the simulations, a fixed dose by weight tier dosing strategy was chosen. Accordingly, doses for subjects will be based on their respective weight tier, namely Weight Tier 1: ≥ 15 to ≤ 40 kg, or Weight Tier 2: > 40 kg. This dosing strategy *was intended to* provide similarity in levels of exposure and target suppression for subjects within a given weight tier targeting the achievement of either ≥ 70% (Dose Level 1) or ≥ 90% (Dose Level 2) suppression of free myostatin at trough.

Doses selected for Study WN40227 are as follows:

Table 3.1.1-1: Dose Level 1, targeting moderate level of suppression: (≥ 70% suppression at trough)

Dose Level	Body Weight tier	Body Weight range	Dose
1	1	≥ 15 to ≤ 40 kg	7.5 mg QW
1	2	> 40 kg	15.0 mg QW

Table 3.1.1-2: Dose Level 2, targeting high level of suppression: (≥ 90% suppression at trough)

Dose Level	Body Weight tier	Body Weight range	Dose
2	1	≥ 15 to ≤ 40 kg	35 mg QW
2	2	> 40 kg	50 mg QW

The dose may be adjusted when a subject's weight exceeds or drops below the dosing weight tier to which he was originally assigned by > 2 kg, confirmed by repeat.

The population PK/PD model was further developed utilizing more observations, including the top dose data from Study WN40226 (see the IB). This model predicts similar reductions of free serum myostatin of over 90% for both doses tested. In healthy subjects, the pre-filled syringe (PFS) achieved approximately 1.6-fold higher exposure than the vial formulation. If this would also be the case in patients with DMD, the exposure achieved in this study with the PFS at the top dose

would still be lower than the exposure achieved with 180 mg in healthy subjects. The drug was well tolerated up to doses of 180 mg QW in healthy volunteers. Although the doses may give higher serum myostatin reduction as initially expected, tissue level myostatin reduction is unknown. There may be meaningful differences in tissue effects, and therefore the continued use of the selected doses is justified.

3.1.2 Rationale for Specific Inclusion/Exclusion Criteria

Age Range:

A minimum age of inclusion of 6 years is planned, as children younger than 6 years are less likely to consistently understand and be able to cooperate with performing the functional endpoints, such as *the NSAA*, reliably. An upper age limit for inclusion of < 12 years is planned to enable a focus on children who are able to perform the *functional endpoints* 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 on ambulatory DMD patients will be before lower extremity muscle fibers have been completely 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.[16,17,18] Key approaches to minimizing this variability include restricting the age range for inclusion, thereby reducing variability stemming from growth.

NSAA Minimum Score:

It was recently reported [26] that in ambulant boys, a baseline total linearized NSAA score of 50 points (□16 of the raw scale) implies that the boys are very unlikely to lose ambulation over the course of a 2-year study. It has also been observed on the basis of emerging data from Study WN40226 in ambulant boys with DMD aged 5-10 years at randomization that there was an increased rate of decline in function and increased rate of loss of ambulation for subjects with at a total score below 15 points. A minimum NSAA score is planned to limit the participation to patients who are not expected to lose ambulation during the study period and who will be able to continue to participate in key functional assessments. The specific cutoff score of 15 points at screening will allow a focus on the inclusion of patients who are able to participate in key study assessments throughout the study, without being too stringent. It is intended to minimize variability of the functional assessments and expected disease progression, given that the patients are very unlikely to lose ambulation over the course of 2 years.

Use of Corticosteroids:

As *CS* have demonstrated benefit on motor function in boys with DMD[19], boys included in this study will be required to be on a *CS* (e.g., prednisone/prednisolone or deflazacort) for at least 6 months prior to the start of study treatment, with no significant change in dosage or dosing regimen for at least *12 weeks* prior to the start of study treatment. The goal of this inclusion criterion is to control variability in motor function and strength stemming from *CS* use.

Both prednisone/prednisolone and deflazacort have been shown to be effective in increasing and preserving muscle strength in DMD[19,20] supporting this pharmacotherapy as an important standard of care. Following initiation of *CS*, 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 patients who have been taking *CS* for at least 6 months, plan to continue *CS* for the duration of the double-blind phase of the clinical trial, and have not had a significant change in dose in the *12 weeks* preceding initiation of study treatment.

Use of Translarna (Ataluren), Exon-Skipping Therapies, or Investigational Agents:

Subjects receiving Translarna (ataluren), exon skipping therapies (e.g., Exondys51 [eteplirsen]), or investigational agents will not be eligible for this study. The magnitude and time course of treatment response to these agents in DMD is not well understood. Similarly, limited data are available regarding the safety and tolerability of investigational agents. These information gaps render controlling for potential effects of these drugs 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, patients receiving this treatment currently or within *12 weeks* prior to the start of study treatment will be excluded from participation. Patients receiving treatment with any (approved or investigational) exon skipping therapies within 6 months prior to the start of study treatment will also be excluded from participation. *Treatment with deflazacort as part of CS dose-finding trials is allowed, as long as the patient has been on treatment for 6 months without significant dose changes 12 weeks prior to study start.*

Heart Failure:

RO7239361 also binds to growth and differentiation factor-11 (GDF-11), which has been implicated in heart failure associated with aging in mice.[41] However, this finding is controversial.[42] [43] [44] Although the mechanism underlying the development of aging-related re-modeling of the heart likely differs from that underlying the development of heart failure in DMD, potential cardiac effects of RO7239361 will be monitored in subjects enrolled in this study using echocardiography. Notably, there have been no cardiac findings in animals that received RO7239361 in completed or ongoing toxicology studies. In order to clearly differentiate any potential effects of RO7239361 on heart function from cardiac manifestations of disease progression, it is prudent to exclude patients with uncontrolled cardiac failure. It is requested that potential participants with ejection fraction <55% at baseline be discussed with the Sponsor's Medical Monitor.

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.[21,22] To avoid potential confounding effects of growth hormone and androgens on the endpoints being measured in this study, patients receiving these treatments currently or within 12 weeks or 5 half-lives (whichever is longer) prior to the start of study treatment will be excluded from participation.

3.1.3 Rationale for Primary Endpoint

The primary endpoint for this study is the change from baseline in the NSAA total score at Week 48 in RO7239361-treated participants compared with participants treated with placebo. The NSAA is a validated clinician-reported outcome instrument that has been widely used in clinical trials. The instrument consists of 17 items designed to measure ambulatory function in boys with DMD. The instrument has a 3-point scale: 2="normal"—no obvious modification of activity, 1=modified method but achieves goal independent of physical assistance from another, 0=unable to achieve independently. Therefore, higher scores represent greater functional abilities.[47]

The NSAA has been demonstrated to have good inter-rater reliability and test–retest reliability.[56] *The NSAA correlates with key disease progression milestones, such as losing the ability to rise independently from the floor (a decline in linearized score from 50 to 40 points), loss of ability to stand still (decline in linearized score from 21 to 11 points), and loss of ambulation.*[25,26] *It has been reported that boys with DMD improve their motor function up to 7 years of age, as seen by an overall gain of 4 points/year in the linearized NSAA score, after which they start to decline an average of 8 linearized units/year.*[25,26] *However, because age alone cannot serve as a predictor of loss of ambulation, it has been reported that the estimated mean linearized NSAA score at 12 and 24 months prior to loss of ambulation (i.e., at ages 12 and 11 years, respectively) is 34 and 42 units (approximately 9 and 13 on the raw scale, respectively).*[26] *In addition, the NSAA has been demonstrated to have moderate to high construct validity based on the 6MWD,* [57, 58] *and it has been shown to detect change in an interventional setting.*[25] *Unlike other functional tests, such as the 6MWD or the 4SC, NSAA is a more reliable and comprehensive scale that captures both motor strength and quality of movement, in addition to being less susceptible to external motivations. Moreover, while TFTs in DMD tend to remain stable until a point when they decline rapidly, the NSAA has been shown to decline more gradually. Thus, the NSAA is a reliable outcome measure to assess the efficacy of RO7239361 treatment in the study population.*

3.1.4 Research Hypothesis

The hypothesis for this study is that in ambulant patients with DMD:

- 48 weeks of treatment with RO7239361, at either or both doses, will be generally safe and well tolerated and will result in improved *NSAA total score* relative to 48 weeks of placebo treatment.

3.2 Background

A detailed description of the chemistry, pharmacology, efficacy, and safety of RO7239361 is provided in the Investigator Brochure.

RO7239361 is expected to preserve and improve muscle strength primarily by increasing the size of existing muscle fibers. Several investigators have demonstrated that either genetic manipulation[27,28,29] or pharmacologic treatments[23,30,31,32,33] that reduce circulating myostatin produce increases in muscle mass and measures of strength and motor function in the mouse mdx model and the golden retriever model of DMD.[34] Importantly, the relationship between myostatin reduction, increased muscle size, and increased muscle strength suggests that muscle volume

increases induced by this mechanism result in increased muscle function. In contrast, the pseudohypertrophic increases in muscle size observed in DMD reflect increases in non-contractile fat and fibrotic tissue within the muscle, *and* do not increase muscle strength.[35]

RO7239361 has potential therapeutic utility for conditions such as DMD as well as other muscle-wasting diseases.

A first-in-human combined single and multiple ascending dose (SAD/MAD) study in normal healthy volunteers (NHVs) with RO7239361 has been completed (CN001001).[36] Data from this study indicate that single doses up to 180 mg and multiple weekly (Q1W) doses up to 180 mg and every-2-week doses of 45 mg RO7239361 are generally safe and well tolerated. PK data indicate that plasma concentrations of total RO7239361 (serum free RO7239361 plus RO7239361-myostatin complex) increase in a dose-related fashion. Similarly, the extent and duration of serum free myostatin suppression increases in a dose-related fashion. Treatment with 5 weekly doses of 45 mg or more of RO7239361 was associated with increases in thigh muscle volume and total lean body mass in NHVs.

A Phase 1b/2, multi-site, randomized, placebo-controlled multiple ascending SC dose study to evaluate the safety, tolerability and PK of RO7239361 in ambulatory boys with DMD is ongoing. Preliminary safety data from this study, which includes ambulatory boys with DMD, age ≥ 5 to < 11 years on stable CS treatment, suggest that RO7239361 is generally safe and well tolerated in this population.

