Study Protocol
A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy

Protocol Ref: DSC/11/2357/43

Protocol Date: 01 August 2012 (final)

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16. APPENDICES

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Italfarmaco S.p.A.

STUDY PROTOCOL

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SYNOPSIS

Title of Study:	A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy
Protocol Number:	DSC/11/2357/43
Eudra CT Number	2012-002566-12
Investigators/ Study Centers:	Children will be enrolled at approximately 5 study sites in Italy.
Phase of Development:	2
Objectives:	The primary objective of this study is as follows: • To establish the histologic effects of Givinostat administered chronically at the selected daily dose The secondary objectives of this study are as follows: • To establish the effects of Givinostat administered chronically at the selected daily dose on functional parameters, such as the 6-Minute Walk Test (6MWT), North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL) • To establish the safety and tolerability of Givinostat administered chronically at the selected daily dose in children with Duchenne muscular dystrophy (DMD) • To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as magnetic resonance imaging (MRI), biomarkers, and cytokines
Design:	This is a 2-part, phase 2 study to assess the effects of Givinostat on muscle histologic parameters and on clinical parameters in ambulant children with DMD. The safety, tolerability, and pharmacokinetics of Givinostat will also be assessed. Children who assent to participate in this study (if capable of doing so) and whose parent/guardian signs the informed consent to participate will undergo pre-study screening assessments up to 4 weeks (±2 weeks) before the first scheduled dose of study drug. Approximately 20 children will be enrolled in the study as follows: the first 4 children will be treated at a low dose level of Givinostat (25 mg BID in children who weigh 20–49 kg and 37.5 mg BID in children who weigh

	≥50 kg). If none of the stopping criteria (see Section 3.3.5) are met after 2 weeks of treatment at the low dose, the review team will determine the escalated dose level (i.e., intermediate dose level) to be used for the treatment of an additional 8 children who will be treated at the intermediate dose. The 4 children previously treated at the low dose level will also be switched to the intermediate dose level. If none of the stopping criteria are met after 2 weeks of treatment at the intermediate dose, the review team will determine the subsequent escalated dose level to be used for the treatment of an additional 8 children who will be treated at the high dose. All children treated at the intermediate dose level will be switched to the high dose level. Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the recommended dose (RD) to be used in Part 2 based on the safety and tolerability profile observed and on the pharmacokinetic (PK) analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2). The additional children (if any) will be enrolled during Part 2 of the study and will receive the RD of Givinostat for 12 months. During Part 1 of the study, children will visit the center once each week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 11 visits, including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 2 weeks after the last dose of study drug as well as the protocol-scheduled follow-up visit. Children who have ongoing adverse events at discontinuation will be followed until resolution or stabilization. Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2). Exploratory efficacy parameters will include changes from baseline in MRI results, PK-pharmacodynamic correlations and measures of cytokines and micro ribonucleic acid (miRNA). Safety will be assessed by number of children experiencing adverse events (AEs); type, incidence, and severity of AEs correlated with dose; and measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies, and urinalysis), echocardiographs, pulmonary function tests (PFTs; forced expiratory volume at 1 second [FEV_1], forced vital capacity [FVC], and FEV_1/FVC) and 12-lead ECGs. Plasma samples for PK measurements will be collected after 1 week of treatment with Givinostat, during Part 1 at pre-dose and 1, 2, 4, and 8 hours

	post-dose. PK samples will also be collected during Part 2 of the study as follows: • At Visit 2, blood samples for PK analysis will be taken pre-dose and between 6 and 8 hours post-dose. • At Visit 3, blood samples for PK analysis will be collected pre-dose in the morning before study drug intake and between 0 and 2 hours post-dose. • At Visit 4, blood samples for PK analysis will be taken pre-dose and between 2 and 4 hours post-dose. • At Visit 6, blood samples for PK analysis will be taken pre-dose and between 4 and 6 hours post-dose.
Planned Sample Size:	Approximately 20 children will be enrolled in Part 1 (dose-finding) of this study. *Muscle fibers:* a sample size of 20 children (from Part 1) completing the treatment period should provide 90% power (at a 2-sided alpha level of 5%) to detect at least a 12.5% increase in muscle fiber area % (MFA%) between pre- and post-treatment (which corresponds approximately to a 26% relative increase given the observed mean in the Desguerre publication of 48% [Desguerre I et al. 2009]), the ―worst case‖ standard deviation of 16%, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment. This sample size is based on a paired t-test. In the event the data is not normally distributed, a Wilcoxon signed rank test will be used. After the first 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability observed in the study will be implemented as follows: the within-subject standard deviation of MFA% will be calculated and the final sample size will be adjusted based on this observed standard deviation. A conservative approach will be adopted where the sample size may be increased but not decreased.
Diagnosis and Key Subject Selection Criteria:	Inclusion criteria: 1. Male children aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD. 2. A parent/guardian and child can comply with all study evaluations/procedures and return for all study activities. 3. Able to complete the 2 screening 6MWTs with a minimal distance of at least 250 m each. In addition, the results of these tests must be within ±30 m of each other.

	4. On a stable dose of systemic corticosteroids for at least 6 months. 5. At least 6 months worth of data on the 6MWT (this will be the "historical" 6MWT). From the moment of the historical 6MWT assessment(s), the child must not have received any compound that could potentially affect the 6MWT, with the exception of the stable steroid treatment. 6. Parent/guardian has signed the informed consent form and child has assented to be in the study (if applicable). Exclusion criteria: 1. Initiation of systemic corticosteroid therapy within 6 months prior to the start of study drug or change in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) within 6 months prior to the start of study drug. 2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and integrators will be allowed. 3. Surgery that might have an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study. 4. Exposure to another investigational drug since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment. 5. History of participation in gene therapy, cell-based therapy or oligonucleotide therapy. 6. Presence of other clinically significant disease that in the opinion of the investigator places the child in unacceptable risk for an adverse outcome or that could affect study results. 7. Symptomatic cardiomyopathy or heart failure. If child has a left ventricular ejection fraction <45% at screening, the investigator should discuss inclusion of child in the study with the medical monitor. 8. Inadequate hematological function

	9. Absolute neutrophil count: <1.5 x 10^9/L 10. Platelets: <100 x 10^9/L 11. Current or history of liver disease or impairment, including but not limited to an elevated total bilirubin. 12. Inadequate renal function, as defined by serum creatinine >2 x the upper limit of normal. 13. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening 14. A baseline QTc >450 msec, (as the mean of 3 consecutive readings 5 minutes apart) or history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome). 15. Psychiatric illness/social situations rendering the potential child unable to understand and comply with the study protocol.
Treatments:	The study drug (Givinostat) will be administered as 2 oral doses daily while the child is in the fed state. Children who enroll during Part 1 of the study will receive their assigned dose until the RD is determined. They will then receive up to 12 months of treatment with Givinostat at the RD. Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who enroll during Part 2 will receive up to 12 months of treatment with Givinostat at the RD. The total duration of the study is anticipated to be 15 months.
Main Parameters of Efficacy:	The primary endpoint of this study is change in the value of MFA% comparing the histology biopsies before and after 12 months of treatment with Givinostat. The secondary efficacy endpoints of this study are as follows: • Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA • Change in muscular function after 12 months of treatment with

	Givinostat at the selected daily dose based the PUL
Main Parameters of Safety:	• Number of children experiencing treatment-emergent AEs and serious adverse events (SAEs) • Type, incidence, and severity of treatment-emergent AEs and SAEs correlated with dose
PK Parameters:	Individual Givinostat concentrations tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2.
Exploratory Endpoints:	Change in muscle, fat and fibrosis content after treatment with Givinostat as measured by MRI • Change in muscle biomarkers (e.g., miRNA) and cytokines following treatment with Givinostat • PK–PD correlations
Key Statistical Considerations	Efficacy analyses will be conducted on the ITT population, which is defined as all children who are enrolled in the Part 1 portion or entered the Part 2 portion of the study. All values will be expressed as means ± standard deviation or standard error of the mean. Each of all efficacy endpoints will be subject to normality test and formal statistical test performed will be based on whether it is normally distributed. If normal, statistical differences will be calculated using the paired t test; if not normal, Wilcoxon signed rank test will be used to assess the statistical significance of differences between groups. $P<0.05$ will be set as significant. *General considerations:* Data will be provided in data listings sorted by treatment group and child number. Tabular summaries will be presented by treatment group for Part 1. Categorical data will be summarized by the number and percentage of children in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided, unless otherwise stated. Details of endpoint analyses will be described in the SAP. For analyses of change from baseline, baseline will generally be defined as the value collected before any Givinostat treatment is administered. If this value is missing, the last non-missing value prior to dosing will be used. Otherwise, missing observations will be treated as missing at random, and no data imputation will be performed. All data from the case report forms,

	as well as any derived variables, will be presented in data listings. All hypothesis tests will be two-sided with a 5% significance level, and 95% CIs will be used, unless stated otherwise. As this is a Phase 2 exploratory study, no adjustments for multiplicity will made.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

6MWT	6-Minute Walk Test
Ab	Antibodies
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the plasma–concentration time curve
BID	twice daily
BUN	blood urea nitrogen
CI	confidence interval
CL	clearance
CL/F	volume of distribution
cm	Centimeter
C_{max}	maximum plasma concentration
CMV	Cytomegalovirus
CPK	creatine phosphokinase
CrCl	creatinine clearance
CRP	C-reactive protein
CSA	cross-sectional area
CSOM	Clinical Study Operations Manual
DMD	Duchenne muscular dystrophy
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ECHO	Echocardiograph
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EOS	end of study
FACS	fluorescence-activated cell sorting
FAP	fibroadipongenic progenitors
FEV_1	forced expiratory volume at 1 second
FU	follow up
FVC	forced vital capacity
GI	Gastrointestinal
h	hour
H&E	hematoxylin and eosin
HbeAg	hepatitis B e antigen
HbsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HDAC	histone deacetylase
HIV	human immunodeficiency virus
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee

IgM	immunoglobulin M
IL	interleukin
IMP	investigational medicinal product
ITT	intent to treat
JIA	juvenile idiopathic arthritis
KA	absorption rate constant
kg	kilogram
L	liter
LDH	lactate dehydrogenase
m	Meter
MFA%	muscle fibers area %
mg	Milligram
miRNA	micro ribonucleic acid
mL	Milliliter
MRI	magnetic resonance imaging
msec	Millisecond
MTD	maximum-tolerated dose
MuSC	muscle satellite cells
ng	nanogram
nmol	nanomole
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
PD	Pharmacodynamic
PDGFR	platelet-derived growth factor receptor
PET	polyethylene terephthalate
PFT	pulmonary function tests
PK	Pharmacokinetic
PUL	performance of upper limb
QT	QT interval
QTc	QT interval – corrected
RBC	red blood cells
RD	recommended dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOJIA	systemic onset juvenile idiopathic arthritis
SWI/SNF	switch/sucrose non-fermentable
TNF-α	tumor necrosis factor-alpha
V/F	plasma volume
V2/F	peripheral volume
WBC	white blood cells
WCT	Worldwide Clinical Trials

1 Introduction

1.1 Background on Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD) is the most common childhood muscular dystrophy, occurring in about 1 of every 3500 male newborns. The disease is inherited as an X-linked recessive disorder and is caused by mutation in the dystrophin gene on the X chromosome, resulting in dystrophin deficiency. The main function of dystrophin is to stabilize and link the muscle fiber cytoskeleton to the membrane. The lack of functional dystrophin results in the loss of dystrophin-glycoprotein complex, thereby rendering the muscle fibers less resistant to mechanical stress.

Faulty muscle structure caused by the absence of extracellular or intracellular structural proteins results in cell membrane instability, initiating a cascade of deleterious events, such as uncontrolled calcium influx, apoptosis and necrosis, inflammation, and replacement of muscle with fibrotic tissue and fat (Consalvi S et al. 2011). The clinical effect of this deficiency can be dramatic and fatal.

Patients begin to show symptoms of the disease between the ages of 3 to 5 years (Emery AEH 2002), which leads to severe muscle wasting and weakness. Patients with DMD usually stop walking by about 12 years of age and usually experience fatal respiratory failure in their early 20s (Eagle M et al. 2002).

Treatment with steroids is currently used in a large portion of DMD patients, but it is palliative and complicated by serious side effects. No current treatment interrupts or halts the progression of DMD.

By acting on muscle resident stem cells, histone deacetylase (HDAC) inhibitors increase skeletal myogenesis in vitro and in vivo (Iezzi S et al. 2002) and restore normal muscle morphology and increase the size and strength of myofibers in *mdx* mice a preclinical model of DMD (Minetti GC et al. 2006).

The beneficial potential of the HDAC inhibitor Givinostat in the treatment of DMD has been studied in the *mdx* mouse disease model. In this model, long-term exposure to Givinostat effectively countered disease progression. In particular, Givinostat dose and concentration dependently increased the cross-sectional area of myofibers, decreased the cellular (inflammatory) infiltrate and prevented the formation of fibrotic scars. Pharmacokinetic (PK)–pharmacodynamic (PD) analysis suggests that exposures of Givinostat of 600 h*nmol/L are required to exert the beneficial effect.

1.2 Background on Givinostat

Givinostat has been tested in long-term repeat-dose toxicology studies in rats, dogs, and monkeys. The main adverse effects (e.g., reduction in white blood cells [WBC], reduced thymus weight, bone marrow atrophy, and liver and possibly kidney function impairments) were observed only at high doses of drug. Most of these changes returned to baseline levels upon drug discontinuation or a trend toward resolution was seen at the end of the recovery period.

In the rat, Givinostat was administered consecutively up to 26 weeks where the no observed adverse effect level (NOAEL) was 10 mg/kg/day. There were no side effects in dogs administered 12.5 mg/kg/day of Givinostat for 4 consecutive weeks. However, liquid feces were found in the animals receiving higher doses with consequent reduced absorption. For these reasons, the dog species was not further considered and general toxicology studies in non-rodents were continued in the monkey. In the monkey, Givinostat was well tolerated and similar toxicity profiles were seen in the 4-, 13-, and 39-week studies, where the NOAELs were 10 mg/kg, 10 mg/kg, and 12 mg/kg, respectively.

Givinostat had a favorable safety pharmacology profile. There was no observation of embryo-fetal toxicity in rats and in rabbits, or potential genotoxicity in mammalian cells in vitro and in vivo. The only sign of a possible adverse effect was seen at relatively high doses (>1μM) of Givinostat in vitro but not in vivo in cardiovascular safety pharmacology studies.

Givinostat was given by oral gavage to juvenile rats starting at the age of weaning (25 days of age) at 4 different dosages: 0, 20, 60, or 180 mg/kg/day once-a day for 4 weeks. Givinostat was well tolerated at the dosage up to 60 mg/kg/day. Treatment-related changes were detected at 180 mg/kg/day in the adrenals, bone marrow, liver, and spleen. The changes had resolved or showed partial recovery at the end of the study (mature animals). The NOAEL was set at 60 mg/kg/day.

Givinostat has been tested in a number of clinical studies that enrolled for the following major indications: inflammation and oncology. In particular:

- 105 healthy volunteers have been enrolled in 3 phase 1 studies (single dose, repeat dose, and food interaction); 18 were treated with placebo.
- 422 patients have been enrolled in the phase 1–2 studies; 62 were treated with placebo. Of these, 33 were children with systemic onset juvenile idiopathic arthritis (SOJIA) or polyarticular juvenile idiopathic arthritis (JIA) treated with Givinostat at the following doses: 0.50 mg/kg twice daily (BID) and 0.75 mg/kg BID for up to 3 months.

Givinostat has shown preliminary signs of clinical activity in subjects with SOJIA (study DSC/05/2357/19) as well as myeloproliferative disease (study DSC/07/2357/28).

The maximum-administered dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in subjects with multiple myeloma. Doses up to approximately 100 mg BID were generally well tolerated. The most common adverse events (AEs) observed were thrombocytopenia as well as gastrointestinal (GI) toxicities. AEs were generally mild to moderate and reversible upon discontinuation of study drug. In particular, thrombocytopenia appeared in just a few days of treatment at thrombocytopenic doses and reverted to normal values within a few days/weeks after treatment discontinuation. Twenty-one AEs of QT interval – corrected (QTc) prolongations have been reported. In one case, the electrocardiogram (ECG) was manually read and the QTc value determined was normal, thus it was considered a spurious finding. Eight AEs of QTc prolongation were reported in subjects with chronic myeloproliferative diseases (study DSC/07/2357/28), 6 in subjects with Hodgkin‘s lymphoma (study DSC/07/2357/26), 2 in subjects with polycythemia vera (study DSC/08/2357/38), 2 in subjects with acute myeloid leukemia (study DSC/05/2357/15), 1 in a subject with Crohn‘s disease (study DSC/06/2357/23), and 1 in a subject with SOJIA (study DSC/05/2357/19). No clear dose dependence was observed. In the 2 studies in healthy volunteers (DM/00/2357/01 and DM/00/2357/03) where ECG and QTc measurements were systematically assessed, no episode of QTc prolongation was observed. Details of each trial are included in the Investigator‘s Brochure (2012).

1.3 Rationale

1.3.1 Study Rationale

Different studies suggest that histone acetylation has a significant role in the pathogenesis of DMD and that inhibition of HDAC leads to a reduction in inflammation and fibrosis and an increase in muscle regeneration. In particular, two preclinical studies with Givinostat have shown that chronic treatment with this compound in a DMD mouse model (*mdx* mouse) determines a dose- and concentration-dependent reduction in inflammation and fibrosis and an increase in muscle regeneration, which in turn determines an improvement in muscular function.

The primary objective of this study is to replicate these findings in humans. In particular, the primary objective of the study will be to demonstrate that Givinostat stimulates muscle regeneration by detecting an increase in the fraction of muscle biopsy occupied by muscle when comparing the muscle biopsy at study end *versus* the muscle biopsy at study start. Other objectives of the study will be to evaluate the effects of Givinostat on other histological parameters (inflammation, necrosis, fibrosis), on functional parameters

such as the 6-Minute Walk Test (6MWT), and on magnetic resonance imaging (MRI) and biomarkers. Finally, the safety and tolerability and pharmacokinetics of Givinostat will also be assessed.

As the currently used dose of Givinostat is considered not sufficient to exert the expected positive effects (see dose rationale), the first part of the study will be used to escalate the dose to one yielding exposures expected to be efficacious and which are well tolerated.

To ensure an adequate assessment of the safety, efficacy, and PK parameters in this study, and considering the expected mechanism of action of Givinostat in DMD, ambulant children aged 7 to <11 years, who have been on a stable steroid dose for at least the last 6 months and who have a 6MWT assessment performed at least 6 months before screening will be selected for participation in this study.

1.3.2 Dose Rationale

Until now, Givinostat has been administered to 87 healthy volunteers and 360 patients enrolled in 17 phase 1–2 studies. Of the 360 patients, 33 were children/adolescents treated with either Givinostat 0.5 mg/kg BID or 0.75 mg/kg BID. The maximum dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in subjects with multiple myeloma. Subjects have been treated in clinical trials with daily doses of Givinostat for up to 6 months. Moreover, 31 patients with myeloproliferative diseases enrolled in a compassionate use program have been receiving givinostat at doses up to 50mg t.i.d for a period up to 4 years and 1 adolescent with JIA enrolled in the extension study has been receiving Givinostat 0.75 mg/kg since December 2011.

Doses of Givinostat up to approximately 100 mg BID have generally been well tolerated. At higher doses of Givinostat, transient reductions hematological parameters (particularly platelets) and diarrhoea as well as nausea and vomiting were observed. AEs were generally mild to moderate and reversible upon discontinuation of study drug (Investigator‘s Brochure 2012). In particular, thrombocytopenia appeared in just a few days of treatment at thrombocytopenic doses and reverted to normal values within a few days/weeks after treatment discontinuation. Twenty AEs of QTc prolongation have been reported and confirmed in the clinical studies. Of these, only 1 episode of QTc prolongation was observed in a pediatric study. No episode of QTc prolongation was observed in the 2 studies of Givinostat in healthy volunteers where ECG and QTc measurements were systematically assessed.

A population PK analysis was conducted using the PK data collected so far in all the clinical trials where PK samples were collected. In particular, PK data from 7 studies have been included (single- and repeat-dose studies in healthy volunteers, 2 studies in

Crohn's disease subjects, 1 study in subjects with SOJIA, 1 study in subjects with JIA, and 1 study in subjects with psoriasis) for a total of 226 subjects. Givinostat pharmacokinetics were described using a 2-compartment model with first order absorption showing clearance (CL/F)=118 L/h, plasma volume (V/F)=155 L, peripheral volume (V2/F)=514 L, and absorption rate constant (KA)=0.243 h^{-1}. The covariate screening indicated weight and steroid co-administration as the most significant covariates on clearance (CL) parameters (no gender, formulation, age, or healthy volunteer vs. patient differences). However, the covariates effects were considered limited on pharmacokinetics (i.e., the presence of steroids was found to determine an increase of <30% in the population CL, and an increase of 10 kg was found to determine an increase of 10%–15% in CL).

The mean exposure in healthy volunteers treated with Givinostat 100mg BID was 1083 ng*h/mL (area under the plasma–concentration time curve from 0 to 12 hours [AUC_{0-12}]) and 181.5 ng/mL (maximum plasma concentration [C_{max}]). The estimated AUC_{0-12} and C_{max} in children treated with Givinostat are reported in Table 1.

Table 1 Concentrations of Givinostat in Pediatric Studies to Date

Disease State		AUC_{0-12} (ng*h/mL)	C_{max} (ng/mL)
SOJIA	Median 5^{th} percentile 95^{th} percentile	234.0 126.4 428.7	31.5 20.0 55.3
JIA	Median 5^{th} percentile 95^{th} percentile	206.4 109.0 492.7	31.0 14.9 69.8

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; C_{max}=maximum plasma concentration; SOJIA=systemic onset juvenile idiopathic arthritis; JIA=juvenile idiopathic arthritis.

Preclinical studies in a mouse model of DMD (*mdx* mouse model) suggest that daily exposures (AUC_{0-24}) of 600 nmol*h/L, i.e., approximately 300 ng*h/mL, are needed to exert beneficial histological and functional effects. As shown in Table 1, doses higher than those administered so far to children are needed to ensure that the majority of children are treated with doses that allow such exposures. Therefore, the first part of the study will escalate the dose to a maximum tolerated dose (MTD) that will then be recommended for the second part of the study.

Because of the limited effect of weight on CL only 2 dose adjustments will be applied as follows: children who weigh 20–49 kg: Dose X; children who weigh ≥50 kg: Dose 1.5X.

The starting dose will be 25 mg BID in children who weigh 20–49 kg and 37.5 mg BID in children who weigh ≥50 kg. Table 2 reports the expected median, 5th, and 95th percentile exposures in children weighing 20, 30, 40, 50, and 60kg.

Table 2 Expected Exposure by Weight

Weight (kg)	**20**	**30**	**40**	**50**	**60**
AUC_{0-12} (ng h/mL)					
Median	263	223	203	283	257
5th percentile	127	107	97	132	123
95th percentile	564	466	426	603	542

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours

Expected exposures fall within the range of exposures safely tested in children with SOJIA and JIA and well within the exposures in adults treated with 100 mg BID.

If safety and tolerability of the starting dose is confirmed in the first group of children treated, the dose will be escalated in the second group of children enrolled. The children treated at the lower dose will also be switched to the escalated dose level. Similarly, if the safety and tolerability of the second dose level is confirmed, the dose will be further escalated in the third group of children enrolled, and the children treated at the second dose level will be switched to the higher dose level.

Dose escalation will be decided based on the safety and tolerability profile observed, and on the PK analyses in the children treated until a dose-escalation decision is made. In any case each dose escalation should not yield more than a doubling of the expected exposure. An example of dose escalation based on the data available so far is provided in Table 3, Table 4, and Table 5 show the starting, second, and third dose, respectively.

Table 3 Starting Dose (25 mg BID in Children Who Weigh 20–49 kg and 37.5 mg BID in Children Who Weigh ≥50 kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	263	223	203	283	257
5th percentile	127	107	97	132	123
95th percentile	564	466	426	603	542

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Table 4 Second Dose (50 mg BID in Children Who Weigh 20–49 kg and 75 mg BID in Children Who Weigh ≥ 50kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	527	445	407	566	515
5th percentile	253	215	194	263	246
95th percentile	1129	932	852	1205	1084

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Table 5 Third Dose (75 mg BID in Children Who Weigh 20–49 kg and 100 mg BID in Children Who Weigh ≥50 kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	790	668	610	755	686
5th percentile	381	322	290	351	329
95th percentile	1693	1398	1278	1607	1446

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Once all 20 children enrolled in Part 1 of the study have been treated for at least 2 weeks, the review team will decide the recommended dose (RD) to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses.

All children will then switch to the RD, which will be administered for the subsequent 12 months of the study (Part 2).

This clinical study has been designed to comply with the Good Clinical Practice guidelines.

2 Study Objectives

Primary Objective

The primary objective of this study is as follows:

- To establish the histologic effects of Givinostat administered chronically at the selected daily dose

Secondary Objectives

The secondary objectives of this study are as follows:

- To establish the effects of Givinostat administered chronically at the selected daily dose on functional parameters, such as the 6MWT, North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL)
- To establish the safety and tolerability of Givinostat administered chronically at the selected daily dose in children with DMD
- To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as MRI, biomarkers, and cytokines

Primary Endpoint

The primary endpoint of this study is as follows:

- Change in the value of muscle fiber area % (MFA%) comparing the histology biopsies before and after 12 months of treatment with Givinostat

Secondary Efficacy Endpoints

The secondary efficacy endpoints of this study are as follows:

- Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA

- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based the PUL

Safety Endpoints

The safety endpoints of this study are as follows:

- Number of children experiencing treatment-emergent AEs and serious AEs (SAEs)
- Type, incidence, and severity of treatment-related AEs and SAEs
- Measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies), echocardiographs (ECHOs), pulmonary function tests (PFTs; forced expiratory volume at 1 second [FEV_1], forced vital capacity [FVC], and FEV_1/FVC), and 12-lead ECGs.

Pharmacokinetic Endpoints

The PK endpoints of this study are as follows:

- Individual Givinostat concentrations tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2.

Exploratory Endpoints

The exploratory endpoints of this study are as follows:

- Change in muscle, fat and fibrosis content after treatment with Givinostat as measured by MRI
- Change in muscle biomarkers (e.g., miRNA) and cytokines following treatment with Givinostat
- PK–PD correlations

3 Investigational Plan

3.1 Description of Overall Study Design and Plan

This is a 2-part, phase 2 study to assess the effects of Givinostat on muscle histologic parameters and on clinical parameters in ambulant children with DMD. The safety, tolerability, and pharmacokinetics of Givinostat will also be assessed.

Children who assent to participate in this study (if capable of doing so) and whose parent/guardian signs the informed consent to participate will undergo pre-study screening assessments up to 4 weeks (±2 weeks) before the first scheduled dose of study drug.

Approximately 20 children will be enrolled in the study as follows: the first 4 children will be treated at a low dose level of Givinostat (25 mg BID in children who weigh 20-49 kg and 37.5 mg BID in children who weigh ≥50 kg).

If none of the stopping criteria (see Section 3.3.5 for stopping criteria) are met after 2 weeks of treatment at the low dose, the review team will determine the escalated dose level (i.e., intermediate dose level) to be used for the treatment of an additional 8 children who will be treated at the intermediate dose. The 4 children previously treated at the low dose level will also be switched to the intermediate dose level.

If none of the stopping criteria are met after 2 weeks of treatment at the intermediate dose, the review team will determine the subsequent escalated dose level to be used for the treatment of an additional 8 children who will be treated at the high dose. All children treated at the intermediate dose level will be switched to the high dose level.

Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the RD to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2).

After the 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability of MFA% observed in the study will be implemented, the within-subject standard deviation of MFA% will be calculated, and the final sample size will be adjusted based on this interim analysis and in particular on the observed standard deviation. A conservative approach will be adopted, where the sample size may be increased but not decreased.

The additional children (if any) will be enrolled during Part 2 of the study and will receive the RD of Givinostat for 12 months.

A treatment table for the study is presented in Table 6.

Table 6 Treatment Table

	PART 1						PART 2									
	Treatment Visit[c]						Treatment Visit[c]									
Subject	**1**	**2[a]**	**3**	**4[a]**	**5**	**6[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[b]**
1–4																
5–12																
13–20																
Additional children (if any)																

[a] At the end of Week 2 (Visit 2) at every dose level, a safety check is foreseen.
[b] At the end of Month 12, an efficacy analysis on biopsy results and functional tests is foreseen.
[c] The visits during Part 1 will be performed every 7 days (±1 day); the visits during Part 2 will be performed periodically every 1 – 1.5 months (± 7 days).

Legend:

	25 – 37.5 mg BID – low dose level
	Intermediate dose level
	High dose level
	Recommended dose level

During Part 1 of the study, children will visit the center once a week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 10 visits, not including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 1 week after the last dose of study drug. Children who have ongoing AEs at discontinuation will be followed until resolution or stabilization.

Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2). Exploratory efficacy parameters will include changes from baseline in MRI results, PK/PD correlations, and measures of cytokines and miRNA. Safety will be assessed by number of children experiencing treatment-emergent AEs and SAEs; type, incidence, and severity of treatment-emergent AEs and SAEs; and measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies), ECHOs, PFTs (FEV_1, FVC, and FEV_1/FVC), and 12-lead ECGs.

Plasma samples for PK measurements will be collected after 1 week of treatment with givinostat, during Part 1: at pre-dose and 1, 2, 4, and 8 hours post-dose.

PK samples will also be collected during Part 2 of the study. At Visit 2, blood samples for PK analysis should be taken pre-dose and between 6 and 8 hours post-dose. At Visit 3, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and between 0 and 2 hours post-dose. At Visit 4, blood samples for PK analysis should be taken pre-dose and between 2 and 4 hours post-dose. At Visit 6, blood samples for PK analysis should be taken pre-dose and between 4 and 6 hours post-dose.

3.2 Selection of Study Population

This study will enroll approximately 20 ambulatory male children with an established diagnosis of DMD who are at least 7 years of age but <11 years of age. Additional children could be enrolled in the second part of the study, after the interim evaluation of baseline biopsies if the observed variability is higher than the one used for the current sample size estimate. Children will be enrolled at approximately 5 study sites in Italy. Specific entry criteria are detailed in Section 3.2.1 and Section 3.2.2.

3.2.1 Inclusion Criteria

Children meeting all of the following inclusion criteria are eligible for inclusion in the study:

1. Male children aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD.
2. Parent/guardian has signed the informed consent form and child has assented to be in the study (if applicable).
3. Able to complete the 2 screening 6MWTs with a minimal distance of at least 250 m each. In addition, the results of theses 2 tests must be within ±30 m of each other.
4. On a stable dose of systemic corticosteroids for at least 6 months.
5. At least 6 months worth of data on the 6MWT (this will be the ‑historical‖ 6MWT). From the moment of the historical 6MWT assessment(s), the child must not have received any compound that could potentially affect the 6MWT, with the exception of the stable steroid treatment.
6. A parent/guardian and child can comply with all study evaluations/procedures and return for all study activities.

3.2.2 Exclusion Criteria

Children meeting any of the following exclusion criteria will not be enrolled in the study.

1. Initiation of systemic corticosteroid therapy within 6 months prior to the start of study drug or change in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) within 6 months prior to the start of study drug.
2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and integrators will be allowed.
3. Surgery that might have an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study.
4. Exposure to another investigational drug since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment.
5. History of participation in gene therapy, cell-based therapy or oligonucleotide therapy.
6. Presence of other clinically significant disease that in the opinion of the investigator places the child in unacceptable risk for an adverse outcome or that could affect study results.
7. Symptomatic cardiomyopathy or heart failure. If child has a left ventricular ejection fraction <45% at screening, the investigator should discuss inclusion of child in the study with the medical monitor.
8. Inadequate hematological function
9. Absolute neutrophil count: $<1.5 \times 10^9/L$
10. Platelets: $<100 \times 10^9/L$
11. Current or history of liver disease or impairment, including but not limited to an elevated total bilirubin.
12. Inadequate renal function, as defined by serum creatinine >2 x the upper limit of normal.

13. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening

14. A baseline QTc >450 msec, (as the mean of 3 consecutive readings 5 minutes apart) or history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome).

15. Psychiatric illness/social situations rendering the potential child unable to understand and comply with the study protocol.

3.2.3 Removal of Children from Therapy or Assessment

A child will be considered to have completed the study when he completes the 12-month/end-of-study visit (Visit 10) in Part 2. If a child is discontinued at any time after entering the study, the investigator will make every effort to see the child and complete the early termination and follow-up assessments as shown in Section 3.4.5. All adverse events should be followed until the child recovers or his condition stabilizes.

A termination electronic case report form (eCRF) should be completed for every child who receives study drug, whether or not the child completes the study. The reason for any early discontinuation should be indicated on this form. The primary reason for a child withdrawing prematurely should be selected from the following standard categories of early termination:

- *Adverse Event (Adverse Reaction)*: Clinical or laboratory events occurred that, in the medical judgment of the investigator for the best interest of the child, are grounds for discontinuation. This includes serious adverse events (SAEs) and non-serious AEs, regardless of relation to study drug.
- *Death:* The child died.
- *Withdrawal of Consent*: The child or his parent/guardian desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If the child or parent/guardian gave a reason for withdrawing, it should be recorded in the eCRF.
- *Protocol Violation*: The child‘s findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g., drug noncompliance, failure to return for defined number of visits). The violation necessitated premature termination from the study.
- *Lost to Follow-Up*: The child stopped coming for visits, and study personnel were unable to contact the child.

- *Other*: The child was terminated for a reason other than those listed above, such as theft or loss of study drugs or termination of study by sponsor.

3.3 Treatments

3.3.1 Details of Study Treatment

Details about study drug are provided in Table 7.