There are no identified risks to date related to RO7239361 administration. Anti-drug antibodies (ADAs) and mild *or moderate* injection-site *reactions* have been observed in *studies of healthy adults and patients with DMD. The presence of ADAs did not appear to impact the safety or exposure of RO7239361.*

RO7239361 also recognizes growth and differentiation factor-11 (GDF-11), which is hypothesized to play roles in inducing mesoderm in early development and in anterior-posterior patterning of axial skeleton.[37,38,39,40] GDF-11 has also been implicated in heart failure associated with aging *in mice*.[41] However, this finding is controversial.[42] [43] [44] While the mechanism underlying the development of aging related re-modeling of the heart likely differs from that underlying the development of heart failure in DMD, potential cardiac effects of RO7239361 will be monitored in this trial using echocardiography. Notably, there have been no cardiac findings in animals *that* received RO7239361 in the completed or ongoing toxicology studies. *No clinically significant treatment-related changes in ECG or echocardiogram parameters have been observed in RO7239361-treated patients with DMD to date.*

Potential risks include hypersensitivity reactions, *injection-site reactions*, immunogenicity*, and findings from nonclinical studies or observations with the use of other anti-myostatin agents*. These potential risks are described in the current Investigator Brochure.[45] Appropriate clinical monitoring will be implemented in all clinical studies. *An independent* Data Monitoring Committee (*i*DMC) will review data at intervals specified in the *i*DMC charter.

3.3 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably anticipated AEs of RO7239361 may be found in the Investigator Brochure.

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

RO7239361 has been tested in animals including rats and monkeys. Serum aminotransferase elevations were observed in rats given RO7239361 for 6 months. However, these elevations were not associated with microscopic evidence of liver or skeletal muscle damage and the animals did not experience muscle weakness. In monkeys that received RO7239361 for 6 months there were no serum transaminase elevations even though systemic exposures were approximately 4× higher than in rats.

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 and bilirubin in serum and evaluations of clinical signs and symptoms. The hepatocyte-specific markers GGT and GLDH will also be monitored.

In some animals treated with RO7239361 a small decrease in the IgG antibody response to primary immunization was observed. IgM antibody responses to primary immunization were not decreased in treated animals. Neither IgG nor IgM antibody responses were decreased in response to subsequent (booster) immunizations in those animals tested. Given the small size 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, any 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.

Among healthy adult humans who received RO7239361 in study CN001001, the most common *AEs* were mild *or moderate* injection-site erythema. These AEs did not require cessation of study drug and were not accompanied by systemic signs or symptoms, such as fever or eosinophilia. In Study CN001001, 45.2% of SAD phase participants developed ADAs at some point during the study, while 27.8% of MAD phase participants developed ADAs. Presence of ADAs did not appear to impact exposure to RO7239361. 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.

A Phase 1b/2 study of RO7239361 in ambulatory boys with DMD (WN40226) is ongoing. *Safety* data from *the completed double-blind and 48-week OL phases* of this study suggest that RO7239361 is generally safe and well tolerated in this population. *The most common related AEs were mild or moderate injection-site reactions that resolved without change to study treatment. Immunogenicity data from 43 patients showed a single value of low positive ADA titer in 1 patient who had received RO7239361.*

All participants who are administered study drug will be closely monitored for possible immunogenicity-related AEs, such as rash, fever, and injection-site reactions. Clinically relevant immune reactions (e.g., hypersensitivity reactions) will be reported as AEs and treated according to

current standard of care medical practice. If present, antibodies will be assessed for neutralizing activity by assessment of serum free myostatin levels and a neutralizing antibody (NAB) assay. Subjects completing clinical trials of RO7239361 who do not enter the Open Label Extension phase will enter the follow-up phase of the study. Subjects who enter the follow-up phase will undergo ADA monitoring during the follow-up visits, which will occur at regular intervals for 24 weeks. Injection sites will be rotated and inspected regularly. Injection sites will be monitored from Day 1 through the end of the study.

Hypersensitivity or acute allergic reactions may occur as a result of the biologic nature of RO7239361. As of *8 February 2018*, no subject receiving RO7239361 has developed an acute hypersensitivity reaction *associated with study drug*. Although the risk of a subject developing an acute hypersensitivity reaction to RO7239361 is considered low, sites should be prepared to manage possible acute hypersensitivity reactions to initial doses of RO7239361 following accepted standards of medical care. Qualified personnel should administer or supervise the administration by the caregiver of the first 3 weekly SC doses of RO7239361, and appropriate emergency equipment should be available in the event of a serious anaphylactic reaction. Participants and their parents/caregivers will be provided with emergency contact information by the site. Participants and their 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.

4 OBJECTIVES AND ENDPOINTS

Table 4-1: Objectives and Endpoints

Objectives	Endpoints
Primary • To compare the efficacy of RO7239361 to placebo in ambulatory boys with Duchenne muscular dystrophy	• The change from baseline in the *North Star Ambulatory Assessment (NSAA) total score* at Week 48 in RO7239361-treated participants compared to placebo-treated participants
Secondary • To compare the efficacy of RO7239361 to placebo using the following tests: – *4 Stair Climb Velocity* – Stand from supine velocity – 10 M walk/run velocity – PODCI transfers and basic mobility subscale – Proximal lower extremity flexor (knee extension and knee flexion) strength, measured using manual myometry – 6 Minute Walk Distance (6MWD) – *Clinical Global Impression of Change (CGI-C)* – *Stride velocity, as recorded by the ActiMyo device*	• Change from baseline at Week 48 in RO7239361-treated participants compared to placebo-treated participants in: – *4 Stair Climb Velocity* – Stand from supine velocity – 10 M walk/run velocity – PODCI transfers and basic mobility subscale – Proximal lower extremity flexor (knee extension and knee flexion) strength, measured using manual myometry – 6 Minute Walk Distance (6MWD) – Clinical Global Impression of Change (CGI-C) – *95th percentile stride velocity, as recorded with the ActiMyo device in a subset of the overall study population*
• To assess the safety and tolerability of RO7239361 in boys with DMD as reflected by new or worsening lab abnormalities (as defined by CTCAE criteria), serious adverse events (SAEs) and adverse events (AEs) leading to discontinuation	• Tabulations of the numbers of unique participants with new or worsening laboratory abnormalities, SAEs and AEs leading to discontinuation, in RO7239361 arms compared to the placebo arm

Tertiary/Exploratory • To assess the safety and tolerability of RO7239361 in boys with DMD as reflected by vital signs, measures of cardiac function.	• Summaries and listings of vital signs, ECG parameters, echocardiogram parameters. – Proportion of participants with on treatment adverse events in RO7239361 arms compared to the placebo arm – Proportion of participants with on treatment decrease in ejection fraction (EF), measured using echocardiography, in RO7239361 arms compared to the placebo arm
• To compare the efficacy of RO7239361 to placebo using the following tests: - Performance of upper limb (PUL) total score	• Change from baseline at Week 48 in RO7239361 treated participants relative to placebo treated participants in: – Performance of upper limb (PUL) total score
• *To evaluate other measures of efficacy, quality of life, and health care utilization*	• Change from baseline at Week 48 in RO7239361 treated participants compared to placebo treated participants: – Pulmonary Function Tests, including forced vital capacity (FVC) % predicted, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), maximal expiratory pressure (MEP), maximal inspiratory pressure (MIP). – PODCI total score – *Caregiver Global Impression of Change (CaGI-C) in a subset of the overall study population at selected sites* – *Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL MFS) in a subset of the overall study population* – Cardiac magnetic resonance imaging (cMRI) measures of ejection fraction, fractional strain, and volume of fibrosis in a subset of the overall study population – Strength of elbow flexors, elbow extensors, and pinch, measured by manual myometry – Health Utilities Index III and PedsQL Family Impact Module scores – Dual x-ray absorptiometry (DXA) measures of lean body mass, fat mass, and bone mineral density

Table 4-1: Objectives and Endpoints

Objectives	Endpoints
	• *Assessments at selected timepoints during the study of:* – *Activity levels in a subset of the overall study population, as recorded with ActiMyo* – *Scoring of functional assessments, as recorded by a subset of parents/caregivers participating in the caregiver video assessment at selected sites* – *Number of falls while on treatment in RO7239361 arms compared with the placebo arm*
• To evaluate *RO7239361 pharmacokinetics, pharmacodynamics*, and immunogenicity	• Assessments at select timepoints during the study of: – Serum RO7239361 concentrations – Serum free myostatin and myostatin drug complex concentrations – Serum ADA concentrations – *Serum creatine kinase level in muscle*

Table 4-1: Objectives and Endpoints

Objectives	Endpoints
• To explore the effect of genetic variation on target engagement, pharmacodynamic (PD) effects or functional test endpoints	• Assess the impact on genetic variation in the *DYSTROPHIN* gene or in genes suspected to impact DMD disease expression (including but not limited to LTBP4, SPP1) in RO7239361- and placebo-treated participants, on target engagement, pharmacodynamic (PD) and functional endpoints

5 STUDY DESIGN

5.1 Overall Design

This is a multi-center, randomized, double-blind, placebo-controlled study to assess the efficacy, safety and tolerability of two different weekly SC doses of RO7239361 in ambulatory boys with Duchenne muscular dystrophy (DMD).

Eligible participants will be randomized to receive doses of RO7239361 or placebo (1:1:1 across the three treatment arms). The randomization will be stratified by:

- Age (6 through 7 and 8 through 11)
- CS regimen: daily (prednisone, prednisolone or deflazacort) vs intermittent (any non-daily dosing of prednisone, prednisolone or deflazacort)

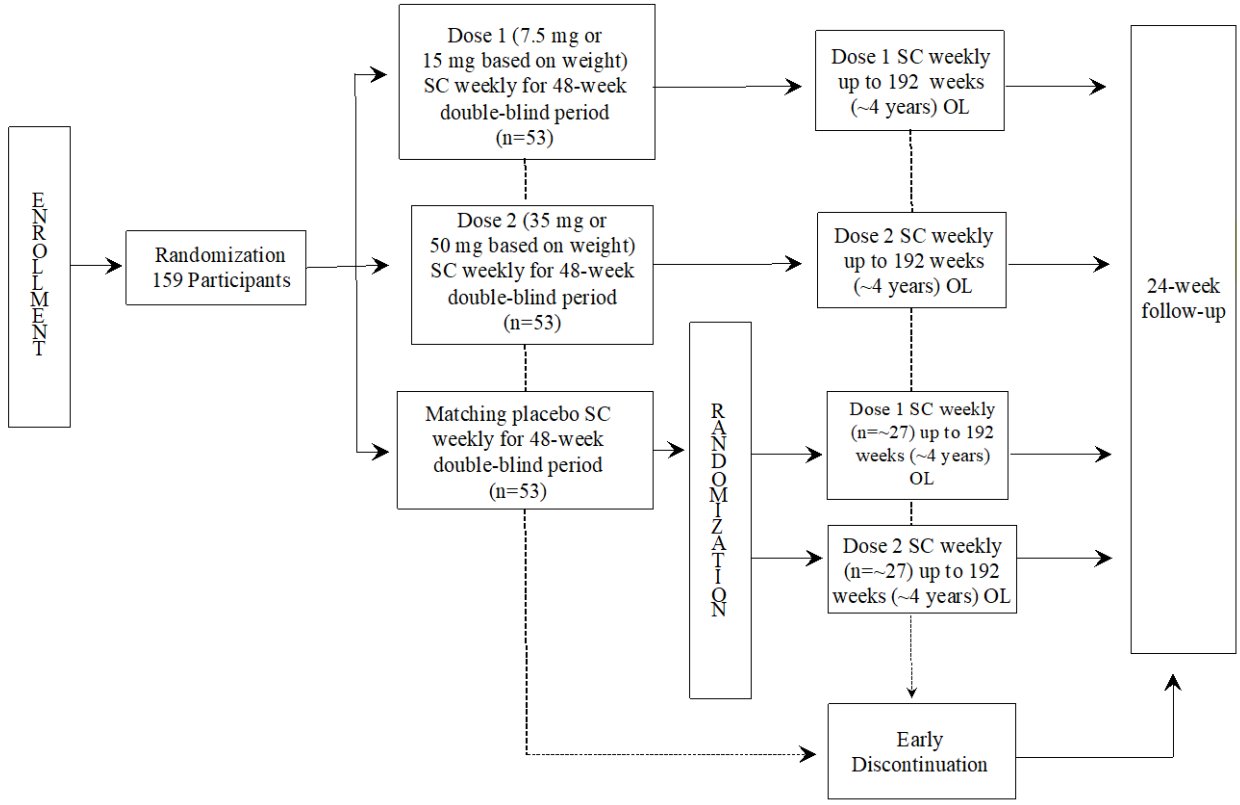
A separate randomization will be conducted for Japan (N~15) with stratification for age only (6 through 7 and 8 through 11), not CS regimen.

RO7239361 or placebo will be administered weekly. The first three doses (Days 1, 8 and 15) of study *drug* must be administered at the site. Parents/caregivers will be trained to administer SC injections during the first three dose administrations. Parents/caregivers who are comfortable with SC administration can administer the injection at home starting at Day 22. Parents/caregivers will be dispensed an approximately 6 week supply of drug in pre-filled syringes (RO7239361 or matching placebo) for weekly study drug/placebo administration to the participant.

After completion of this 48-week double-blind phase, participants may enter the *OL* phase in which all participants will receive active RO7239361 study drug. Participants originally randomized to active drug will continue to receive the dose to which they were originally randomized. Participants originally randomized to placebo will be randomized to the high or low dose of RO7239361 on Day 1 of the *OL* phase. Dose assignment in the *OL* phase may be modified based upon emerging safety findings. Subjects entering the *OL* phase will remain blinded to their original dose assignment. *Participants* who discontinue study *treatment at any timepoint* (i.e., early *discontinuation for patients* who do not enter the *OL* phase *or at termination of the OL phase)* will enter the follow-up phase of the study. The follow-up phase of the study is 24 weeks. Participants will be monitored for safety at three visits in the follow-up phase. Participants should provide immunogenicity samples at all follow-up visits.

The study design schematic is presented in Figure 5.1-1.

Figure 5.1-1: Study Design Schematic



OL=open label; SC=subcutaneous.

Study visits and endpoint measurements will occur as indicated in Table 2-1 through Table 2-4.

5.1.1 Independent Data Monitoring Committee and Other External Committees

There will be an *i*DMC for this study. The *i*DMC scope, frequency of review, membership, activities and other specifications are detailed in the *i*DMC charter.

The *i*DMC shall have access to partially unblinded data (coded treatment assignments) including but not limited to the following: the frequency and spectrum of SAEs; Grades 3 to 4 laboratory abnormalities; occurrence of malignancies, if any; and other select AEs of interest. The *i*DMC shall also review aggregate data results from *any interim analyses performed prior to the end of the double-blind treatment period*. The *i*DMC shall have the right to request full unblinding of the data (codes will be decoded to reveal actual treatment assignments). The *i*DMC shall act as an advisor to the sponsor and have responsibility for safeguarding the subjects' interests. The *i*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.

5.2 Number of Participants

A total of approximately n=159 participants randomized will provide for slightly more than 80% power at the primary analysis. This allows for roughly a 5% dropout rate by Week 48.

Some of the details behind sample size calculations are in Section 10.1.

5.3 End of Study Definition

The start of the trial is defined as the first visit for the first participant screened. End of trial is defined as the last visit or scheduled procedure shown in the Schedule of Activities for the last participant.

5.4 Scientific Rationale for Study Design

See Section 3.1

5.5 Justification for Dose

See Section 3.1.1

6 STUDY POPULATION

For entry into the study, the following criteria MUST be met.

6.1 Inclusion Criteria

1) Signed Written Informed Consent

a) Prior to study participation, written informed consent from subjects, *and* in the case of minors, written permission (informed consent) *also* 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.

2) Type of Participant and Target Disease Characteristics

a) Males, ≥ 6 to < 12 years of age at time of randomization

b) Diagnosis of *DMD*, confirmed by medical history (e.g., onset of clinical signs or symptoms before 5 years of age together with an elevated serum creatine kinase level observed before or after initial diagnosis) and by genotyping

c) Participants ≥ 15 kg

d) Ambulatory without assistance

e) Participants 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 prednisone or > 0.24 mg/kg deflazacort) or dosing regimen for at least 12 weeks 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.

f) NSAA score ≥15 points at screening

g) 4SC ≤ 8 seconds at screening

h) Subjects must agree to avoid major changes in their physical or respiratory therapy regimen during the double-blind phase, to the extent possible

3) Age and Reproductive Status

a) Males, ≥ 6 to < 12 years on day of randomization

b) *Subjects* who are sexually active must agree to follow instructions for method(s) of contraception for the duration of treatment with study treatment(s) plus 5 half-lives of the study treatment [RO7239361; 50 days] plus 90 days (duration of sperm turnover) for a total of 140 days (5 months) post-treatment completion.

c) Azoospermic *subjects* are exempt from contraceptive requirements.

d) *Subjects* must be willing to refrain from sperm donation during the entire study and for 5 half-lives of study treatment plus 90 days (duration of sperm turnover) 140 days after dosing has been completed.

Investigators shall provide appropriate and patient-centered counseling of adolescent trial subjects who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy. Investigators shall advise *subjects* who are sexually active with WOCBP on the use of highly effective methods of contraception in non-pregnant partner (Appendix 4), and *on the* use of condom in subjects with a pregnant partner. Highly effective methods of contraception have a failure rate of < 1% when used consistently and correctly.

6.2 Exclusion Criteria

1) Medical Conditions

a) Participants with cognitive impairment or behavioral issues that, in the judgement of the investigator, will compromise their ability to comply with study procedures.

b) Participants with a FVC of < 50% of predicted value (in participants able to produce a valid FVC, as judged by the clinical evaluator or respiratory therapist)

c) Cutaneous AEs sustained during participation in a prior clinical trial that resolved less than *12 weeks* prior to the start of study drug administration.

d) History of lower limb fracture within 12 weeks prior to the start of study drug administration.

e) History of upper limb fracture within *8* weeks prior to the start of study drug administration.

f) Any injury *that* may impact functional testing. Previous injuries must be fully healed prior to consenting.

g) Expectation of major surgical procedure, such as scoliosis surgery, during the double-blind phase of this study.

h) Requirement of daytime ventilator assistance

i) Initiation of nighttime ventilation less than 4 weeks prior to the start of study drug administration

j) Expectation that daytime or nighttime ventilation may be initiated during the double-blind phase of this study.

k) Clinical signs or symptoms of *uncontrolled* congestive heart failure (American College of

Cardiology/American Heart Associated Stage C or Stage D)

l) For participants participating in cMRI substudy: 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 cMRI)

2) Prior/Concomitant Therapy

a) Any change (initiation, change in drug class, dose modification unrelated to change in body weight, interruption or re-initiation) in prophylaxis/treatment for CHF within 12 weeks prior to start of study treatment

b) 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 *12 weeks* prior to start of study treatment

c) Participants on intermittent *CS* regimens with off periods of 20 days or longer (e.g., 10 days on, 20 days off)

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

e) Treatment with ataluren *currently or within 12 weeks prior to the start of study drug administration*

f) *Treatment with* any other investigational drug (excluding deflazacort *in CS dose-finding trials*) currently or within *12 weeks* prior to the start of study drug administration

g) Current or prior treatment within *12 weeks* prior to the start of study drug administration with androgens or human growth hormone.

h) *Concurrent or previous participation at any time in a gene therapy study*

i) Prior treatment with RO7239361 or any other anti-myostatin agent

3) Physical and Laboratory Test Findings

a) 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

4) Allergies and Adverse Drug Reaction

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

b) History of hypersensitivity to components of the study drug (histidine, trehalose, diethylenetriaminepentaacetic acid, polysorbate 80)

5) Other Exclusion Criteria

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

b) Unwilling or unable to administer study drug at home

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

6.3 Lifestyle Restrictions

Not applicable

6.3.1 Meals and Dietary Restrictions

Not applicable

6.3.2 Caffeine, Alcohol and Tobacco

Not applicable

6.3.3 Activity

Not applicable

6.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities. Minimal information includes date of consent, demography, screen failure details, eligibility criteria, and any serious AEs.

6.4.1 Rescreening and Retesting during Screening Period

Participant re-*screening*: This study permits one-time re-*screening* of a participant once that participant has discontinued the study as a *screen* failure (i.e., participant has not been randomized). *Laboratory testing that is repeated because of administrative or technical issues (e.g., breakage of a sample vial during transit to the central laboratory or degradation of a sample during transportation) is not considered to be rescreening.* If re-*screened,* the participant must be re-consented.

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).

The most current result prior to randomization is the value by which study inclusion will be assessed, as it represents the participant's most current, clinical state.

Screening Procedural Outline may be repeated in an effort to find all possible well-qualified participants. Consultation with the Medical Monitor may be needed to identify whether repeat testing of any particular parameter is clinically relevant.

7 TREATMENT

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo or medical device intended to be administered to a study participant according to the study randomization or treatment allocation

Study treatment 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.

An investigational product, also known as investigational medicinal product in some regions, is defined 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.

In this protocol, the investigational product is RO7239361 *(previously, BMS-986089)* solution for injection and matching placebo solution for injection, dispensed in pre-filled syringes.

Other medications used as support medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, may be considered as non-investigational products. In this study, *CS* are considered non-investigational product and will not be provided by the sponsor.