Table 7 Details of Study Treatment

	Givinostat^
Drug name	Givinostat
Manufacturer	Italfarmaco S.p.A.
Dose(s)	1st dose level: 25 mg (children 20–49 kg) 37.5 mg (children ≥50 kg) 2nd dose level: 50 mg (children 20–49 kg) 75 mg (children ≥ 50 kg)* 3rd dose level: 75 mg (children 20–49 kg) 100 mg (children ≥ 50 kg)*
Dose frequency	BID
Route	Oral under fed conditions
Formulation	Oral suspension and/or capsules

^Givinostat is used to indicate the whole study drug name Givinostat hydrochloride monohydrate. The dosages / concentrations of the study drug are expressed as Givinostat hydrochloride monohydrate.
* Estimated doses based on current knowledge.
BID=twice daily

3.3.2 Dosage Schedule

Study drug will be administered as 2 oral doses daily while the child is in the fed state. Children who enroll during Part 1 of the study will receive their assigned dose until the RD is determined. They will then receive up to 12 months of treatment with Givinostat

at the RD. Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who enroll during Part 2 will receive up to 12 months of treatment with Givinostat at the RD.

3.3.3 Treatment Assignment

All children will receive study drug. Children enrolled in Part 1 of the study will start to take either the low, intermediate, or high dose, depending on the needs of the study at the time of enrollment and they will switch to the RD when the review team decide that this dose is safe (see Table 6).

When the safety review team has determined the RD, all children currently on study drug will be switched to that dose (the RD), and Part 2 of the study will commence. All children who enroll during Part 2 (if applicable) of the study will be given the RD of Givinostat.

3.3.4 Drug Packaging, Labeling, Storage, Dispensing, Investigational Medicinal Product Accountability, and Blinding

Drug Packaging - Oral Suspension

The primary packaging will consist of an amber plastic bottle containing the suspension. The secondary packaging will be a carton box containing 1 amber bottle and a syringe dosing system for dispensing the suspension.

All the bottles will contain 120 mL of suspension.

Drug Packaging - Capsules

Capsules will be supplied as 50 mg hard gelatin capsules for oral administration in white plastic bottles containing 30 capsules each.

Labeling

The primary and secondary labels will show all the information requested according to the Annex 13 of the Good Manufacturing Practice. Bottles containing Givinostat will be labeled in local language (i.e., Italian).

Storage

The investigational medicinal product (IMP; Givinostat) will be stored at Italfarmaco until distribution to the investigational sites. The investigational site will store the IMP under the conditions specified in the label (i.e., 5 ±3°C for the oral suspension and <30°C for the capsules), ensuring that it is not accessible to unauthorized persons until it is dispensed to the child‘s parents/legal guardians.

Dispensing

The investigational site will be supplied initially with a congruous number of suspension bottles and capsule bottles, in order to have sufficient supply of study drug to treat the children who are enrolled in the study.

All IMP supplies are to be used only for this protocol and for no other purpose.

The investigator will be responsible for the delivery of IMP to the child‘s parents/legal guardians according to the protocol.

Children will be administered the IMP on an outpatient basis.

At each scheduled visit, the investigator will supply the children with the appropriate number of suspension bottles and/or capsules bottles, sufficient to cover the treatment until the following visit (i.e., 1 week of treatment during Part 1 of the study and 1 to 1.5 months, during Part 2 of the study).

Italfarmaco will provide to the investigator a table with volume of suspension and/or number of capsules to be administered according to body weight and dose level.

The investigator will provide to the child‘s parent/legal guardian written instruction on the dosage and corresponding volume in milliliters of suspension and/or number of capsules to be taken at each administration. Refer to the relevant Clinical Study Operations Manual (CSOM) for more detailed information.

Blinding

Not applicable. This is an open-label study.

Emergency Procedure for Unblinding

Not applicable. This is an open-label study.

3.3.5 Dose Modifications

3.3.5.1 Child Stopping and Dose Reduction Safety Rules

Permanent Stopping Rule

Study drug should be permanently stopped if any of the following occur:

- Severe diarrhea (i.e., increase of ≥7 stools per day)
- Any drug-related SAE
- QTc >500 msec
- Platelets ≤50 x 10^9/L

Temporary Stopping and Dose Reduction Safety Rule

Study drug should be temporarily stopped if any of the following occur:

- Platelets ≤75 x 10^9/L but >50 x 10^9/L
- Moderate diarrhea (i.e., increase of 4 to 6 stools per day)

Study drug may be resumed at a reduced dose level once the event resolves. Such a dose reduction can happen only once per child. The treatment can be temporarily interrupted for a maximum of 4 weeks. If the child has not recovered from the AE after this period, the treatment should be permanently discontinued.

If a child has a medical event not necessarily drug related that requires interruption of study drug dosing for >4 weeks, the review team will determine if the child may resume study drug treatment.

Cohort Expansion Safety Rules

The review team can decide to expand the first cohort of children and enroll additional 4 children (for a total of 8 children treated at the first dose level), on the basis of safety, tolerability, and PK results in the children treated in that cohort, according but not limited to the following rules:

- If 1 child experiences any type of stopping criterion (as defined in the permanent and temporary stopping rules), or
- If 2 children experience different types of stopping criterion (e.g., 1 child experiences hematological toxicity and another child experiences GI toxicity).

3.3.5.2 Cohort Abandoned Safety Rules

The review team can also decide to abandon a given dose level on the basis of safety, tolerability and PK results in the children treated at that dose level, according to but not limited to the following rules:

- If >2 children experience any type of stopping criterion, or
- If ≥2 or more children experience the same type of stopping criterion

3.3.5.3 Cohort Dose Escalation Safety Rules

During Part 1 of the study, each child will receive study drug at a specific dose level. Once the first 4 children have been treated for at least 2 weeks, the review team will examine safety and tolerability data and PK results and decide if the children can be switched to the escalated dose level and if the second group of children can start the treatment at the escalated dose level.

After a further 2 weeks, the review team will decide if the already treated children at the lower and intermediate dose levels can switch to the highest dose and if the third and last group of children can start the treatment at the highest dose level.

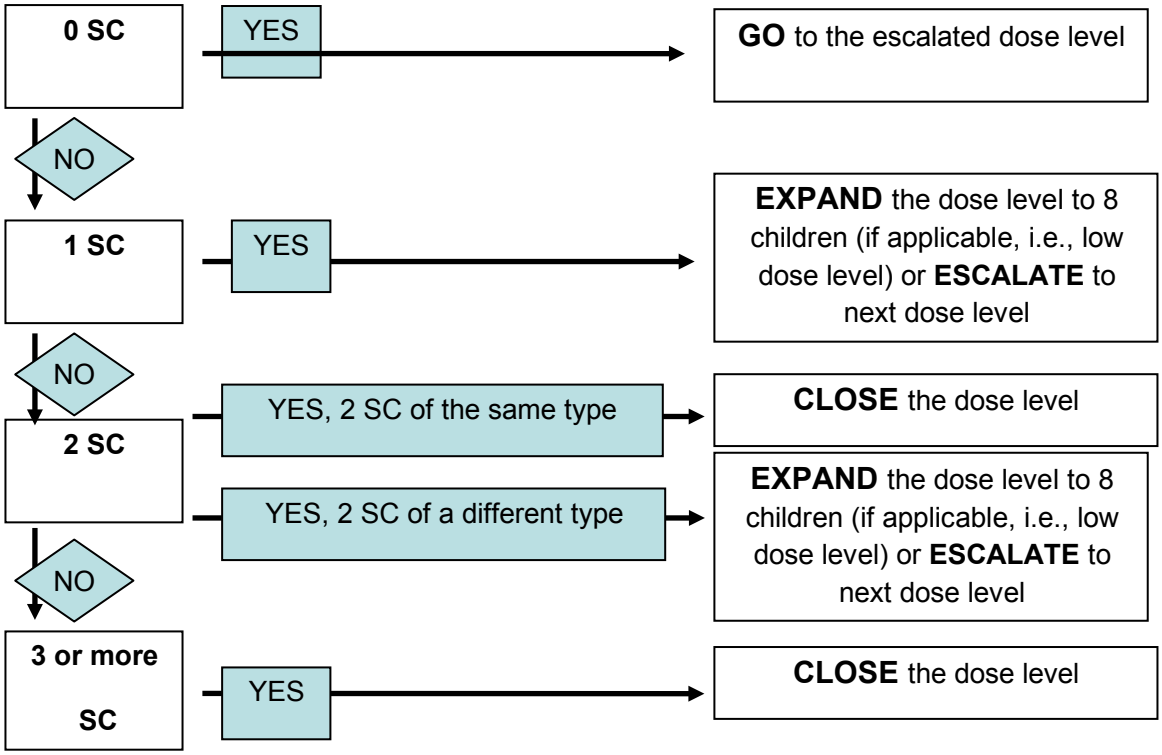
The children should be switched to the escalated dose level (i.e., intermediate or high dose level) only if no or only 1 stopping criterion per dose level occurred.

For more details, see Figure 1.

3.3.5.4 Study Stopping Safety Rules

If 2 or more stopping criteria occur in the lower dose, the study will be temporarily stopped to allow a reassessment of the risks and benefits of the compound.

Figure 1 Cohort expansion, Cohort Abandoned and Dose Escalation Safety Rules for Part 1



SC=stopping criterion, as defined under permanent and temporary stopping rules.

The review team will include: the Principal Investigators, the Study Chair, the Medical Monitor and other Italfarmaco representatives.

3.3.6 Treatment Compliance and IMP Accountability

At each visit, the child‘s parents/legal guardians will bring back to the study site all the bottles previously received (used, partially used, and unused) and receive a new supply of the IMP.

All unused IMP will be not re-dispensed by the investigator to another child.

The investigator will maintain accurate records of the disposition of all IMP received, distributed to children (including date and time), and accidentally lost or destroyed.

When dispensing the bottles to the children, the investigator will attach the relevant tear-off label to the relevant form.

Oral Suspension Accountability

The residual volume of suspension in the bottles will be measured by the investigator by means of a calibrated glass cylinder supplied by Italfarmaco and reported in the Drug Accountability Form and in the eCRF.

Capsule Accountability

The investigator will count the capsules unused for each bottle and insert the number in the Drug Accountability Form and in the eCRF.

Periodically throughout and at the conclusion of the study, a representative of Italfarmaco S.p.A. or its delegate will conduct an inventory of all study drug supplies and the bottles of oral suspension and/or capsules used, partially used, and unused will be destroyed at the site, if possible, or sent back to Italfarmaco.

Missed doses are not to be recovered and they should be recorded in the eCRF and in the Drug Accountability Form, specifying the reason for any missed dose.

For more detailed procedures, please refer to the relevant CSOM.

3.3.7 Prior and Concomitant Illnesses and Treatments

Prior and Concomitant Illnesses

Investigators should document all significant illnesses that the child has experienced within 6 months of screening. Additional illnesses present at the time informed consent is given are to be regarded as concomitant illnesses. Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs in the eCRF.

Prior and Concomitant Treatments

Prior treatments, defined as those taken within 6 months prior to screening, should be recorded in the eCRF as prior medications.

Concomitant treatments are defined as treatments taken after study drug administration.

Children should be on stable systemic corticosteroid therapy for at least 6 months prior to initiation of study drug. That is, there have been no changes in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) during the previous 6 months.

Supportive treatments, such as anti-emetics, anti-diarrheals, anti-pyretics, anti-allergics, analgesics, and antibiotics are allowed.

Use of Vitamin D, calcium and integrators if clinically indicated before enrolment and for duration of the trial are allowed.

The following medications are prohibited prior to (as noted below) and during study treatment:

- Other investigational agents within 3 months of start of study drug, since time historical 6MWT data was obtained, or while on study
- Prior gene therapy or cell-based therapy or oligonucleotide therapy prior to study treatment or while on study
- Any pharmacologic treatments (other than stable doses of corticosteroids) that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). or while on study
- Drugs that prolong the QTc interval (see Appendix 1: Drugs Known to Prolong the QTc Interval)

3.4 Assessments

3.4.1 Schedule of Assessments

The procedures to be performed during the study are outlined in the Schedule of Assessments (Table 8 and Table 9). A detailed description of each assessment may be found in Section 3.4.2.

Table 8 Schedule of Assessments: Part 1 (Dose Escalation)

Assessment	Screen/ Pre-Study[a]	Part 1 (Approximately 20 Children)					
Week	**-4 (±2 weeks)**	**0**	**1**	**2**	**3**	**4**	**5**
Visit (1-day window)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Informed consent and assent	X						
Medical history/ eligibility	X						
Concomitant medications	X[b]	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X
Height	X						
12-lead ECG[c]	X	X	X	X	X	X	X
ECHO[c]	X						
PFTs (FEV_1, FVC, FEV_1/FVC)[c]	X						
Clinical laboratory tests[c,d]	X	X	X	X	X	X	X
Urinalysis[b,c]	X	X	X	X	X	X	X
Cytokine	X						
miRNA	X						
Serology[e]	X						
Quality of Life test	X						
Muscle evaluations (6MWT[f], NSAA[g], PUL[g])	X						
Muscle biopsy	X						
MRI	X						
PK assessment[h]			X[i]		X[i]		X[i]
Adverse events	X	X	X	X	X	X	X
Study drug administration		X	X	X	X	X	X

6MWT=6-Minute Walk Test; CMV=cytomegalovirus; EBV=Epstein Barr virus; ECG=electrocardiogram; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MRI=magnetic resonance imaging; NSAA=North Star Ambulatory assessment; PFTs=pulmonary function tests; PK=pharmacokinetic.

[a] During the pre-study visit, historical function data (from 6 months before the pre-study visit) will be collected.
[b] Obtain prior medications at screening as well.
[c] To be performed more frequently, if clinically indicated.
[d] The following laboratory parameters will be assessed: hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, ANC, and abnormal cells; blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium,

potassium, chloride, calcium, glucose, creatinine, BUN, and CPK (CrCl will be calculated by the Cockcroft and Gault formula); urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.
[e] The following serology tests will be conducted: ELISA for HIV-Ab, HCV-Ab, HbsAg, HbeAg, anti-EBV, and anti-CMV IgM.
[f] During the pre-study visit, the historical function data relevant to 6MWTs will be collected: (1) result from 6MWT performed at least 6 months before the pre-study visit, and (2) result from 2 6MWT performed within 4 weeks prior to treatment start will be necessary and inserted into the eCRF.
[g] During the pre-study visit, the functional data relevant to the NSAA and PUL will be collected: results performed within 4 weeks prior to treatment start will be necessary and inserted into the eCRF.
[h] At Visit 2, 4, and 6, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose. It is important to record the time of PK assessments and the time of the last drug intake in the evening before the PK assessment.
[i] At Visit 2, the first 4 patients will have PK assessments performed; at Visit 4, the next 8 patients will have PK assessments performed; at Visit 6, the next set of 8 patients will have PK assessments performed.

Table 9 Schedule of Assessments: Part 2 (Proof of Mechanism)

Assessment	Part 2 (All Children)[j]									12/ EOS Visit[i]	FU Visit
	Month of Study:										
	0	1	2	3	4.5	6	7.5	9	10.5	12	--
Visit (1 week window)	**1[a]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X
Height										X	
Weight	X	X	X	X	X	X	X	X	X	X	
12-lead ECG[c]	X	X	X	X	X	X	X	X	X	X	X
ECHO[c]										X	
PFTs (FEV_1, FVC, FEV_1/FVC)[c]										X	
Clinical laboratory tests[c,d]	X	X	X	X	X	X	X	X	X	X	X
Urinalysis[c,d]	X	X	X	X	X	X	X	X	X	X	X
Serology[e]											
Quality of life test										X	
Muscle evaluations (6MWT[f], NSAA[g], PUL[g])	X			X		X				X	
Muscle biopsy										X	
MRI										X	
Cytokine	X			X		X				X	
miRNA	X			X		X				X	
PK assessment[h]		X	X	X		X					
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Study drug administration	X	X	X	X	X	X	X	X	X		

6MWT=6-Minute Walk Test; CMV=cytomegalovirus; EBV=Epstein Barr virus; ECG=electrocardiogram; EOS=end of study; FU=follow up; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MRI=magnetic resonance imaging; NSAA=North Star Ambulatory assessment; PK=pharmacokinetic.

[a] The screening visit is only for children newly enrolled in Part 2. Children who were enrolled in Part 1 do not need to repeat the screening visit. For the relevant assessment, see Table 3 (screen/Pre study visit).
[b] Obtain prior medications at screening as well.
[c] To be performed more frequently, if clinically indicated.

[d] The following laboratory parameters will be assessed: Hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, ANC, and abnormal cells; Blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, troponin 1, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CPK (CrCl will be calculated by the Cockcroft and Gault formula); Urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.
[e] The following serology tests will be conducted: ELISA for HIV-Ab, HCV-Ab, HbsAg, HbeAg, anti-EBV, and anti-CMV IgM.
[f] During the pre-study visit, the historical function data relevant to 3 6MWTs will be collected: (1) result from 6MWT performed at least 6 months before the pre-study visit, and (2) result from 6MWT performed within 4 ±2 weeks prior to treatment start will be necessary and inserted into the eCRF.
[g] During the pre-study visit, the functional data relevant to the NSAA and PUL will be collected: results performed within 4±2 weeks prior to treatment start will be necessary and inserted into the eCRF.
[h] At Visit 2, blood samples for PK analysis should be taken pre-dose and between 6 and 8 hours post-dose. At Visit 3, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and between 0 and 2 hours post-dose. At Visit 4, blood samples for PK analysis should be taken pre-dose and between 2 and 4 hours post-dose. At Visit 6, blood samples for PK analysis should be taken pre-dose and between 4 and 6 hours post-dose. It is important to record the time of PK assessments.
[i] Children who discontinue participation prior to completion the study should perform the Early Termination Visit within 2 weeks after the last drug intake. For the assessments to be performed, see –12/EOS visit.‖
[j] Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the RD to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2).

3.4.2 Study Procedures

3.4.2.1 Pre-Study (Screening)—(Day -28 [±2 Weeks])

The assessments during the pre-study phase will determine the child's eligibility for the study and also their ability to comply with protocol requirements by completing all screening assessments. All children will undergo all screening assessments, regardless of whether they are enrolled during Part 1 or Part 2 of the study.

The following procedures will be performed and recorded during the screening period:

- Obtain written informed consent from the child's parent/legal guardian and assent from the child, if applicable.
- Collect medical history.
- Review inclusion and exclusion criteria.
- Obtain and record current and prior medications (taken in the past 6 months).
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC.
- Obtain blood samples for hematology, blood chemistry, and disease serology tests.
- Obtain a urine sample for urinalysis.
- Obtain blood samples for cytokine measurements and miRNA.
- Perform a quality of life test
- Perform the muscle evaluations:
    - 6MWT (collect historical data on tests performed at least 6 months prior to the pre-study visit and within 4 ± 2 weeks of pre-study visit).
    - NSAA and PUL (collect results from within 4 ± 2 weeks prior to treatment start).
- Obtain a muscle biopsy (brachial biceps; please refer to Section 3.4.6 for instructions; obtained within 4 ± 2 weeks prior to the start of treatment).
- Obtain an MRI of dystrophic muscle (quadriceps femoris; does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment).
- Assess AEs

3.4.2.2 Weekly Visits (Part 1; Weeks 0 to 6)

Children will visit the study center weekly during Part 1 of the study. Children will continue to come in weekly (i.e., every 7 ± 1 days) until they are switched to Part 2 of the study when the RD is selected. At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain blood for PK assessments at Visit 2 only as noted in the schedule of assessments.
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.2.3 Part 2 Visits (Months 1 to 10.5)

Children will visit the study center as noted in the schedule of assessments during Part 2 of the study (i.e., every 1 to 1.5 months ± 1 week). At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain blood samples for cytokine and miRNA (Visits 4 and 6 only).
- Obtain a urine sample for urinalysis.
- Obtain blood for a PK assessments at Visits 2, 3, 4, and 6 only as noted in the schedule of assessments.
- Obtain muscle evaluations (6MTW, NSAA, and PUL; Visits 4 and 6 only).
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.3 Assessments for End of Study (Visit 10 [Month 12])

Children who remain in the study will continue to take study drug through Visit 10. The end-of-study visit overlaps with the 12-month visit, if the child completes treatment.

The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Perform a quality of life test.
- Obtain muscle evaluations (6MWT, NSAA, PUL).
- Perform a muscle biopsy (please refer to Section 3.4.6 for instructions).
- Obtain an MRI of dystrophic muscle.
- Obtain blood samples for cytokines and miRNA.
- Perform accountability.
- Assess AEs.

3.4.4 Early Termination Visit

Children who discontinue participation prior to completion of all study drug administration (i.e., 12 months of treatment) will be asked to return to the hospital within 2 weeks after the last dose of study drug for completion of the same assessments given at the End of Study visit (for details, see Section 3.4.3).

3.4.5 Follow-up Visit

All children, regardless of whether they complete the study or terminate early in Part 1 or Part 2 should return to the study center within 4 weeks of the last dose of study drug for the follow-up visit.

The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.

- Obtain a 12-lead ECG in triplicate.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Assess AEs.

3.4.6 Description of Assessments

Unless otherwise indicated, all assessments will be performed by the investigator or other regular study personnel. Assessments are to be performed according to the schedule shown in Section 3.4.1. The assessments at the end-of-study and follow-up visits should also be performed if the child terminates the study early.

Efficacy Assessments

Muscle Biopsies: A first brachial biceps biopsy (baseline) will be taken prior to the first dose of study drug. A second brachial biceps biopsy will be taken at the end of study from the opposite arm.

The muscle biopsy samples from the biceps muscle will be collected by open biopsy according to standard hospital procedures for obtaining muscle biopsies from children. The minimum amount of muscle tissue required is a small piece of muscle of at least 0.5 x 0.5 x 0.5 cm. The muscle sample, embedded with tragacanth gum on a piece of cork, must be frozen in liquid nitrogen-cooled 2-methylbutane and stored at -80°C or -70ºC until shipment.

The collection, processing, and shipment of these muscle biopsy samples to the Ospedale Pediatrico Bambino Gesù laboratory will be described in detail in the study-specific laboratory manual.

6-Minute Walk Test: A modified version of the 6MWT recommended by American Thoracic Society (2002) for use in adults will be performed.

North Star Ambulatory Assessment: The NSAA will be graded using the standard scorecard with each assessment rated as 0 – unable to achieve independently, 1 – modified method but achieves goal independent of physical assistance from another, or 2 – normal with no obvious modification of activity.

Performance of Upper Limb: The PUL was devised to assess motor performance in the upper limb for patients with Becker and Duchenne muscular dystrophy. The purpose is to assess change that occurs in motor performance of the upper limb over time from when a child is still ambulant until he loses all arm function when non-ambulant. The PUL will

be administered according to the guidelines developed by the Physiotherapy Working Group (Mayhew A et al. 2012, Mercuri E et al. 2012).

MRIs: MRIs will be taken at quadriceps femoris and evaluated per the specifications in the Clinical Study Operation Manual (CSOM).

Cytokines and miRNA: The following cytokines may be evaluated: tumor-necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-6. In addition, miR-1, miR-133, and miR-206 may be evaluated. Additional cytokines and miRNA parameters may be evaluated if warranted. Evaluation of cytokines and miRNA will be documented in a separate report.

Other efficacy assessments – exploratory analysis of muscle biopsies: Muscle biopsies will be analyzed in 2 distinct steps, by using different complementary approaches (fluorescence-activated cell sorting [FACS]-mediated isolation of cells and functional characterization ex vivo and staining of frozen sections) that are finalized to the identification and functional/molecular characterization of specific cell types that contribute to the regeneration or fibro-adipogenic degeneration of dystrophic muscles. These cells include muscle satellite cells (MuSCs) and a heterogeneous population that is referred to as ―fibro-adipogenic progenitors‖ (FAPs). These cell types can be isolated by FACS in mouse and human muscle samples as distinct populations, based on specific combination of surface antigens.

In the mouse system, MuSCs can be isolated from skeletal muscles by FACS, as $CD34_{pos}/\alpha7\text{-integrin}_{pos}/Sca1_{neg}$ cells (Sacco A et al. 2008), while FAPs are isolated either as $CD34_{pos}/\alpha7integrin_{neg}/Sca1_{pos}$ cells or platelet-derived growth factor receptor (PDGFR) PDGFR- α_{pos} cells (Joe AW et al. 2010; Uezumi A et al. 2010). In muscle biopsies of human patients or normal individuals, MuSCs are isolated as PDGFR- $\alpha_{neg}/\alpha7\text{-integrin}_{pos}$/N-CAM $_{pos}$ cells, while FAPs can be isolated as PDGFR- $\alpha_{neg}/\alpha7\text{-integrin}_{pos}$/N-CAM $_{pos}$ cells.

Pre-clinical studies have demonstrated that functional interactions between these cell types contributes to the disease progression in mouse models of DMD (*mdx* mice) and that HDAC inhibitors promote the FAP property of stimulating muscle regeneration at the expense of fibro-adipogenic degeneration. The sponsor has identified a novel nuclear network that regulates FAP lineage identity and ability to support regeneration or fibro-adipogenic degeneration in dystrophic muscles. This network consists of an HDAC-repressed miRNA (the myomiRs 1.2, 133a and 206) that target 2 specific sub-units (BAF60a and b) of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex, which promotes the expression of fibro-adipogenic genes. Upon treatment with HDAC inhibitors, de-repression of myomiRs 1.2, 133a and 206 causes the

down regulation of BAF60a and b, and the simultaneous activation of BAF60c, leading to the formation of BAF60c-based SWI/SNF complex that promotes skeletal myogenesis in FAPs. The sponsor has found that *mdx* mice that respond to HDAC inhibitors, such as Givinostat, show increased levels of BAF60c and myomiRs 1.2, 133a and 206.

1. FACS-sorting of MuSCs and FAPs from biopsies of children before and at the end of the treatment will be used to measure: a) the relative amount of these cell populations; b) their ability to differentiate in culture, in myogenic and adipogenic media, and their functional interactions by co-culture experiments; c) gene and miRNA expression analysis by quantitative polymerase chain reaction.

   It should be emphasized that in vitro exposure to Givinostat of MuSCs and FAPs from biopsies of children before the treatment might provide a useful measure predictive of child response that can be used to better select children in a follow-up trial.

2. Histologic analysis of muscle sections from biopsies of children before and at the end of the treatment will be used to measure: a) the expression levels of BAF60 a, b, and c variants, and myomiRs 1.2, 133a and 206 in MuSCs and FAPs, which will be identified as $Pax7_{pos}$ or PDGFR- α_{pos}, respectively.

Safety Assessments

Physical Examination: Physical examination will include examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system. An AE form must be completed for all changes identified as clinically noteworthy. Height without shoes and weight will be recorded as noted in the schedule of assessments.

Vital Signs: Vital signs will include body temperature (ºC), pulse rate, and blood pressure.

Electrocardiogram: Standard 12-lead ECGs will be conducted in triplicate per the schedule of assessments and more often if clinically indicated. ECG will be acquired at the sites, then ECG data will be transmitted and a central reading will be performed. For details, see the relevant CSOM.

ECHOs: Standard ECHOs will be conducted as per the schedule of assessments and more often if clinically indicated.

Pulmonary function tests: FEV_1, FVC, and FEV_1/FVC, will be collected per the site‘s standard process as noted in the schedule of assessments and more often if clinically indicated.

Laboratory Parameters: The following laboratory tests are to be performed as indicated in the schedule of assessments and more often if clinically indicated:

- Hematology: red blood cell (RBC) count, hemoglobin, hematocrit, WBC count with differential, platelets, ANC, and abnormal cells
- Blood chemistry: total bilirubin, alkaline phosphatase, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, blood urea nitrogen (BUN), and creatine phosphokinase (CPK) (creatinine clearance [CrCl] will be calculated by the Cockcroft and Gault formula.)
- Urinalysis: pH of freshly voided specimen, specific gravity, protein, glucose, ketones, cytology, and myoglobin
- Other: enzyme-linked immunosorbent assay (ELISA) for HIV- Ab, HCV-Ab, HbsAg, hepatitis B e antigen (HbeAg), anti-EBV, and anti-cytomegalovirus (CMV) immunoglobulin M (IgM).

Lab tests will be performed and analyzed by a local laboratory to ensure consistent interpretation of results. In the event of an unexplained clinically noteworthy abnormal laboratory test value, the test should be repeated immediately and followed up until it has returned to the normal range and/or an adequate explanation of the abnormality is found.

Adverse Events: An AE is any is –any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (International Conference on Harmonisation [ICH] E2A).‖ All medical and psychiatric conditions (except those related to the indication under study) present at screening will be documented on the Prior Illnesses eCRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE eCRF during the rest of the study. Laboratory abnormalities should be recorded as AEs only if they meet the criteria for an SAE, result in discontinuation of the study drug, require medical intervention, or are judged by the investigator to be clinically significant changes from baseline values. See Section 4, for additional information.

Pharmacokinetic Assessments

Blood samples for PK assessments will be collected as indicated in the schedule of assessments. Additional information about the PK time points will be provided in the relevant CSOM.

Quality of Life Assessment

The PedsQL Test will be performed before treatment start and at the end of the study.

3.4.7 Appropriateness of Measurements

All assessments to be used in this study are commonly used, standard measurements frequently seen in DMD studies.

4 Adverse Event Reporting

Throughout the course of the study, all AEs will be monitored and reported in the AE eCRF, including the event‘s seriousness, severity, action taken, and relationship to the IMP. If AEs occur, the first concern will be the safety of the children. All AEs will be followed until resolved or stable and the outcome documented on the appropriate eCRF.

In order to avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology rather than the child‘s or parent‘s/legal guardian‘s own words.

4.1 Definitions and Criteria

4.1.1 Adverse Events

An AE is ―any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (ICH E2A)‖.

AEs include:

- Onset of any clinical sign or symptom
- Worsening (change in nature, severity or frequency) of conditions present at the start of the trial
- Subject deterioration due to the primary illness
- Intercurrent illness(es)
- Drug interactions
- Events related or possibly related to concomitant medications
- Abnormal laboratory values, as well as significant shifts from baseline within the range of normal that the investigator considers to be clinically significant

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

4.1.2 Adverse Drug Reactions

In the pre-approval clinical experience with a new medicinal product: –all noxious and unintended responses to a medicinal product related to any dose should be considered Adverse Drug Reaction.

4.1.3 Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction is an event in which the nature or severity is not consistent with the applicable product information (e.g., Investigator‘s Brochure for an unapproved IMP).

4.1.4 Serious Adverse Events

An SAE (experience) or reaction is any untoward medical occurrence that at any dose:

- Is fatal (results in the outcome death)
- Is life-threatening*
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above

*The term life-threatening refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the child or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An SAE is not necessarily severe; e.g., an overnight hospitalization for a diagnostic procedure must be reported as an SAE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: nausea of several hours‘ duration may be rated as severe but may not be considered serious.

4.1.5 Assessing Intensity and Relationship

All AEs will be assessed on 2 descriptive parameters: intensity and relationship to study drug:

Intensity refers to the ‒severity‖ of an event and references impact on a child‘s functioning.

Relationship refers to the likelihood that the event being assessed was caused by the study drug.

Intensity

Each AE will be classified according to the following criteria:

Mild:	The AE does not interfere in a significant manner with the child‘s normal functioning level.
Moderate:	The AE produces some impairment of functioning, but is not hazardous to health.
Severe:	The AE produces significant impairment of functioning or incapacitation and is a definite hazard to the child‘s health.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience should be noted. If the intensity category changes over a number of days, those changes should be recorded separately (with distinct onset dates).

Relationship

Each AE will be assessed as to its relationship to study drug based on the following criteria. Although investigator attribution will be collected for reported events, for analytic purposes a temporal association with the use of study drug will be assumed sufficient for at least plausible association.

Not related:	No causal relationship exists between the investigational product and the AE, but an obvious alternative cause exists, e.g., the child‘s underlying medical condition or concomitant therapy.
Related:	There is a reasonable/plausible possibility that the AE may have been caused by the investigational product.

When assessing the relationship to study drug, the follow criteria will be considered:

- Positive rechallenge
- Positive dechallenge (resolution upon stopping suspect product, in absence of other intervention or treatment)
- Known class effect
- Biological plausibility
- Lack of alternative explanation—concomitant drug or disease

Factors to be considered in assessing the relationship of the AE to study drug include:

- The temporal sequence from IMP administration
- The recovery on discontinuation and recurrence on reintroduction
- The concomitant diseases
- The evolution of the treated disease
- The concomitant medication(s)
- The pharmacology and pharmacokinetics of the IMP

4.2 Reporting Procedures and Requirements

4.2.1 Adverse Events

The investigator or his/her designees are requested to collect and assess any spontaneous AE reported by the child and to question the child about AEs and intercurrent illnesses at each visit during the treatment period and follow-up. The questioning of children regarding AEs is generalized such as ―How have you been feeling since your last visit?‖ Any AE occurring after the informed consent form/assent has been signed and up to the follow-up study visit, whether volunteered by the child; discovered during general questioning by the investigators; or detected through physical examination, laboratory test, or other means will be recorded on the specific section of the eCRF. Each AE will be described by:

- Seriousness
- Duration (start and end dates)
- Severity
- Relationship to the IMP
- Action taken
- Outcome

The severity of AE should be assessed and graded according to the most recently published National Cancer Institute Common Terminology Criteria for AEs (v. 4.0).

The relationship to the investigational drug should be assessed as:

- Related to IMP
- Not related to IMP
- Unknown

The assessment of the relationship of an AE to the administration of IMP is a clinical decision based on all available information at the time of the completion of the eCRF.

An assessment of ‗not related' would include the existence of a clear alternative explanation, or non-plausibility.

An assessment of ‗related' indicates that there is a reasonable suspicion that the AE is associated with the use of the IMP.

4.2.1.1 Abnormal Laboratory Findings and Other Objective Measurements

Abnormal laboratory findings and other objective measurements should not be routinely captured and reported as AEs in the eCRF as they will be collected and analyzed separately. However, abnormal laboratory findings and other objective measurements that meet the criteria for an SAE, result in discontinuation of the IMP, require medical intervention, or are judged by the investigator to be clinically significant changes from baseline values should be captured and reported as AEs in the eCRF.

When reporting an abnormal laboratory finding as an AE in the eCRF, a clinical diagnosis should be recorded in addition to the abnormal value itself, if this is available (for example ―anemia‖ in addition to ―hemoglobin = 10.5 g/dL‖).