Table 7-1: Study Treatments

Product Description /Class and Dosage Form	Potency	IP/Non-IMP	Blinded or Open Label	Packaging / Appearance	Storage Conditions (per label)
RO7239361 injection 7.5 mg/syringe	10.7 mg/mL	IP	Blinded (during *the 48-week double*-blind phase) and Open Label (during *the 192*-week OL phase)	Solution packaged in a 1 cc glass syringe equipped with a safety syringe device Secondary packaging is either a 1 syringe carton or 4 syringe carton	2-8° C (36-46° F), protect from light, protect from freezing
RO7239361 injection 15 mg/syringe	21.4 mg/mL	IP	Blinded (during *the 48-week double*-blind phase) and Open Label (during *the 192*-week OL phase)	Solution packaged in a 1 cc glass syringe equipped with a safety syringe device Secondary packaging is either a 1 syringe carton or 4 syringe carton	2-8° C (36-46° F), protect from light, protect from freezing
RO7239361 injection 35 mg/syringe	50 mg/mL	IP	Blinded (during *the 48-week double*-blind phase) and Open Label (during *the 192*-week OL phase)	Solution packaged in a 1 cc glass syringe equipped with a safety syringe device Secondary packaging is either a 1 syringe carton or 4 syringe carton	2-8° C (36-46° F), protect from light, protect from freezing
RO7239361 injection 50 mg/syringe	71.4 mg/mL	IP	Blinded (during *the 48-week double*-blind phase) and Open Label (during *the 192*-week OL phase)	Solution packaged in a 1 cc glass syringe equipped with a safety syringe device Secondary packaging is either a 1 syringe carton or 4 syringe carton	2-8° C (36-46° F), protect from light, protect from freezing
Placebo for RO7239361 injection	0 mg/mL	IP	Blinded	Solution packaged in a 1 cc glass syringe equipped with a safety syringe device Secondary packaging is either a 1 syringe carton or 4 syringe carton	2-8° C (36-46° F), protect from light, protect from freezing

Oral *CS* will be obtained by standard prescribing procedures.

7.1 Treatments Administered

The selection and timing of dose for each participant is as follows:

Table 7.1-1: Selection and Timing of Dose

Study Treatment	Unit dose strength(s)/Dosage level(s)	Dosage formulation Frequency of Administration	Route of Administration
RO7239361 injection	7.5 mg, 15 mg, 35 mg, or 50 mg syringe	Weekly	SC
Placebo for RO7239361 injection	0mg syringe	Weekly	SC

Table 7.1-2: Dose Level 1, targeting moderate level of suppression: (≥ 70% suppression at trough)

Dose Level	Body Weight tier	Body Weight range	Dose
1	1	≥ 15 to ≤ 40 kg	7.5 mg QW
1	2	> 40 kg	15.0 mg QW

Table 7.1-3: Dose Level 2, targeting high level of suppression: (90% suppression at trough)

Dose Level	Body Weight tier	Body Weight range	Dose
2	1	≥ 15 to ≤ 40 kg	35 mg QW
2	2	> 40 kg	50 mg QW

RO7239361 or placebo will be administered as a SC injection *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 IB for PK and safety data) following single-dose administration across these injection sites in healthy subjects (Study WP40225) and for details on the recommended storage and use conditions for the PFS.

Refer to current version of the IB for details on the recommended storage of and use conditions for the PFS. Do not use the PFS after the labeled expiry date.

Subjects whose weight exceeds or drops below the dosing weight tier to which they were assigned by > 2 kg (confirmed by repeat) should receive the new weight-based dose in the subject's assigned dose level.

7.2 Method of Treatment Assignment

Subjects will be randomized to receive either low or high dose of 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 [redacted], (e.g., [redacted]). [redacted] 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.

Study treatment will be dispensed at the study visits as listed in Schedule of Activities (Section 2).

7.3 Blinding

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

Before breaking the blind of an individual participant's treatment, the investigator should determine that the unblinded information is necessary, i.e., that it will alter the participant'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 participant 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 participant has been made.

In case of an emergency, the investigator(s) has unrestricted access to randomization information via the Interactive Web Response System (IWRS) and is capable of breaking the blind through the IWRS system without prior approval from sponsor. Following the unblinding the Investigator shall notify the medical monitor and/or study director.

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.

As this is a placebo-controlled, double-blind study, all efforts must be maintained by site staff, subjects and caregivers in not making any assumptions on potential treatment assignments based on clinical presentations.

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

7.4 Dosage Modification

Dose modifications are not allowed.

7.5 Preparation/Handling/Storage/Accountability

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

The product storage manager should ensure that the study treatment 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 treatment arise, the study treatment should not be dispensed and contact Roche immediately.

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

Investigational product 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).

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 allowed starting on Day 22 of double-blind phase and at Week 4 of the *OL* phase), the parents/caregivers must be trained on proper administration of study drug. The site staff will be provided with a pharmacy manual that includes parent/caregiver training guidance and a training checklist. The parents/caregivers will be provided with IFU on administration of study drug. 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, time, date, etc. of each SC injection. Parents/caregivers must be instructed to bring the used and/or empty syringes in a Sharps container and the dosing diary to each study visit; this will allow site staff to assess treatment compliance and study drug accountability. A diary will also be provided to the parent/caregiver to record the subject's *CS* therapy, AEs, *concomitant medicines*, and falls. 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 or designee.

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.

- Further guidance and information for final disposition of unused study treatment are provided

in Appendix 2 and the pharmacy manual.

7.5.1 Retained Samples for Bioavailability/Bioequivalence

Not applicable.

7.6 Treatment Compliance

Study treatment compliance will be monitored by review of dosing diary cards. Drug accountability should be reviewed by the site study staff at each visit to confirm treatment compliance. Sites should discuss discrepancies with the participant at each on-treatment study visit.

Any overdose or incorrect administration of study drug should be reported as an adverse event and recorded on the Adverse Event electronic Case Report Form (eCRF), and the Medical Monitor should be informed.

7.7 Concomitant Therapy

7.7.1 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, including *CS*, should be reported on the CRF, regardless of when the prior therapy was administered. Routine standard of care immunizations should be maintained throughout the study.

Experimental medications to treat DMD may be permitted during the *OL* phase. This will be dependent upon results from the double-blind phase.

1) Prior exposure to RO7239361 or any other anti-myostatin agent.
2) Treatment with ataluren *within 12 weeks prior to the start of study drug administration*.
3) *Treatment with* any *other* investigational drug (excluding deflazacort *in CS dose-finding trials*) 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.
4) Treatment with exon skipping therapies within 6 months prior to the start of study drug administration
5) Current or prior treatment within 12 weeks of study drug administration with androgens or human growth hormone.
6) Concomitant or previous participation at any time in a gene therapy study.
7) 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.

Any concomitant therapies must be recorded on the CRF.

7.7.2 Other Restrictions and Precautions

7.7.2.1 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, and 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, 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 go to their local emergency care facility 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 antihistamine, 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.

7.7.2.2 Imaging Restriction and Precautions

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 2-2 and Table 2-3). 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.

7.8 Treatment after the End of the Study

At the conclusion of the study, participants who continue to demonstrate clinical benefit will be eligible to receive Roche-supplied study treatment. Study treatment will be provided via an extension of the study, a rollover study requiring approval by responsible health authority and ethics committee or through another mechanism at the discretion of Roche and according to local legislation.

Roche reserves the right to terminate access to Roche-supplied study treatment if any of the following occur: a) the study is terminated due to safety concerns; b) the development of RO7239361 is terminated for other reasons, including but not limited to lack of efficacy and/or not meeting the study objectives; c) the participant can obtain medication *commercially,* from a government-sponsored *program,* or *from a* private health program. In all cases, Roche will follow local regulations.

8 DISCONTINUATION CRITERIA

Clinical Criteria for Dose Discontinuation:

- Dosing 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 *AE* 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 within a subject 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 for *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.

8.1 Discontinuation from Study Treatment

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

- Participant's request to stop study treatment. Participants who request to discontinue study treatment 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 participant specifically withdraws consent for any further contact with him/her or persons previously authorized by participant to provide this information
- 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 participant
- Termination of the study by Roche
- Loss of ability 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

Refer to the Schedule of Activities for data to be collected at the time of treatment discontinuation and follow-up and for any further evaluations that can be completed

All participants who discontinue study treatment should comply with protocol specified follow-up procedures as outlined in Section 2. The only exception to this requirement is when a participant withdraws consent for all study procedures including post-treatment study follow-up 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 treatment is discontinued prior to the participant's completion of the study, the reason for the discontinuation must be documented in the participant's medical records and entered on the appropriate *eCRF*.

8.1.1 Post Study Treatment Study Follow-up

In this study, safety and the *NSAA* are key endpoints of the study. Post study follow-up is of critical importance and is essential to preserving participant safety and the integrity of the study. Participants who discontinue study treatment 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 the last dose of study drug (Table 2-4)

8.2 Discontinuation from the Study

Participants who request to discontinue study treatment will remain in the study and must continue to be followed for protocol specified follow-up procedures (Table 2-4). The only exception to this is when a participant specifically withdraws consent for any further contact with him/her or persons previously authorized by participant to provide this information.

- Participants 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 treatment only or also from study procedures and/or post treatment study follow-up, and entered on the appropriate CRF page.
- In the event that vital status (whether the participant 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.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

8.3 Lost to Follow-Up

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

9 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and timing are summarized in the Schedule of Activities (Table 2-1, Table 2-2, Table 2-3, and Table 2-4).
- Protocol waivers or exemptions are not allowed.
- All immediate safety concerns must be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue treatment.
- Adherence to the study design requirements, including those specified in the Schedule of Activities, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria before randomization. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

9.1 Efficacy Assessments

9.1.1 Functional Endpoint Assessment

Measurements of function *will be assessed at the timepoints specified in* Table 2-1 *through* Table 2-4 *and* include *the following*:

- TFTs (4SC, 10 m walk/run, stand from supine)
- 6 minute walk distance (6MWD)
- NSAA scale
- Performance of Upper Limb Scale (PUL)

Detailed instructions for performing each of these assessments are provided in the Clinical Evaluator (CE) Manual. Functional endpoints will be measured by a clinical evaluator who has undergone study specific training on the administration of these assessments. The clinical evaluators who assess functional endpoints must be distinct from the clinicians who assess AEs of the study subjects. To ensure that clinical evaluators remain blind to study drug assignment, clinicians evaluating AEs should not communicate the presence, absence or severity of AEs with clinical evaluators at the site unless such communication is required to ensure subject safety.

TFTs include *4SC*, 10-meter walk/run and stand from supine. The 6MWD measures the distance a patient is able to traverse while walking for 6 minutes. *TFTs, such as the 4SC and the 6MWD, are well validated and have been widely used in clinical trials.*[19, 23, 24, 46] *Data delineating the natural history of performance of the 4SC and other TFTs in boys with DMD have been published.*[18, 25] A 2.2-second difference in 4SCV corresponds to a 30-meter difference on the 6MWD. A 30-meter difference on the 6MWD has been shown to be associated with a significantly heightened risk of loss of ambulation within the ensuing 2 years.[26] Unlike the 6MWD, the 4SC is less reliant on endurance and is most reflective of lower extremity muscle power. The 4SC velocity (the number of stairs climbed per second) is calculated as the ratio of the number of stairs climbed (4) divided by the number of seconds taken to complete the 4-stair climb. Because children who are

or have become unable to perform this task, the time cannot be measured, conventionally a time equal or bigger than the worst performance in the group is subjectively given to indicate poor performance. In order to improve the *possibility of performing statistical analysis, the results will be converted into velocity (distance/time) rather than the conventional time as this would allow conversion of all the poor or failed results from a subjectively chosen number into a meaningful number.*

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.[48]

Some of the functional endpoint assessment including the TFTs, NSAA, 6MWD and upper and lower extremity myometry will be video recorded at select study visits while the subject is completing the assessments. The videos will be uploaded by the site to a secure database, maintained by a separate vendor, and reviewed by master physiotherapists to assure quality or identify potential issues requiring re-training of clinical evaluators at the site. In addition the videos may be used to explore additional assessment of timing of TFTs, and to grade the quality of the patient's functional abilities, which could inform the feasibility of potential new outcome measures focused on movement quality.

9.1.2 Myometry and Ankle Range of Motion (ROM)

Muscle strength will be assessed using hand-held dynamometry at the time points specified in Table 2-1 through Table 2-3, following procedures outlined in the CE 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 CE Manual. These assessments will be performed by a clinical evaluator who has undergone study specific training on the administration of these assessments.

9.1.3 Imaging Assessment for the Study

Images will be submitted to an imaging core lab. Sites should be trained prior to scanning the first study participant. Image acquisition guidelines and submission process will be outlined in the Imaging Manual provided by the core lab.

9.1.3.1 DXA

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

DXA acquisition guidelines and submission processes will be outlined in the DXA Scanning Guide.

Adequacy of DXA scans should be confirmed by the central imaging vendor prior to randomization.

Screening DXA should be obtained between Day -42 and Day -14 to allow for central imaging vendor review.

DXA is not required for subjects enrolled at sites in Germany.

DXA is not required at the early termination visit.

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.[49]

9.1.3.2 Cardiac MRI (cMRI)

cMRI with gadolinium enhancement will be performed in a subset of participants at select U.S. centers at the time points indicated in Table 2-1 through Table 2-3. Subjects enrolled at select U.S. centers participating in the cMRI substudy will be required to attempt to complete the cMRI. Subjects participating at sites where the cMRI substudy is being performed who have an allergy to gadolinium may undergo cMRI scanning without gadolinium.

cMRI acquisition guidelines, protocols, contrast agent type and dose, and image submission processes are outlined in the imaging manual provided by the core imaging lab.

Adequacy of cMRI scans should be confirmed by the central imaging vendor prior to randomization.

Screening cMRI should be obtained between Day -*42* and Day -14 to allow for central imaging vendor review. cMRI will be conducted at early termination visits if ≥ 24 weeks has elapsed from previous cMRI.

Re-enrollment: Repeat of cMRI is not required if previously obtained within 6 months of Day 1/randomization.

9.1.4 Exploratory Genotyping

A 2-mL whole blood *9.1.7 s*ample will be drawn (as indicated in Table 2-2) 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.

9.1.5 ActiMyo

Subjects enrolled at selected sites will be asked to wear a *device* called ActiMyo. *The w*earing *of the* ActiMyo is optional. *Hence, refusal to wear ActiMyo will not affect the eligibility of a subject to be enrolled in the study. The purpose of the ActiMyo device is to measure the daily movement and activity levels of subjects with a degree of high accuracy. Unlike consumer-grade activity trackers and mobile phone applications, the endpoints of the ActiMyo device are clinically validated. Therefore, the activity data collected by ActiMyo will be analyzed to provide deep*

insights into the ability of subjects to ambulate (i.e., walking distance, walking speed, ability to climb stairs, speed of climbing stairs, etc.) and allows for the determination of the clinical meaningfulness of RO7239361 in the treatment of DMD, and refusal will not exclude a *subject* from the study. The purpose of the ActiMyo device is to accurately measure the daily movement and activity levels of the subject. The device consists of two sensors, to be worn on each ankle. Subjects will wear the devices each day during specified periods of the study and during TFTs and 6MWT at specified *site* visits. As the ActiMyo device battery requires recharging overnight, the device will not be worn at night. The subject and parent/caregiver will be trained *on how* to use the device, including correct fitting and wearing of the device, how to dock the device on the battery charging station, and when to return ActiMyo materials to the site. *As a CE-marked device, the possibility of allergic reactions and skin irritation has been addressed and are thus unlikely to occur. Nevertheless, should any skin reaction be observed, the subject should remove the strap and sensor and contact the study team as soon as possible*. Detailed instructions as to use of the ActiMyo device are provided in the ActiMyo User Manual and Quick Start Guide.

9.1.6 Caregiver Video Assessment

Subjects enrolled at selected sites will be invited to participate in the caregiver video assessment. Participation is optional, and refusal to participate will not exclude a subject from the main study. The aim of the caregiver video assessment is to provide additional insight into a subject's physical functioning and motor strength during the study. A video application will be used to capture a range of standardized tasks in a real-world environment, external to the clinic.

The video application will ask caregivers to record the subject completing selected tasks every 12 weeks according to pre-specified instructions read aloud by the caregiver. These tasks include sitting up, standing up, walking, running, climbing stairs, and one additional task personally chosen by the caregiver. The caregiver is allowed to skip the recording of any of these tasks if not appropriate or applicable. In addition, the application will allow caregivers to record a new ability at any point during the study.

If the subject and caregiver agree to participate, they will sign a separate Informed Consent Form at baseline (for newly enrolled subjects) or at a future study visit (for subjects who are already enrolled in the main study only). Detailed instructions regarding the caregiver video assessment are provided in the parent/caregiver manual.

9.1.7 Clinical Global Impression of Change (CGI-C)

The CGI-C is a single item that clinicians will complete at the end of the double-blind phase (*Week 48 of the study*) and *at Week 48 of the* OL phase (*Week 96 of the study*) to rate change in the patient's global impression of change in DMD from *the start of each phase (i.e., from baseline for the double-blind phase and from Week 49 for the OL phase)*. There are seven response options: "Very much improved", "Much improved", "Minimally improved", "No change", "Minimally worse", "Much worse", and "Very much worse". It is a widely used endpoint in clinical trials across a variety of disease areas. To enhance inter-rater consistency, an instruction document (developed with input from clinical experts) will be provided in the user manual, which includes examples for each of the response options.

The item should be completed by a clinician (e.g., Principal Investigator or study coordinator) who

has interacted with the individual since baseline. The same clinician should complete the CGI-C at all specified visits for an individual patient. If there is no clinician at the site who is able to reflect on the patient's health status, an alternative clinician should make the rating using existing study notes, and sites should record this.

9.1.8 *Caregiver Global Impression of Change (CaGI-C)*

The CaGI-C comprises six items that caregivers will complete at the end of the double-blind phase (Week 48 of the study) and at Week 48 of the OL phase (Week 96 of the study) to rate the change in a subject's symptoms, physical abilities, daily activities, social life, emotions and mental well-being, and overall health from the start of each phase (i.e., from baseline of the double-blind phase and from Week 49 of the OL phase). There are seven response options: "Very much improved," "Much improved," "Minimally improved," "No change," "Minimally worse," "Much worse," and "Very much worse." It is a widely used endpoint in clinical trials across a variety of disease areas.

To enhance intra- and inter-consistency, descriptions of each level of change have been developed and domain level global impression items have been created with input from patients with DMD and their caregivers. The items should be completed by the parent or caregiver who has been involved in the study since baseline. The same parent or caregiver should complete the CaGI-C for his or her child at the end of each study phase to reflect the subject's health status from baseline for the double-blind phase and Week 49 of the OL phase. The CaGI-C will be assessed at selected sites only.

9.1.9 *Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL MFS)*

The PedsQL MFS parent proxy measures the severity and impact of fatigue on the subject from the caregiver's perspective. The recall period is the last 4 weeks. The instrument is appropriate for individuals aged 2-18 years old and captures six items on general fatigue, six items on sleep/rest fatigue, and six items on cognitive fatigue. A total fatigue score can be calculated from the 18 items that ranges from 0 to 100, with higher scores indicating less fatigue. The PedsQL MFS takes approximately 10 minutes to complete. The PedsQL MFS will be assessed at selected sites only.

9.2 Adverse Events

The definitions of an AE or SAE can be found in Appendix 3.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

Malfunctions of the pre-filled syringes containing study drug should be reported to the sponsor via designated form (electronic system) in accordance with local regulations.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that

are serious, considered related to the study treatment or the study, or that caused the participant to discontinue before completing the study.

The clinical evaluators who assess functional endpoints must be distinct from the clinicians who assess AEs of the study subjects. Clinicians evaluating AEs should not communicate the presence, absence or severity of AEs with clinical evaluators at the site unless required to do so to ensure subject safety.

Contacts for SAE reporting *are* specified in Appendix 3.

9.2.1 Time Period and Frequency for Collecting AE and SAE Information

The collection of nonserious AE information should begin at initiation of study treatment until *50 days of discontinuation of dosing*. Nonserious 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 participants.

Section *6.4* in the IB represent the Reference Safety Information to determine expectedness of serious *AEs* for expedited reporting. Following the participant's written consent 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 (5 half-lives) of discontinuation of dosing. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy).

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.

- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the appropriate section of the eCRF section.
- All SAEs will be recorded and reported to Sponsor or designee within 24 hours, as indicated in Appendix 3.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of this being available.

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.

The method of evaluating and assessing *the seriousness, severity, and* causality of AEs and the procedures for completing and reporting/transmitting SAE reports are provided in Appendix 3. *Guidance for assessing severity of AEs is also provided in Section 8.*

9.2.2 Method of Detecting AEs and SAEs

Adverse events 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.)

9.2.3 Follow-up of AEs and SAEs

- Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious (see Appendix 3).
- Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study treatment and for those present at the end of study treatment as appropriate.
- All identified nonserious AEs must be recorded and described on the nonserious 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.

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the participant is lost to follow-up (as defined in Section 8.3).

Further information on follow-up procedures is given in Appendix 3.

9.2.4 Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the Sponsor of SAEs is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a product under clinical investigation are met.
- An investigator who receives an investigator safety report describing SAEs or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will file it along with the Investigator Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

Sponsor or designee will be reporting *AEs* to regulatory authorities and ethics committees according to local applicable laws including European Directive 2001/20/EC and FDA Code of Federal Regulations 21 CFR Parts 312 and 320. A SUSAR (Suspected, Unexpected Serious Adverse Reaction) is a subset of SAEs and will be reported to the appropriate regulatory authorities and investigators following local and global guidelines and requirements.