4.2.1.2 Baseline Medical Conditions

Medical conditions present at the screening visit that do not worsen in severity or frequency during the study are defined as baseline medical conditions and are not AEs. These medical conditions should be adequately documented on the appropriate page of the eCRF (i.e., the medical history page). However, medical conditions present at the initial study visit that worsen in severity or frequency during the study should be recorded and reported as AEs.

4.2.2 Serious Adverse Events

Any SAE, including death from any cause that occurs after a child has signed the informed consent /assent and up to the final follow-up visit (regardless of relationship to study drug) must be reported by the investigators to the sponsor within 24 hours of learning of its occurrence.

Related SAEs ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

The investigators are required to complete the SAE form provided by the sponsor. Sufficient details must be provided to allow for a complete medical assessment of the AE and independent determination of possible causality. The investigators are obliged to pursue and provide additional information as requested by the sponsor‘s drug safety manager, or study director, or designee.

The investigator must send notification of the SAE to the sponsor‘s Drug Safety Unit by faxing the SAE form, within 24 hours of a SAE, at the number specified below; then, the investigator must confirm any SAE notifications by mailing to the mail address or phoning to the phone number specified below:

Aurelio Scotti
Drug Safety Unit, Chemi S.p.A.
Via dei Lavoratori
54 20092 Cinisello Balsamo (MI), Italy
Fax: 02 6610 6538
phone: +39 02 6443 2510, mobile +39 333 9262611
e-mail: drug-safety@italfarmaco.com
a. scotti@italfarmaco.com

The same procedure must be applied to the SAE follow-up information.

The sponsor‘s drug safety manager will report all serious and unexpected AE that are related to the use of the study drug to the competent authority within the required time and following procedures required by applicable laws. It is imperative that the sponsor be informed as soon as possible, so that reporting can be done within the required time frame.

The SAEs will also be recorded in the AE section of the eCRF.

Overdose and Other Situations Putting the Child at Risk of an Adverse Reaction

Any instance of overdose (suspected or confirmed) must be reported to the sponsor within 24 hours and be fully documented as a SAE. Details of any signs or symptoms and their management should be recorded including details of any antidote(s) or systematic treatment administered. Any signs or symptoms of overdose will be treated symptomatically.

Any other situations putting the child at risk of adverse reaction, such as misuse and abuse, medication errors, suspicion of transmission of infective agents must be reported to the sponsor within 24 hours and be fully documented as a SAE.

5 Data Management and Statistical Analysis

5.1 Data Management Considerations

Electronic CRFs will be employed for this study. Completed eCRFs for this study will be forwarded to the sponsor or its representative where editing and construction of a quality-assured database will occur. The statistical analysis of these data will be performed by the sponsor or its representative.

Data Analysis of the Biopsies

All images will be digitally captured, using both light microscopy (hematoxylin and eosin [H&E] and Gomori trichrome stain) and fluorescence microscopy coupled to the Olympus Fluoview FV1000 confocal microscope. Fields for fluorescent imaging will be randomly selected while viewing the laminin-dystrophin signal.

In the only step involving operator discretion, all image parameters including pinhole size, detector gain, amplifier offset, amplifier gain, and laser intensity will be first set for the dystrophin and laminin channels using normal control tissue, and the same setting used for all samples imaged on a given day. Frame size, scan speed, and averaging will be the same for all images. For each sample 4 non-overlapping images for each channel will be acquired and stored as 12-bit fluorescent images (.TIFF) for analysis. A single technician will perform all sectioning, staining, and morphometry steps, while another operator will execute the confocal imaging.

Image processing and quantitative analyses will be done using Metamorph (Molecular Devices, Inc.) software program using a custom script. Additional details are provided in the relevant CSOM.

5.2 Statistical Considerations

The statistical analysis will be undertaken by the contract research organization Worldwide Clinical Trials (WCT) in collaboration with the sponsor.

Any deviations from the analyses described below will be included in the Statistical Analysis Plan (SAP).

5.2.1 General Considerations

Data will be provided in data listings sorted by treatment group and child number. Tabular summaries will be presented by treatment group for Part 1. Categorical data will be summarized by the number and percentage of children in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided, unless otherwise stated. Details of endpoint analyses will be described in the SAP.

For analyses of change from baseline, baseline will generally be defined as the value collected before any Givinostat treatment is administered. If this value is unavailable, the last non-missing value prior to dosing will be used. Otherwise, missing observations will be treated as missing at random, and no data imputation will be performed. All data from the CRFs, as well as any derived variables, will be presented in data listings.

All hypothesis tests will be two-sided with a 5% significance level, and 95% CIs will be used, unless stated otherwise. As this is a Phase 2 exploratory study, no adjustments for multiplicity will made.

5.2.2 Sample Size Justification

A minimum of 20 evaluable children will be enrolled in this study.

5.2.2.1 Part 1

Approximately 20 children will be enrolled in Part 1 (dose-finding) of this study.

5.2.2.2 Part 2

Muscle fibers: a sample size of 20 children from part 1 completing the treatment period of part 2 should provide 90% power (at a 2-sided alpha level of 5%) to detect at least a 12.5% increase in MFA% between pre- and post-treatment (which corresponds approximately to a 26% relative increase given the observed mean in the Desguerre publication of 48% [Desguerre I et al. 2009]), the ‑worst case‖ standard deviation of 16%, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment. This sample size is based on a paired t-test and the assumption of normal distribution of MFA%.

After the first 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability observed in the study will be implemented: the within-subject standard deviation of MFA% will be calculated, the actual distribution of MFA% will be checked and the final sample size will be adjusted based on the observed standard deviation and actual distribution of MFA%. Results by Al-Sunduqchi and Guenther (1990) indicate that power calculations for the Wilcoxon test may be made using the

standard t-test formulations with a simple adjustment to the sample size. The size of the adjustment depends upon the actual distribution of the data. They give sample size adjustment factors for four distributions. These are 1 for the uniform distribution, 2/3 for the double exponential distribution, $9/\pi^2$ for the logistic distribution, and $\pi/3$ for the normal distribution. So depending on the actual distribution of MFA%, sample size re-calculation will be based on a Wilcoxon Signed Rank test with the corresponding adjustment if the observed distribution of MFA% is not normal.

5.2.3 Analysis Populations

The intent-to-treat (ITT) population includes all children who are enrolled in the Part 1 portion or entered the Part 2 portion of the study. The efficacy analysis will be conducted based on the ITT population.

The evaluable population will include all children who are in the Part 2 of the study, receive Givinostat of at least 80% dose, have at least one baseline and one post-baseline assessment of biopsies, and have no major protocol violations. The evaluable population will be identified prior to database lock. A sensitivity analysis may be conducted based on the evaluable population.

The safety population will include all children who receive any investigational product. The safety analysis will be conducted based on the safety population.

The PK population will include all children with at least one quantifiable post-dose concentration datum available. All statistical analyses of PK data will be performed using the PK population.

5.2.4 Primary Endpoint

The primary endpoint of this study is change in the value of MFA% comparing the histology biopsies before and after 12 months of treatment with Givinostat.

5.2.5 Secondary Efficacy Endpoints

The secondary efficacy endpoints are the following:

- Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA

- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based the PUL

Additional details on secondary efficacy endpoints will be provided in the SAP.

5.2.6 Secondary Safety Endpoints

The secondary safety endpoints will include the following:

- The number of children experiencing AEs
- The type, incidence, and severity of AEs correlated with dose
- ECG, ECHO, vital sign, physical examination, PFTs, and clinical laboratory parameter findings

5.2.7 Secondary Pharmacokinetic Endpoints

Individual Givinostat concentrations will be tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2. Noncompartmental PK data analysis will be performed for data obtained from each dose cohort with scheduled PK sample collection. If data allows, descriptive statistics of noncompartmental PK parameters (area under the plasma–concentration time curve, maximum plasma concentration, clearance, terminal elimination half-life) will be provided.

5.2.8 Exploratory Endpoints

The exploratory endpoints will include the following:

- Changes in muscle, fat, and fibrosis content after treatment with Givinostat as measured by MRI
- Change biomarkers (e.g., miRNA) and cytokine following treatment with Givinostat
- PK–PD correlations

5.2.8.1 Magnetic Resonance Imaging

MRI will be collected via T1w imaging and quantitative Dixon imaging (the fat content of the image as a percentage of the total signal per voxel). Additional details will be provided in the SAP.

5.2.8.2 miRNA and Cytokine Parameters

miR-1, miR-133, and miR-206 as well as TNF-α, IL-1, and IL-6 will be evaluated. Other miRNA and cytokine parameters may be evaluated as appropriate. Additional details will be provided in the SAP.

5.2.9 Efficacy Analyses

Efficacy analyses will be conducted on the evaluable population.

All values will be expressed as means ± standard deviation or standard error of the mean.

Each of all efficacy endpoints will be subject to normality test and formal statistical test performed will be based on whether it is normally distributed. If normal, statistical differences will be calculated using the paired t test; if not normal, Wilcoxon signed rank test will be used to assess the statistical significance of differences between groups. $P<0.05$ will be set as significant.

5.2.10 Safety Analyses

Safety analyses will be conducted on the safety population.

The safety of Givinostat will be assessed primarily by summarizing treatment-emergent AEs and SAEs. Other safety data (e.g., laboratory, ECG, PFTs, physical examination, and vital sign findings) will be summarized. Treatment-emergent AEs and SAEs that occur after administration of Givinostat will be summarized by system organ class and preferred terms, by severity, and by relationship to investigational product. Change from baseline in laboratory values, ECG findings, PFTs, physical examination findings, and vital signs measurements will be summarized.

5.2.11 Pharmacokinetic Analyses

The PK analysis will be conducted on the PK population.

Plasma concentrations from Part 1 will be listed and tabulated by dose and time point for all children and time points with at least 1 PK assessment. Plasma concentrations from Part 2 will be listed and tabulated by time point for all children and time points with at least 1 PK assessment.

Descriptive statistics for all PK parameters for Part 1 will be calculated by treatment. Descriptive statistics for all PK parameters for Part 2 will also be calculated. These tables will include number of observations, mean, standard deviation, median, minimum and maximum and additionally the geometric mean and coefficient of variation (not for time to maximum plasma concentration).

5.2.12 Interim Analyses

The following interim analyses will be conducted:

- After the first 20 baseline biopsies are collected, the between-subject standard deviation of MFA% fraction will be calculated, the actual distribution of MFA% will be checked. The within-subject standard deviation will be estimated by between-subject standard deviation under the ―worst case‖ scenario, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment.
- Based on the standard deviation obtained above, the final sample size will be adjusted. A conservative approach will be adopted, where the sample size may be increased but not decreased.

6 Study Management

6.1 Approval and Consent

6.1.1 Regulatory Guidelines

The study will be performed in accordance with local national laws (as applicable), the guidelines of the ICH, and the guidelines of the Declaration of Helsinki adopted by the 18th World Medical Assembly in Helsinki, Finland in 1964 and amended by subsequent assemblies in Tokyo, Japan in 1975; Venice, Italy in 1983; Hong Kong in 1989; Somerset West, South Africa in 1996, and in Edinburgh, Scotland in October 2000. These guidelines are on file at WCT.

This clinical study has been designed to comply with the Good Clinical Practice guidelines.

6.1.2 Institutional Review Board/Independent Ethics Committees

This study will be undertaken only after approval of the protocol has been obtained from the appropriate Independent Ethics Committee (IEC), and a copy of the approval has been received by Italfarmaco S.p.A.

The IEC must be informed of all subsequent protocol amendments and should be asked whether a re-evaluation of the ethical aspects of the study is necessary.

If applicable, interim reports on the study and reviews of its progress will be submitted to the IEC by the investigator at intervals stipulated in their guidelines.

6.1.3 Informed Consent

For each trial subject, written informed consent from the legally accepted representative will be obtained prior to any protocol-related activities. Informed assent may be obtained from children who are capable of providing assent. As part of this procedure, the

principal investigator or a designated representative must explain orally and in writing the nature, duration, and purpose of the study, and the action of the drug in such a manner that the child and (if applicable) appointed guardian are aware of the potential risks, inconveniences, or adverse effects that may occur. Children and their legally accepted representatives should be given ample time and opportunity to inquire about the details of the study prior to deciding whether to participate in the study. It is the responsibility of the investigator to ensure that all questions about the study are answered to the satisfaction of the children and their legally accepted representatives.

Children and their legally accepted representatives should be informed that children may withdraw from the study at any time. They will receive all information that is required by local regulations and ICH guidelines. The principal investigator or a designated representative will provide the sponsor or its representative with a copy of the IEC-approved informed consent form prior to the start of the study.

The informed consent form should be signed and dated by the child‘s legally accepted representative and the investigator on the same day. If the child and/or legally accepted representative are not able to read, an impartial witness should be present during the informed consent discussion, and the witness must co-sign and date the informed consent form. The child‘s legally accepted representative and/or impartial witness should receive a copy of the signed documents.

For details of the information provided, refer to the informed consent form.

6.2 Discontinuation of the Study by the Sponsor

The sponsor reserves the right to discontinue the study at this site or at multiple sites for safety or administrative reasons at any time. In particular, a site that does not recruit at a reasonable rate may be discontinued. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to the sponsor or its representative.

6.3 Study Documentation

The investigator will supply the sponsor with:

- Curricula vitae for all investigators
- Signed protocol signature page
- List of IEC members and their occupations/affiliations or multiple assurance number
- Letter indicating IEC approval to conduct the protocol
- Copy of IEC-approved informed consent form
- Laboratory certification records and reference ranges

The main documents that will be supplied by the Sponsor to investigator are:

- Clinical study protocol
- Investigational drug brochure
- Sample informed consent form
- eCRFs/instruction manual
- Insurance letter

6.4 Study Monitoring and Auditing

This study will be monitored at all stages of its development by the clinical research personnel employed by the sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to protocol and in order to comply with guidelines of Good Clinical Practice. On-site review of eCRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each child. Note that a variety of original documents, data, and records will be considered as source documents in this trial.

The eCRF itself is not to be used as a source document under any circumstances.

Medical advisors and clinical research associates or assistants may request to witness child evaluations occurring as part of this protocol. The investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the sponsor to assure acceptable protocol execution. The study may be subject to audit by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required child records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in eCRF generation, where clinically appropriate.

6.5 Retention of Records

The investigator must arrange for retention of study records at the site. The nature of the records and the duration of the retention period must meet the requirements of the relevant regulatory authority. In addition, because this is an international study, the retention period must meet the requirements of the most stringent authority. The investigator should take measures to prevent accidental or premature destruction of these documents.

6.6 Use of Study Findings

By signing the study protocol, the investigator agrees to the use of results of the study for the purposes of national and international registration. If necessary, the authorities will

be notified of the investigator‘s name, address, qualifications, and extent of involvement. Reports covering clinical and biometric aspects of the study will be prepared by the sponsor or its representative.

6.7 Publications

The sponsor assures that the key design elements of this protocol will be posted in a publicly accessible database, such as clinicaltrials.gov. In addition, upon study completion and finalization of the study report the results of this study will be either submitted for publication and/or posted in a publicly accessible database of clinical study results.

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8 Appendices

Appendix 1: Drugs Known to Prolong the QTc Interval

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Albuterol (Proventil®;Ventolin®)	ß2-receptor agonist/Asthma		Congenital QT Avoid
Alfuzosin (Uroxatral®)	Alpha1-blocker/Benign prostatic hyperplasia		Possible Risk of TdP
Amantadine (Symmetrel®)	Dopaminergic/Anti-viral/Anti-infective/ Parkinson's Disease		Possible Risk of TdP
Amiodarone (Cordarone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	Risk of TdP
Amiodarone (Pacerone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	Risk of TdP
Amitriptyline (Elavil®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	Conditional TdP Risk
Amphetamine (Dexedrine®)	CNS stimulant/ADHD	This includes dextroamphetamine and amphetamine / dextroamphetamine combinations.	Congenital QT Avoid
Amphetamine (Adderall®)	CNS stimulant/ADHD	This includes dextroamphetamine and amphetamine / dextroamphetamine combinations.	Congenital QT Avoid
Arsenic trioxide (Trisenox®)	Anti-cancer/Leukemia		Risk of TdP
Astemizole (Hismanal®)	Antihistamine/Allergic rhinitis	No Longer available in U.S.	Risk of TdP
Atazanavir (Reyataz®)	Protease inhibitor/HIV		Possible Risk of TdP
Atomoxetine (Strattera®)	norepinephrine reuptake inhibitor /ADHD		Congenital QT Avoid
Azithromycin (Zithromax®)	Antibiotic/bacterial infection		Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Bepridil (Vascor®)	Anti-anginal/heart pain	Females>Males	Risk of TdP
Chloral hydrate (Noctec®)	Sedative/sedation/ insomnia		Possible Risk of TdP
Chloroquine (Aralen®)	Anti-malarial/malaria infection		Risk of TdP
Chlorpromazine (Thorazine®)	Anti-psychotic/ Anti-emetic/schizophrenia/ nausea		Risk of TdP
Ciprofloxacin (Cipro®)	Antibiotic/bacterial infection	Drug metabolism inhibitor-Risk for drug interactions	Conditional TdP Risk
Cisapride (Propulsid®)	GI stimulant/heartburn	No longer available in the U.S.; available in Mexico	Risk of TdP
Citalopram (Celexa®)	Anti-depressant/depression		Risk of TdP
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection		Risk of TdP
Clomipramine (Anafranil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Clozapine (Clozaril®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Cocaine (Cocaine)	Local anesthetic/	Cardiac stimulant	Congenital QT Avoid
Desipramine (Pertofrane®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	Conditional TdP Risk
Dexmethylphenidate (Focalin®)	CNS stimulant/ADHD		Congenital QT Avoid
Diphenhydramine (Benadryl®)	Antihistamine/Allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	Conditional TdP Risk
Diphenhydramine (Nytol®)	Antihistamine/Allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	Conditional TdP Risk
Disopyramide (Norpace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Dobutamine (Dobutrex®)	Catecholamine/heart failure and		Congenital

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
	shock		QT Avoid
Dofetilide (Tikosyn®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Dolasetron (Anzemet®)	Anti-nausea/nausea, vomiting		Possible Risk of TdP
Domperidone (Motilium®)	Anti-nausea/nausea	Not available in the U.S.	Risk of TdP
Dopamine (Intropine®)	Inotropic agent/heart failure; hypotension; shock		Congenital QT Avoid
Doxepin (Sinequan®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Dronedarone (Multaq®)	Anti-arrhythmic/Atrial Fibrillation		Possible Risk of TdP
Droperidol (Inapsine®)	Sedative;Anti-nausea/anesthesia adjunct, nausea		Risk of TdP
Ephedrine (Broncholate®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma		Congenital QT Avoid
Ephedrine (Rynatuss®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma		Congenital QT Avoid
Epinephrine (Primatene®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	Cardiac stimulant	Congenital QT Avoid
Epinephrine (Bronkaid®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	Cardiac stimulant	Congenital QT Avoid
Eribulin (Halaven®)	Anti-cancer/metastatic breast neoplasias		Possible Risk of TdP
Erythromycin (E.E.S.®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	Risk of TdP
Erythromycin (Erythrocin®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	Risk of TdP
Escitalopram (Cipralex®)	Anti-depressant/Major depression/		Possible

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
	Anxiety disorders		Risk of TdP
Escitalopram (Lexapro®)	Anti-depressant/Major depression/ Anxiety disorders		Possible Risk of TdP
Famotidine (Pepcid®)	H2-receptor antagonist/Peptic ulcer/ GERD		Possible Risk of TdP
Felbamate (Felbatrol®)	Anti-convulsant/seizure		Possible Risk of TdP
Fenfluramine (Pondimin®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Fingolimod (Gilenya®)	Immunosuppressant/Multiple Sclerosis		Possible Risk of TdP
Flecainide (Tambocor®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Fluconazole (Diflucan®)	Anti-fungal/fungal infection	Drug metabolism inhibitor-Risk for drug interactions	Conditional TdP Risk
Fluoxetine (Prozac®)	Anti-depressant/depression		Conditional TdP Risk
Fluoxetine (Sarafem®)	Anti-depressant/depression		Conditional TdP Risk
Foscarnet (Foscavir®)	Anti-viral/HIV infection		Possible Risk of TdP
Fosphenytoin (Cerebyx®)	Anti-convulsant/seizure		Possible Risk of TdP
Galantamine (Reminyl®)	Cholinesterase inhibitor/ Dementia, Alzheimer's		Conditional TdP Risk
Gatifloxacin (Tequin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Gemifloxacin (Factive®)	Antibiotic/bacterial infection		Possible Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Granisetron (Kytril®)	Anti-nausea/nausea and vomiting		Possible Risk of TdP
Halofantrine (Halfan®)	Anti-malarial/malaria infection	Females>Males	Risk of TdP
Haloperidol (Haldol®)	Anti-psychotic/schizophrenia, agitation	When given intravenously or at higher-than- recommended doses, risk of sudden death, QT prolongation and torsades increases.	Risk of TdP
Ibutilide (Corvert®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Iloperidone (Fanapt®)	Antipsychotic, atypical/Schizophrenia		Possible Risk of TdP
Imipramine (Norfranil®)	Tricyclic Antidepressant/depression	Risk of TdP in overdosage	Conditional TdP Risk
Indapamide (Lozol®)	Diuretic/stimulate urine & salt loss		Possible Risk of TdP
Isoproterenol (Isupres®)	Catecholamine/allergic reaction		Congenital QT Avoid
Isoproterenol (Medihaler-Iso®)	Catecholamine/allergic reaction		Congenital QT Avoid
Isradipine (Dynacirc®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Itraconazole (Sporanox®)	Anti-fungal/fungal infection	Drug metabolism inhibitor-Risk for drug interactions	Conditional TdP Risk
Ketoconazole (Nizoral®)	Anti-fungal/fungal infection	Drug metabolism inhibitor	Conditional TdP Risk
Lapatinib (Tykerb®)	Anti-cancer/breast cancer, metastatic		Possible Risk of TdP
Lapatinib (Tyverb®)	Anti-cancer/breast cancer, metastatic		Possible Risk of TdP
Levalbuterol (Xopenex®)	Bronchodilator/asthma		Congenital

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
			QT Avoid
Levofloxacin (Levaquin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	Not available in the U.S.	Risk of TdP
Lisdexamfetamine (Vyvanse®)	CNS stimulant/ADHD		Congenital QT Avoid
Lithium (Eskalith®)	Anti-mania/bipolar disorder		Possible Risk of TdP
Lithium (Lithobid®)	Anti-mania/bipolar disorder		Possible Risk of TdP
Mesoridazine (Serentil®)	Anti-psychotic/schizophrenia		Risk of TdP
Metaproterenol (Alupent®)	Bronchodilator/asthma		Congenital QT Avoid
Metaproterenol (Metaprel®)	Bronchodilator/asthma		Congenital QT Avoid
Methadone (Dolophine®)	Opiate agonist/pain control, narcotic dependence	Females>Males	Risk of TdP
Methadone (Methadose®)	Opiate agonist/pain control, narcotic dependence	Females>Males	Risk of TdP
Methylphenidate (Ritalin®)	CNS stimulant/ADHD		Congenital QT Avoid
Methylphenidate (Concerta®)	CNS stimulant/ADHD		Congenital QT Avoid
Midodrine (ProAmatine®)	Vasoconstrictor/low blood pressure, fainting		Congenital QT Avoid
Moexipril/HCTZ (Uniretic®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection		Risk of TdP
Nicardipine (Cardene®)	Anti-hypertensive/high blood		Possible

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
	pressure		Risk of TdP
Nilotinib (Tasigna®)	Anti-cancer/Leukemia		Possible Risk of TdP
Norepinephrine (Levophed®)	Vasconstrictor, Inotrope/shock, low blood pressure		Congenital QT Avoid
Nortriptyline (Pamelor®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Octreotide (Sandostatin®)	Endocrine/acromegaly, carcinoid diarrhea		Possible Risk of TdP
Ofloxacin (Floxin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Ondansetron (Zofran®)	Anti-emetic/nausea and vomiting		Possible Risk of TdP
Oxytocin (Pitocin®)	Oxytocic/Labor stimulation		Possible Risk of TdP
Paliperidone (Invega®)	Antipsychotic, atypical/Schizophrenia		Possible Risk of TdP
Paroxetine (Paxil®)	Anti-depressant/depression		Conditional TdP Risk
Pentamidine (NebuPent®)	Anti-infective/pneumocystis pneumonia	Females>Males	Risk of TdP
Pentamidine (Pentam®)	Anti-infective/pneumocystis pneumonia	Females>Males	Risk of TdP
Perflutren lipid microspheres (Definity®)	Imaging contrast agent/Echocardiography		Possible Risk of TdP
Phentermine (Fastin®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Phentermine (Adipex®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Phenylephrine (Neosynephrine®)	Vasoconstrictor, decongestant/low blood pressure, allergies, sinusitis, asthma		Congenital QT Avoid
Phenylpropanolamine (Acutrim®)	Decongestant/allergies, sinusitis, asthma	No longer available in the U.S.	Congenital QT Avoid
Phenylpropanolamine (Dexatrim®)	Decongestant/allergies, sinusitis, asthma	No longer available in the U.S.	Congenital QT Avoid
Pimozide (Orap®)	Anti-psychotic/Tourette's tics	Females>Males	Risk of TdP
Probucol (Lorelco®)	Antilipemic/Hypercholesterolemia	No longer available in U.S.	Risk of TdP
Procainamide (Pronestyl®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Procainamide (Procan®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Protriptyline (Vivactil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Pseudoephedrine (PediaCare®)	Decongestant/allergies, sinusitis, asthma		Congenital QT Avoid
Pseudoephedrine (Sudafed®)	Decongestant/allergies, sinusitis, asthma		Congenital QT Avoid
Quetiapine (Seroquel®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Quinidine (Quinaglute®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Quinidine (Cardioquin®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Ranolazine (Ranexa®)	Anti-anginal/chronic angina		Possible Risk of TdP
Risperidone (Risperdal®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Ritodrine (Yutopar®)	Uterine relaxant/prevent premature labor		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Ritonavir (Norvir®)	Protease inhibitor/HIV		Conditional TdP Risk
Roxithromycin* (Rulide®)	Antibiotic/bacterial infection	*not available in the United States	Possible Risk of TdP
Salmeterol (Serevent®)	Sympathomimetic/asthma, COPD		Congenital QT Avoid
Sertindole (Serdolect®)	Antipsychotic, atypical/Anxiety, Schizophrenia		Possible Risk of TdP
Sertindole (Serlect®)	Antipsychotic, atypical/Anxiety, Schizophrenia		Possible Risk of TdP
Sertraline (Zoloft®)	Anti-depressant/depression		Conditional TdP Risk
Sibutramine (Meridia®)	Appetitie suppressant/dieting, weight loss		Congenital QT Avoid
Solifenacin (VESIcare®)	muscarinic receptor anatagonist/treatment of overactive bladder		Conditional TdP Risk
Sotalol (Betapace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection		Risk of TdP
Sunitinib (Sutent®)	Anti-cancer/RCC, GIST		Possible Risk of TdP
Tacrolimus (Prograf®)	Immunosuppressant/Immune suppression		Possible Risk of TdP
Tamoxifen (Nolvadex®)	Anti-cancer/breast cancer		Possible Risk of TdP
Telithromycin (Ketek®)	Antibiotic/bacterial infection		Possible Risk of TdP
Terbutaline (Brethine®)	Bronchodilator/asthma		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Terfenadine (Seldane®)	Antihistamine/Allergic rhinitis	No longer available in U.S.	Risk of TdP
Thioridazine (Mellaril®)	Anti-psychotic/schizophrenia		Risk of TdP
Tizanidine (Zanaflex®)	Muscle relaxant/		Possible Risk of TdP
Tolterodine (Detrol®)	Bladder Antispasmodic/		Congenital QT Avoid
Tolterodine (Detrol LA®)	Bladder Antispasmodic/		Congenital QT Avoid
Trazodone (Desyrel®)	Anti-depressant/Depression, insomnia		Conditional TdP Risk
Trimethoprim-Sulfa (Bactrim®)	Antibiotic/bacterial infection		Conditional TdP Risk
Trimethoprim-Sulfa (Sulfa®)	Antibiotic/bacterial infection		Conditional TdP Risk
Trimipramine (Surmontil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Vandetanib (Caprelsa®)	Anti-cancer/Thyroid cancer		Risk of TdP
Vardenafil (Levitra®)	phosphodiesterase inhibitor/vasodilator		Possible Risk of TdP
Venlafaxine (Effexor®)	Anti-depressant/depression		Possible Risk of TdP
Voriconazole (VFend®)	Anti-fungal/anti-fungal		Possible Risk of TdP
Ziprasidone (Geodon®)	Anti-psychotic/schizophrenia		Possible Risk of TdP

Source: Arizona Center for Education and Research on Therapeutics. Updated 17 May 2012. http://www.azcert.org/medical-pros/drug-lists/printable-drug-list.cfm. Accessed 15 June 2012.

Italfarmaco S.p.A.

STUDY PROTOCOL

Protocol Title:	A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy
Protocol Number:	DSC/11/2357/43
Clinical Phase:	2
Protocol Date **Amendment 1**	01 Aug 2012 05 Dec 2012
EudraCT Number:	2012-002566-12
Sponsor	Italfarmaco S.p.A. Via dei Lavoratori, 54 20092 Cinisello Balsamo (MI) Italy Tel: +39 02 6443 2512 Fax: +39 02 6443 3554

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SYNOPSIS

Title of Study:	A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy
Protocol Number:	DSC/11/2357/43
Eudra CT Number	2012-002566-12
Investigators/ Study Centers:	Children will be enrolled at approximately 5 study sites in Italy.
Phase of Development:	2
Objectives:	The primary objective of this study is as follows: • To establish the histologic effects of Givinostat administered chronically at the selected daily dose The secondary objectives of this study are as follows: • To establish the effects of Givinostat administered chronically at the selected daily dose on functional parameters, such as the 6-Minute Walk Test (6MWT), North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL) • To establish the safety and tolerability of Givinostat administered chronically at the selected daily dose in children with Duchenne muscular dystrophy (DMD) • To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as magnetic resonance imaging (MRI), biomarkers, and cytokines
Design:	This is a 2-part, phase 2 study to assess the effects of Givinostat on muscle histologic parameters and on clinical parameters in ambulant children with DMD. The safety, tolerability, and pharmacokinetics of Givinostat will also be assessed. Children who assent to participate in this study (if capable of doing so) and whose parent/guardian signs the informed consent to participate will undergo pre-study screening assessments up to 4 weeks (±2 weeks) before the first scheduled dose of study drug. Approximately 20 children will be enrolled in the study as follows: the first 4 children will be treated at a low dose level of Givinostat (25 mg twice daily [BID] in children who weigh 20–49 kg and 37.5 mg BID in

	children who weigh ≥50 kg). If none of the stopping criteria (see Section 3.3.5) are met after 2 weeks of treatment at the low dose, the review team will determine the escalated dose level (i.e., intermediate dose level) to be used for the treatment of an additional 8 children who will be treated at the intermediate dose. The 4 children previously treated at the low dose level will also be switched to the intermediate dose level. If none of the stopping criteria are met after 2 weeks of treatment at the intermediate dose, the review team will determine the subsequent escalated dose level to be used for the treatment of an additional 8 children who will be treated at the high dose. All children treated at the intermediate dose level will be switched to the high dose level. Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the recommended dose (RD) to be used in Part 2 based on the safety and tolerability profile observed and on the pharmacokinetic (PK) analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2). The additional children (if any) will be enrolled during Part 2 of the study and will receive the RD of Givinostat for 12 months. During Part 1 of the study, children will visit the center once each week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 11 visits, including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 2 weeks after the last dose of study drug as well as the protocol-scheduled follow-up visit. Children who have ongoing adverse events (AEs) at discontinuation will be followed until resolution or stabilization. Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2). Exploratory efficacy parameters will include changes from baseline in MRI results, PK-pharmacodynamic correlations and measures of cytokines and micro ribonucleic acid (miRNA). Safety will be assessed by number of children experiencing AEs; type, incidence, and severity of AEs correlated with dose; and measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies, and urinalysis), echocardiographs, pulmonary function tests (PFTs; forced expiratory volume at 1 second [FEV_1], forced vital capacity [FVC], and FEV_1/FVC) and 12-lead electrocardiograms. Plasma samples for PK measurements will be collected after 1 week of treatment with Givinostat, during Part 1 at pre-dose in the morning before

	drug intake and at 1, 2, 4, and 8 hours post-dose. PK samples will also be collected during Part 2 of the study as follows: • At Visit 2, blood samples for PK analysis will be taken pre-dose and between 6 and 8 hours post-dose. • At Visit 3, blood samples for PK analysis will be collected pre-dose in the morning before study drug intake and between 0 and 2 hours post-dose. • At Visit 4, blood samples for PK analysis will be taken pre-dose and between 2 and 4 hours post-dose. • At Visit 6, blood samples for PK analysis will be taken pre-dose and between 4 and 6 hours post-dose.
Planned Sample Size:	Approximately 20 children will be enrolled in Part 1 (dose-finding) of this study. *Muscle fibers:* a sample size of 20 children (from Part 1) completing the treatment period should provide 90% power (at a 2-sided alpha level of 5%) to detect at least a 12.5% increase in muscle fiber area % (MFA%) between pre- and post-treatment (which corresponds approximately to a 26% relative increase given the observed mean in the Desguerre publication of 48% [Desguerre I et al. 2009]), the ―worst case‖ standard deviation of 16%, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment. This sample size is based on a paired t-test. In the event the data is not normally distributed, a Wilcoxon signed rank test will be used. After the first 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability observed in the study will be implemented as follows: the within-subject standard deviation of MFA% will be calculated and the final sample size will be adjusted based on this observed standard deviation. A conservative approach will be adopted where the sample size may be increased but not decreased.
Diagnosis and Key Subject Selection Criteria:	Inclusion criteria: 1. Male children aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD. 2. A parent/guardian and child can comply with all study evaluations/procedures and return for all study activities. 3. Able to complete the 2 screening 6MWTs with a minimal distance of at least 250 m each. In addition, the results of these tests must be within ±30 m of each other.