9.2.5 Pregnancy

Any pregnancy that occurs in a female partner of a male study participant should be reported to Sponsor or designee. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

9.2.6 Laboratory Test Result Abnormalities

The following laboratory test result abnormalities should be *reported as an AE and* captured on the nonserious AE eCRF page or SAE Report Form electronic, as appropriate. Paper forms are only intended as a back-up option when the electronic system is not functioning.

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

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

Laboratory abnormalities that are reported as AEs or SAEs should be reported utilizing the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

9.2.7 Potential Drug Induced Liver Injury (DILI)

- 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.

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs (see Section 9.2 and Appendix 3 for reporting details).

Criteria for initiating an evaluation for potential DILI are defined in Section 8.

9.2.8 Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiogram, x-ray filming, any other potential safety assessment required or not required by protocol should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

9.2.8.1 Injection Site Assessments

Subjects will be monitored for injection-site reactions from Day 1 through the last study visit. Report injection-site reactions as AEs, as appropriate. It is strongly recommended that non-subject identifying photographs of injection site reactions be taken for submission to the sponsor or designee. Some subjects with injection-site reaction or rash may be asked to participate in additional procedures to determine the type of immune reaction associated with the *injection-site*

reaction or rash. Potential subjects will be identified in consultation with the medical monitor. The additional procedures include a blood sample and skin biopsy to assess the type of immune reaction. The blood sample will *be* used to assess ADA titers, serum mast cell tryptase/histamine, and compliment activation products (e.g., C3D, C3G). The skin biopsy should be collected in a sterile manner in accordance with local procedures, using a 3 mm punch biopsy instrument and formalin fixation, and will assess mast cell, eosinophil, and neutrophil infiltration. The Draize Scale for erythema and edema will be used as a guide for reporting injection site reaction AEs[50] (see Table 9.2.8.1-1).

Table 9.2.8.1-1: 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	Severe edema, raised more than 1mm and beyond exposure area	Severe

9.2.9 Overdose or Incorrect Administration of Study Drug

An overdose is defined as the accidental or intentional administration of the drug in an amount higher than the *assigned* dose. *Definitions of other incorrect administration of study drug (including medication error, drug abuse, and drug misuse) are provided in Appendix 3.*

Any overdose or incorrect administration of study drug must be reported as an AE.

If the associated AE fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event). Any AE associated with an overdose or incorrect administration of study drug should be reported as a separate AE as well (see Section 9.2).

9.3 Safety

Planned time points for all safety assessments are listed in the Schedule of Activities.

The data from the safety assessments required in this protocol (laboratory tests, ECGs, echocardiograms) will be transferred to Roche or designee from the relevant vendor. The site staff are not required to report these procedures in 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.

9.3.1 Physical Examinations

Targeted physical exams to be guided by review of systems and include assessment of heart, lungs and abdomen.

9.3.2 Vital Signs

Refer to Schedule of Activities.

9.3.3 Electrocardiograms and Echocardiograms

Refer to Schedule of Activities.

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 (electrocardiogram and 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.

Electrocardiograms and echocardiogram should be repeated in subjects with new cardiac symptoms.

9.3.4 Clinical Safety Laboratory Assessments

Investigators must document their review of each laboratory safety report.

Table 9.3.4-1: 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 -1 or -2)*	X	*X*
Urine test			
Urinalysis (including protein, glucose, blood, leukocyte esterase, specific gravity)	X	X (only required at visits specified in Table 2-2 and Table 2-3)	

Table 9.3.4-1: Laboratory Assessments

	Screening	On Treatment	Follow-up
Other sample collections			
Serum Biomarkers		X (see Table 2-2 and Table 2-3)	
Immunogenicity		X (see Table 2-2 and Table 2-3)	X (see Table 2-4)
Free and myostatin drug complex		X (see Table 2-2 and Table 2-3)	
Exploratory genotyping		X (see Table 2-2)	
Creatine kinase-muscle (CK-MM)		X (Day 1, Weeks 12, 24 and 48 in double-blind phase)	
GDF-11		X (see myostatin collection schedule in Table 2-2 and Table 2-3)	

9.3.5 Imaging Safety Assessment

Any incidental 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.

9.4 Pharmacokinetics

The PK data obtained in this study will be combined with data from other studies in the clinical development program to develop a population PK model. This model will be used to evaluate the effects of intrinsic and extrinsic covariates on the PK of RO7239361 and to determine measures of individual exposure (such as steady-state trough, and time-averaged concentration). Model determined exposures will be used for exposure-response analyses of selected efficacy and safety end points. Results of population PK and exposure-response analyses will be reported separately.

Table 9.4-1 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.

Serum samples will be analyzed for RO7239361 using a validated ligand binding assay. Pharmacokinetic samples collected from a subject who received placebo will not be analyzed.

Table 9.4-1: Pharmacokinetic Sampling Schedule for RO7239361

Study Day of Sample Collection	Event	Time (Relative To RO7239361 Dose) Hour: Min	RO7239361 Blood Sample for Serum
Day 1 *of the* double-blind *phase*	predose	00:00	X
Day 8 *of the* double-blind *phase*	predose	00:00	X
Day 15 *of the* double-blind *phase*	predose	00:00	X
Day 29 *of the* double-blind *phase*	predose	00:00	X
Week 12 *of the* double-blind *phase*	predose	00:00	X
Week 24 *of the* double-blind *phase*	predose	00:00	X
Week 36 *of the* double-blind *phase*	predose	00:00	X
Week 48 *of the* double-blind *phase*	predose	00:00	X
Week 1 *of the* open-label *phase*	predose	00:00	X
Week 2 *of the* open-label *phase*	predose	00:00	X
Week 3 *of the* open-label *phase*	predose	00:00	X
Week 12 *of the* open-label *phase*	predose	00:00	X
Week 24 *of the* open-label *phase*	predose	00:00	X
Week 36 *of the* open-label *phase*	predose	00:00	X
Week 48 *of the* open-label *phase*	predose	00:00	X

9.5 Pharmacodynamics

Not applicable

9.6 Pharmacogenomics

Not applicable.

9.7 Biomarkers

Blood will be drawn at the times indicated in Table 2-1 through Table 2-3 for the measurement of target engagement biomarkers (free myostatin and myostatin-drug complex).

Blood will be drawn at the times indicated in Table 2-1 through Table 2-3 for the measurement of exploratory biomarkers. Serum samples *may* be used to evaluate the levels of exploratory biomarkers known to be involved in muscle growth*, function,* and/or myostatin signaling (e.g., *sTNI, MYL3, FABP3*).

Creatine kinase-muscle (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.

9.7.1 Additional Research Collection

Additional research collections and retention are optional for all subjects, except where prohibited by local laws or regulations.

This protocol will include both sample collection and residual sample storage for additional research (AR).

This serum collection for additional research is intended to expand the translational R&D capability at Roche, and will support as yet undefined research aims that will advance our understanding of disease and options for treatment. For example, this sample may have potential in exploration of diagnostic or prognostic biomarkers contingent on scientific developments in the field of myostatin biology, DMD pathophysiology or unanticipated results in the study.

This collection for additional research may also be used to support health authority requests for analysis, and advancement of pharmacodiagnostic development to better target drugs to the right patients. This may also include genetic/genomic exploration aimed at exploring disease pathways, progression and response to treatment etc.

All requests for access to samples or data for additional research will be vetted through a diverse committee of the study sponsor's senior leaders in Research and Development to ensure the research supports appropriate and well-defined scientific research activities.

- Prospective samples of serum for Additional Research will be collected at selected time points (see Table 9.7.1-1)
- Residual serum from serum biomarker collections (see Table 9.7.1-2) will also be retained for additional research purposes
- Residual whole blood or DNA derived from the whole blood sample from exploratory genotyping analysis (Table 9.7.1-2) will also be retained for additional research purposes

Samples will be securely stored by the Roche Research Biosample Repository or at a Roche approved third party storage management facility.

Samples will be stored in a coded fashion, and no researcher will have access to the key. The key is securely held by the investigator at the clinical site, so there is no direct ability for a researcher to connect a sample to a specific individual

Additional research samples will be retained for 15 years or the maximum allowed by applicable law. No additional sampling is required for residual collections.

Further details of sample collection and processing will be provided to the site in the procedure manual.

Table 9.7.1-1: Additional Research Sample Schedule

Study Day	Time (Event) Hour	Time (Relative To Dosing) Hour: Min	Blood Sample
Baseline Day 1	0 (predose)	00:00	X
Week 24	0	00:00	X
Week 48	0	00:00	X

Table 9.7.1-2: Residual Sample Retention for Additional Research Schedule

Sample Type	Timepoints for which residual samples will be retained
PK	All
Residual serum from serum biomarkers	All
Residual whole blood or DNA derived from the whole blood sample from exploratory genotyping	Day 1
Residual serum from ADA analysis	All
Residual serum from myostatin and myostatin-drug complex	All

9.7.2 Immunogenicity Assessments

Serum samples from all treated subjects will be analyzed by a validated immunogenicity assay. Individual time points are considered positive if confirmed as specific against RO7239361. The subject will be called positive for immunogenicity as defined in the Statistical Analysis Plan (SAP). 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. In the event a subject has a positive immunogenicity response, additional NAB analyses may be conducted.

9.7.3 RNA Transcriptome Research

Not applicable.

9.7.4 RNA Expression Research of a Subset of RNA Species

Not applicable.

9.7.5 Proteome Research

Not applicable.

9.7.6 Metabolomic Research

Not applicable.

9.7.7 Other Assessments

Not applicable.

9.8 Health Economics or Medical Resource Utilization and Health Economics

This study includes a number of patient reported outcomes, such as pediatric data collection instrument (PODCI), health utilities index (HUI III), and PedsQL family impact module (PedsQL FIM) that provide additional information to the clinician-reported outcomes. In addition, several questions will be included to collect presenteeism and absenteeism data, along with health care resource utilization.

Health care resource utilization data associated with medical encounters, will be collected in the CRF by the investigator and study-site personnel for all participants throughout the study. Protocol-mandated procedures, tests, and encounters are excluded.