	4. On a stable dose of systemic corticosteroids for at least 6 months. 5. At least 6 months worth of data on the 6MWT (this will be the "historical" 6MWT). From the moment of the historical 6MWT assessment(s), the child must not have received any compound that could potentially affect the 6MWT, with the exception of the stable steroid treatment. 6. Parent/guardian has signed the informed consent form and child has assented to be in the study (if applicable). Exclusion criteria: 1. Initiation of systemic corticosteroid therapy within 6 months prior to the start of study drug or change in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) within 6 months prior to the start of study drug. 2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and integrators will be allowed. 3. Surgery that might have an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study. 4. Exposure to another investigational drug since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment. 5. History of participation in gene therapy, cell-based therapy, or oligonucleotide therapy. 6. Presence of other clinically significant disease that in the opinion of the investigator places the child in unacceptable risk for an adverse outcome or that could affect study results. 7. Symptomatic cardiomyopathy or heart failure. If child has a left ventricular ejection fraction <45% at screening, the investigator should discuss inclusion of child in the study with the medical monitor. 8. Inadequate hematological function

	9. Absolute neutrophil count: <1.5 x 10^9/L 10. Platelets: <100 x 10^9/L 11. Current or history of liver disease or impairment, including but not limited to an elevated total bilirubin. 12. Inadequate renal function, as defined by serum creatinine >2 x the upper limit of normal. 13. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening 14. A baseline QTc >450 msec, (as the mean of 3 consecutive readings 5 minutes apart) or history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome). 15. Psychiatric illness/social situations rendering the potential child unable to understand and comply with the study protocol.
Treatments:	The study drug (Givinostat) will be administered as 2 oral doses daily while the child is in the fed state. Children who enroll during Part 1 of the study will receive their assigned dose until the RD is determined. They will then receive up to 12 months of treatment with Givinostat at the RD. Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who enroll during Part 2 will receive up to 12 months of treatment with Givinostat at the RD. The total duration of the study is anticipated to be 15 months.
Main Parameters of Efficacy:	The primary endpoint of this study is change in the value of MFA% comparing the histology biopsies before and after 12 months of treatment with Givinostat. The secondary efficacy endpoints of this study are as follows: • Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA • Change in muscular function after 12 months of treatment with

	Givinostat at the selected daily dose based the PUL
Main Parameters of Safety:	• Number of children experiencing treatment-emergent AEs and serious adverse events (SAEs) • Type, incidence, and severity of treatment-emergent AEs and SAEs correlated with dose
PK Parameters:	Individual Givinostat concentrations tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2.
Exploratory Endpoints:	Change in muscle, fat and fibrosis content after treatment with Givinostat as measured by MRI • Change in muscle biomarkers (e.g., miRNA) and cytokines following treatment with Givinostat • PK–PD correlations
Key Statistical Considerations	Efficacy analyses will be conducted on the intent-to-treat (ITT) population, which is defined as all children who are enrolled in the Part 1 portion or entered the Part 2 portion of the study. All values will be expressed as means ± standard deviation or standard error of the mean. Each of all efficacy endpoints will be subject to normality test and formal statistical test performed will be based on whether it is normally distributed. If normal, statistical differences will be calculated using the paired t test; if not normal, Wilcoxon signed rank test will be used to assess the statistical significance of differences between groups. $P<0.05$ will be set as significant. *General considerations:* Data will be provided in data listings sorted by treatment group and child number. Tabular summaries will be presented by treatment group for Part 1. Categorical data will be summarized by the number and percentage of children in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided, unless otherwise stated. Details of endpoint analyses will be described in the Statistical Analysis Plan. For analyses of change from baseline, baseline will generally be defined as the value collected before any Givinostat treatment is administered. If this value is missing, the last non-missing value prior to dosing will be used. Otherwise, missing observations will be treated as missing at random, and no data imputation will be performed. All data from the case report forms,

	as well as any derived variables, will be presented in data listings. All hypothesis tests will be two-sided with a 5% significance level, and 95% CIs will be used, unless stated otherwise. As this is a Phase 2 exploratory study, no adjustments for multiplicity will made.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

6MWT	6-Minute Walk Test
Ab	Antibodies
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the plasma–concentration time curve
BID	twice daily
BUN	blood urea nitrogen
CI	confidence interval
CL	clearance
CL/F	volume of distribution
cm	Centimeter
C_{max}	maximum plasma concentration
CMV	Cytomegalovirus
CPK	creatine phosphokinase
CrCl	creatinine clearance
CRP	C-reactive protein
CSA	cross-sectional area
CSOM	Clinical Study Operations Manual
DMD	Duchenne muscular dystrophy
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ECHO	Echocardiograph
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EOS	end of study
FACS	fluorescence-activated cell sorting
FAP	fibroadipongenic progenitors
FEV_1	forced expiratory volume at 1 second
FU	follow up
FVC	forced vital capacity
GI	Gastrointestinal
h	hour
H&E	hematoxylin and eosin
HbeAg	hepatitis B e antigen
HbsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HDAC	histone deacetylase
HIV	human immunodeficiency virus
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee

IgM	immunoglobulin M
IL	interleukin
IMP	investigational medicinal product
ITT	intent to treat
JIA	juvenile idiopathic arthritis
KA	absorption rate constant
kg	kilogram
L	liter
LDH	lactate dehydrogenase
m	Meter
MFA%	muscle fibers area %
mg	Milligram
miRNA	micro ribonucleic acid
mL	Milliliter
MRI	magnetic resonance imaging
msec	Millisecond
MTD	maximum-tolerated dose
MuSC	muscle satellite cells
ng	nanogram
nmol	nanomole
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
PD	Pharmacodynamic
PDGFR	platelet-derived growth factor receptor
PET	polyethylene terephthalate
PFT	pulmonary function tests
PK	Pharmacokinetic
PUL	performance of upper limb
QT	QT interval
QTc	QT interval – corrected
RBC	red blood cells
RD	recommended dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOJIA	systemic onset juvenile idiopathic arthritis
SWI/SNF	switch/sucrose non-fermentable
TNF-α	tumor necrosis factor-alpha
V/F	plasma volume
V2/F	peripheral volume
WBC	white blood cells
WCT	Worldwide Clinical Trials

1 Introduction

1.1 Background on Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD) is the most common childhood muscular dystrophy, occurring in about 1 of every 3500 male newborns. The disease is inherited as an X-linked recessive disorder and is caused by mutation in the dystrophin gene on the X chromosome, resulting in dystrophin deficiency. The main function of dystrophin is to stabilize and link the muscle fiber cytoskeleton to the membrane. The lack of functional dystrophin results in the loss of dystrophin-glycoprotein complex, thereby rendering the muscle fibers less resistant to mechanical stress.

Faulty muscle structure caused by the absence of extracellular or intracellular structural proteins results in cell membrane instability, initiating a cascade of deleterious events, such as uncontrolled calcium influx, apoptosis and necrosis, inflammation, and replacement of muscle with fibrotic tissue and fat (Consalvi S et al. 2011). The clinical effect of this deficiency can be dramatic and fatal.

Patients begin to show symptoms of the disease between the ages of 3 to 5 years (Emery AEH 2002), which leads to severe muscle wasting and weakness. Patients with DMD usually stop walking by about 12 years of age and usually experience fatal respiratory failure in their early 20s (Eagle M et al. 2002).

Treatment with steroids is currently used in a large portion of DMD patients, but it is palliative and complicated by serious side effects. No current treatment interrupts or halts the progression of DMD.

By acting on muscle resident stem cells, histone deacetylase (HDAC) inhibitors increase skeletal myogenesis in vitro and in vivo (Iezzi S et al. 2002) and restore normal muscle morphology and increase the size and strength of myofibers in *mdx* mice a preclinical model of DMD (Minetti GC et al. 2006).

The beneficial potential of the HDAC inhibitor Givinostat in the treatment of DMD has been studied in the *mdx* mouse disease model. In this model, long-term exposure to Givinostat effectively countered disease progression. In particular, Givinostat dose and concentration dependently increased the cross-sectional area of myofibers, decreased the cellular (inflammatory) infiltrate and prevented the formation of fibrotic scars. Pharmacokinetic (PK)–pharmacodynamic (PD) analysis suggests that exposures of Givinostat of 600 h*nmol/L are required to exert the beneficial effect.

1.2 Background on Givinostat

Givinostat has been tested in long-term repeat-dose toxicology studies in rats, dogs, and monkeys. The main adverse effects (e.g., reduction in white blood cells [WBC], reduced thymus weight, bone marrow atrophy, and liver and possibly kidney function impairments) were observed only at high doses of drug. Most of these changes returned to baseline levels upon drug discontinuation or a trend toward resolution was seen at the end of the recovery period.

In the rat, Givinostat was administered consecutively up to 26 weeks where the no observed adverse effect level (NOAEL) was 10 mg/kg/day. There were no side effects in dogs administered 12.5 mg/kg/day of Givinostat for 4 consecutive weeks. However, liquid feces were found in the animals receiving higher doses with consequent reduced absorption. For these reasons, the dog species was not further considered and general toxicology studies in non-rodents were continued in the monkey. In the monkey, Givinostat was well tolerated and similar toxicity profiles were seen in the 4-, 13-, and 39-week studies, where the NOAELs were 10 mg/kg, 10 mg/kg, and 12 mg/kg, respectively.

Givinostat had a favorable safety pharmacology profile. There was no observation of embryo-fetal toxicity in rats and in rabbits, or potential genotoxicity in mammalian cells in vitro and in vivo. The only sign of a possible adverse effect was seen at relatively high doses (>1μM) of Givinostat in vitro but not in vivo in cardiovascular safety pharmacology studies.

Givinostat was given by oral gavage to juvenile rats starting at the age of weaning (25 days of age) at 4 different dosages: 0, 20, 60, or 180 mg/kg/day once-a day for 4 weeks. Givinostat was well tolerated at the dosage up to 60 mg/kg/day. Treatment-related changes were detected at 180 mg/kg/day in the adrenals, bone marrow, liver, and spleen. The changes had resolved or showed partial recovery at the end of the study (mature animals). The NOAEL was set at 60 mg/kg/day.

Givinostat has been tested in a number of clinical studies that enrolled for the following major indications: inflammation and oncology. In particular:

- 105 healthy volunteers have been enrolled in 3 phase 1 studies (single dose, repeat dose, and food interaction); 18 were treated with placebo.
- 422 patients have been enrolled in the phase 1–2 studies; 62 were treated with placebo. Of these, 33 were children with systemic onset juvenile idiopathic arthritis (SOJIA) or polyarticular juvenile idiopathic arthritis (JIA) treated with Givinostat at the following doses: 0.50 mg/kg twice daily (BID) and 0.75 mg/kg BID for up to 3 months.

Givinostat has shown preliminary signs of clinical activity in subjects with SOJIA (study DSC/05/2357/19) as well as myeloproliferative disease (study DSC/07/2357/28).

The maximum-administered dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in subjects with multiple myeloma. Doses up to approximately 100 mg BID were generally well tolerated. The most common adverse events (AEs) observed were thrombocytopenia as well as gastrointestinal (GI) toxicities. AEs were generally mild to moderate and reversible upon discontinuation of study drug. In particular, thrombocytopenia appeared in just a few days of treatment at thrombocytopenic doses and reverted to normal values within a few days/weeks after treatment discontinuation. Twenty-one AEs of QT interval – corrected (QTc) prolongations have been reported. In one case, the electrocardiogram (ECG) was manually read and the QTc value determined was normal, thus it was considered a spurious finding. Eight AEs of QTc prolongation were reported in subjects with chronic myeloproliferative diseases (study DSC/07/2357/28), 6 in subjects with Hodgkin‘s lymphoma (study DSC/07/2357/26), 2 in subjects with polycythemia vera (study DSC/08/2357/38), 2 in subjects with acute myeloid leukemia (study DSC/05/2357/15), 1 in a subject with Crohn‘s disease (study DSC/06/2357/23), and 1 in a subject with SOJIA (study DSC/05/2357/19). No clear dose dependence was observed. In the 2 studies in healthy volunteers (DM/00/2357/01 and DM/00/2357/03) where ECG and QTc measurements were systematically assessed, no episode of QTc prolongation was observed. Details of each trial are included in the Investigator‘s Brochure (2012).

1.3 Rationale

1.3.1 Study Rationale

Different studies suggest that histone acetylation has a significant role in the pathogenesis of DMD and that inhibition of HDAC leads to a reduction in inflammation and fibrosis and an increase in muscle regeneration. In particular, two preclinical studies with Givinostat have shown that chronic treatment with this compound in a DMD mouse model (*mdx* mouse) determines a dose- and concentration-dependent reduction in inflammation and fibrosis and an increase in muscle regeneration, which in turn determines an improvement in muscular function.

The primary objective of this study is to replicate these findings in humans. In particular, the primary objective of the study will be to demonstrate that Givinostat stimulates muscle regeneration by detecting an increase in the fraction of muscle biopsy occupied by muscle when comparing the muscle biopsy at study end *versus* the muscle biopsy at study start. Other objectives of the study will be to evaluate the effects of Givinostat on other histological parameters (inflammation, necrosis, fibrosis), on functional parameters

such as the 6-Minute Walk Test (6MWT), and on magnetic resonance imaging (MRI) and biomarkers. Finally, the safety and tolerability and pharmacokinetics of Givinostat will also be assessed.

As the currently used dose of Givinostat is considered not sufficient to exert the expected positive effects (see dose rationale), the first part of the study will be used to escalate the dose to one yielding exposures expected to be efficacious and which are well tolerated.

To ensure an adequate assessment of the safety, efficacy, and PK parameters in this study, and considering the expected mechanism of action of Givinostat in DMD, ambulant children aged 7 to <11 years, who have been on a stable steroid dose for at least the last 6 months and who have a 6MWT assessment performed at least 6 months before screening will be selected for participation in this study.

1.3.2 Dose Rationale

Until now, Givinostat has been administered to 87 healthy volunteers and 360 patients enrolled in 17 phase 1–2 studies. Of the 360 patients, 33 were children/adolescents treated with either Givinostat 0.5 mg/kg BID or 0.75 mg/kg BID. The maximum dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in subjects with multiple myeloma. Subjects have been treated in clinical trials with daily doses of Givinostat for up to 6 months. Moreover, 31 patients with myeloproliferative diseases enrolled in a compassionate use program have been receiving givinostat at doses up to 50 mg 3 times daily for a period up to 4 years and 1 adolescent with JIA enrolled in the extension study has been receiving Givinostat 0.75 mg/kg since December 2011.

Doses of Givinostat up to approximately 100 mg BID have generally been well tolerated. At higher doses of Givinostat, transient reductions hematological parameters (particularly platelets) and diarrhoea as well as nausea and vomiting were observed. AEs were generally mild to moderate and reversible upon discontinuation of study drug (Investigator‘s Brochure 2012). In particular, thrombocytopenia appeared in just a few days of treatment at thrombocytopenic doses and reverted to normal values within a few days/weeks after treatment discontinuation. Twenty AEs of QTc prolongation have been reported and confirmed in the clinical studies. Of these, only 1 episode of QTc prolongation was observed in a pediatric study. No episode of QTc prolongation was observed in the 2 studies of Givinostat in healthy volunteers where ECG and QTc measurements were systematically assessed.

A population PK analysis was conducted using the PK data collected so far in all the clinical trials where PK samples were collected. In particular, PK data from 7 studies have been included (single- and repeat-dose studies in healthy volunteers, 2 studies in

Crohn's disease subjects, 1 study in subjects with SOJIA, 1 study in subjects with JIA, and 1 study in subjects with psoriasis) for a total of 226 subjects. Givinostat pharmacokinetics were described using a 2-compartment model with first order absorption showing clearance (CL/F)=118 L/h, plasma volume (V/F)=155 L, peripheral volume (V2/F)=514 L, and absorption rate constant (KA)=0.243 h^{-1}. The covariate screening indicated weight and steroid co-administration as the most significant covariates on clearance (CL) parameters (no gender, formulation, age, or healthy volunteer vs. patient differences). However, the covariates effects were considered limited on pharmacokinetics (i.e., the presence of steroids was found to determine an increase of <30% in the population CL, and an increase of 10 kg was found to determine an increase of 10%–15% in CL).

The mean exposure in healthy volunteers treated with Givinostat 100 mg BID was 1083 ng*h/mL (area under the plasma–concentration time curve from 0 to 12 hours [AUC_{0-12}]) and 181.5 ng/mL (maximum plasma concentration [C_{max}]). The estimated AUC_{0-12} and C_{max} in children treated with Givinostat are reported in Table 1.

Table 1 Concentrations of Givinostat in Pediatric Studies to Date

Disease State		AUC_{0-12} (ng*h/mL)	C_{max} (ng/mL)
SOJIA	Median 5th percentile 95th percentile	234.0 126.4 428.7	31.5 20.0 55.3
JIA	Median 5th percentile 95th percentile	206.4 109.0 492.7	31.0 14.9 69.8

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; C_{max}=maximum plasma concentration; SOJIA=systemic onset juvenile idiopathic arthritis; JIA=juvenile idiopathic arthritis.

Preclinical studies in a mouse model of DMD (*mdx* mouse model) suggest that daily exposures (AUC_{0-24}) of 600 nmol*h/L, i.e., approximately 300 ng*h/mL, are needed to exert beneficial histological and functional effects. As shown in Table 1, doses higher than those administered so far to children are needed to ensure that the majority of children are treated with doses that allow such exposures. Therefore, the first part of the study will escalate the dose to a maximum tolerated dose (MTD) that will then be recommended for the second part of the study.

Because of the limited effect of weight on CL only 2 dose adjustments will be applied as follows: children who weigh 20–49 kg: Dose X; children who weigh ≥50 kg: Dose 1.5X.

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The starting dose will be 25 mg BID in children who weigh 20–49 kg and 37.5 mg BID in children who weigh ≥50 kg. Table 2 reports the expected median, 5th, and 95th percentile exposures in children weighing 20, 30, 40, 50, and 60 kg.

Table 2 Expected Exposure by Weight

Weight (kg)	**20**	**30**	**40**	**50**	**60**
AUC_{0-12} (ng h/mL)					
Median	263	223	203	283	257
5th percentile	127	107	97	132	123
95th percentile	564	466	426	603	542

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours

Expected exposures fall within the range of exposures safely tested in children with SOJIA and JIA and well within the exposures in adults treated with 100 mg BID.

If safety and tolerability of the starting dose is confirmed in the first group of children treated, the dose will be escalated in the second group of children enrolled. The children treated at the lower dose will also be switched to the escalated dose level. Similarly, if the safety and tolerability of the second dose level is confirmed, the dose will be further escalated in the third group of children enrolled, and the children treated at the second dose level will be switched to the higher dose level.

Dose escalation will be decided based on the safety and tolerability profile observed, and on the PK analyses in the children treated until a dose-escalation decision is made. In any case each dose escalation should not yield more than a doubling of the expected exposure. An example of dose escalation based on the data available so far is provided in Table 3, Table 4, and Table 5 show the starting, second, and third dose, respectively.

Table 3 Starting Dose (25 mg BID in Children Who Weigh 20–49 kg and 37.5 mg BID in Children Who Weigh ≥50 kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	263	223	203	283	257
5th percentile	127	107	97	132	123
95th percentile	564	466	426	603	542

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Table 4 Second Dose (50 mg BID in Children Who Weigh 20–49 kg and 75 mg BID in Children Who Weigh ≥ 50kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	527	445	407	566	515
5th percentile	254	215	194	263	246
95th percentile	1129	932	852	1205	1084

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Table 5 Third Dose (75 mg BID in Children Who Weigh 20–49 kg and 100 mg BID in Children Who Weigh ≥50 kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	790	668	610	755	686
5th percentile	381	322	290	351	329
95th percentile	1693	1398	1278	1607	1446

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Once all 20 children enrolled in Part 1 of the study have been treated for at least 2 weeks, the review team will decide the recommended dose (RD) to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses.

All children will then switch to the RD, which will be administered for the subsequent 12 months of the study (Part 2).

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This clinical study has been designed to comply with the Good Clinical Practice guidelines.

2 Study Objectives

Primary Objective

The primary objective of this study is as follows:

- To establish the histologic effects of Givinostat administered chronically at the selected daily dose

Secondary Objectives

The secondary objectives of this study are as follows:

- To establish the effects of Givinostat administered chronically at the selected daily dose on functional parameters, such as the 6MWT, North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL)
- To establish the safety and tolerability of Givinostat administered chronically at the selected daily dose in children with DMD
- To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as MRI, biomarkers, and cytokines

Primary Endpoint

The primary endpoint of this study is as follows:

- Change in the value of muscle fiber area % (MFA%) comparing the histology biopsies before and after 12 months of treatment with Givinostat

Secondary Efficacy Endpoints

The secondary efficacy endpoints of this study are as follows:

- Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA

- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based the PUL

Safety Endpoints

The safety endpoints of this study are as follows:

- Number of children experiencing treatment-emergent AEs and serious AEs (SAEs)
- Type, incidence, and severity of treatment-related AEs and SAEs
- Measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies), echocardiographs (ECHOs), pulmonary function tests (PFTs; forced expiratory volume at 1 second [FEV_1], forced vital capacity [FVC], and FEV_1/FVC), and 12-lead ECGs.

Pharmacokinetic Endpoints

The PK endpoints of this study are as follows:

- Individual Givinostat concentrations tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2.

Exploratory Endpoints

The exploratory endpoints of this study are as follows:

- Change in muscle, fat, and fibrosis content after treatment with Givinostat as measured by MRI
- Change in muscle biomarkers (e.g., miRNA) and cytokines following treatment with Givinostat
- PK–PD correlations

3 Investigational Plan

3.1 Description of Overall Study Design and Plan

This is a 2-part, phase 2 study to assess the effects of Givinostat on muscle histologic parameters and on clinical parameters in ambulant children with DMD. The safety, tolerability, and pharmacokinetics of Givinostat will also be assessed.

Children who assent to participate in this study (if capable of doing so) and whose parent/guardian signs the informed consent to participate will undergo pre-study screening assessments up to 4 weeks (±2 weeks) before the first scheduled dose of study drug.

Approximately 20 children will be enrolled in the study as follows: the first 4 children will be treated at a low dose level of Givinostat (25 mg BID in children who weigh 20-49 kg and 37.5 mg BID in children who weigh ≥50 kg).

If none of the stopping criteria (see Section 3.3.5 for stopping criteria) are met after 2 weeks of treatment at the low dose, the review team will determine the escalated dose level (i.e., intermediate dose level) to be used for the treatment of an additional 8 children who will be treated at the intermediate dose. The 4 children previously treated at the low dose level will also be switched to the intermediate dose level.

If none of the stopping criteria are met after 2 weeks of treatment at the intermediate dose, the review team will determine the subsequent escalated dose level to be used for the treatment of an additional 8 children who will be treated at the high dose. All children treated at the intermediate dose level will be switched to the high dose level.

Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the RD to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2).

After the 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability of MFA% observed in the study will be implemented, the within-subject standard deviation of MFA% will be calculated, and the final sample size will be adjusted based on this interim analysis and in particular on the observed standard deviation. A conservative approach will be adopted, where the sample size may be increased but not decreased.

The additional children (if any) will be enrolled during Part 2 of the study and will receive the RD of Givinostat for 12 months.

A treatment table for the study is presented in Table 6.

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Table 6 Treatment Table

	PART 1						PART 2									
	Treatment Visit[c]						Treatment Visit[c]									
Subject	**1**	**2[a]**	**3**	**4[a]**	**5**	**6[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[b]**
1–4																
5–12																
13–20																
Additional children (if any)																

[a] At the end of Week 2 (Visit 2) at every dose level, a safety check is foreseen.
[b] At the end of Month 12, an efficacy analysis on biopsy results and functional tests is foreseen.
[c] The visits during Part 1 will be performed every 7 days (±1 day); the visits during Part 2 will be performed periodically every 1 – 1.5 months (± 7 days).

Legend:

	25 – 37.5 mg BID – low dose level
	Intermediate dose level
	High dose level
	Recommended dose level

During Part 1 of the study, children will visit the center once a week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 10 visits, not including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 1 week after the last dose of study drug. Children who have ongoing AEs at discontinuation will be followed until resolution or stabilization.

Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2). Exploratory efficacy parameters will include changes from baseline in MRI results, PK–PD correlations, and measures of cytokines and miRNA. Safety will be assessed by number of children experiencing treatment-emergent AEs and SAEs; type, incidence, and severity of treatment-emergent AEs and SAEs; and measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies), ECHOs, PFTs (FEV_1, FVC, and FEV_1/FVC), and 12-lead ECGs.

After 1 week of treatment, blood samples for PK measurements will be collected pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose.

PK samples will also be collected during Part 2 of the study. At Visit 2, blood samples for PK analysis should be taken pre-dose and between 6 and 8 hours post-dose. At Visit 3, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and between 0 and 2 hours post-dose. At Visit 4, blood samples for PK analysis should be taken pre-dose and between 2 and 4 hours post-dose. At Visit 6, blood samples for PK analysis should be taken pre-dose and between 4 and 6 hours post-dose.

3.2 Selection of Study Population

This study will enroll approximately 20 ambulatory male children with an established diagnosis of DMD who are at least 7 years of age but <11 years of age. Additional children could be enrolled in the second part of the study, after the interim evaluation of baseline biopsies if the observed variability is higher than the one used for the current sample size estimate. Children will be enrolled at approximately 5 study sites in Italy. Specific entry criteria are detailed in Section 3.2.1 and Section 3.2.2.

3.2.1 Inclusion Criteria

Children meeting all of the following inclusion criteria are eligible for inclusion in the study:

1. Male children aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD.
2. Parent/guardian has signed the informed consent form and child has assented to be in the study (if applicable).
3. Able to complete the 2 screening 6MWTs with a minimal distance of at least 250 m each. In addition, the results of theses 2 tests must be within ±30 m of each other.
4. On a stable dose of systemic corticosteroids for at least 6 months.
5. At least 6 months worth of data on the 6MWT (this will be the ‑historical‖ 6MWT). From the moment of the historical 6MWT assessment(s), the child must not have received any compound that could potentially affect the 6MWT, with the exception of the stable steroid treatment.
6. A parent/guardian and child can comply with all study evaluations/procedures and return for all study activities.

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3.2.2 Exclusion Criteria

Children meeting any of the following exclusion criteria will not be enrolled in the study.

1. Initiation of systemic corticosteroid therapy within 6 months prior to the start of study drug or change in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) within 6 months prior to the start of study drug.
2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and integrators will be allowed.
3. Surgery that might have an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study.
4. Exposure to another investigational drug since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment.
5. History of participation in gene therapy, cell-based therapy or oligonucleotide therapy.
6. Presence of other clinically significant disease that in the opinion of the investigator places the child in unacceptable risk for an adverse outcome or that could affect study results.
7. Symptomatic cardiomyopathy or heart failure. If child has a left ventricular ejection fraction <45% at screening, the investigator should discuss inclusion of child in the study with the medical monitor.
8. Inadequate hematological function
9. Absolute neutrophil count: $<1.5 \times 10^9/L$
10. Platelets: $<100 \times 10^9/L$
11. Current or history of liver disease or impairment, including but not limited to an elevated total bilirubin.
12. Inadequate renal function, as defined by serum creatinine >2 x the upper limit of normal.

13. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening

14. A baseline QTc >450 msec, (as the mean of 3 consecutive readings 5 minutes apart) or history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome).

15. Psychiatric illness/social situations rendering the potential child unable to understand and comply with the study protocol.

3.2.3 Removal of Children from Therapy or Assessment

A child will be considered to have completed the study when he completes the 12-month/end-of-study visit (Visit 10) in Part 2. If a child is discontinued at any time after entering the study, the investigator will make every effort to see the child and complete the early termination and follow-up assessments as shown in Section 3.4.5. All AEs should be followed until the child recovers or his condition stabilizes.

A termination electronic case report form (eCRF) should be completed for every child who receives study drug, whether or not the child completes the study. The reason for any early discontinuation should be indicated on this form. The primary reason for a child withdrawing prematurely should be selected from the following standard categories of early termination:

- *Adverse Event (Adverse Reaction)*: Clinical or laboratory events occurred that, in the medical judgment of the investigator for the best interest of the child, are grounds for discontinuation. This includes serious adverse events (SAEs) and non-serious AEs, regardless of relation to study drug.
- *Death:* The child died.
- *Withdrawal of Consent*: The child or his parent/guardian desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If the child or parent/guardian gave a reason for withdrawing, it should be recorded in the eCRF.
- *Protocol Violation*: The child‘s findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g., drug noncompliance, failure to return for defined number of visits). The violation necessitated premature termination from the study.
- *Lost to Follow-Up*: The child stopped coming for visits, and study personnel were unable to contact the child.

- *Other*: The child was terminated for a reason other than those listed above, such as theft or loss of study drugs or termination of study by sponsor.

3.3 Treatments

3.3.1 Details of Study Treatment

Details about study drug are provided in Table 7.

Table 7 Details of Study Treatment

	Givinostat^
Drug name	Givinostat
Manufacturer	Italfarmaco S.p.A.
Dose(s)	1st dose level: 25 mg (children 20–49 kg) 37.5 mg (children ≥50 kg) 2nd dose level: 50 mg (children 20–49 kg) 75 mg (children ≥ 50 kg)* 3rd dose level: 75 mg (children 20–49 kg) 100 mg (children ≥ 50 kg)*
Dose frequency	BID
Route	Oral under fed conditions
Formulation	Oral suspension and/or capsules

^Givinostat is used to indicate the whole study drug name Givinostat hydrochloride monohydrate. The dosages / concentrations of the study drug are expressed as Givinostat hydrochloride monohydrate.
* Estimated doses based on current knowledge.
BID=twice daily

3.3.2 Dosage Schedule

Study drug will be administered as 2 oral doses daily while the child is in the fed state. Children who enroll during Part 1 of the study will receive their assigned dose until the RD is determined. They will then receive up to 12 months of treatment with Givinostat

at the RD. Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who enroll during Part 2 will receive up to 12 months of treatment with Givinostat at the RD.

3.3.3 Treatment Assignment

All children will receive study drug. Children enrolled in Part 1 of the study will start to take either the low, intermediate, or high dose, depending on the needs of the study at the time of enrollment and they will switch to the RD when the review team decide that this dose is safe (see Table 6).

When the safety review team has determined the RD, all children currently on study drug will be switched to that dose (the RD), and Part 2 of the study will commence. All children who enroll during Part 2 (if applicable) of the study will be given the RD of Givinostat.

3.3.4 Drug Packaging, Labeling, Storage, Dispensing, Investigational Medicinal Product Accountability, and Blinding

Drug Packaging - Oral Suspension

The primary packaging will consist of an amber plastic bottle containing the suspension. The secondary packaging will be a carton box containing 1 amber bottle and a syringe dosing system for dispensing the suspension.

All the bottles will contain 120 mL of suspension.

Drug Packaging - Capsules

Capsules will be supplied as 50 mg hard gelatin capsules for oral administration in white plastic bottles containing 30 capsules each.

Labeling

The primary and secondary labels will show all the information requested according to the Annex 13 of the Good Manufacturing Practice. Bottles containing Givinostat will be labeled in local language (i.e., Italian).

Storage

The investigational medicinal product (IMP; Givinostat) will be stored at Italfarmaco until distribution to the investigational sites. The investigational site will store the IMP under the conditions specified in the label (i.e., 5 ±3°C for the oral suspension and <30°C for the capsules), ensuring that it is not accessible to unauthorized persons until it is dispensed to the child‘s parents/legal guardians.

Dispensing

The investigational site will be supplied initially with a congruous number of suspension bottles and capsule bottles, in order to have sufficient supply of study drug to treat the children who are enrolled in the study.

All IMP supplies are to be used only for this protocol and for no other purpose.

The investigator will be responsible for the delivery of IMP to the child‘s parents/legal guardians according to the protocol.

Children will be administered the IMP on an outpatient basis.

At each scheduled visit, the investigator will supply the children with the appropriate number of suspension bottles and/or capsules bottles, sufficient to cover the treatment until the following visit (i.e., 1 week of treatment during Part 1 of the study and 1 to 1.5 months, during Part 2 of the study).

Italfarmaco will provide to the investigator a table with volume of suspension and/or number of capsules to be administered according to body weight and dose level.

The investigator will provide to the child‘s parent/legal guardian written instruction on the dosage and corresponding volume in milliliters of suspension and/or number of capsules to be taken at each administration. Refer to the relevant Clinical Study Operations Manual (CSOM) for more detailed information.

Blinding

Not applicable. This is an open-label study.

Emergency Procedure for Unblinding

Not applicable. This is an open-label study.

3.3.5 Dose Modifications

3.3.5.1 Child Stopping and Dose Reduction Safety Rules

Permanent Stopping Rule

Study drug should be permanently stopped if any of the following occur:

- Severe diarrhea (i.e., increase of ≥7 stools per day)
- Any drug-related SAE
- QTc >500 msec
- Platelets ≤50 x 10^9/L

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Temporary Stopping and Dose Reduction Safety Rule

Study drug should be temporarily stopped if any of the following occur:

- Platelets ≤75 x 10^9/L but >50 x 10^9/L
- Moderate diarrhea (i.e., increase of 4 to 6 stools per day)

Study drug may be resumed at a reduced dose level once the event resolves. Such a dose reduction can happen only once per child. The treatment can be temporarily interrupted for a maximum of 4 weeks. If the child has not recovered from the AE after this period, the treatment should be permanently discontinued.

If a child has a medical event not necessarily drug related that requires interruption of study drug dosing for >4 weeks, the review team will determine if the child may resume study drug treatment.

Cohort Expansion Safety Rules

The review team can decide to expand the first cohort of children and enroll additional 4 children (for a total of 8 children treated at the first dose level), on the basis of safety, tolerability, and PK results in the children treated in that cohort, according but not limited to the following rules:

- If 1 child experiences any type of stopping criterion (as defined in the permanent and temporary stopping rules), or
- If 2 children experience different types of stopping criterion (e.g., 1 child experiences hematological toxicity and another child experiences GI toxicity).

3.3.5.2 Cohort Abandoned Safety Rules

The review team can also decide to abandon a given dose level on the basis of safety, tolerability and PK results in the children treated at that dose level, according to but not limited to the following rules:

- If >2 children experience any type of stopping criterion, or
- If ≥2 or more children experience the same type of stopping criterion

3.3.5.3 Cohort Dose Escalation Safety Rules

During Part 1 of the study, each child will receive study drug at a specific dose level. Once the first 4 children have been treated for at least 2 weeks, the review team will examine safety and tolerability data and PK results and decide if the children can be switched to the escalated dose level and if the second group of children can start the treatment at the escalated dose level.

After a further 2 weeks, the review team will decide if the already treated children at the lower and intermediate dose levels can switch to the highest dose and if the third and last group of children can start the treatment at the highest dose level.

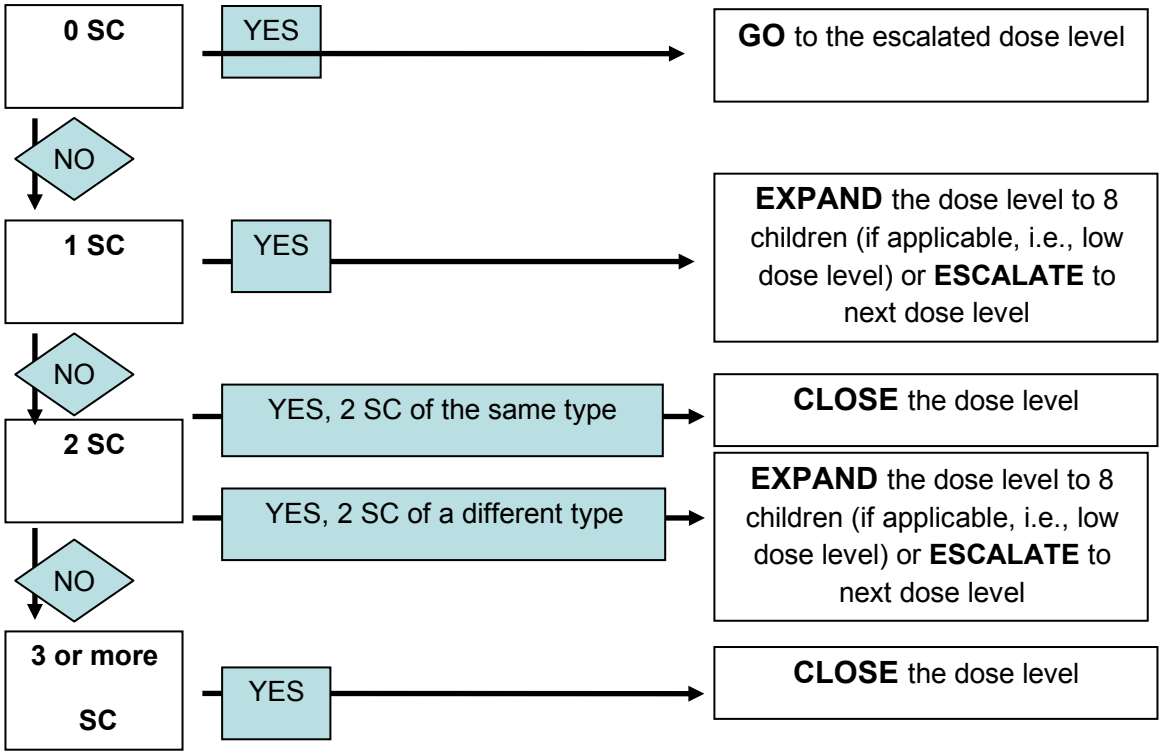
The children should be switched to the escalated dose level (i.e., intermediate or high dose level) only if no or only 1 stopping criterion per dose level occurred.

For more details, see Figure 1.

3.3.5.4 Study Stopping Safety Rules

If 2 or more stopping criteria occur in the lower dose, the study will be temporarily stopped to allow a reassessment of the risks and benefits of the compound.

Figure 1 Cohort expansion, Cohort Abandoned and Dose Escalation Safety Rules for Part 1



SC=stopping criterion, as defined under permanent and temporary stopping rules.

The review team will include: the Principal Investigators, the Study Chair, the Medical Monitor and other Italfarmaco representatives.

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3.3.6 Treatment Compliance and IMP Accountability

At each visit, the child‘s parents/legal guardians will bring back to the study site all the bottles previously received (used, partially used, and unused) and receive a new supply of the IMP.

All unused IMP will be not re-dispensed by the investigator to another child.

The investigator will maintain accurate records of the disposition of all IMP received, distributed to children (including date and time), and accidentally lost or destroyed.

When dispensing the bottles to the children, the investigator will attach the relevant tear-off label to the relevant form.

Oral Suspension Accountability

The residual volume of suspension in the bottles will be measured by the investigator by means of a calibrated glass cylinder supplied by Italfarmaco and reported in the Drug Accountability Form and in the eCRF.

Capsule Accountability

The investigator will count the capsules unused for each bottle and insert the number in the Drug Accountability Form and in the eCRF.

Periodically throughout and at the conclusion of the study, a representative of Italfarmaco S.p.A. or its delegate will conduct an inventory of all study drug supplies and the bottles of oral suspension and/or capsules used, partially used, and unused will be destroyed at the site, if possible, or sent back to Italfarmaco.

Missed doses are not to be recovered and they should be recorded in the eCRF and in the Drug Accountability Form, specifying the reason for any missed dose.

For more detailed procedures, please refer to the relevant CSOM.

3.3.7 Prior and Concomitant Illnesses and Treatments

Prior and Concomitant Illnesses

Investigators should document all significant illnesses that the child has experienced within 6 months of screening. Additional illnesses present at the time informed consent is given are to be regarded as concomitant illnesses. Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs in the eCRF.

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Prior and Concomitant Treatments

Prior treatments, defined as those taken within 6 months prior to screening, should be recorded in the eCRF as prior medications.

Concomitant treatments are defined as treatments taken after study drug administration.

Children should be on stable systemic corticosteroid therapy for at least 6 months prior to initiation of study drug. That is, there have been no changes in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) during the previous 6 months.

Supportive treatments, such as anti-emetics, anti-diarrheals, anti-pyretics, anti-allergics, analgesics, and antibiotics are allowed.

Use of Vitamin D, calcium and integrators if clinically indicated before enrolment and for duration of the trial are allowed.

The following medications are prohibited prior to (as noted below) and during study treatment:

- Other investigational agents within 3 months of start of study drug, since time historical 6MWT data was obtained, or while on study
- Prior gene therapy or cell-based therapy or oligonucleotide therapy prior to study treatment or while on study
- Any pharmacologic treatments (other than stable doses of corticosteroids) that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone) or while on study
- Drugs that prolong the QTc interval (see Appendix 1: Drugs Known to Prolong the QTc Interval)

3.4 Assessments

3.4.1 Schedule of Assessments

The procedures to be performed during the study are outlined in the Schedule of Assessments (Table 8 and Table 9). A detailed description of each assessment may be found in Section 3.4.2.

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Table 8 Schedule of Assessments: Part 1 (Dose Escalation)

Assessment	Screen/ Pre-Study[a]	Part 1 (Approximately 20 Children)					
Week	**-4 (±2 weeks)**	**0**	**1**	**2**	**3**	**4**	**5**
Visit (1-day window)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Informed consent and assent	X						
Medical history/ eligibility	X						
Concomitant medications	X[b]	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X
Height	X						
12-lead ECG[c]	X	X	X	X	X	X	X
ECHO[c]	X						
PFTs (FEV_1, FVC, FEV_1/FVC)[c]	X						
Clinical laboratory tests[c,d]	X	X	X	X	X	X	X
Urinalysis[b,c]	X	X	X	X	X	X	X
Cytokine	X						
miRNA	X						
Serology[e]	X						
Quality of Life test	X						
Muscle evaluations (6MWT[f], NSAA[g], PUL[g])	X						
Muscle biopsy	X						
MRI	X[i]						
PK assessment[h]			X				
Adverse events	X	X	X	X	X	X	X
Study drug administration		X	X	X	X	X	X

6MWT=6-Minute Walk Test; CMV=cytomegalovirus; EBV=Epstein Barr virus; ECG=electrocardiogram; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MRI=magnetic resonance imaging; NSAA=North Star Ambulatory assessment; PFTs=pulmonary function tests; PK=pharmacokinetic.

[a] During the pre-study visit, historical function data (from 6 months before the pre-study visit) will be collected.
[b] Obtain prior medications at screening as well.
[c] To be performed more frequently, if clinically indicated.
[d] The following laboratory parameters will be assessed: hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, and abnormal cells; blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium, potassium,

chloride, calcium, glucose, creatinine, BUN, and CPK (CrCl will be calculated by the Cockcroft and Gault formula); urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.
[e] The following serology tests will be conducted: ELISA for HIV-Ab, HCV-Ab, HbsAg, HbeAg, anti-EBV, and anti-CMV IgM.
[f] During the pre-study visit, the historical function data relevant to 6MWTs will be collected: (1) result from 6MWT performed at least 6 months before the pre-study visit, and (2) result from 2 6MWT performed within 4 weeks prior to treatment start will be necessary and inserted into the eCRF.
[g] During the pre-study visit, the functional data relevant to the NSAA and PUL will be collected: results performed within 4 weeks prior to treatment start will be necessary and inserted into the eCRF.
[h] After 1 week of treatment, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose. It is important to record the time of PK assessments and the time of the last drug intake in the evening before the PK assessment.
[i] Obtain an MRI of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb). MRI does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment.

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Table 9 Schedule of Assessments: Part 2 (Proof of Mechanism)

Assessment	Part 2 (All Children)[j]									12/ EOS Visit[i]	FU Visit
	Month of Study:										
	0	1	2	3	4.5	6	7.5	9	10.5	12	--
Visit (1 week window)	**1[a]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X
Height										X	
Weight	X	X	X	X	X	X	X	X	X	X	
12-lead ECG[c]	X	X	X	X	X	X	X	X	X	X	X
ECHO[c]										X	
PFTs (FEV_1, FVC, FEV_1/FVC)[c]										X	
Clinical laboratory tests[c,d]	X	X	X	X	X	X	X	X	X	X	X
Urinalysis[c,d]	X	X	X	X	X	X	X	X	X	X	X
Serology[e]											
Quality of life test										X	
Muscle evaluations (6MWT[f], NSAA[g], PUL[g])	X			X		X				X	
Muscle biopsy										X	
MRI										X	
Cytokine	X			X		X				X	
miRNA	X			X		X				X	
PK assessment[h]		X	X	X		X					
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Study drug administration	X	X	X	X	X	X	X	X	X		

6MWT=6-Minute Walk Test; CMV=cytomegalovirus; EBV=Epstein Barr virus; ECG=electrocardiogram; EOS=end of study; FU=follow up; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MRI=magnetic resonance imaging; NSAA=North Star Ambulatory assessment; PK=pharmacokinetic.

[a] The screening visit is only for children newly enrolled in Part 2. Children who were enrolled in Part 1 do not need to repeat the screening visit. For the relevant assessment, see Table 3 (screen/Pre study visit).
[b] Obtain prior medications at screening as well.
[c] To be performed more frequently, if clinically indicated.

[d] The following laboratory parameters will be assessed: Hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, ANC, and abnormal cells; Blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CPK (CrCl will be calculated by the Cockcroft and Gault formula); Urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.
[e] The following serology tests will be conducted: ELISA for HIV-Ab, HCV-Ab, HbsAg, HbeAg, anti-EBV, and anti-CMV IgM.
[f] During the pre-study visit, the historical function data relevant to 3 6MWTs will be collected: (1) result from 6MWT performed at least 6 months before the pre-study visit, and (2) result from 6MWT performed within 4 ±2 weeks prior to treatment start will be necessary and inserted into the eCRF.
[g] During the pre-study visit, the functional data relevant to the NSAA and PUL will be collected: results performed within 4±2 weeks prior to treatment start will be necessary and inserted into the eCRF.
[h] At Visit 2, blood samples for PK analysis should be taken pre-dose and between 6 and 8 hours post-dose. At Visit 3, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and between 0 and 2 hours post-dose. At Visit 4, blood samples for PK analysis should be taken pre-dose and between 2 and 4 hours post-dose. At Visit 6, blood samples for PK analysis should be taken pre-dose and between 4 and 6 hours post-dose. It is important to record the time of PK assessments.
[i] Children who discontinue participation prior to completion the study should perform the Early Termination Visit within 2 weeks after the last drug intake. For the assessments to be performed, see –12/EOS visit.‖
[j] Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the RD to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2).

3.4.2 Study Procedures

3.4.2.1 Pre-Study (Screening)—(Day -28 [±2 Weeks])

The assessments during the pre-study phase will determine the child's eligibility for the study and also their ability to comply with protocol requirements by completing all screening assessments. All children will undergo all screening assessments, regardless of whether they are enrolled during Part 1 or Part 2 of the study.

The following procedures will be performed and recorded during the screening period:

- Obtain written informed consent from the child's parent/legal guardian and assent from the child, if applicable.
- Collect medical history.
- Review inclusion and exclusion criteria.
- Obtain and record current and prior medications (taken in the past 6 months).
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC.
- Obtain blood samples for hematology, blood chemistry, and disease serology tests.
- Obtain a urine sample for urinalysis.
- Obtain blood samples for cytokine measurements and miRNA.
- Perform a quality of life test
- Perform the muscle evaluations:
    - 6MWT (collect historical data on tests performed at least 6 months prior to the pre-study visit and within 4 ± 2 weeks of pre-study visit).
    - NSAA and PUL (collect results from within 4 ± 2 weeks prior to treatment start).
- Obtain a muscle biopsy (brachial biceps; please refer to Section 3.4.6 for instructions; obtained within 4 ± 2 weeks prior to the start of treatment).
- Obtain an MRI of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb; MRI does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment).
- Assess AEs

3.4.2.2 Weekly Visits (Part 1; Weeks 0 to 6)

Children will visit the study center weekly during Part 1 of the study. Children will continue to come in weekly (i.e., every 7 ± 1 days) until they are switched to Part 2 of the study when the RD is selected. At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain blood for PK assessments at Visit 2 only as noted in the schedule of assessments.
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.2.3 Part 2 Visits (Months 1 to 10.5)

Children will visit the study center as noted in the schedule of assessments during Part 2 of the study (i.e., every 1 to 1.5 months ± 1 week). At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain blood samples for cytokine and miRNA (Visits 4 and 6 only).
- Obtain a urine sample for urinalysis.
- Obtain blood for a PK assessments at Visits 2, 3, 4, and 6 only as noted in the schedule of assessments.
- Obtain muscle evaluations (6MTW, NSAA, and PUL; Visits 4 and 6 only).
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.3 Assessments for End of Study (Visit 10 [Month 12])

Children who remain in the study will continue to take study drug through Visit 10. The end-of-study visit overlaps with the 12-month visit, if the child completes treatment.

The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Perform a quality of life test.
- Obtain muscle evaluations (6MWT, NSAA, PUL).
- Perform a muscle biopsy (please refer to Section 3.4.6 for instructions).
- Obtain an MRI of dystrophic muscle.
- Obtain blood samples for cytokines and miRNA.
- Perform accountability.
- Assess AEs.

3.4.4 Early Termination Visit

Children who discontinue participation prior to completion of all study drug administration (i.e., 12 months of treatment) will be asked to return to the hospital within 2 weeks after the last dose of study drug for completion of the same assessments given at the End of Study visit (for details, see Section 3.4.3).

3.4.5 Follow-up Visit

All children, regardless of whether they complete the study or terminate early in Part 1 or Part 2 should return to the study center within 4 weeks of the last dose of study drug for the follow-up visit.

The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.

- Obtain a 12-lead ECG in triplicate.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Assess AEs.

3.4.6 Description of Assessments

Unless otherwise indicated, all assessments will be performed by the investigator or other regular study personnel. Assessments are to be performed according to the schedule shown in Section 3.4.1. The assessments at the end-of-study and follow-up visits should also be performed if the child terminates the study early.

Efficacy Assessments

Muscle Biopsies: A first brachial biceps biopsy (baseline) will be taken prior to the first dose of study drug. A second brachial biceps biopsy will be taken at the end of study from the opposite arm.

The muscle biopsy samples from the biceps muscle will be collected by open biopsy according to standard hospital procedures for obtaining muscle biopsies from children. The minimum amount of muscle tissue required is a small piece of muscle of at least 0.5 x 0.5 x 0.5 cm. The muscle sample, embedded with tragacanth gum on a piece of cork, must be frozen in liquid nitrogen-cooled 2-methylbutane and stored at -80°C or -70ºC until shipment.

The collection, processing, and shipment of these muscle biopsy samples to the Ospedale Pediatrico Bambino Gesù laboratory will be described in detail in the study-specific laboratory manual.

6-Minute Walk Test: A modified version of the 6MWT recommended by American Thoracic Society (2002) for use in adults will be performed.

North Star Ambulatory Assessment: The NSAA will be graded using the standard scorecard with each assessment rated as 0 – unable to achieve independently, 1 – modified method but achieves goal independent of physical assistance from another, or 2 – normal with no obvious modification of activity.

Performance of Upper Limb: The PUL was devised to assess motor performance in the upper limb for patients with Becker and Duchenne muscular dystrophy. The purpose is to assess change that occurs in motor performance of the upper limb over time from when a child is still ambulant until he loses all arm function when non-ambulant. The PUL will

be administered according to the guidelines developed by the Physiotherapy Working Group (Mayhew A et al. 2012, Mercuri E et al. 2012).

MRIs: MRIs will be taken of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb) and evaluated per the specifications in the CSOM.

Cytokines and miRNA: The following cytokines may be evaluated: tumor-necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-6. In addition, miR-1, miR-133, and miR-206 may be evaluated. Additional cytokines and miRNA parameters may be evaluated if warranted. Evaluation of cytokines and miRNA will be documented in a separate report.

Other efficacy assessments – exploratory analysis of muscle biopsies: Muscle biopsies will be analyzed in 2 distinct steps, by using different complementary approaches (fluorescence-activated cell sorting [FACS]-mediated isolation of cells and functional characterization ex vivo and staining of frozen sections) that are finalized to the identification and functional/molecular characterization of specific cell types that contribute to the regeneration or fibro-adipogenic degeneration of dystrophic muscles. These cells include muscle satellite cells (MuSCs) and a heterogeneous population that is referred to as ―fibro-adipogenic progenitors‖ (FAPs). These cell types can be isolated by FACS in mouse and human muscle samples as distinct populations, based on specific combination of surface antigens.

In the mouse system, MuSCs can be isolated from skeletal muscles by FACS, as $CD34^{pos}/\alpha7\text{-integrin}^{pos}/Sca1^{neg}$ cells (Sacco A et al. 2008), while FAPs are isolated either as $CD34^{pos}/\alpha7\text{integrin}^{neg}/Sca1^{pos}$ cells or platelet-derived growth factor receptor (PDGFR) PDGFR- α^{pos} cells (Joe AW et al. 2010; Uezumi A et al. 2010). In muscle biopsies of human patients or normal individuals, MuSCs are isolated as PDGFR- $\alpha^{neg}/\alpha7\text{-integrin}^{pos}$/N-CAM ^{pos} cells, while FAPs can be isolated as PDGFR- $\alpha^{neg}/\alpha7\text{-integrin}^{pos}$/N-CAM ^{pos} cells.

Pre-clinical studies have demonstrated that functional interactions between these cell types contributes to the disease progression in mouse models of DMD (*mdx* mice) and that HDAC inhibitors promote the FAP property of stimulating muscle regeneration at the expense of fibro-adipogenic degeneration. The sponsor has identified a novel nuclear network that regulates FAP lineage identity and ability to support regeneration or fibro-adipogenic degeneration in dystrophic muscles. This network consists of an HDAC-repressed miRNA (the myomiRs 1.2, 133a and 206) that target 2 specific sub-units (BAF60a and b) of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex, which promotes the expression of fibro-adipogenic genes. Upon

treatment with HDAC inhibitors, de-repression of myomiRs 1.2, 133a and 206 causes the down regulation of BAF60a and b, and the simultaneous activation of BAF60c, leading to the formation of BAF60c-based SWI/SNF complex that promotes skeletal myogenesis in FAPs. The sponsor has found that *mdx* mice that respond to HDAC inhibitors, such as Givinostat, show increased levels of BAF60c and myomiRs 1.2, 133a and 206.

1. FACS-sorting of MuSCs and FAPs from biopsies of children before and at the end of the treatment will be used to measure: a) the relative amount of these cell populations; b) their ability to differentiate in culture, in myogenic and adipogenic media, and their functional interactions by co-culture experiments; c) gene and miRNA expression analysis by quantitative polymerase chain reaction.

   It should be emphasized that in vitro exposure to Givinostat of MuSCs and FAPs from biopsies of children before the treatment might provide a useful measure predictive of child response that can be used to better select children in a follow-up trial.

2. Histologic analysis of muscle sections from biopsies of children before and at the end of the treatment will be used to measure: a) the expression levels of BAF60 a, b, and c variants, and myomiRs 1.2, 133a and 206 in MuSCs and FAPs, which will be identified as $Pax7_{pos}$ or PDGFR- α_{pos}, respectively.

Safety Assessments

Physical Examination: Physical examination will include examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system. An AE form must be completed for all changes identified as clinically noteworthy. Height without shoes and weight will be recorded as noted in the schedule of assessments.

Vital Signs: Vital signs will include body temperature (ºC), pulse rate, and blood pressure.

Electrocardiogram: Standard 12-lead ECGs will be conducted in triplicate per the schedule of assessments and more often if clinically indicated. ECG will be acquired at the sites, then ECG data will be transmitted and a central reading will be performed. For details, see the relevant CSOM.

ECHOs: Standard ECHOs will be conducted as per the schedule of assessments and more often if clinically indicated.

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Pulmonary function tests: FEV_1, FVC, and FEV_1/FVC, will be collected per the site's standard process as noted in the schedule of assessments and more often if clinically indicated.

Laboratory Parameters: The following laboratory tests are to be performed as indicated in the schedule of assessments and more often if clinically indicated:

- Hematology: red blood cell (RBC) count, hemoglobin, hematocrit, WBC count with differential, platelets, ANC, and abnormal cells
- Blood chemistry: total bilirubin, alkaline phosphatase, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, blood urea nitrogen (BUN), and creatine phosphokinase (CPK) (creatinine clearance [CrCl] will be calculated by the Cockcroft and Gault formula.)
- Urinalysis: pH of freshly voided specimen, specific gravity, protein, glucose, ketones, cytology, and myoglobin
- Other: enzyme-linked immunosorbent assay (ELISA) for HIV- Ab, HCV-Ab, HbsAg, hepatitis B e antigen (HbeAg), anti-EBV, and anti-cytomegalovirus (CMV) immunoglobulin M (IgM).

Lab tests will be performed and analyzed by a local laboratory to ensure consistent interpretation of results. In the event of an unexplained clinically noteworthy abnormal laboratory test value, the test should be repeated immediately and followed up until it has returned to the normal range and/or an adequate explanation of the abnormality is found.

Adverse Events: An AE is any is –any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (International Conference on Harmonisation [ICH] E2A).‖ All medical and psychiatric conditions (except those related to the indication under study) present at screening will be documented on the Prior Illnesses eCRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE eCRF during the rest of the study. Laboratory abnormalities should be recorded as AEs only if they meet the criteria for an SAE, result in discontinuation of the study drug, require medical intervention, or are judged by the investigator to be clinically significant changes from baseline values. See Section 4, for additional information.

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Pharmacokinetic Assessments

Blood samples for PK assessments will be collected as indicated in the schedule of assessments. Additional information about the PK time points will be provided in the relevant CSOM.

Quality of Life Assessment

The PedsQL Test will be performed before treatment start and at the end of the study.

3.4.7 Appropriateness of Measurements

All assessments to be used in this study are commonly used, standard measurements frequently seen in DMD studies.

4 Adverse Event Reporting

Throughout the course of the study, all AEs will be monitored and reported in the AE eCRF, including the event‘s seriousness, severity, action taken, and relationship to the IMP. If AEs occur, the first concern will be the safety of the children. All AEs will be followed until resolved or stable and the outcome documented on the appropriate eCRF.

In order to avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology rather than the child‘s or parent‘s/legal guardian‘s own words.

4.1 Definitions and Criteria

4.1.1 Adverse Events

An AE is ―any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (ICH E2A)‖.

AEs include:

- Onset of any clinical sign or symptom
- Worsening (change in nature, severity or frequency) of conditions present at the start of the trial
- Subject deterioration due to the primary illness
- Intercurrent illness(es)
- Drug interactions
- Events related or possibly related to concomitant medications

- Abnormal laboratory values, as well as significant shifts from baseline within the range of normal that the investigator considers to be clinically significant

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

4.1.2 Adverse Drug Reactions

In the pre-approval clinical experience with a new medicinal product: ‒all noxious and unintended responses to a medicinal product related to any dose should be considered Adverse Drug Reaction.‖

4.1.3 Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction is an event in which the nature or severity is not consistent with the applicable product information (e.g., Investigator‘s Brochure for an unapproved IMP).

4.1.4 Serious Adverse Events

An SAE (experience) or reaction is any untoward medical occurrence that at any dose:

- Is fatal (results in the outcome death)
- Is life-threatening*
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above

*The term life-threatening refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the child or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An SAE is not necessarily severe; e.g., an overnight hospitalization for a diagnostic procedure must be reported as an SAE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: nausea of several hours‘ duration may be rated as severe but may not be considered serious.

4.1.5 Assessing Intensity and Relationship

All AEs will be assessed on 2 descriptive parameters: intensity and relationship to study drug:

Intensity refers to the ―severity‖ of an event and references impact on a child‘s functioning.

Relationship refers to the likelihood that the event being assessed was caused by the study drug.

Intensity

Each AE will be classified according to the following criteria:

Mild:	The AE does not interfere in a significant manner with the child‘s normal functioning level.
Moderate:	The AE produces some impairment of functioning, but is not hazardous to health.
Severe:	The AE produces significant impairment of functioning or incapacitation and is a definite hazard to the child‘s health.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience should be noted. If the intensity category changes over a number of days, those changes should be recorded separately (with distinct onset dates).

Relationship

Each AE will be assessed as to its relationship to study drug based on the following criteria. Although investigator attribution will be collected for reported events, for analytic purposes a temporal association with the use of study drug will be assumed sufficient for at least plausible association.

Not	No causal relationship exists between the investigational product and the AE, but an obvious alternative cause

related: exists, e.g., the child‘s underlying medical condition or concomitant therapy.

Related: There is a reasonable/plausible possibility that the AE may have been caused by the investigational product.

When assessing the relationship to study drug, the follow criteria will be considered:

- Positive rechallenge
- Positive dechallenge (resolution upon stopping suspect product, in absence of other intervention or treatment)
- Known class effect
- Biological plausibility
- Lack of alternative explanation—concomitant drug or disease

Factors to be considered in assessing the relationship of the AE to study drug include:

- The temporal sequence from IMP administration
- The recovery on discontinuation and recurrence on reintroduction
- The concomitant diseases
- The evolution of the treated disease
- The concomitant medication(s)
- The pharmacology and pharmacokinetics of the IMP

4.2 Reporting Procedures and Requirements

4.2.1 Adverse Events

The investigator or his/her designees are requested to collect and assess any spontaneous AE reported by the child and to question the child about AEs and intercurrent illnesses at each visit during the treatment period and follow-up. The questioning of children regarding AEs is generalized such as ―How have you been feeling since your last visit?‖ Any AE occurring after the informed consent form/assent has been signed and up to the follow-up study visit, whether volunteered by the child; discovered during general questioning by the investigators; or detected through physical examination, laboratory test, or other means will be recorded on the specific section of the eCRF. Each AE will be described by:

- Seriousness
- Duration (start and end dates)
- Severity
- Relationship to the IMP

- Action taken
- Outcome

The severity of AE should be assessed and graded according to the most recently published National Cancer Institute Common Terminology Criteria for AEs (v. 4.0).

The relationship to the investigational drug should be assessed as:

- Related to IMP
- Not related to IMP
- Unknown

The assessment of the relationship of an AE to the administration of IMP is a clinical decision based on all available information at the time of the completion of the eCRF.

An assessment of ‗not related' would include the existence of a clear alternative explanation, or non-plausibility.

An assessment of ‗related' indicates that there is a reasonable suspicion that the AE is associated with the use of the IMP.

4.2.1.1 Abnormal Laboratory Findings and Other Objective Measurements

Abnormal laboratory findings and other objective measurements should not be routinely captured and reported as AEs in the eCRF as they will be collected and analyzed separately. However, abnormal laboratory findings and other objective measurements that meet the criteria for an SAE, result in discontinuation of the IMP, require medical intervention, or are judged by the investigator to be clinically significant changes from baseline values should be captured and reported as AEs in the eCRF.

When reporting an abnormal laboratory finding as an AE in the eCRF, a clinical diagnosis should be recorded in addition to the abnormal value itself, if this is available (for example ―anemia‖ in addition to ―hemoglobin = 10.5 g/dL‖).

4.2.1.2 Baseline Medical Conditions

Medical conditions present at the screening visit that do not worsen in severity or frequency during the study are defined as baseline medical conditions and are not AEs. These medical conditions should be adequately documented on the appropriate page of the eCRF (i.e., the medical history page). However, medical conditions present at the initial study visit that worsen in severity or frequency during the study should be recorded and reported as AEs.

4.2.2 Serious Adverse Events

Any SAE, including death from any cause that occurs after a child has signed the informed consent /assent and up to the final follow-up visit (regardless of relationship to study drug) must be reported by the investigators to the sponsor within 24 hours of learning of its occurrence.

Related SAEs ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

The investigators are required to complete the SAE form provided by the sponsor. Sufficient details must be provided to allow for a complete medical assessment of the AE and independent determination of possible causality. The investigators are obliged to pursue and provide additional information as requested by the sponsor‘s drug safety manager, or study director, or designee.

The investigator must send notification of the SAE to the sponsor‘s Drug Safety Unit by faxing the SAE form, within 24 hours of a SAE, at the number specified below; then, the investigator must confirm any SAE notifications by mailing to the mail address or phoning to the phone number specified below:

Aurelio Scotti
Drug Safety Unit, Chemi S.p.A.
Via dei Lavoratori
54 20092 Cinisello Balsamo (MI), Italy
Fax: 02 6610 6538
phone: +39 02 6443 2510, mobile +39 333 9262611
e-mail: drug-safety@italfarmaco.com
a. scotti@italfarmaco.com

The same procedure must be applied to the SAE follow-up information.

The sponsor‘s drug safety manager will report all serious and unexpected AE that are related to the use of the study drug to the competent authority within the required time and following procedures required by applicable laws. It is imperative that the sponsor be informed as soon as possible, so that reporting can be done within the required time frame.

The SAEs will also be recorded in the AE section of the eCRF.

Overdose and Other Situations Putting the Child at Risk of an Adverse Reaction

Any instance of overdose (suspected or confirmed) must be reported to the sponsor within 24 hours and be fully documented as a SAE. Details of any signs or symptoms and their management should be recorded including details of any antidote(s) or systematic treatment administered. Any signs or symptoms of overdose will be treated symptomatically.

Any other situations putting the child at risk of adverse reaction, such as misuse and abuse, medication errors, suspicion of transmission of infective agents must be reported to the sponsor within 24 hours and be fully documented as a SAE.

5 Data Management and Statistical Analysis

5.1 Data Management Considerations

Electronic CRFs will be employed for this study. Completed eCRFs for this study will be forwarded to the sponsor or its representative where editing and construction of a quality-assured database will occur. The statistical analysis of these data will be performed by the sponsor or its representative.

Data Analysis of the Biopsies

All images will be digitally captured, using both light microscopy (hematoxylin and eosin [H&E] and Gomori trichrome stain) and fluorescence microscopy coupled to the Olympus Fluoview FV1000 confocal microscope. Fields for fluorescent imaging will be randomly selected while viewing the laminin-dystrophin signal.

In the only step involving operator discretion, all image parameters including pinhole size, detector gain, amplifier offset, amplifier gain, and laser intensity will be first set for the dystrophin and laminin channels using normal control tissue, and the same setting used for all samples imaged on a given day. Frame size, scan speed, and averaging will be the same for all images. For each sample 4 non-overlapping images for each channel will be acquired and stored as 12-bit fluorescent images (.TIFF) for analysis. A single technician will perform all sectioning, staining, and morphometry steps, while another operator will execute the confocal imaging.

Image processing and quantitative analyses will be done using Metamorph (Molecular Devices, Inc.) software program using a custom script. Additional details are provided in the relevant CSOM.

5.2 Statistical Considerations

The statistical analysis will be undertaken by the contract research organization Worldwide Clinical Trials (WCT) in collaboration with the sponsor.

Any deviations from the analyses described below will be included in the Statistical Analysis Plan (SAP).

5.2.1 General Considerations

Data will be provided in data listings sorted by treatment group and child number. Tabular summaries will be presented by treatment group for Part 1. Categorical data will be summarized by the number and percentage of children in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided, unless otherwise stated. Details of endpoint analyses will be described in the SAP.

For analyses of change from baseline, baseline will generally be defined as the value collected before any Givinostat treatment is administered. If this value is unavailable, the last non-missing value prior to dosing will be used. Otherwise, missing observations will be treated as missing at random, and no data imputation will be performed. All data from the CRFs, as well as any derived variables, will be presented in data listings.

All hypothesis tests will be two-sided with a 5% significance level, and 95% CIs will be used, unless stated otherwise. As this is a Phase 2 exploratory study, no adjustments for multiplicity will made.

5.2.2 Sample Size Justification

A minimum of 20 evaluable children will be enrolled in this study.

5.2.2.1 Part 1

Approximately 20 children will be enrolled in Part 1 (dose-finding) of this study.