The data collected may be used to conduct exploratory economic analyses and will include:

- Number and duration of medical care encounters, including surgeries, and other selected procedures (inpatient and outpatient)
- Duration of hospitalization (total days length of stay, including duration by wards, e.g., intensive care unit)
- Number and character of diagnostic and therapeutic tests and procedures
- Outpatient medical encounters and treatments (including physician or emergency room visits, tests and procedures, and medications)

10 STATISTICAL CONSIDERATIONS

10.1 Sample Size Determination

Statistical Considerations: Sample Size

The sample size of 159 patients (53 patients per arm) will provide 80% power for testing the null hypothesis of no difference between a dose of RO7239361 and placebo in the change from baseline in the NSAA total score at 48 weeks under the following assumptions:

- *A true treatment difference (RO7239361 minus placebo) of 2.5 points*
- *A standard deviation of 4.4 points*
- *A treatment discontinuation rate of 5%*

10.2 Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign informed consent and are assigned a participant identification number.
Randomized	Enrolled participants who receive a randomization treatment assignment.
Treated	Enrolled subjects who receive at least 1 dose of study therapy. • If a subject received the same incorrect treatment throughout the study, then the participant will be analyzed based on the treatment received. • If a subject received study drug from more than one treatment group, and none of the administrations were consistent with the assigned randomized treatment group, then the participant will be analyzed based on the first treatment received.
Modified Intention to Treat (mITT)	Randomized subjects that received at least one dose of study *drug*. For change from baseline analyses, this includes only those subjects that had at least one efficacy assessment.
Per-Protocol	mITT subjects with no major protocol violations*, as described in the Statistical Analysis Plan*

10.3 Statistical Analyses

The *SAP* will be developed and finalized before database lock and will describe the selection of participants to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. Below is a summary of planned statistical analyses of the primary and secondary endpoints.

10.3.1 Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary	The primary analysis, on the change from baseline in *NSAA total score*, will be conducted using a Mixed Model Repeated Measures (MMRM) analysis with treatment, visit, and the treatment-by-visit interaction entered into the model as fixed effects, and participant entered as a random effect. The baseline *NSAA total score* will be entered into the model as a covariate. The stratification factors will be entered into the model as blocks. *For each RO7239361 dose group, the null hypothesis of the no treatment difference between the dose group and placebo will be tested using the estimates from this MMRM model at the end of the 48-week double-blind treatment period. In order to control the overall type I error rate for the analysis, the following hierarchical testing of doses will be implemented:* 1. *Comparison of the RO7239361 high dose (35/50 mg) to placebo at a two-sided α level of 0.05 is performed.* 2. *If the null hypothesis for the high dose is rejected (comparison of the high dose to placebo in the previous step is statistically significant), then a comparison of the RO7239361 low dose (7.5/15 mg) to placebo at a two-sided α level of 0.05 will be performed. If the null hypothesis for the high dose is not rejected, then the testing of the low dose versus placebo will not be performed.* Sensitivity analyses will include the following: an analysis of covariance (ANCOVA) using the mITT data set; an ANCOVA on the mITT data set with missing data imputed using multiple imputations; and an MMRM analysis using the per-protocol data.
Secondary	*Following testing of the primary endpoint,* the key secondary endpoints will be analyzed *using a hierarchical testing procedure in order to maintain the overall type I error rate at* 0.05. *This procedure will be specified in the Statistical Analysis Plan prior to database lock.* Most secondary endpoints will be tested using a MMRM that is similar in structure to the primary analysis. Proximal lower extremity flexor strength will be tested using a MANOVA of the 2 component variables: knee extension and knee flexion. If a dose fails to reach significance against placebo in any stage of the testing hierarchy, any further tests will be considered as exploratory.
Exploratory	Will be described in the Statistical Analysis Plan finalized before database lock

10.3.2 Safety Analyses

All safety analyses will be performed using treated subjects.

Endpoint	**Statistical Analysis Methods**
Primary	Not applicable
Secondary	Tabular summaries of SAEs, AEs leading to discontinuation and Grade 3 to 4 laboratory abnormalities will present the number and percentage of unique subjects with treatment emergent events. Adverse event summaries will be presented by system organ class (SOC) and preferred term (PT). All SAEs since enrollment will be listed. Grade 3 to 4 laboratory abnormalities will be summarized as proportions by baseline toxicity grade.
Endpoint	**Statistical Analysis Methods**
Exploratory	Will be described in the Statistical Analysis Plan finalized before database lock

10.3.3 Other Analyses

PK, *PD*, biomarker and exploratory analyses will be described in the *SAP* finalized before database lock. The population *PK* analysis and PD analyses will be presented separately from the main clinical study report.

10.3.4 Interim Analyses

Schedule of Analyses

*In addition to the primary analysis (to be conducted when all patients have completed Week 48, the end of the double-blind treatment period), t*he following analyses may be conducted during the course this study:

- *When at least 20% of the total number of patients have completed 24 weeks of double-blind, placebo-controlled treatment*
- *When at least 20% of the total number of patients has completed 48 weeks of double-blind, placebo-controlled treatment*
- An analysis conducted *at Week 96 (*at the end of the *first 48 weeks of the OL phase of the study)*
- *A final data analysis when the study has been completed*

The *SAP* will further describe the planned interim analyses.

Interim SAPs (iSAPs) will contain detailed information about the conduct of the futility analyses, which will be performed by an independent data coordinating center with results reviewed by the iDMC. Because these futility analyses will be conducted prior to the primary efficacy analysis, the unblinded data will not be shared with the Sponsor in order to maintain the integrity of the study blind.

For the primary efficacy endpoint (the change from baseline in NSAA total score), additional analyses may be performed, incorporating the first 96 weeks of exposure to RO7239361 (48 weeks of double-blind treatment) followed by 48 weeks of OL treatment). The data will be compared with data from one or more control cohorts from external data sources (e.g., disease registries) that include at least 96 weeks of follow-up data.

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12. APPENDICES

APPENDIX 1 ABBREVIATIONS AND TRADEMARKS

Term	Definition
4SC	4 stair climb
6MWD	6 minute walk distance
AE	adverse event
ACLS	advanced cardiac life support
ADA	anti-drug antibody
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
AST	aspartate aminotransferase
AT	amino transaminases
ATOM	*Advancing Trial Outcome Measures International, Ltd.*
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
BID, bid	bis in die, twice daily
BLQ	below limit of quantification
BMI	body mass index
BMS	Bristol-Myers Squibb
BP	blood pressure
BUN	blood urea nitrogen
C	Celsius
C12	concentration at 12 hours
24	concentration at 24 hours
Ca^{++}	calcium

Term	Definition
Cavg	average concentration
CBC	complete blood count
Cexpected-tau	expected concentration in a dosing interval
CFR	Code of Federal Regulations
CGI-C	Clinical Global Impression of Change
CHF	Congestive heart failure
CI	confidence interval
$C1^-$	chloride
CLcr	creatinine clearance
cm	centimeter
Cmax, CMAX	maximum observed concentration
Cmin, CMIN	minimum observed concentration
cMRI	Cardiac magnetic resonance imaging
CPF	Cough peak flow
CRC	Clinical Research Center
CRF	Case Report Form, paper or electronic
CS	Corticosteroid
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.)
CTCAE	Common Terminology Criteria for Adverse Events
Ctrough	Trough observed plasma concentration
CV	coefficient of variation
D/C	discontinue
dL	deciliter
DMD	Duchenne muscular dystrophy
DXA	Dual-energy X-ray absorptiometry
ECG	electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EF	Ejection fraction
e.g.	exempli gratia (for example)
EOSI	events of special interest
ESR	Expedited Safety Report

Term	Definition
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FVC	Forced Vital Capacity
g	Gram
GCP	Good Clinical Practice
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
HUI-3	Health Utility Index-3
IA	interim analysis
IB	Investigator's Brochure
ICD	International Classification of Diseases
ICH	International Conference on Harmonisation
iDMC	*independent Data Monitoring Committee*
i.e.	id est (that is)
IEC	Independent Ethics Committee
IFU	*instructions for use*
IMP	investigational medicinal product
IND	Investigational New Drug
IP	Investigational product
IRB	Institutional Review Board
IRT	Interactive Response Technology
IU	International Unit
IV	Intravenous
IWRS	Interactive web response system
K	slope of the terminal phase of the log concentration-time curve

Term	Definition
K_3EDTA	potassium ethylenediaminetetraacetic acid
K^+	Potassium
kg	Kilogram
L	Liter
LC	liquid chromatography
LDH	lactate dehydrogenase
ln	natural logarithm
LTBP4	Latent transforming growth factor beta binding protein 4
MAD	Multiple ascending dose
MEP	Maximum expiratory pressure
MIP	Maximum inspiratory pressure
mg	Milligram
Mg^{++}	Magnesium
min	Minute
mITT	Modified intent to treat
mL	Milliliter
mmHg	millimeters of mercury
MMRM	Mixed model repeated measures
MR	medical research
MRI	Magnetic resonance imaging
MRT	mean residence time
MS	Mass spectrometry
MTD	maximum tolerated dose
µg	Microgram
N	number of subjects or observations
Na^+	Sodium
NA	not applicable
NAB	Neutralizing antibodies
ng	Nanogram
NIMP	non-investigational medicinal products
NOAEL	No observed adverse event level
NSAA	North Star Ambulatory Assessment Scale
NSAID	non-steroidal anti-inflammatory drug
OL	Open label
pAUCe	Extrapolated partial AUC from last quantifiable concentration to infinity

Term	Definition
Pb	percent of bound drug
PD	pharmacodynamic
PE	Physical exam
PedsQL FIM	Pediatric Quality of Life Family Impact Module
PFR	Peak flow rate
PFS	*pre-filled syringe*
PFTs	Pulmonary function tests
PK	pharmacokinetic
PODCI	Pediatric Outcome Data Collection Instrument
PO	per os (by mouth route of administration)
PT	prothrombin time
PTT	partial thromboplastin time
Pu	percent of unbound drug
PUL	Performance of Upper Limb
QC	quality control
QD, qd	quaque die, once daily
R^2	coefficient of determination
RBC	red blood cell
SAE	serious adverse event
SAP	*Statistical Analysis Plan*
SC	Subcutaneous
SOP	Standard Operating Procedures
SPP1	Secreted phosphoprotein 1
t	Temperature
T	Time
TE	Target engagement
TFTs	Timed function tests
TGF-β	transforming growth factor-β
T-HALF	Half life
Tmax, TMAX	time of maximum observed concentration
Vss/F (or Vss)	apparent volume of distribution at steady state
W	Washout
WBC	white blood cell
WHO	World Health Organization
WOCBP	women of childbearing potential

Term	Definition
x g	times gravity

APPENDIX 2 STUDY GOVERANCE CONSIDERATIONS

The term "Participant" is used in the protocol to refer to a person who has consented to participate in the clinical research study. The term "Subject" used in the eCRF is intended to refer to a person (Participant) who has consented to participate in the clinical research study.