5.2.2.2 Part 2

Muscle fibers: a sample size of 20 children from part 1 completing the treatment period of part 2 should provide 90% power (at a 2-sided alpha level of 5%) to detect at least a 12.5% increase in MFA% between pre- and post-treatment (which corresponds approximately to a 26% relative increase given the observed mean in the Desguerre publication of 48% [Desguerre I et al. 2009]), the ‑worst case‖ standard deviation of 16%, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment. This sample size is based on a paired t-test and the assumption of normal distribution of MFA%.

After the first 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability observed in the study will be implemented: the within-subject standard deviation of MFA% will be calculated, the actual distribution of MFA% will be checked and the final sample size will be adjusted based on the observed standard

deviation and actual distribution of MFA%. Results by Al-Sunduqchi and Guenther (1990) indicate that power calculations for the Wilcoxon test may be made using the standard t-test formulations with a simple adjustment to the sample size. The size of the adjustment depends upon the actual distribution of the data. They give sample size adjustment factors for four distributions. These are 1 for the uniform distribution, 2/3 for the double exponential distribution, $9/\pi_2$ for the logistic distribution, and $\pi/3$ for the normal distribution. So depending on the actual distribution of MFA%, sample size re-calculation will be based on a Wilcoxon Signed Rank test with the corresponding adjustment if the observed distribution of MFA% is not normal.

5.2.3 Analysis Populations

The intent-to-treat (ITT) population includes all children who are enrolled in the Part 1 portion or entered the Part 2 portion of the study. The efficacy analysis will be conducted based on the ITT population.

The evaluable population will include all children who are in the Part 2 of the study, receive Givinostat of at least 80% dose, have at least one baseline and one post-baseline assessment of biopsies, and have no major protocol violations. The evaluable population will be identified prior to database lock. A sensitivity analysis may be conducted based on the evaluable population.

The safety population will include all children who receive any investigational product. The safety analysis will be conducted based on the safety population.

The PK population will include all children with at least one quantifiable post-dose concentration datum available. All statistical analyses of PK data will be performed using the PK population.

5.2.4 Primary Endpoint

The primary endpoint of this study is change in the value of MFA% comparing the histology biopsies before and after 12 months of treatment with Givinostat.

5.2.5 Secondary Efficacy Endpoints

The secondary efficacy endpoints are the following:

- Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT

- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based the PUL

Additional details on secondary efficacy endpoints will be provided in the SAP.

5.2.6 Secondary Safety Endpoints

The secondary safety endpoints will include the following:

- The number of children experiencing AEs
- The type, incidence, and severity of AEs correlated with dose
- ECG, ECHO, vital sign, physical examination, PFTs, and clinical laboratory parameter findings

5.2.7 Secondary Pharmacokinetic Endpoints

Individual Givinostat concentrations will be tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2. Noncompartmental PK data analysis will be performed for data obtained from each dose cohort with scheduled PK sample collection. If data allows, descriptive statistics of noncompartmental PK parameters (area under the plasma–concentration time curve, maximum plasma concentration, clearance, terminal elimination half-life) will be provided.

5.2.8 Exploratory Endpoints

The exploratory endpoints will include the following:

- Changes in muscle, fat, and fibrosis content after treatment with Givinostat as measured by MRI
- Change biomarkers (e.g., miRNA) and cytokine following treatment with Givinostat
- PK–PD correlations

5.2.8.1 Magnetic Resonance Imaging

MRI will be collected via T1w imaging and quantitative Dixon imaging (the fat content of the image as a percentage of the total signal per voxel). Additional details will be provided in the SAP.

5.2.8.2 miRNA and Cytokine Parameters

miR-1, miR-133, and miR-206 as well as TNF-α, IL-1, and IL-6 will be evaluated. Other miRNA and cytokine parameters may be evaluated as appropriate. Additional details will be provided in the SAP.

5.2.9 Efficacy Analyses

Efficacy analyses will be conducted on the evaluable population.

All values will be expressed as means ± standard deviation or standard error of the mean.

Each of all efficacy endpoints will be subject to normality test and formal statistical test performed will be based on whether it is normally distributed. If normal, statistical differences will be calculated using the paired t test; if not normal, Wilcoxon signed rank test will be used to assess the statistical significance of differences between groups. $P<0.05$ will be set as significant.

5.2.10 Safety Analyses

Safety analyses will be conducted on the safety population.

The safety of Givinostat will be assessed primarily by summarizing treatment-emergent AEs and SAEs. Other safety data (e.g., laboratory, ECG, PFTs, physical examination, and vital sign findings) will be summarized. Treatment-emergent AEs and SAEs that occur after administration of Givinostat will be summarized by system organ class and preferred terms, by severity, and by relationship to investigational product. Change from baseline in laboratory values, ECG findings, PFTs, physical examination findings, and vital signs measurements will be summarized.

5.2.11 Pharmacokinetic Analyses

The PK analysis will be conducted on the PK population.

Plasma concentrations from Part 1 will be listed and tabulated by dose and time point for all children and time points with at least 1 PK assessment. Plasma concentrations from Part 2 will be listed and tabulated by time point for all children and time points with at least 1 PK assessment.

Descriptive statistics for all PK parameters for Part 1 will be calculated by treatment. Descriptive statistics for all PK parameters for Part 2 will also be calculated. These tables will include number of observations, mean, standard deviation, median, minimum and maximum and additionally the geometric mean and coefficient of variation (not for time to maximum plasma concentration).

5.2.12 Interim Analyses

The following interim analyses will be conducted:

- After the first 20 baseline biopsies are collected, the between-subject standard deviation of MFA% fraction will be calculated, the actual distribution of MFA% will be checked. The within-subject standard deviation will be estimated by between-subject standard deviation under the ‒worst case‖ scenario, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment.
- Based on the standard deviation obtained above, the final sample size will be adjusted. A conservative approach will be adopted, where the sample size may be increased but not decreased.

6 Study Management

6.1 Approval and Consent

6.1.1 Regulatory Guidelines

The study will be performed in accordance with local national laws (as applicable), the guidelines of the ICH, and the guidelines of the Declaration of Helsinki adopted by the 18th World Medical Assembly in Helsinki, Finland in 1964 and amended by subsequent assemblies in Tokyo, Japan in 1975; Venice, Italy in 1983; Hong Kong in 1989; Somerset West, South Africa in 1996, and in Edinburgh, Scotland in October 2000. These guidelines are on file at WCT.

This clinical study has been designed to comply with the Good Clinical Practice guidelines.

6.1.2 Institutional Review Board/Independent Ethics Committees

This study will be undertaken only after approval of the protocol has been obtained from the appropriate Independent Ethics Committee (IEC), and a copy of the approval has been received by Italfarmaco S.p.A.

The IEC must be informed of all subsequent protocol amendments and should be asked whether a re-evaluation of the ethical aspects of the study is necessary.

If applicable, interim reports on the study and reviews of its progress will be submitted to the IEC by the investigator at intervals stipulated in their guidelines.

6.1.3 Informed Consent

For each trial subject, written informed consent from the legally accepted representative will be obtained prior to any protocol-related activities. Informed assent may be obtained from children who are capable of providing assent. As part of this procedure, the principal investigator or a designated representative must explain orally and in writing the nature, duration, and purpose of the study, and the action of the drug in such a manner that the child and (if applicable) appointed guardian are aware of the potential risks, inconveniences, or adverse effects that may occur. Children and their legally accepted representatives should be given ample time and opportunity to inquire about the details of the study prior to deciding whether to participate in the study. It is the responsibility of the investigator to ensure that all questions about the study are answered to the satisfaction of the children and their legally accepted representatives.

Children and their legally accepted representatives should be informed that children may withdraw from the study at any time. They will receive all information that is required by local regulations and ICH guidelines. The principal investigator or a designated representative will provide the sponsor or its representative with a copy of the IEC-approved informed consent form prior to the start of the study.

The informed consent form should be signed and dated by the child‘s legally accepted representative and the investigator on the same day. If the child and/or legally accepted representative are not able to read, an impartial witness should be present during the informed consent discussion, and the witness must co-sign and date the informed consent form. The child‘s legally accepted representative and/or impartial witness should receive a copy of the signed documents.

For details of the information provided, refer to the informed consent form.

6.2 Discontinuation of the Study by the Sponsor

The sponsor reserves the right to discontinue the study at this site or at multiple sites for safety or administrative reasons at any time. In particular, a site that does not recruit at a reasonable rate may be discontinued. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to the sponsor or its representative.

6.3 Study Documentation

The investigator will supply the sponsor with:

- Curricula vitae for all investigators
- Signed protocol signature page

- List of IEC members and their occupations/affiliations or multiple assurance number
- Letter indicating IEC approval to conduct the protocol
- Copy of IEC-approved informed consent form
- Laboratory certification records and reference ranges

The main documents that will be supplied by the Sponsor to investigator are:

- Clinical study protocol
- Investigational drug brochure
- Sample informed consent form
- eCRFs/instruction manual
- Insurance letter

6.4 Study Monitoring and Auditing

This study will be monitored at all stages of its development by the clinical research personnel employed by the sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to protocol and in order to comply with guidelines of Good Clinical Practice. On-site review of eCRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each child. Note that a variety of original documents, data, and records will be considered as source documents in this trial.

The eCRF itself is not to be used as a source document under any circumstances.

Medical advisors and clinical research associates or assistants may request to witness child evaluations occurring as part of this protocol. The investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the sponsor to assure acceptable protocol execution. The study may be subject to audit by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required child records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in eCRF generation, where clinically appropriate.

6.5 Retention of Records

The investigator must arrange for retention of study records at the site. The nature of the records and the duration of the retention period must meet the requirements of the relevant regulatory authority. In addition, because this is an international study, the retention period must meet the requirements of the most stringent authority. The

investigator should take measures to prevent accidental or premature destruction of these documents.

6.6 Use of Study Findings

By signing the study protocol, the investigator agrees to the use of results of the study for the purposes of national and international registration. If necessary, the authorities will be notified of the investigator‘s name, address, qualifications, and extent of involvement. Reports covering clinical and biometric aspects of the study will be prepared by the sponsor or its representative.

6.7 Publications

The sponsor assures that the key design elements of this protocol will be posted in a publicly accessible database, such as clinicaltrials.gov. In addition, upon study completion and finalization of the study report the results of this study will be either submitted for publication and/or posted in a publicly accessible database of clinical study results.

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Italfarmaco S.p.A. Protocol DSC/11/2357/4 Amendment 1, 05 Dec 2012

8 Appendices

Appendix 1: Drugs Known to Prolong the QTc Interval

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Albuterol (Proventil®;Ventolin®)	ß2-receptor agonist/Asthma		Congenital QT Avoid
Alfuzosin (Uroxatral®)	Alpha1-blocker/Benign prostatic hyperplasia		Possible Risk of TdP
Amantadine (Symmetrel®)	Dopaminergic/Anti-viral/Anti-infective/ Parkinson's Disease		Possible Risk of TdP
Amiodarone (Cordarone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	Risk of TdP
Amiodarone (Pacerone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	Risk of TdP
Amitriptyline (Elavil®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	Conditional TdP Risk
Amphetamine (Dexedrine®)	CNS stimulant/ADHD	This includes dextroamphetamine and amphetamine / dextroamphetamine combinations.	Congenital QT Avoid
Amphetamine (Adderall®)	CNS stimulant/ADHD	This includes dextroamphetamine and amphetamine / dextroamphetamine combinations.	Congenital QT Avoid
Arsenic trioxide (Trisenox®)	Anti-cancer/Leukemia		Risk of TdP
Astemizole (Hismanal®)	Antihistamine/Allergic rhinitis	No Longer available in U.S.	Risk of TdP
Atazanavir (Reyataz®)	Protease inhibitor/HIV		Possible Risk of TdP
Atomoxetine (Strattera®)	norepinephrine reuptake inhibitor /ADHD		Congenital QT Avoid
Azithromycin (Zithromax®)	Antibiotic/bacterial infection		Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Bepridil (Vascor®)	Anti-anginal/heart pain	Females>Males	Risk of TdP
Chloral hydrate (Noctec®)	Sedative/sedation/ insomnia		Possible Risk of TdP
Chloroquine (Aralen®)	Anti-malarial/malaria infection		Risk of TdP
Chlorpromazine (Thorazine®)	Anti-psychotic/ Anti-emetic/schizophrenia/ nausea		Risk of TdP
Ciprofloxacin (Cipro®)	Antibiotic/bacterial infection	Drug metabolism inhibitor-Risk for drug interactions	Conditional TdP Risk
Cisapride (Propulsid®)	GI stimulant/heartburn	No longer available in the U.S.; available in Mexico	Risk of TdP
Citalopram (Celexa®)	Anti-depressant/depression		Risk of TdP
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection		Risk of TdP
Clomipramine (Anafranil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Clozapine (Clozaril®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Cocaine (Cocaine)	Local anesthetic/	Cardiac stimulant	Congenital QT Avoid
Desipramine (Pertofrane®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	Conditional TdP Risk
Dexmethylphenidate (Focalin®)	CNS stimulant/ADHD		Congenital QT Avoid
Diphenhydramine (Benadryl®)	Antihistamine/Allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	Conditional TdP Risk
Diphenhydramine (Nytol®)	Antihistamine/Allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	Conditional TdP Risk
Disopyramide (Norpace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Dobutamine (Dobutrex®)	Catecholamine/heart failure and		Congenital

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
	shock		QT Avoid
Dofetilide (Tikosyn®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Dolasetron (Anzemet®)	Anti-nausea/nausea, vomiting		Possible Risk of TdP
Domperidone (Motilium®)	Anti-nausea/nausea	Not available in the U.S.	Risk of TdP
Dopamine (Intropine®)	Inotropic agent/heart failure; hypotension; shock		Congenital QT Avoid
Doxepin (Sinequan®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Dronedarone (Multaq®)	Anti-arrhythmic/Atrial Fibrillation		Possible Risk of TdP
Droperidol (Inapsine®)	Sedative;Anti-nausea/anesthesia adjunct, nausea		Risk of TdP
Ephedrine (Broncholate®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma		Congenital QT Avoid
Ephedrine (Rynatuss®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma		Congenital QT Avoid
Epinephrine (Primatene®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	Cardiac stimulant	Congenital QT Avoid
Epinephrine (Bronkaid®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	Cardiac stimulant	Congenital QT Avoid
Eribulin (Halaven®)	Anti-cancer/metastatic breast neoplasias		Possible Risk of TdP
Erythromycin (E.E.S.®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	Risk of TdP
Erythromycin (Erythrocin®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	Risk of TdP
Escitalopram (Cipralex®)	Anti-depressant/Major depression/		Possible

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
	Anxiety disorders		Risk of TdP
Escitalopram (Lexapro®)	Anti-depressant/Major depression/ Anxiety disorders		Possible Risk of TdP
Famotidine (Pepcid®)	H2-receptor antagonist/Peptic ulcer/ GERD		Possible Risk of TdP
Felbamate (Felbatrol®)	Anti-convulsant/seizure		Possible Risk of TdP
Fenfluramine (Pondimin®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Fingolimod (Gilenya®)	Immunosuppressant/Multiple Sclerosis		Possible Risk of TdP
Flecainide (Tambocor®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Fluconazole (Diflucan®)	Anti-fungal/fungal infection	Drug metabolism inhibitor-Risk for drug interactions	Conditional TdP Risk
Fluoxetine (Prozac®)	Anti-depressant/depression		Conditional TdP Risk
Fluoxetine (Sarafem®)	Anti-depressant/depression		Conditional TdP Risk
Foscarnet (Foscavir®)	Anti-viral/HIV infection		Possible Risk of TdP
Fosphenytoin (Cerebyx®)	Anti-convulsant/seizure		Possible Risk of TdP
Galantamine (Reminyl®)	Cholinesterase inhibitor/ Dementia, Alzheimer's		Conditional TdP Risk
Gatifloxacin (Tequin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Gemifloxacin (Factive®)	Antibiotic/bacterial infection		Possible Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Granisetron (Kytril®)	Anti-nausea/nausea and vomiting		Possible Risk of TdP
Halofantrine (Halfan®)	Anti-malarial/malaria infection	Females>Males	Risk of TdP
Haloperidol (Haldol®)	Anti-psychotic/schizophrenia, agitation	When given intravenously or at higher-than- recommended doses, risk of sudden death, QT prolongation and torsades increases.	Risk of TdP
Ibutilide (Corvert®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Iloperidone (Fanapt®)	Antipsychotic, atypical/Schizophrenia		Possible Risk of TdP
Imipramine (Norfranil®)	Tricyclic Antidepressant/depression	Risk of TdP in overdosage	Conditional TdP Risk
Indapamide (Lozol®)	Diuretic/stimulate urine & salt loss		Possible Risk of TdP
Isoproterenol (Isupres®)	Catecholamine/allergic reaction		Congenital QT Avoid
Isoproterenol (Medihaler-Iso®)	Catecholamine/allergic reaction		Congenital QT Avoid
Isradipine (Dynacirc®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Itraconazole (Sporanox®)	Anti-fungal/fungal infection	Drug metabolism inhibitor-Risk for drug interactions	Conditional TdP Risk
Ketoconazole (Nizoral®)	Anti-fungal/fungal infection	Drug metabolism inhibitor	Conditional TdP Risk
Lapatinib (Tykerb®)	Anti-cancer/breast cancer, metastatic		Possible Risk of TdP
Lapatinib (Tyverb®)	Anti-cancer/breast cancer, metastatic		Possible Risk of TdP
Levalbuterol (Xopenex®)	Bronchodilator/asthma		Congenital

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
			QT Avoid
Levofloxacin (Levaquin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	Not available in the U.S.	Risk of TdP
Lisdexamfetamine (Vyvanse®)	CNS stimulant/ADHD		Congenital QT Avoid
Lithium (Eskalith®)	Anti-mania/bipolar disorder		Possible Risk of TdP
Lithium (Lithobid®)	Anti-mania/bipolar disorder		Possible Risk of TdP
Mesoridazine (Serentil®)	Anti-psychotic/schizophrenia		Risk of TdP
Metaproterenol (Alupent®)	Bronchodilator/asthma		Congenital QT Avoid
Metaproterenol (Metaprel®)	Bronchodilator/asthma		Congenital QT Avoid
Methadone (Dolophine®)	Opiate agonist/pain control, narcotic dependence	Females>Males	Risk of TdP
Methadone (Methadose®)	Opiate agonist/pain control, narcotic dependence	Females>Males	Risk of TdP
Methylphenidate (Ritalin®)	CNS stimulant/ADHD		Congenital QT Avoid
Methylphenidate (Concerta®)	CNS stimulant/ADHD		Congenital QT Avoid
Midodrine (ProAmatine®)	Vasoconstrictor/low blood pressure, fainting		Congenital QT Avoid
Moexipril/HCTZ (Uniretic®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection		Risk of TdP
Nicardipine (Cardene®)	Anti-hypertensive/high blood		Possible

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
	pressure		Risk of TdP
Nilotinib (Tasigna®)	Anti-cancer/Leukemia		Possible Risk of TdP
Norepinephrine (Levophed®)	Vasconstrictor, Inotrope/shock, low blood pressure		Congenital QT Avoid
Nortriptyline (Pamelor®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Octreotide (Sandostatin®)	Endocrine/acromegaly, carcinoid diarrhea		Possible Risk of TdP
Ofloxacin (Floxin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Ondansetron (Zofran®)	Anti-emetic/nausea and vomiting		Possible Risk of TdP
Oxytocin (Pitocin®)	Oxytocic/Labor stimulation		Possible Risk of TdP
Paliperidone (Invega®)	Antipsychotic, atypical/Schizophrenia		Possible Risk of TdP
Paroxetine (Paxil®)	Anti-depressant/depression		Conditional TdP Risk
Pentamidine (NebuPent®)	Anti-infective/pneumocystis pneumonia	Females>Males	Risk of TdP
Pentamidine (Pentam®)	Anti-infective/pneumocystis pneumonia	Females>Males	Risk of TdP
Perflutren lipid microspheres (Definity®)	Imaging contrast agent/Echocardiography		Possible Risk of TdP
Phentermine (Fastin®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Phentermine (Adipex®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Phenylephrine (Neosynephrine®)	Vasoconstrictor, decongestant/low blood pressure, allergies, sinusitis, asthma		Congenital QT Avoid
Phenylpropanolamine (Acutrim®)	Decongestant/allergies, sinusitis, asthma	No longer available in the U.S.	Congenital QT Avoid
Phenylpropanolamine (Dexatrim®)	Decongestant/allergies, sinusitis, asthma	No longer available in the U.S.	Congenital QT Avoid
Pimozide (Orap®)	Anti-psychotic/Tourette's tics	Females>Males	Risk of TdP
Probucol (Lorelco®)	Antilipemic/Hypercholesterolemia	No longer available in U.S.	Risk of TdP
Procainamide (Pronestyl®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Procainamide (Procan®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Protriptyline (Vivactil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Pseudoephedrine (PediaCare®)	Decongestant/allergies, sinusitis, asthma		Congenital QT Avoid
Pseudoephedrine (Sudafed®)	Decongestant/allergies, sinusitis, asthma		Congenital QT Avoid
Quetiapine (Seroquel®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Quinidine (Quinaglute®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Quinidine (Cardioquin®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Ranolazine (Ranexa®)	Anti-anginal/chronic angina		Possible Risk of TdP
Risperidone (Risperdal®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Ritodrine (Yutopar®)	Uterine relaxant/prevent premature labor		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Ritonavir (Norvir®)	Protease inhibitor/HIV		Conditional TdP Risk
Roxithromycin* (Rulide®)	Antibiotic/bacterial infection	*not available in the United States	Possible Risk of TdP
Salmeterol (Serevent®)	Sympathomimetic/asthma, COPD		Congenital QT Avoid
Sertindole (Serdolect®)	Antipsychotic, atypical/Anxiety, Schizophrenia		Possible Risk of TdP
Sertindole (Serlect®)	Antipsychotic, atypical/Anxiety, Schizophrenia		Possible Risk of TdP
Sertraline (Zoloft®)	Anti-depressant/depression		Conditional TdP Risk
Sibutramine (Meridia®)	Appetitie suppressant/dieting, weight loss		Congenital QT Avoid
Solifenacin (VESIcare®)	muscarinic receptor anatagonist/treatment of overactive bladder		Conditional TdP Risk
Sotalol (Betapace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection		Risk of TdP
Sunitinib (Sutent®)	Anti-cancer/RCC, GIST		Possible Risk of TdP
Tacrolimus (Prograf®)	Immunosuppressant/Immune suppression		Possible Risk of TdP
Tamoxifen (Nolvadex®)	Anti-cancer/breast cancer		Possible Risk of TdP
Telithromycin (Ketek®)	Antibiotic/bacterial infection		Possible Risk of TdP
Terbutaline (Brethine®)	Bronchodilator/asthma		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Terfenadine (Seldane®)	Antihistamine/Allergic rhinitis	No longer available in U.S.	Risk of TdP
Thioridazine (Mellaril®)	Anti-psychotic/schizophrenia		Risk of TdP
Tizanidine (Zanaflex®)	Muscle relaxant/		Possible Risk of TdP
Tolterodine (Detrol®)	Bladder Antispasmodic/		Congenital QT Avoid
Tolterodine (Detrol LA®)	Bladder Antispasmodic/		Congenital QT Avoid
Trazodone (Desyrel®)	Anti-depressant/Depression, insomnia		Conditional TdP Risk
Trimethoprim-Sulfa (Bactrim®)	Antibiotic/bacterial infection		Conditional TdP Risk
Trimethoprim-Sulfa (Sulfa®)	Antibiotic/bacterial infection		Conditional TdP Risk
Trimipramine (Surmontil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Vandetanib (Caprelsa®)	Anti-cancer/Thyroid cancer		Risk of TdP
Vardenafil (Levitra®)	phosphodiesterase inhibitor/vasodilator		Possible Risk of TdP
Venlafaxine (Effexor®)	Anti-depressant/depression		Possible Risk of TdP
Voriconazole (VFend®)	Anti-fungal/anti-fungal		Possible Risk of TdP
Ziprasidone (Geodon®)	Anti-psychotic/schizophrenia		Possible Risk of TdP

Source: Arizona Center for Education and Research on Therapeutics. Updated 17 May 2012. http://www.azcert.org/medical-pros/drug-lists/printable-drug-list.cfm. Accessed 15 June 2012.

AMENDMENT 1 RATIONALE

Protocol DSC/11/2357/4 has been amended for the following reasons:

- To add the magnetic resonance imaging (MRI) of muscle on the upper limb, if it is possible and if the child is compliant.
- To better describe the muscles to be observed during the MRI, inserting "muscle of lower limb" instead of "quadriceps femoris" and "muscle of upper limb" instead of "brachial biceps.
- To clarify the timing around the collection of pharmacokinetic (PK) blood samples.

The following is the rationale for the amendment:

- During the study, the children will perform functional test to evaluate the muscular strength of the upper limb (i.e., performance of upper limb [PUL]). It is advisable to also collect the data from the MRI on upper limbs to compare the functional and radiological results.
- The acquisition of MRI imaging is relevant not only to a single muscle (e.g., quadriceps or biceps) but a number of muscles should be observed and analysed. It is preferable to insert in the protocol the generic definition of "upper limb muscles" and "lower limb muscles."

The changes relevant to "muscle of lower limbs" instead of "quadriceps femoris" and "muscle of the upper limbs" instead of "brachial biceps" do not modify the MRI duration and acquisition time and, consequently, do not modify the impact the study will have on the children who participate.

In addition, minor typographical and formatting corrections have been made, as appropriate.

AMENDMENT 1 SUMMARY OF CHANGES

Substantive additions to the protocol are denoted in **bold**. Substantive deletions are in ~~strikethrough~~.

Section 3.1 Description of Overall Study Design and Plan

Plasma **After 1 week of treatment, blood** samples for PK measurements will be collected after 1 week of treatment with givinostat during Part 1: at pre-dose **in the morning before drug intake** and **at** 1, 2, 4, and 8 hours post-dose.

Section 3.4.1 Schedule of Assessments

Table 8 Schedule of Assessments: Part 1 (Dose Escalation)

"ANC" has been removed from footnote of the table.

Footnote h has been modified for clarity and consistency: "At Visit 2, 4, and 6 **After 1 week of treatment**, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose. It is important to record the time of PK assessments and the time of the last drug intake in the evening before the PK assessment."

The original footnote i has been removed from the table.

Footnote i text has been added replaced with: "**Obtain an MRI of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb). MRI**

Assessments

MRIs: MRIs will be taken at **of muscle (initially on the** quadriceps femoris **muscles of the lower limb and, if possible, does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment.**"

Table 9 Schedule of Assessments: Part 2 (Proof of Mechanism)

"Troponin 1" has been removed from footnote d.

Section 3.4.2.1 Pre-Study (Screening)—(Day -28 [±2 Weeks])

- Obtain an MRI of dystrophic muscle (quadriceps femoris **initially on the muscles of the lower limb, and, if possible, on the muscles of the upper limb**; **MRI** does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment).

Section 3.4.6 Description of **on the muscles of the upper limb)** and evaluated per the specifications in the CSOM.

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STUDY PROTOCOL

Protocol Title:	A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy
Protocol Number:	DSC/11/2357/43
Clinical Phase:	2
Original Protocol Date	01 Aug 2012
Amendment 1	05 Dec 2012
Amendment 2	25 Mar 2013
Amendment 3	23 Oct 2013
Amendment 4	17 Apr 2014
Amendment 5	16 Mar 2015
Amendment 6	06 Jan 2016
Amendment 7	11 Apr 2017

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EudraCT Number: 2012-002566-12

Sponsor

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SYNOPSIS

Title of Study:	A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy
Protocol Number:	DSC/11/2357/43
Eudra CT Number	2012-002566-12
Investigators/ Study Centers:	Children will be enrolled at approximately 5 study sites in Italy.
Phase of Development:	2

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Objectives:	The primary objective of this study is as follows: • To establish the histologic effects of Givinostat administered chronically at the selected daily dose The secondary objectives of this study are as follows: • To establish the effects of Givinostat administered chronically at the selected daily dose on functional parameters, such as the 6-Minute Walk Test (6MWT), North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL) • To establish the safety and tolerability of Givinostat administered chronically at the selected daily dose in children with Duchenne muscular dystrophy (DMD) • To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as magnetic resonance imaging (MRI) and biomarkers • To explore the acceptability/palatability of the oral suspension • To explore whether the effects of Givinostat on disease progression may be related to the type of DMD mutation Extension Phases: The primary objective is to evaluate the safety and tolerability of long-term administration of Givinostat administered chronically at the selected daily dose in children with DMD. The secondary objectives are: • To establish the effects of Givinostat administered chronically at the selected daily dose on other functional parameters, such as the 6MWT, NSAA, and PUL (Extension 1, 2, 3) • To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as MRI (Extension 1)

	• To collect information related to 2 biomarkers, LTBP4 and osteopontin genotype (at the beginning of Extension 2 only) • To collect information related to time to wheelchair and how much time the children spend in wheelchair (Extension 3 – only for the children that are not able to complete the 6MWT)
Design:	This is a 2-part, phase 2 study to assess the effects of Givinostat on muscle histologic parameters and on clinical parameters in ambulant children with DMD. The safety, tolerability, and pharmacokinetics of Givinostat will also be assessed. Children who assent to participate in this study (if capable of doing so) and whose parent/guardian signs the informed consent to participate will undergo pre-study screening assessments up to 4 weeks (±2 weeks) before the first scheduled dose of study drug. In addition, patients will be asked to share information concerning the type of mutation they carry in relation to DMD. PART 1: Approximately 20 children will be enrolled in the study as follows: the first 4 children will be treated at a low dose level of Givinostat (25 mg twice daily [BID] in children who weigh 20–49 kg and 37.5 mg BID in children who weigh ≥50 kg). If none of the stopping criteria (see Section 3.3.5) are met after 2 weeks of treatment at the low dose, the review team will determine the escalated dose level (i.e., intermediate dose level) to be used for the treatment of an additional 8 children who will be treated at the intermediate dose. The 4 children previously treated at the low dose level will also be switched to the intermediate dose level. If none of the stopping criteria are met after 2 weeks of treatment at the intermediate dose, the review team will determine the subsequent escalated dose level to be used for the treatment of an additional 8 children who will be treated at the high dose. All children treated at the intermediate dose level will be switched to the high dose level. PART 2: Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the recommended dose (RD) to be used in Part 2 based on the safety and tolerability profile observed and on the pharmacokinetic (PK) analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2). As of July 2013, the safety and tolerability assessments of Givinostat from Part 1 of the study were completed and the Safety Review Team determined the RD to be 37.5 mg, BID. The Safety Review Team also

	recommended performing additional platelet count assessments every week, to maintain a stricter control and to monitor this parameter for safety reasons. In addition, as of September 2013, after assessing approximately 2 months of data on the platelet counts, the Safety Review Team decided to reduce the RD in subjects with platelet count ≤150 x 10^9/L to 25-mg BID Givinostat. Subjects with platelet count >150 x 10^9/L will continue on 37.5-mg Givinostat. EXTENSION 1 A first extension phase study treatment is foreseen in order to allow the patients to continue study treatment at least until the final analysis is performed (Part 2; after 12 months of treatment). Part 2 was originally planned with a 12-month study drug treatment. At 12 months, an efficacy and safety analysis will be conducted. If the results show a positive effect on patients, and no safety concern is raised, then patients will continue study treatment for up to another 12 months to evaluate the safety and tolerability of long-term administration of Givinostat and to evaluate the effect of treatment on muscle function. EXTENSION 2 Since the histology results of the first 12 months of treatment are positive, a second extension phase is foreseen in order to allow the patients to continue a study treatment that supports a beneficial effect of Givinostat in DMD. The children on treatment in the Extension 1 will be asked to continue in the Extension 2 and will receive Givinostat at the same ongoing dose for a maximum of an additional 12 months. During Extension 2, the dose will be adjusted based on the weight of the children. During Part 1 of the study, children will visit the center once each week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 11 visits, or 17 visits if they will continue treatment in Extension 1 of the study, and 23 visits if they continue treatment in Extension 2 of the study, including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 2 weeks after the last dose of study drug as well as the protocol-scheduled follow-up visit. Children who have ongoing adverse events (AEs) at discontinuation will be followed until resolution or stabilization. Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2), at 2 years (Extension 1), and at 3 years (Extension 2). Exploratory efficacy parameters will include changes from baseline in MRI results, PK-pharmacodynamic correlations, and measures of micro ribonucleic acid (miRNA). In addition, exploratory analysis will be

	performed to investigate whether the effects of Givinostat on disease progression may be related to the type of DMD mutation. Safety will be assessed by number of children experiencing treatment-emergent AEs and serious adverse events (SAEs); type, incidence, and severity of treatment-emergent AEs and SAEs; and measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies, and urinalysis), echocardiographs, pulmonary function tests (PFTs; forced expiratory volume at 1 second [FEV_1], forced vital capacity [FVC], FEV_1/FVC, and peak expiratory flow [PEF]) and 12-lead electrocardiograms (ECG). In Part 2 and during the extension phases, additional safety evaluations (i.e, laboratory re-testing, AE assessments), or additional medical evaluations may be performed at unscheduled visit(s) at the discretion of the Investigator. Plasma samples for PK measurements will be collected after 1 week of treatment with Givinostat, during Part 1 at pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose. PK samples will also be collected during Part 2 of the study as follows: • At Visit 2, blood samples for PK analysis will be taken pre-dose and between 6 and 8 hours post-dose. • At Visit 3, blood samples for PK analysis will be collected pre-dose in the morning before study drug intake and between 0 and 2 hours post-dose. • At Visit 4, blood samples for PK analysis will be taken pre-dose and between 2 and 4 hours post-dose. • At Visit 6, blood samples for PK analysis will be taken pre-dose and between 4 and 6 hours post-dose. • At Visit 10, blood samples for PK analysis will be taken at pre-dose and at 1, 2, 4, and 8 hours post-dose. EXTENSION 3 A third extension phase is foreseen in order to allow the patients to continue a study treatment that supports a beneficial effect of Givinostat in DMD. The children on treatment in the Extension 2 will be asked to continue in the Extension 3 and will receive Givinostat at the same ongoing dose for a maximum of an additional 12 months. During Extension 3, the dose will be adjusted based on the weight of the children. This part of the study will be additionally extended until the activation of the Long Term Safety Study (Study NDSC/14/2357/51 - EUDRACT: 2017-000397-10). During Part 1 of the study, children will visit the center once each week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 11 visits, or 17 visits if

	they continue treatment in Extension 1 of the study, 23 visits if they continue treatment in Extension 2 of the study and 27 visits if they continue treatment in Extension 3, including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 2 weeks after the last dose of study drug as well as the protocol-scheduled follow-up visit. Children who have ongoing adverse events (AEs) at discontinuation will be followed until resolution or stabilization. Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2), at 2 years (Extension 1), at 3 years (Extension 2) and at 4 years (Extension 3). Exploratory efficacy parameters will include changes from baseline in MRI results, and measures of miRNA. In addition, exploratory analysis will be performed to investigate whether the effects of Givinostat on disease progression may be related to the type of DMD mutation. Safety will be assessed by number of children experiencing treatment-emergent AEs and SAEs; type, incidence, and severity of treatment-emergent AEs and SAEs; and measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies, and urinalysis), echocardiographs, pulmonary function tests (PFTs; FEV_1, FVC, FEV_1/FVC, and PEF) and 12-lead ECGs. In Part 2 and during the extension phases, additional safety evaluations (i.e., laboratory re-testing, AE assessments), or additional medical evaluations may be performed at unscheduled visit(s) at the discretion of the Investigator.
Planned Sample Size:	Approximately 20 children will be enrolled in Part 1 (dose-finding) of this study. *Muscle fibers:* a sample size of 20 children (from Part 1) completing the treatment period should provide 90% power (at a 2-sided alpha level of 5%) to detect at least a 12.5% increase in muscle fiber area % (MFA%) between pre- and post-treatment (which corresponds approximately to a 26% relative increase given the observed mean in the Desguerre publication of 48% [Desguerre I et al. 2009]), the “worst case” standard deviation of 16%, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment. This sample size is based on a paired t-test. In the event the data is not normally distributed, a Wilcoxon signed rank test will be used. After the first 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability observed in the study will be implemented as follows: the within-subject standard deviation of MFA% will be calculated and the final sample size will be adjusted based on this observed standard deviation. A conservative approach will be adopted where the sample size may be increased but not decreased.

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Diagnosis and Key Subject Selection Criteria:	Inclusion criteria: 1. Male children aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD. 2. A parent/guardian and child can comply with all study evaluations/procedures and return for all study activities. 3. Able to complete the 2 screening 6MWTs with a minimal distance of at least 250 m each. In addition, the results of these tests must be within ±30 m of each other. 4. On a stable dose of systemic corticosteroids for at least 6 months. 5. At least 6 months worth of data on the 6MWT (this will be the "historical" 6MWT). From the moment of the historical 6MWT assessment(s), the child must not have received any compound that could potentially affect the 6MWT, with the exception of the stable steroid treatment. 6. Parent/guardian has signed the informed consent form and child has assented to be in the study (if applicable). Exclusion criteria: 1. Initiation of systemic corticosteroid therapy within 6 months prior to the start of study drug or change in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) within 6 months prior to the start of study drug. 2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and integrators will be allowed. 3. Surgery that might have an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study. 4. Exposure to another investigational drug since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment. 5. History of participation in gene therapy, cell-based therapy, or oligonucleotide therapy. 6. Presence of other clinically significant disease that in the opinion of the investigator places the child in unacceptable risk for an adverse outcome or that could affect study results. 7. Symptomatic cardiomyopathy or heart failure. If child has a left ventricular ejection fraction <45% at screening, the

	investigator should discuss inclusion of child in the study with the medical monitor. 8. Inadequate hematological function 9. Absolute neutrophil count: <1.5 x 10^9/L 10. Platelets: <100 x 10^9/L 11. Current or history of liver disease or impairment, including but not limited to an elevated total bilirubin. 12. Inadequate renal function, as defined by serum creatinine >2 x the upper limit of normal. 13. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening 14. A baseline QTc >450 msec, (as the mean of 3 consecutive readings 5 minutes apart) or history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome). 15. Psychiatric illness/social situations rendering the potential child unable to understand and comply with the study protocol.
Treatments:	The study drug (Givinostat) will be administered as 2 oral doses daily while the child is in the fed state. Children who enroll during Part 1 of the study will receive their assigned dose until the RD is determined. They will then receive up to 12 months of treatment with Givinostat at the RD. Moreover, children who will be enrolled in the extension phases will receive Givinostat at the same ongoing dose (during the last visit planned at 12 months of treatment) for a maximum of an additional 40 months (12 months for Extension 1, 12 months for Extension 2, and 16 months for Extension 3). Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who enroll during Part 2 will receive up to 12 or 48 months (if they will be enrolled in the extension phases) of treatment with Givinostat at the RD. During Extensions 2 and 3, the dose will be adjusted based on the weight of the children. The total duration of the study is anticipated to be 15 months, and an additional 40 months for Extension 1 (12 months), Extension 2 (12 months), and Extension 3 (16 months).
Main Parameters of Efficacy:	The primary endpoint of this study is change in the value of MFA% comparing the histology biopsies before and after 12 months of treatment with Givinostat. The secondary efficacy endpoints of this study are as follows: • Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle

	regeneration) after 12 months of treatment with Givinostat at the selected daily dose • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA • Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based the PUL Extension Phases: The primary endpoints for the extension phases are the following: • Number of children experiencing treatment-emergent AEs and SAEs • Type, incidence, and severity of treatment-emergent AEs and SAEs correlated with dose As secondary endpoints, the change in muscular function after 24 months, 36 months, and 52 months (Extension 1, Extension 2, and Extension 3, respectively) of treatment with Givinostat as assessed by 6MWT, NSAA, and PUL will be investigated. For the children that are not able to complete the 6MWT, the time to wheelchair and how much time the children spend in wheelchair were assessed.
Main Parameters of Safety:	• Number of children experiencing treatment-emergent AEs and SAEs • Type, incidence, and severity of treatment-emergent AEs and SAEs correlated with dose
PK Parameters:	• Individual Givinostat concentrations tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistics for Part 2.
Exploratory Endpoints:	• Change in muscle, fat and fibrosis content after treatment with Givinostat as measured by MRI (also evaluated during Extension 1) • Change in muscle biomarkers (e.g., miRNA) following treatment with Givinostat • PK–PD correlations • Evaluation of acceptability/palatability of the oral suspension • Evaluation of any correlation between the effects of Givinostat on disease progression and the type of DMD mutation patients may have in relation to their disease
Key Statistical Considerations	Efficacy analyses will be conducted on the intent-to-treat (ITT) population, which is defined as all children who are enrolled in the Part 1 portion or entered the Part 2 portion of the study.

	All values will be expressed as means ± standard deviation or standard error of the mean. Each of all efficacy endpoints will be subject to normality test and formal statistical test performed will be based on whether it is normally distributed. If normal, statistical differences will be calculated using the paired t test; if not normal, Wilcoxon signed rank test will be used to assess the statistical significance of differences between groups. $P<0.05$ will be set as significant. *General considerations:* Data will be provided in data listings sorted by treatment group and child number. Tabular summaries will be presented by treatment group for Part 1. Categorical data will be summarized by the number and percentage of children in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided, unless otherwise stated. Details of endpoint analyses will be described in the Statistical Analysis Plan. For analyses of change from baseline, baseline will generally be defined as the value collected before any Givinostat treatment is administered. If this value is missing, the last non-missing value prior to dosing will be used. Otherwise, missing observations will be treated as missing at random, and no data imputation will be performed. All data from the case report forms, as well as any derived variables, will be presented in data listings. All hypothesis tests will be two-sided with a 5% significance level, and 95% Cis will be used, unless stated otherwise. As this is a Phase 2 exploratory study, no adjustments for multiplicity will be made.

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TABLES AND FIGURES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

6MWT	6-Minute Walk Test
6MWD	6-Minute Walk Distance
Ab	Antibodies
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the plasma–concentration time curve
BID	twice daily
BUN	blood urea nitrogen
CI	confidence interval
CL	clearance
CL/F	volume of distribution
cm	Centimeter
C_{max}	maximum plasma concentration
CMV	Cytomegalovirus
CPK	creatine phosphokinase
CrCl	creatinine clearance
CRP	C-reactive protein
CSA	cross-sectional area
CSOM	Clinical Study Operations Manual
DMD	Duchenne muscular dystrophy
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ECHO	Echocardiograph
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EOS	end of study
FACS	fluorescence-activated cell sorting
FAP	fibroadipongenic progenitors
FEV_1	forced expiratory volume at 1 second
FU	follow up
FVC	forced vital capacity
GI	Gastrointestinal
h	hour
H&E	hematoxylin and eosin
HbeAg	hepatitis B e antigen
HbsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HDAC	histone deacetylase
HIV	human immunodeficiency virus
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee

IgM	immunoglobulin M
IL	interleukin
IMP	investigational medicinal product
ITT	intent to treat
JIA	juvenile idiopathic arthritis
KA	absorption rate constant
kg	kilogram
L	liter
LDH	lactate dehydrogenase
LTBP4	latent TGFβ binding protein 4
m	Meter
MFA%	muscle fibers area %
mg	Milligram
miRNA	micro ribonucleic acid
mL	Milliliter
MRI	magnetic resonance imaging
msec	Millisecond
MTD	maximum-tolerated dose
MuSC	muscle satellite cells
ng	nanogram
nmol	nanomole
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
PD	Pharmacodynamic
PDGFR	platelet-derived growth factor receptor
PEF	peak expiratory flow
PET	polyethylene terephthalate
PFT	pulmonary function tests
PK	Pharmacokinetic
PUL	performance of upper limb
QT	QT interval
QTc	QT interval – corrected
RBC	red blood cells
RD	recommended dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOJIA	systemic onset juvenile idiopathic arthritis
SWI/SNF	switch/sucrose non-fermentable
TNF-α	tumor necrosis factor-alpha
V/F	plasma volume
V2/F	peripheral volume
WBC	white blood cells
WCT	Worldwide Clinical Trials

1 Introduction

1.1 Background on Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD) is the most common childhood muscular dystrophy, occurring in about 1 of every 3500 male newborns. The disease is inherited as an X-linked recessive disorder and is caused by mutation in the dystrophin gene on the X chromosome, resulting in dystrophin deficiency. The main function of dystrophin is to stabilize and link the muscle fiber cytoskeleton to the membrane. The lack of functional dystrophin results in the loss of dystrophin-glycoprotein complex, thereby rendering the muscle fibers less resistant to mechanical stress.

Faulty muscle structure caused by the absence of extracellular or intracellular structural proteins results in cell membrane instability, initiating a cascade of deleterious events, such as uncontrolled calcium influx, apoptosis and necrosis, inflammation, and replacement of muscle with fibrotic tissue and fat (Consalvi S et al. 2011). The clinical effect of this deficiency can be dramatic and fatal.

Patients begin to show symptoms of the disease between the ages of 3 to 5 years (Emery AE 2002), which leads to severe muscle wasting and weakness. Patients with DMD usually stop walking by about 12 years of age and usually experience fatal respiratory failure in their early 20s (Eagle M et al. 2002).

Treatment with steroids is currently used in a large portion of DMD patients, but it is palliative and complicated by serious side effects. No current treatment interrupts or halts the progression of DMD.

By acting on muscle resident stem cells, histone deacetylase (HDAC) inhibitors increase skeletal myogenesis in vitro and in vivo (Iezzi S et al. 2002) and restore normal muscle morphology and increase the size and strength of myofibers in *mdx* mice a preclinical model of DMD (Minetti GC et al. 2006).

The beneficial potential of the HDAC inhibitor Givinostat in the treatment of DMD has been studied in the *mdx* mouse disease model. In this model, long-term exposure to Givinostat effectively countered disease progression. In particular, Givinostat dose and concentration dependently increased the cross-sectional area of myofibers, decreased the cellular (inflammatory) infiltrate and prevented the formation of fibrotic scars. Pharmacokinetic (PK)–pharmacodynamic (PD) analysis suggests that exposures of Givinostat of 600 h*nmol/L are required to exert the beneficial effect.

1.2 Background on Givinostat

Givinostat has been tested in long-term repeat-dose toxicology studies in rats, dogs, and monkeys. The main adverse effects (e.g., reduction in white blood cells [WBC], reduced

thymus weight, bone marrow atrophy, and liver and possibly kidney function impairments) were observed only at high doses of drug. Most of these changes returned to baseline levels upon drug discontinuation or a trend toward resolution was seen at the end of the recovery period.

In the rat, Givinostat was administered consecutively up to 26 weeks where the no observed adverse effect level (NOAEL) was 10 mg/kg/day. There were no side effects in dogs administered 12.5 mg/kg/day of Givinostat for 4 consecutive weeks. However, liquid feces were found in the animals receiving higher doses with consequent reduced absorption. For these reasons, the dog species was not further considered and general toxicology studies in non-rodents were continued in the monkey. In the monkey, Givinostat was well tolerated and similar toxicity profiles were seen in the 4-, 13-, and 39-week studies, where the NOAELs were 10 mg/kg, 10 mg/kg, and 12 mg/kg, respectively.

Givinostat had a favorable safety pharmacology profile. There was no observation of embryo-fetal toxicity in rats and in rabbits, or potential genotoxicity in mammalian cells in vitro and in vivo. The only sign of a possible adverse effect was seen at relatively high doses (>1μM) of Givinostat in vitro but not in vivo in cardiovascular safety pharmacology studies.

Givinostat was given by oral gavage to juvenile rats starting at the age of weaning (25 days of age) at 4 different dosages: 0, 20, 60, or 180 mg/kg/day once-a day for 4 weeks. Givinostat was well tolerated at the dosage up to 60 mg/kg/day. Treatment-related changes were detected at 180 mg/kg/day in the adrenals, bone marrow, liver, and spleen. The changes had resolved or showed partial recovery at the end of the study (mature animals). The NOAEL was set at 60 mg/kg/day.

Givinostat has been tested in a number of clinical studies that enrolled for the following major indications: inflammation and oncology. In particular:

- 105 healthy volunteers have been enrolled in 3 phase 1 studies (single dose, repeat dose, and food interaction); 18 were treated with placebo.
- 422 patients have been enrolled in the phase 1–2 studies; 62 were treated with placebo. Of these, 33 were children with systemic onset juvenile idiopathic arthritis (SOJIA) or polyarticular juvenile idiopathic arthritis (JIA) treated with Givinostat at the following doses: 0.50 mg/kg twice daily (BID) and 0.75 mg/kg BID for up to 3 months.

Givinostat has shown preliminary signs of clinical activity in subjects with SOJIA (study DSC/05/2357/19) as well as myeloproliferative disease (study DSC/07/2357/28).

The maximum-administered dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in subjects with multiple myeloma. Doses up to approximately 100 mg BID were generally well tolerated. The most common adverse events (AEs) observed were thrombocytopenia as well as gastrointestinal (GI) toxicities. AEs were generally mild to moderate and reversible upon discontinuation of study drug. In particular, thrombocytopenia appeared in just a few days of treatment at thrombocytopenic doses and reverted to normal values within a few days/weeks after treatment discontinuation. Twenty-one AEs of QT interval – corrected (QTc) prolongations have been reported. In one case, the electrocardiogram (ECG) was manually read and the QTc value determined was normal, thus it was considered a spurious finding. Eight AEs of QTc prolongation were reported in subjects with chronic myeloproliferative diseases (study DSC/07/2357/28), 6 in subjects with Hodgkin's lymphoma (study DSC/07/2357/26), 2 in subjects with polycythemia vera (study DSC/08/2357/38), 2 in subjects with acute myeloid leukemia (study DSC/05/2357/15), 1 in a subject with Crohn's disease (study DSC/06/2357/23), and 1 in a subject with SOJIA (study DSC/05/2357/19). No clear dose dependence was observed. In the **2** studies in healthy volunteers (DM/00/2357/01 and DM/00/2357/03) where ECG and QTc measurements were systematically assessed, no episode of QTc prolongation was observed. Details of each trial are included in the Investigator's Brochure (2012).

1.3 Rationale

1.3.1 Study Rationale

Different studies suggest that histone acetylation has a significant role in the pathogenesis of DMD and that inhibition of HDAC leads to a reduction in inflammation and fibrosis and an increase in muscle regeneration. In particular, two preclinical studies with Givinostat have shown that chronic treatment with this compound in a DMD mouse model (*mdx* mouse) determines a dose- and concentration-dependent reduction in inflammation and fibrosis and an increase in muscle regeneration, which in turn determines an improvement in muscular function.

The primary objective of this study is to replicate these findings in humans. In particular, the primary objective of the study will be to demonstrate that Givinostat stimulates muscle regeneration by detecting an increase in the fraction of muscle biopsy occupied by muscle when comparing the muscle biopsy at study end *versus* the muscle biopsy at study start. Other objectives of the study will be to evaluate the effects of Givinostat on other histological parameters (inflammation, necrosis, fibrosis), on functional parameters such as the 6-Minute Walk Test (6MWT), and on magnetic resonance imaging (MRI) and biomarkers. Finally, the safety and tolerability and pharmacokinetics of Givinostat will also be assessed.

As the currently used dose of Givinostat is considered not sufficient to exert the expected positive effects (see dose rationale), the first part of the study will be used to escalate the dose to one yielding exposures expected to be efficacious and which are well tolerated.

To ensure an adequate assessment of the safety, efficacy, and PK parameters in this study, and considering the expected mechanism of action of Givinostat in DMD, ambulant children aged 7 to <11 years, who have been on a stable steroid dose for at least the last 6 months and who have a 6MWT assessment performed at least 6 months before screening will be selected for participation in this study.

1.3.2 Dose Rationale – Part 1 and Part 2

Until now, Givinostat has been administered to 87 healthy volunteers and 360 patients enrolled in 17 phase 1–2 studies. Of the 360 patients, 33 were children/adolescents treated with either Givinostat 0.5 mg/kg BID or 0.75 mg/kg BID. The maximum dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in subjects with multiple myeloma. Subjects have been treated in clinical trials with daily doses of Givinostat for up to 6 months. Moreover, 31 patients with myeloproliferative diseases enrolled in a compassionate use program have been receiving Givinostat at doses up to 50 mg 3 x daily for a period up to 4 years and 1 adolescent with JIA enrolled in the extension study has been receiving Givinostat 0.75 mg/kg since December 2011.

Doses of Givinostat up to approximately 100 mg BID have generally been well tolerated. At higher doses of Givinostat, transient reductions hematological parameters (particularly platelets) and diarrhoea as well as nausea and vomiting were observed. AEs were generally mild to moderate and reversible upon discontinuation of study drug (Investigator's Brochure 2012). In particular, thrombocytopenia appeared in just a few days of treatment at thrombocytopenic doses and reverted to normal values within a few days/weeks after treatment discontinuation. Twenty AEs of QTc prolongation have been reported and confirmed in the clinical studies. Of these, only 1 episode of QTc prolongation was observed in a pediatric study. No episode of QTc prolongation was observed in the 2 studies of Givinostat in healthy volunteers where ECG and QTc measurements were systematically assessed.

A population PK analysis was conducted using the PK data collected so far in all the clinical trials where PK samples were collected. In particular, PK data from 7 studies have been included (single- and repeat-dose studies in healthy volunteers, 2 studies in Crohn's disease subjects, 1 study in subjects with SOJIA, 1 study in subjects with JIA, and 1 study in subjects with psoriasis) for a total of 226 subjects. Givinostat pharmacokinetics were described using a 2-compartment model with first order absorption showing clearance (CL/F)=118 L/h, plasma volume (V/F)=155 L, peripheral volume (V2/F)=514 L, and absorption rate constant (KA)=0.243 h^{-1}. The covariate

screening indicated weight and steroid co-administration as the most significant covariates on clearance (CL) parameters (no gender, formulation, age, or healthy volunteer vs. patient differences). However, the covariates effects were considered limited on pharmacokinetics (i.e., the presence of steroids was found to determine an increase of <30% in the population CL, and an increase of 10 kg was found to determine an increase of 10%–15% in CL).

The mean exposure in healthy volunteers treated with Givinostat 100 mg BID was 1083 ng*h/mL (area under the plasma–concentration time curve from 0 to 12 hours [AUC_{0-12}]) and 181.5 ng/mL (maximum plasma concentration [C_{max}]). The estimated AUC_{0-12} and C_{max} in children treated with Givinostat are reported in Table 1.

Table 1 Concentrations of Givinostat in Pediatric Studies to Date

Disease State		AUC_{0-12} (ng*h/mL)	C_{max} (ng/mL)
SOJIA	Median 5th percentile 95th percentile	234.0 126.4 428.7	31.5 20.0 55.3
JIA	Median 5th percentile 95th percentile	206.4 109.0 492.7	31.0 14.9 69.8

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; C_{max}=maximum plasma concentration; SOJIA=systemic onset juvenile idiopathic arthritis; JIA=juvenile idiopathic arthritis.

Preclinical studies in a mouse model of DMD (*mdx* mouse model) suggest that daily exposures (AUC_{0-24}) of 600 nmol*h/L, i.e., approximately 300 ng*h/mL, are needed to exert beneficial histological and functional effects. As shown in Table 1, doses higher than those administered so far to children are needed to ensure that the majority of children are treated with doses that allow such exposures. Therefore, the first part of the study will escalate the dose to a maximum tolerated dose (MTD) that will then be recommended for the second part of the study.

Because of the limited effect of weight on CL, only 2 dose adjustments will be applied as follows: children who weigh 20–49 kg: 25 mg BID; children who weigh ≥50 kg: 37.5 mg BID.

The starting dose will be 25 mg BID in children who weigh 20–49 kg and 37.5 mg BID in children who weigh ≥50 kg. Table 2 reports the expected median, 5th, and 95th percentile exposures in children weighing 20, 30, 40, 50, and 60 kg.

Table 2 Expected Exposure by Weight

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	263	223	203	283	257
5th percentile	127	107	97	132	123
95th percentile	564	466	426	603	542

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours

Expected exposures fall within the range of exposures safely tested in children with SOJIA and JIA and well within the exposures in adults treated with 100 mg BID.

If safety and tolerability of the starting dose is confirmed in the first group of children treated, the dose will be escalated in the second group of children enrolled. The children treated at the lower dose will also be switched to the escalated dose level. Similarly, if the safety and tolerability of the second dose level is confirmed, the dose will be further escalated in the third group of children enrolled, and the children treated at the second dose level will be switched to the higher dose level.

Dose escalation will be decided based on the safety and tolerability profile observed, and on the PK analyses in the children treated until a dose-escalation decision is made. In any case, each dose escalation should not yield more than a doubling of the expected exposure. An example of dose escalation based on the data available so far is provided in Table 3, Table 4, and Table 5, which show the starting, second, and third dose, respectively.

Table 3 Starting Dose (25 mg BID in Children Who Weigh 20–49 kg and 37.5 mg BID in Children Who Weigh ≥50 kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	263	223	203	283	257
5th percentile	127	107	97	132	123
95th percentile	564	466	426	603	542

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Table 4 Second Dose (50 mg BID in Children Who Weigh 20–49 kg and 75 mg BID in Children Who Weigh ≥ 50kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	527	445	407	566	515
5th percentile	254	215	194	263	246
95th percentile	1129	932	852	1205	1084

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Table 5 Third Dose (75 mg BID in Children Who Weigh 20–49 kg and 100 mg BID in Children Who Weigh ≥50 kg)

Weight (kg)	20	30	40	50	60
AUC_{0-12} (ng h/mL)					
Median	790	668	610	755	686
5th percentile	381	322	290	351	329
95th percentile	1693	1398	1278	1607	1446

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily.

Once all 20 children enrolled in Part 1 of the study have been treated for at least 2 weeks, the review team will decide the recommended dose (RD) to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses.

All children will then switch to the RD, which will be administered for the subsequent 12 months of the study (Part 2).

As of July 2013, the safety and tolerability assessments of Givinostat from Part 1 of the study were completed and the Safety Review Team determined the RD to be 37.5 mg BID. The Safety Review Team also recommended performing additional platelet count assessments every week to maintain a stricter control and to monitor this parameter for safety reasons.

In addition, as of September 2013, after assessing approximately 2 months of data on the platelet counts, the Safety Review Team decided to reduce the RD in subjects with platelet count ≤150 x 10^9/L to 25-mg BID Givinostat. Subjects with platelet count >150 x 10^9/L will continue on 37.5-mg Givinostat.

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This clinical study has been designed to comply with the Good Clinical Practice guidelines.

1.3.3 Dose Rationale - Extension Phases

Extension 1: An extension study phase is foreseen in order to allow the patients to continue study treatment at least until the final analysis is performed (Part 2; after 12 months of treatment).

All children treated in Part 2 will be asked to continue in the extension phase and if no safety concern is raised, they will receive Givinostat at the same ongoing dose (during the last visit planned at 12 months) for a maximum of up to an additional 12 months (Extension 1).

Extension 2: A second extension study phase is foreseen because of the positive histology results.

All children treated in Extension 1 will be asked to continue in the extension phase and if no safety concern is raised, they will receive Givinostat at the appropriate dose for a maximum of up to an additional 12 months.

A recent PK population analysis was performed with the aim to develop a population PK model and support dosing recommendation in DMD clinical development plan (for more details, see the IB n. 14 – par 6.3.2.1).

Pharmacokinetic parameter estimates were used to simulate exposure in children across a wide weight range following different doses of Givinostat. The aim was to identify doses of the compound that warranted comparable exposures with acceptable efficacy and safety profiles. The dose increase of 5 mg was considered feasible in clinical practice of the compound. The results are shown in Table 6 and Table 7.

Extension 3: All children treated in Extension 2 will be asked to continue in the extension phase and if no safety concern is raised, they will receive Givinostat at the appropriate dose for a maximum of up to an additional 12 months.

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Table 6 Simulation C_{max} (ng/mL) After Different Oral Doses of Givinostat to Patients Weighing 10-70 kg (1200 Simulations for Each Patient Weight)

	C_{max} (ng/mL) Weight (kg)									
	10	12.5	15	20	25	30	40	50	60	70
Dose (mg) BID	**20**	**25**	**25**	**30**	**35**	**40**	**50**	**55**	**60**	**70**
Mean	74.5	81.2	76.8	84.7	84.9	90.3	105	101	101	114
Median	69.5	74.9	70.4	80.6	77.8	84.5	97.5	94.0	96.3	106
Standard deviation	28.5	32.6	30.9	32.0	34.1	35.6	41.7	40.0	38.5	43.4
CV%	38.3	40.2	40.2	37.8	40.2	39.4	39.6	39.8	38.0	38.2
5P	37.9	39.1	37.7	40.7	42.4	44.5	50.6	50.2	49	59.2
25P	54.5	57.2	54.7	61.7	60.6	63.7	75.1	72.3	74	82.0
75P	89.8	99.4	93.6	102	103	111	128	121	122	139
95P	127	143	135	144	152	156	183	174	174	195

BID=twice daily; C_{max}=maximum plasma concentration; CV%=coefficient of variation percent.

Note: Compound administered as an oral suspension at the doses of 20 (10 kg), 25 (12.5 kg), 25 (15 kg), 30 (20 kg), 35 (25 kg), 40 (30 kg), 50 (40 kg), 55 mg (50 kg), 60 mg (60 kg) and 70 mg bid (70 kg).

Table 7 Simulation AUC_{0-12} (ng h/mL) after Different Oral Doses of Givinostat to Patients Weighing 10-70 kg (1200 Simulations for Each Patient Weight)

	AUC_{0-12} (ng h/mL)									
	Weight (kg)									
	10	12.5	15	20	25	30	40	50	60	70
Dose (mg) BID	**20**	**25**	**25**	**30**	**35**	**40**	**50**	**55**	**60**	**70**
Mean	595	624	571	598	562	579	627	588	572	600
Median	531	561	502	539	507	520	558	522	512	534
Standard deviation	289	305	292	288	273	286	316	293	281	298
CV %	48.6	48.9	51.1	48.2	48.5	49.4	50.4	49.9	49.2	49.6
5P	254	248	235	243	229	236	253	242	241	252
25P	393	408	366	387	363	370	412	381	378	385
75P	738	769	693	752	687	717	780	722	708	743
95P	1141	1209	1128	1155	1099	1122	1226	1145	1090	1187

AUC_{0-12}=area under the plasma–concentration time curve from 0 to 12 hours; BID=twice daily; CV%=coefficient of variation percent.
Note: Compound administered as an oral suspension at the doses of 20 (10 kg), 25 (12.5 kg), 25 (15 kg), 30 (20 kg), 35 (25 kg), 40 (30 kg), 50 (40 kg), 55 (50 kg), 60 (60 kg) and 70 mg BID (70 kg)

During the Extension 2, the dose administered will be adjusted depending on the weight of the child, according the tables above (Table 6 and Table 7).

2 Study Objectives

Primary Objective

The primary objective of this study is as follows:

- To establish the histologic effects of Givinostat administered chronically at the selected daily dose

Secondary Objectives

The secondary objectives of this study are as follows:

- To establish the effects of Givinostat administered chronically at the selected daily dose on functional parameters, such as the 6MWT, North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL)
- To establish the safety and tolerability of Givinostat administered chronically at the selected daily dose in children with DMD
- To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as MRI and biomarkers
- To explore the acceptability/palatability of the oral suspension
- To explore whether the effects of Givinostat on disease progression may be related to the type of DMD mutation (Pane M et al. 2014)

Extension phases:

The primary objective is to evaluate the safety and tolerability of long-term administration of Givinostat administered chronically at the selected daily dose in children with DMD.

The secondary objectives are:

- To establish the effects of Givinostat administered chronically at the selected daily dose on other functional parameters, such as the 6MWT, NSAA, and PUL (Extension 1, 2, 3)
- To explore the effects of Givinostat administered chronically at the selected daily dose on parameters such as MRI (Extension 1)
- To collect information related to 2 biomarkers, LTBP4 and osteopontin genotype (at the beginning of Extension 2 only)
- To collect information related to time to wheelchair and how much time the children spend in wheelchair (Extension 3 – only for the children that are not able to complete the 6MWT)

Primary Endpoint

The primary endpoint of this study is as follows:

- Change in the value of muscle fiber area % (MFA%) comparing the histology biopsies before and after 12 months of treatment with Givinostat

The primary endpoints of the extension phases are:

- Number of children experiencing treatment-emergent AEs and serious adverse events (SAEs)
- Type, incidence, and severity of treatment-emergent AEs and SAEs correlated with dose

Secondary Efficacy Endpoints

The secondary efficacy endpoints of this study are as follows:

- Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based the PUL

The secondary efficacy endpoints of the extension phases are as follows:

- Change of muscular function (including loss of ambulation) after 24, 36, and 52 months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 24, 36, and 52 months of treatment with Givinostat at the selected daily dose based on the NSAA
- Change in muscular function after 24, 36, and 52 months of treatment with Givinostat at the selected daily dose based on the PUL
- Time to wheelchair and how much time the children spend in wheelchair (Extension 3 – only for the children that are not able to complete the 6MWT)

Safety Endpoints

The safety endpoints of this study are as follows:

- Number of children experiencing treatment-emergent AEs and SAEs
- Type, incidence, and severity of treatment-related AEs and SAEs
- Measures of vital signs, clinical laboratory tests (blood chemistry, hematology, disease serologies), echocardiographs (ECHOs), pulmonary function tests (PFTs; forced expiratory volume at 1 second [FEV_1], forced vital capacity [FVC], FEV_1/FVC, and peak expiratory flow [PEF])

(Suárez AA et al. 2002), and 12-lead ECGs.

Pharmacokinetic Endpoints

The PK endpoints of this study are as follows:

- Individual Givinostat concentrations tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistics for Part 2.

Exploratory Endpoints

The exploratory endpoints of this study are as follows:

- Change in muscle, fat, and fibrosis content after treatment with Givinostat as measured by MRI (also evaluated during Extension 1)
- Change in muscle biomarkers (e.g., miRNA) following treatment with Givinostat (Part 1 and Part 2 of the study)
- Evaluation of LTBP4 and osteopontin genotype (at the beginning of Extension 2 only)
- PK–PD correlations
- Evaluation of any correlation between the effects of Givinostat on disease progression and the type of DMD mutation patients may have in relation to their disease

3 Investigational Plan

3.1 Description of Overall Study Design and Plan

This is a 2-part, phase 2 study to assess the effects of Givinostat on muscle histologic parameters and on clinical parameters in ambulant children with DMD. The safety, tolerability, and pharmacokinetics of Givinostat will also be assessed.

Children who assent to participate in this study (if capable of doing so) and whose parent/guardian signs the informed consent to participate will undergo pre-study screening assessments up to 4 weeks (±2 weeks) before the first scheduled dose of study drug. In addition, patients will be asked to share information concerning the type of mutation they carry in relation to DMD.

Approximately 20 children will be enrolled in the study as follows: the first 4 children will be treated at a low dose level of Givinostat (25 mg BID in children who weigh 20-49 kg and 37.5 mg BID in children who weigh ≥50 kg).

If none of the stopping criteria (see Section 3.3.5 for stopping criteria) are met after 2 weeks of treatment at the low dose, the review team will determine the escalated dose level (i.e., intermediate dose level) to be used for the treatment of an additional 8 children who will be treated at the intermediate dose. The 4 children previously treated at the low dose level will also be switched to the intermediate dose level.

If none of the stopping criteria are met after 2 weeks of treatment at the intermediate dose, the review team will determine the subsequent escalated dose level to be used for the treatment of an additional 8 children who will be treated at the high dose. All children treated at the intermediate dose level will be switched to the high dose level.

Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the RD to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2).

As of July 2013, the safety and tolerability assessments of Givinostat from Part 1 of the study were completed and the Safety Review Team determined the RD to be 37.5 mg BID. The Safety Review Team also recommended performing additional platelet count assessments every week to maintain a stricter control and to monitor this parameter for safety reasons.

In addition, as of September 2013, after assessing approximately 2 months of data on the platelet counts, the Safety Review Team decided to reduce the RD in subjects with platelet count $\leq 150 \times 10^9$/L to 25mg BID Givinostat. Subjects with platelet count $>150 \times 10^9$/L will continue on 37.5mg Givinostat.