REGULATORY and ETHICAL CONSIDERATIONS

GOOD CLINICAL PRACTICE

This study will be conducted in accordance with:

- Good Clinical Practice (GCP),
- as defined by the International Council on Harmonisation (ICH)
- in accordance with the ethical principles underlying European Union Directive 2001/20/EC
- United States Code of Federal Regulations, Title 21, Part 50 (21CFR50)
- applicable local requirements.

The study will be conducted in compliance with the protocol. The protocol and any amendments and the participant informed consent will receive approval/favorable opinion by Institutional Review Board/Independent Ethics Committee (IRB/IEC), and regulatory authorities according to applicable local regulations prior to initiation of the study.

All potential serious breaches must be reported to Sponsor or designee 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).

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, consent form, participant 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 or product labeling information to be provided to subjects and any updates.

The investigator, Sponsor or designee 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.

COMPLIANCE WITH THE PROTOCOL AND PROTOCOL REVISIONS

The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion of an amendment from the IRB/IEC (and if applicable, also by local health authority) 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 relevant approval/favorable opinion(s) the deviation or change will be submitted, as soon as possible to:

- IRB/IEC for
- Regulatory Authority(ies), if applicable by local regulations (per national requirements)

Documentation of approval/favorable opinion signed by the chairperson or designee of the IRB(s)/IEC(s) and if applicable, also by local health authority must be sent to Roche.

If an amendment substantially alters the study design or increases the potential risk to the participant: (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).

FINANCIAL DISCLOSURE

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

INFORMED CONSENT PROCESS

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.

In situations where consent cannot be given to subjects, their legally acceptable representatives (as per country guidelines) are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which the participant volunteers to participate.

Sponsor or designee 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:

- Provide a copy of the consent form and written information about the study in the language in which the participant is most proficient prior to clinical study participation. The language must be non-technical and easily understood.
- Allow time necessary for participant or participant's legally acceptable representative to inquire about the details of the study.
- Obtain an informed consent signed and personally dated by the participant or the participant's legally acceptable representative and by the person who conducted the informed consent discussion.
- 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.

If informed consent is initially given by a participant's legally acceptable representative or legal guardian, and the participant subsequently becomes capable of making and communicating his or her informed consent during the study, consent must additionally be obtained from the participant.

Revise the informed consent whenever important new information becomes available that is relevant to the participant's consent. The investigator, or a person designated by the investigator, should fully inform the participant or the participant's legally acceptable representative or legal guardian, of all pertinent aspects of the study and of any new information relevant to the participant'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 U.S., the subjects' signed HIPAA Authorization.

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

For minors, according to local legislation, one or both parents or a legally acceptable representative must be informed of the study procedures and must sign the informed consent form approved for the study prior to clinical study participation. The explicit wish of a minor, who is capable of forming an opinion and assessing this 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 must also give their written assent.

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

SOURCE DOCUMENTS

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.

STUDY TREATMENT RECORDS

Records for study treatments (whether supplied by Roche, its vendors, or the site) must substantiate study treatment integrity and traceability from receipt, preparation, administration, and through destruction or return. Records must be made available for review at the request of Roche/designee or a Health Authority.

If	Then
Supplied by Roche (or its vendors):	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 participant, including unique participant identifiers • amount transferred to another area/site for dispensing or storage • nonstudy disposition (e.g., lost, wasted) • amount destroyed at study site, if applicable • amount returned to Roche • retain samples for bioavailability/bioequivalence, if applicable • dates and initials of person responsible for Investigational Product dispensing/accountability, as per the Delegation of Authority Form.
Sourced by site, and not supplied by Roche or its vendors (examples include IP sourced from the sites stock or commercial supply, or a specialty pharmacy)	The investigator or designee accepts responsibility for documenting traceability and study drug integrity in accordance with requirements applicable under law and the SOPs/standards of the sourcing pharmacy. These records should include: • label identification number or batch number • amount dispensed to and returned by each participant, including unique participant identifiers • dates and initials of person responsible for Investigational Product dispensing/accountability, as per the Delegation of Authority Form.

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

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 Sponsor or designee 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 electronic SAE form and Pregnancy Surveillance form, respectively. If electronic SAE form is not available, a paper SAE form can be used. Spaces may be left blank only in those circumstances permitted by study-specific CRF completion guidelines provided by Sponsor or designee.

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, 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. Subinvestigators in Japan may not be delegated the CRF approval task. 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 Sponsor or designee training requirements and must only access the Roche electronic data capture tool using the unique user account provided by Sponsor or designee. User accounts are not to be shared or reassigned to other individuals.

MONITORING

Sponsor or designee representatives will review data centrally to identify potential issues to determine a schedule of on-site visits for targeted review of study records.

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. Certain CRF pages and/or electronic files may serve as the source documents such as quality of life questionnaires.

In addition, the study may be evaluated by Sponsor or designee 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 Sponsor or designee.

RECORDS RETENTION

The investigator (or head of the study site in Japan) 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 or designee, whichever is longer. The investigator (or head of the study site in Japan) must contact Roche prior to destroying any records associated with the study.

Roche or designee will notify the investigator (or head of the study site in Japan) 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, study site, IRB). Notice of such transfer will be given in writing to Roche or designee.

RETURN OF STUDY TREATMENT

For this study, study treatments (those supplied by Roche, a vendor or sourced by the investigator) such as partially used study treatment *kits*, vials and syringes may be destroyed on site.

If	Then
Study treatments supplied by Roche (including its vendors)	Any unused study treatments supplied by Roche can only be destroyed after being inspected and reconciled by the responsible Study Monitor unless study treatments *kits* must be immediately destroyed as required for safety, or to meet local regulations (e.g., cytotoxics or biologics). If study treatments will be returned, the return will be arranged by the responsible Study Monitor.
Study treatments sourced by site, not supplied by Roche (or its vendors) (examples include study treatments sourced from the sites stock or commercial supply, or a specialty pharmacy)	It is the investigator's or designee's responsibility to dispose of all *kits* according to the institutional guidelines and procedures.

It is the investigator's or designee's responsibility to arrange for disposal, 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. The following minimal standards must be 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 *kits*. The method of disposal, 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.

It is the investigator's or designee's responsibility to arrange for disposal of all empty *kits*.

If conditions for destruction cannot be met the responsible Study Monitor will make arrangements for return of study treatments provided by Roche (or its vendors). Destruction of non- study treatments sourced by the site, not supplied by Roche, is solely the responsibility of the investigator or designee.

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
- National Coordinating Investigator
- Study Steering Committee chair or their designee
- Participant recruitment (e.g., among the top quartile of enrollers)
- Involvement in trial design
- Regional representation (e.g., among top quartile of enrollers from a specified region or country)
- Other criteria (as determined by the study team)

The data collected during this study are confidential and proprietary to Sponsor or designee. Any publications or abstracts arising from this study must adhere to the publication requirements set forth in the clinical trial agreement (CTA) governing [Study site or Investigator] participation in the study. These requirements include, but are not limited to, submitting proposed publications to Sponsor or designee at the earliest practicable time prior to submission or presentation and otherwise within the time period set forth in the CTA.

APPENDIX 3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS: DEFINITIONS AND PROCEDURES FOR RECORDING, EVALUATING, FOLLOW UP AND REPORTING

ADVERSE EVENTS

Adverse Event Definition:
An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant 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.

SERIOUS ADVERSE EVENTS

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:
Results in death
Is life-threatening (defined as an event in which the participant 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) NOTE: The following hospitalizations are not considered SAEs in Roche clinical studies: o 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) o elective surgery, planned prior to signing consent o admissions as per protocol for a planned medical/surgical procedure o routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy) o 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)
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 participant 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 drug induced liver injury (DILI) is also considered an important medical event. (See Section 9.2.7 for the definition of potential DILI.)

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

Although pregnancy, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs. (See Section 9.2.5 for reporting pregnancies).

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

EVALUATING AEs AND SAEs

Assessment of Causality
The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). 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.

Assessment of severity
The following categories and definitions of severity as determined by a physician should be used for assessing severity of adverse events: • *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*

Follow-up of AEs and SAEs
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.

OVERDOSE, MEDICATION ERROR, DRUG MISUSE AND DRUG ABUSE

Definition:
Accidental overdose*: accidental administration of a drug in a quantity that is higher than the assigned dose.* ***Intentional overdose****: intentional administration of a drug in a quantity that is higher than the assigned dose.* ***Medication error****: accidental deviation in the administration of a drug (in some cases, a medication error may be intercepted prior to administration of the drug).* ***Drug abuse****: intentional excessive use of a drug that may lead to addiction or dependence, physical harm, and/or psychological harm.* ***Drug misuse****: intentional deviation in the administration of a drug that does not qualify as drug abuse (drug misuse could involve the drug being administered to someone other than the patient).*

- 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; pregnancies on a Pregnancy Surveillance Form (electronic or paper forms).
- The preferred method for SAE data reporting collection is through the eCRF.
- The paper SAE/pregnancy surveillance 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 and pregnancy reporting): Refer to Contact Information list

APPENDIX 4 WOMEN OF CHILDBEARING POTENTIAL DEFINITIONS AND METHODS OF CONTRACEPTION

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILD BEARING POTENTIAL.

Appropriate patient-centered counseling of adolescent trial subjects about contraception and sexual health topics should be provided, including acceptable, highly effective methods as described below.

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

Highly effective methods of contraception have a failure rate of < 1% when used consistently and correctly. *Female* partners of male subjects, who are WOCBP, are expected to use one of the highly effective methods of contraception listed below. Male subjects must inform their female partners who are WOCBP of the contraceptive requirements of the protocol and are expected to adhere to using contraception with their partner. Contraception methods are as follows:

1. Progestogen only hormonal contraception associated with inhibition of ovulation.
2. Hormonal methods of contraception including oral contraceptive pills containing combined estrogen + progesterone, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena®

Male participants with female partners of childbearing potential are eligible to participate if they agree to the following during the treatment and until the end of relevant systemic exposure.

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator.
- Males who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception for the duration of treatment with study treatment(s) plus 5 half-lives of the study treatment [RO7239361; 50 days] plus 90 days (duration of sperm turnover) for a total of 140 days (5 months) post-treatment completion.
- Azoospermic males are exempt from contraceptive requirements; however, use of condom in male subjects *(including azoospermic males) who are sexually active* with a pregnant partner is required.
- Male participants must be willing to refrain from sperm donation during the entire study and for 5 half-lives of study treatment plus 90 days (duration of sperm turnover) 140 days after dosing has been completed.

2. COLLECTION OF PREGNANCY INFORMATION

Guidance for collection of Pregnancy Information and outcome of pregnancy on the Pregnancy Surveillance Form is provided in Section 9.2.5 and the Appendix for Adverse Events and Serious Adverse Events Definitions and procedures for Evaluating, Follow-up and Reporting.