After the 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability of MFA% observed in the study will be implemented, the within-subject standard deviation of MFA% will be calculated, and the final sample size will be adjusted based on this interim analysis and in particular on the observed standard deviation. A conservative approach will be adopted, where the sample size may be increased but not decreased.

The additional children (if any) will be enrolled during Part 2 of the study and will receive the RD of Givinostat up to 40 months.

A treatment table for the study is presented in Table 8.

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Table 8 Treatment Table – Part 1 and Part 2

	PART 1						PART 2									
	Treatment Visit[c]						Treatment Visit[c]									
Subject	**1**	**2[a]**	**3**	**4[a]**	**5**	**6[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[b]**
1–4																
5–12																
13–20																
Additional children (if any)																

[a] At the end of Week 2 (Visit 2) at every dose level, a safety check is foreseen.
[b] At the end of Month 12, an efficacy analysis on biopsy results and functional tests is foreseen, and a decision to consider the study complete or to continue the study for up to another 40 months (extension phases [Extension 1, 12 months and Extension 2, 12 months and Extension 3, 16 months]) will be taken.
[c] The visits during Part 1 will be performed every 7 days (±1 day); the visits during Part 2 will be performed periodically every 1 – 1.5 months (±7 days).

Legend:

	25 – 37.5 mg BID – low dose level
	Intermediate dose level
	High dose level
	Recommended dose level

During Part 1 of the study, children will visit the center once a week. During Part 2 of the study, children will visit the study center per the schedule of assessments for a total of 10 visits, not including follow up. Children may be evaluated more often if necessary for safety reasons. Children who discontinue the study drug early will be asked to come in for an end-of-study visit within 2 weeks after the last dose of study drug. Children who have ongoing AEs at discontinuation will be followed until resolution or stabilization.

Efficacy will be assessed through changes from baseline in biopsy at 1 year (Part 2) and changes from baseline in the 6MWT, PUL, and NSAA at 1 year (Part 2); at 2 years (for Extension 1), at 3 years (for Extension 2), and at 4 years (for Extension 3). Exploratory efficacy parameters will include changes from baseline in MRI results, PK–PD correlations, and measures of miRNA. In addition, exploratory analysis will be performed to investigate whether the effects of Givinostat on disease progression may be related to the type of DMD mutation. Safety will be assessed by number of children experiencing treatment-emergent AEs and SAEs; type, incidence, and severity of treatment-emergent AEs and SAEs; and measures of vital signs, clinical laboratory tests

(blood chemistry, hematology, disease serologies, and urinalysis), ECHOs, PFTs (FEV_1, FVC, FEV_1/FVC, and PEF), and 12-lead ECGs.

During Part 1, after 1 week of treatment, blood samples for PK measurements will be collected pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose.

PK samples will also be collected during Part 2 of the study. At Visit 2, blood samples for PK analysis should be taken pre-dose and between 6 and 8 hours post-dose. At Visit 3, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and between 0 and 2 hours post-dose. At Visit 4, blood samples for PK analysis should be taken pre-dose and between 2 and 4 hours post-dose. At Visit 6, blood samples for PK analysis should be taken pre-dose and between 4 and 6 hours post-dose. At Visit 10, blood samples for PK analysis should be taken pre-dose and at 1, 2, 4, and 8 hours post-dose.

3.1.1 Extension Phases

EXTENSION 1

A first extension phase study treatment is foreseen in order to allow the patients to continue study treatment at least until the final analysis is performed (Part 2; after 12 months of treatment). Part 2 was originally planned with a 12-month study drug treatment. At 12 months, an efficacy and safety analysis will be conducted. If the results show a positive effect on patients, and no safety concern is raised, then patients will continue study treatment for up to another 12 months to evaluate the safety and tolerability of long-term administration of Givinostat and to evaluate the effect of treatment on muscle function.

EXTENSION 2

In February 2015, the results were available and they have shown that the treatment with Givinostat significantly increases the amount of muscle in the biopsies and significantly reduces the amount of fibrotic tissue. Moreover treatment with Givinostat significantly reduces tissue necrosis and fatty replacement, 2 other parameters related to disease progression. Function tests have shown overall stability, although the sample size of the study is too small to draw definitive conclusions. Finally, the drug was well tolerated.

Based on these results, a second extension phase is foreseen in order to allow the patients to continue a study treatment that supports a beneficial effect of Givinostat in DMD. The children on treatment in the Extension 1 will be asked to continue in the Extension 2 and will receive Givinostat at the same ongoing dose for a maximum of an additional 12 months.

The patient will receive Givinostat at the same ongoing dose, during the last visit planned at 12 months, and will be treated for a maximum of an additional 28 months (Month 40). The dose can be reduced or increased according to the dose modification criteria, specified below (Section 3.3.5).

EXTENSION 3

In February 2015, the results were available and they have shown that the treatment with Givinostat significantly increases the amount of muscle in the biopsies and significantly reduces the amount of fibrotic tissue. Moreover treatment with Givinostat significantly reduces tissue necrosis and fatty replacement, 2 other parameters related to disease progression. Function tests have shown overall stability, although the sample size of the study is too small to draw definitive conclusions. Finally, the drug was well tolerated.

Based on these results, a third extension phase is foreseen in order to allow the patients to continue a study treatment that supports a beneficial effect of Givinostat in DMD. The children on treatment in the Extension 2 will be asked to continue in the Extension 3 and will receive Givinostat at the same ongoing dose for a maximum of an additional16 months.

The extension 3 will be additionally extended by 4 months, until the activation of the Long Term Safety Study (Study NDSC/14/2357/51 - EUDRACT: 2017-000397-10).

The patient will receive Givinostat at the same ongoing dose, during the last visit planned at 12 months (at PART 2), and will be treated for a maximum of an additional 40 months (Month 52). The dose can be reduced or increased according to the dose modification criteria, specified below (Section 3.3.5).

During the extension phases 1 and 2 of the study, children will visit the center every 2 months, for a total of 6 visits during Extension 1 (from Visit 11 to Visit 16) and a further 6 visits for Extension 2 (Visit 17 to Visit 22), and every 4 months during Extension 3 for 4 visits (Visit 23 to Visit 26).

3.2 Selection of Study Population

This study will enroll approximately 20 ambulatory male children with an established diagnosis of DMD who are at least 7 years of age but <11 years of age. Additional children could be enrolled in the second part of the study, after the interim evaluation of baseline biopsies if the observed variability is higher than the one used for the current sample size estimate. Children will be enrolled at approximately 5 study sites in Italy. Specific entry criteria are detailed in Section 3.2.1 and Section 3.2.2.

3.2.1 Inclusion Criteria

Children meeting all of the following inclusion criteria are eligible for inclusion in the study:

1. Male children aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD.
2. Parent/guardian has signed the informed consent form and child has assented to be in the study (if applicable).
3. Able to complete the 2 screening 6MWTs with a minimal distance of at least 250 m each. In addition, the results of theses 2 tests must be within ±30 m of each other.
4. On a stable dose of systemic corticosteroids for at least 6 months.
5. At least 6 months worth of data on the 6MWT (this will be the "historical" 6MWT). From the moment of the historical 6MWT assessment(s), the child must not have received any compound that could potentially affect the 6MWT, with the exception of the stable steroid treatment.
6. A parent/guardian and child can comply with all study evaluations/procedures and return for all study activities.

3.2.2 Exclusion Criteria

Children meeting any of the following exclusion criteria will not be enrolled in the study.

1. Initiation of systemic corticosteroid therapy within 6 months prior to the start of study drug or change in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) within 6 months prior to the start of study drug.
2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and integrators will be allowed.
3. Surgery that might have an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study.
4. Exposure to another investigational drug since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment.

5. History of participation in gene therapy, cell-based therapy or oligonucleotide therapy.

6. Presence of other clinically significant disease that in the opinion of the investigator places the child in unacceptable risk for an adverse outcome or that could affect study results.

7. Symptomatic cardiomyopathy or heart failure. If child has a left ventricular ejection fraction <45% at screening, the investigator should discuss inclusion of child in the study with the medical monitor.

8. Inadequate hematological function.

9. Absolute neutrophil count: $<1.5 \times 10^9$/L.

10. Platelets: $<100 \times 10^9$/L.

11. Current or history of liver disease or impairment, including but not limited to an elevated total bilirubin.

12. Inadequate renal function, as defined by serum creatinine >2 x the upper limit of normal.

13. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening

14. A baseline QTc >450 msec, (as the mean of 3 consecutive readings 5 minutes apart) or history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome).

15. Psychiatric illness/social situations rendering the potential child unable to understand and comply with the study protocol.

3.2.3 Removal of Children from Therapy or Assessment

A child will be considered to have completed the study when he completes the 12-month/end-of-study visit (Visit 10) in Part 2. If a child is discontinued at any time after entering the study, the investigator will make every effort to see the child and complete the early termination and follow-up assessments as shown in Section 3.4.10. All AEs should be followed until the child recovers or his condition stabilizes.

A termination electronic case report form (eCRF) should be completed for every child who receives study drug, whether or not the child completes the study. The reason for any early discontinuation should be indicated on this form. The primary reason for a child

withdrawing prematurely should be selected from the following standard categories of early termination:

- *Adverse Event (Adverse Reaction)*: Clinical or laboratory events occurred that, in the medical judgment of the investigator for the best interest of the child, are grounds for discontinuation. This includes serious adverse events (SAEs) and non-serious AEs, regardless of relation to study drug.
- *Death:* The child died.
- *Withdrawal of Consent*: The child or his parent/guardian desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If the child or parent/guardian gave a reason for withdrawing, it should be recorded in the eCRF.
- *Protocol Violation*: The child's findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g., drug noncompliance, failure to return for defined number of visits). The violation necessitated premature termination from the study.
- *Lost to Follow-Up*: The child stopped coming for visits, and study personnel were unable to contact the child.
- *Other*: The child was terminated for a reason other than those listed above, such as theft or loss of study drugs or termination of study by sponsor.

The children included in the extension phases will be considered to have completed the study when they complete the 12-month end-of-extension study visit (Visit 16) for Extension phase 1, the 36-month end-of-extension study visit (Visit 22) for Extension phase 2, and the 52-month-end-of-extension study visit (Visit 26) for Extension phase 3. If the extension phases are stopped due to negative results of Part 2 or the child is discontinued from the study treatment, the child will be asked to attend the early termination and follow-up assessments as shown in Section 3.4.6 and Section 3.4.10, respectively.

3.3 Treatments

3.3.1 Details of Study Treatment

Details about study drug are provided in Table 9.

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Table 9 Details of Study Treatment

	Givinostat^
Drug name	Givinostat
Manufacturer	Italfarmaco S.p.A.
Dose(s)	1st dose level: 25 mg (children 20–49 kg) 37.5 mg (children ≥50 kg) 2nd dose level: 50 mg (children 20–49 kg) 75 mg (children ≥ 50 kg)* 3rd dose level: 75 mg (children 20–49 kg) 100 mg (children ≥ 50 kg)*
Dose frequency	BID
Route	Oral under fed conditions
Formulation	Oral suspension and/or capsules

^Givinostat is used to indicate the whole study drug name Givinostat hydrochloride monohydrate. The dosages / concentrations of the study drug are expressed as Givinostat hydrochloride monohydrate.
* Estimated doses based on current knowledge.
BID=twice daily

3.3.2 Dosage Schedule

Study drug will be administered as 2 oral doses daily while the child is in the fed state. Children who enroll during Part 1 of the study will receive their assigned dose until the RD is determined. They will then receive up to 12 months of treatment with Givinostat at the RD. Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who will be included in the extension phase, will continue Givinostat up to a total of 48 months of treatment. The RD should be reduced in case of platelet count ≤150 x 10^9/L as described in Section 3.3.5.1.

3.3.3 Treatment Assignment

All children will receive study drug. Children enrolled in Part 1 of the study will start to take either the low, intermediate, or high dose, depending on the needs of the study at the time of enrollment and they will switch to the RD when the review team decide that this dose is safe (see Table 8).

When the safety review team has determined the RD, all children currently on study drug will be switched to that dose (the RD), and Part 2 of the study will commence. All children who enroll during Part 2 (if applicable) of the study will be given the RD of Givinostat.

Children who will be included in the extension phases will be treated at the same ongoing dose during the last visit planned at 12 months and will be treated for a maximum of an additional 40 months (Month 52). During Extension 2 and Extension 3, the dose will be adjusted based on the weight of the children.

3.3.4 Drug Packaging, Labeling, Storage, Dispensing, Investigational Medicinal Product Accountability, and Blinding

Drug Packaging - Oral Suspension

The primary packaging will consist of an amber plastic bottle containing the suspension. The secondary packaging will be a carton box containing 1 amber bottle and a syringe dosing system for dispensing the suspension.

Drug Packaging - Capsules

Capsules will be supplied as 50 mg and 25 mg hard gelatin capsules for oral administration in white plastic bottles containing 30 capsules each.

Labeling

The primary and secondary labels will show all the information requested according to the Annex 13 of the Good Manufacturing Practice. Bottles containing Givinostat will be labeled in local language (i.e., Italian).

Storage

The investigational medicinal product (IMP; Givinostat) will be stored at Italfarmaco until distribution to the investigational sites. The investigational site will store the IMP under the conditions specified in the label (i.e., 5 ± 3°C for the oral suspension and <30°C for the capsules), ensuring that it is not accessible to unauthorized persons until it is dispensed to the child's parents/legal guardians.

Dispensing

The investigational site will be supplied initially with a congruous number of suspension bottles and capsule bottles, in order to have sufficient supply of study drug to treat the children who are enrolled in the study.

All IMP supplies are to be used only for this protocol and for no other purpose.

The investigator will be responsible for the delivery of IMP to the child's parents/legal guardians according to the protocol.

Children will be administered the IMP on an outpatient basis.

At each scheduled visit, the investigator will supply the children with the appropriate number of suspension bottles and/or capsules bottles, sufficient to cover the treatment until the following visit (i.e., 1 week of treatment during Part 1 of the study and 1 to 1.5 months, during Part 2 of the study).

Italfarmaco will provide to the investigator a table with volume of suspension and/or number of capsules to be administered according to body weight and dose level.

The investigator will provide to the child's parent/legal guardian written instruction on the dosage and corresponding volume in milliliters of suspension and/or number of capsules to be taken at each administration. Refer to the relevant Clinical Study Operations Manual (CSOM) for more detailed information.

For the extension phases (Extension 1 [12 months], Extension 2 [12 months], and Extension 3 [16 months]), at each scheduled visit, the investigator will supply the children with the appropriate number of suspension bottles sufficient to cover the treatment for 2 months during Extensions 1 and 2 and every 4 months during Extension 3.

Blinding

Not applicable. This is an open-label study.

Emergency Procedure for Unblinding

Not applicable. This is an open-label study.

3.3.5 Dose Modifications

3.3.5.1 Child Stopping and Dose Reduction Safety Rules

Permanent Stopping Rule

Study drug should be permanently stopped if any of the following occur:

- Severe diarrhea (i.e., increase of ≥7 stools per day)
- Any drug-related SAE
- QTc >500 msec
- Platelets ≤50 x 10^9/L

Temporary Stopping and Dose Reduction Safety Rule

Study drug should be temporarily stopped if any of the following occur:

- Platelets ≤75 x 10^9/L but >50 x 10^9/L
- Moderate diarrhea (i.e., increase of 4 to 6 stools per day)

Study drug may be resumed at a reduced dose level once the event resolves. Such a dose reduction can happen only once per child. The treatment can be temporarily interrupted for a maximum of 4 weeks. If the child has not recovered from the AE after this period, the treatment should be permanently discontinued.

If a child has a medical event not necessarily drug related that requires interruption of study drug dosing for >4 weeks, the review team will determine if the child may resume study drug treatment.

Cohort Expansion Safety Rules

The review team can decide to expand the first cohort of children and enroll additional 4 children (for a total of 8 children treated at the first dose level), on the basis of safety, tolerability, and PK results in the children treated in that cohort, according to but not limited to the following rules:

- If 1 child experiences any type of stopping criterion (as defined in the permanent and temporary stopping rules), or
- If 2 children experience different types of stopping criterion (e.g., 1 child experiences hematological toxicity and another child experiences GI toxicity).

3.3.5.2 Cohort Abandoned Safety Rules

The review team can also decide to abandon a given dose level on the basis of safety, tolerability and PK results in the children treated at that dose level, according to but not limited to the following rules:

- If >2 children experience any type of stopping criterion, or
- If ≥2 or more children experience the same type of stopping criterion

3.3.5.3 Cohort Dose Escalation Safety Rules

During Part 1 of the study, each child will receive study drug at a specific dose level. Once the first 4 children have been treated for at least 2 weeks, the review team will examine safety and tolerability data and PK results and decide if the children can be switched to the escalated dose level and if the second group of children can start the treatment at the escalated dose level.

After a further 2 weeks, the review team will decide if the already treated children at the lower and intermediate dose levels can switch to the highest dose and if the third and last group of children can start the treatment at the highest dose level.

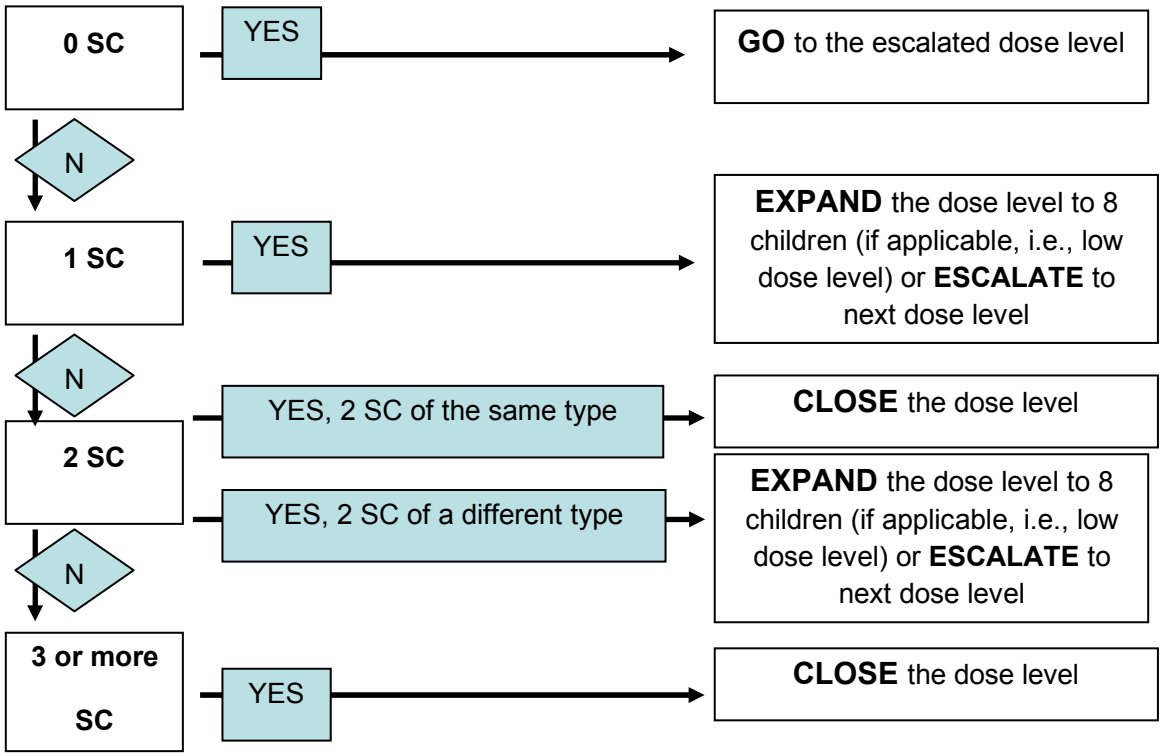
The children should be switched to the escalated dose level (i.e., intermediate or high dose level) only if no or only 1 stopping criterion per dose level occurred.

For more details, see Figure 1.

3.3.5.4 Study Stopping Safety Rules

If 2 or more stopping criteria occur in the lower dose, the study will be temporarily stopped to allow a reassessment of the risks and benefits of the compound.

Figure 1 Cohort expansion, Cohort Abandoned and Dose Escalation Safety Rules for Part 1



SC=stopping criterion, as defined under permanent and temporary stopping rules.

The review team will include: the Principal Investigators, the Study Chair, the Medical Monitor, and other Italfarmaco representatives.

Dose Increase Rule – Extension 2 and Extension 3

Study drug should be increased if the following occur:

- Weight gain
  And
- Platelets >150 x 10^9/L in the last 4 months of treatment;
- No severe drug-related AEs occurred;
- No drug-related SAE occurred.

The dose should be increased only after the discussion of the data and approval by the Medical Monitor, according to Table 10 and Table 11, below.

Table 10 Starting Dose: 37.5 mg BID

Starting Dose – Extension 2: 37.5 mg BID							
Weight (Kg)	20	25	30	40	50	60	70
Dose (BID)			40 mg	50 mg	55 mg	60 mg	70 mg

BID=twice daily.

Table 11 Starting Dose: 25 mg BID

Starting Dose – Extension 2: 25 mg BID							
Weight (Kg)	20	25	30	40	50	60	70
Dose (BID)			27 mg	33 mg	37 mg	40 mg	47 mg

BID=twice daily.

3.3.6 Treatment Compliance and IMP Accountability

At each visit, the child's parents/legal guardians will bring back to the study site all the bottles previously received (used, partially used, and unused) and receive a new supply of the IMP.

All unused IMP will be not re-dispensed by the investigator to another child.

The investigator will maintain accurate records of the disposition of all IMP received, distributed to children (including date and time), and accidentally lost or destroyed.

When dispensing the bottles to the children, the investigator will attach the relevant tear-off label to the relevant form.

Oral Suspension Accountability

The investigator will count the bottles of suspension used and empty, partially used and unused and insert this information in the Drug Accountability Form and in the eCRF.

Capsule Accountability

The investigator will count the capsules unused for each bottle and insert the number in the Drug Accountability Form and in the eCRF.

Periodically throughout and at the conclusion of the study, a representative of Italfarmaco S.p.A. or its delegate will conduct an inventory of all study drug supplies and the bottles of oral suspension and/or capsules used, partially used, and unused will be destroyed at the site, if possible, or sent back to Italfarmaco.

Missed doses are not to be recovered and they should be recorded in the eCRF and in the Drug Accountability Form, specifying the reason for any missed dose.

For more detailed procedures, please refer to the relevant CSOM.

3.3.7 Prior and Concomitant Illnesses and Treatments

Prior and Concomitant Illnesses

Investigators should document all significant illnesses that the child has experienced within 6 months of screening. Additional illnesses present at the time informed consent is given are to be regarded as concomitant illnesses. Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs in the eCRF.

Prior and Concomitant Treatments

Prior treatments, defined as those taken within 6 months prior to screening, should be recorded in the eCRF as prior medications.

Concomitant treatments are defined as treatments taken after study drug administration.

Children should be on stable systemic corticosteroid therapy for at least 6 months prior to initiation of study drug. That is, there have been no changes in systemic corticosteroid therapy (e.g., initiation, change in type of drug, dose modification not related to body weight change, schedule modification, interruption, discontinuation, or re-initiation) during the previous 6 months.

Supportive treatments, such as anti-emetics, anti-diarrheals, anti-pyretics, anti-allergics, analgesics, and antibiotics are allowed.

Use of Vitamin D, calcium and integrators if clinically indicated before enrolment and for duration of the trial are allowed.

The following medications are prohibited prior to (as noted below) and during study treatment:

- Other investigational agents within 3 months of start of study drug, since time historical 6MWT data was obtained, or while on study
- Prior gene therapy or cell-based therapy or oligonucleotide therapy prior to study treatment or while on study
- Any pharmacologic treatments (other than stable doses of corticosteroids) that might have an effect on muscle strength since the time of the historical 6MWT and in any case within 3 months prior to the start of study treatment (e.g., growth hormone) or while on study
- Drugs that prolong the QTc interval (see Appendix 1: Drugs Known to Prolong the QTc Interval)

3.4 Assessments

3.4.1 Schedule of Assessments

The procedures to be performed during the study are outlined in the Schedule of Assessments (Table 12, Table 13, and Table 14). A detailed description of each assessment may be found in Section 3.4.2.

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Table 12 Schedule of Assessments: Part 1 (Dose Escalation)

Assessment	Screen/ Pre-Study[a]	Part 1 (Approximately 20 Children)					
Week	**-4 (±2 weeks)**	**0**	**1**	**2**	**3**	**4**	**5**
Visit (1-day window)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Informed consent and assent	X						
Medical history/ eligibility	X						
Concomitant medications	X[b]	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X
Height	X						
12-lead ECG[c]	X	X	X	X	X	X	X
ECHO[c]	X						
PFTs (FEV_1, FVC, FEV_1/FVC)[c]	X						
Clinical laboratory tests[c,d]	X	X	X	X	X	X	X
Urinalysis[b,c]	X	X	X	X	X	X	X
miRNA	X						
Serology[e]	X						
Quality of Life test	X						
Muscle evaluations (6MWT[f], NSAA[g], PUL[g])	X						
Muscle biopsy	X						
MRI	X[i]						
PK assessment[h]			X				
Adverse events	X	X	X	X	X	X	X
Study drug administration		X	X	X	X	X	X

6MWT=6-Minute Walk Test; CMV=cytomegalovirus; EBV=Epstein Barr virus; ECG=electrocardiogram; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MRI=magnetic resonance imaging; NSAA=North Star Ambulatory assessment; PFTs=pulmonary function tests; PK=pharmacokinetic.

[a] During the pre-study visit, historical function data (from 6 months before the pre-study visit) will be collected.
[b] Obtain prior medications at screening as well.
[c] To be performed more frequently, if clinically indicated.
[d] The following laboratory parameters will be assessed: hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, and abnormal cells; blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CrCl (CrCl will be calculated by the Cockcroft and Gault formula); urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.
[e] The following serology tests will be conducted: ELISA for HIV-Ab, HCV-Ab, HbsAg, HbeAg, anti-EBV, and anti-CMV IgM.

[f] During the pre-study visit, the historical function data relevant to 6MWTs will be collected: (1) result from 6MWT performed at least 6 months before the pre-study visit, and (2) result from 2 6MWT performed within 4 weeks prior to treatment start will be necessary and inserted into the eCRF.
[g] During the pre-study visit, the functional data relevant to the NSAA and PUL will be collected: results performed within 4 weeks prior to treatment start will be necessary and inserted into the eCRF.
[h] After 1 week of treatment, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and at 1, 2, 4, and 8 hours post-dose. It is important to record the time of PK assessments and the time of the last drug intake in the evening before the PK assessment.
[i] Obtain an MRI of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb). MRI does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment.

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Table 13 Schedule of Assessments: Part 2 (Proof of Mechanism)

Assessment	Part 2 (All Children)[j]									12/ EOS Visit[i]
	Month of Study:									
	0	1	2	3	4.5	6	7.5	9	10.5	12
Visit (1 week window)	**1[a]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Informed Consent and assent										X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Height										X
Weight	X	X	X	X	X	X	X	X	X	X
12-lead ECG[c]	X	X	X	X	X	X	X	X	X	X
ECHO[c]										X
PFTs (FEV_1, FVC, FEV_1/FVC, PEF)[c]										X
Clinical laboratory tests[c,d]	X	X	X	X	X	X	X	X	X	X
Urinalysis[c,d]	X	X	X	X	X	X	X	X	X	X
Serology[e]										
Quality of life test										X
Acceptability/ Palatability evaluation								X		X
Muscle evaluations (6MWT[f], NSAA[g], PUL[g])	X			X		X				X
Muscle biopsy										X
MRI										X
miRNA	X			X		X				X
PK assessment[h]		X	X	X		X				X
Adverse events	X	X	X	X	X	X	X	X	X	X
Study drug administration	X	X	X	X	X	X	X	X	X	X

6MWT=6-Minute Walk Test; CMV=cytomegalovirus; EBV=Epstein Barr virus; ECG=electrocardiogram; EOS=end of study; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MRI=magnetic resonance imaging; PEF=peak expiratory flow; NSAA=North Star Ambulatory assessment; PK=pharmacokinetic.

Note: A subject may return for an unscheduled visit at the discretion of Investigator to undergo additional safety evaluations (i.e., laboratory re-testing, AE assessments), or for additional medical evaluations. Platelet count may be assessed at an unscheduled visit if deemed necessary by the Investigator.

The Safety Review Team recommended performing additional platelet count assessment each week, other than the scheduled Part 2 assessments, to maintain a strict control and monitor this parameter for safety reasons.

[a] Children newly enrolled in Part 2 have to perform the screening visit (see Table 8 – screening/pre-study).
[b] Obtain prior medications at screening as well.
[c] To be performed more frequently, if clinically indicated.
[d] The following laboratory parameters will be assessed: Hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, ANC, and abnormal cells; Blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CrCl (CrCl will be calculated by the Cockcroft and Gault formula). Urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin
[e] The following serology tests will be conducted: ELISA for HIV-Ab, HCV-Ab, HbsAg, HbeAg, anti-EBV, and anti-CMV IgM.
[f] During the pre-study visit, the historical function data relevant to 3 6MWTs will be collected: (1) result from 6MWT performed at least 6 months before the pre-study visit, and (2) result from 6MWT performed within 4 ± 2 weeks prior to treatment start will be necessary and inserted into the eCRF.
[g] During the pre-study visit, the functional data relevant to the NSAA and PUL will be collected: results performed within 4 ± 2 weeks prior to treatment start will be necessary and inserted into the eCRF.
[h] At Visit 2, blood samples for PK analysis should be taken pre-dose and between 6 and 8 hours post-dose. At Visit 3, blood samples for PK analysis should be collected pre-dose in the morning before drug intake and between 0 and 2 hours post-dose. At Visit 4, blood samples for PK analysis should be taken pre-dose and between 2 and 4 hours post-dose. At Visit 6, blood samples for PK analysis should be taken pre-dose and between 4 and 6 hours post-dose. At Visit 10, blood samples for PK analysis should be taken pre-dose and at 1, 2, 4, and 8 hours post-dose. It is important to record the time of PK assessments.
[i] Children who discontinue participation prior to completion the study should perform the Early Termination Visit within 2 weeks after the last drug intake. For the assessments to be performed, see "12/EOS visit."
[j] Once all 20 children enrolled during the Part 1 of the study have been treated for at least 2 weeks, the review team will determine the RD to be used in Part 2 based on the safety and tolerability profile observed and on the PK analyses. All the children enrolled will switch to the RD level, which will be administered for the subsequent 12 months of the study (Part 2).

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Table 14 Schedule of Assessments: Extension Phases

Assessment	Extension Phase 1							Extension Phase 2							Extension Phase 3				
Months	**14**	**16**	**18**	**20**	**22**	**24/ EOS[c]**	**FU***	**26**	**28**	**30**	**32**	**34**	**36/ EOS[c]**	**FU***	**40**	**44**	**48**	**52/ EOS[c]**	**FU**
Visit N. (1-week window for V11-V24; 1 month window for V25 and V26)	**11**	**12**	**13**	**14**	**15**	**16**	**NA**	**17**	**18**	**19**	**20**	**21**	**22**	**NA**	**23**	**24**	**25**	**26**	**NA**
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height						X							X				X	X	
Weight	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	
12-lead ECG[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECHO[a]						X							X					X	
PFTs (FEV_1, FVC, FEV_1/FVC, PEF)[a]						X							X					X	
Clinical laboratory tests[a,b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis[a,b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[d]	X[d]	X[d]	X[d]	X[d]
Quality of life test						X							X					X	
Muscle evaluations (6MWT, NSAA, PUL)		X		X		X			X		X		X		X	X	X	X	
MRI						X													
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study drug administration	X	X	X	X	X			X	X	X	X	X			X	X	X		

AE=adverse event; 6MWT=6-Minute Walk Test; ECG=electrocardiogram; EOS=end of study; FEV_1= forced expiratory volume at 1 second; FU=follow up; FVC= forced vital capacity; MRI=magnetic resonance imaging; NA=Not applicable; NSAA=North Star Ambulatory assessment; PEF=peak expiratory flow; PFT=pulmonary function test; PUL= performance of upper limb

* To be performed only if the child does not continue the treatment in Extension 2 after Extension 1 and Extension 3 after Extension 2.

** LTBP4 and osteopontin genotype information Note: A subject may return for an unscheduled visit at the discretion of Investigator to undergo additional safety evaluations (i.e., laboratory re-testing, AE assessments), or for additional medical evaluations. Platelet count may be assessed at an unscheduled visit if deemed necessary by the Investigator.

[a] To be performed more frequently, if clinically indicated.

[b] The following laboratory parameters will be assessed: Hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, ANC, and abnormal cells; Blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CrCl (CrCl will be calculated by the Cockcroft and Gault formula). Urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.

[c] If the child is discontinued from the study treatment, the child will be asked to return for the early termination procedures. For the assessments to be performed, see “EOS visit”. Discontinued children will also have follow-up procedures performed within 4 weeks of the last dose of study drug.

[d] Urinalysis: pH, specific gravity, protein, glucose, ketones and cytology.

3.4.2 Study Procedures

3.4.2.1 Pre-Study (Screening)—(Day -28 [±2 Weeks])

The assessments during the pre-study phase will determine the child's eligibility for the study and also their ability to comply with protocol requirements by completing all screening assessments. All children will undergo all screening assessments, regardless of whether they are enrolled during Part 1 or Part 2 of the study.

The following procedures will be performed and recorded during the screening period:

- Obtain written informed consent from the child's parent/legal guardian and assent from the child, if applicable.
- Collect medical history.
- Review inclusion and exclusion criteria.
- Obtain and record current and prior medications (taken in the past 6 months).
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC.
- Obtain blood samples for hematology, blood chemistry, and disease serology tests.
- Obtain a urine sample for urinalysis.
- Obtain blood sample for miRNA.
- Perform a quality of life test
- Perform the muscle evaluations:
    - 6MWT (collect historical data on tests performed at least 6 months prior to the pre-study visit and within 4 ± 2 weeks of pre-study visit).
    - NSAA and PUL (collect results from within 4 ± 2 weeks prior to treatment start).
- Obtain a muscle biopsy (brachial biceps; please refer to Section 3.4.11 for instructions; obtained within 4 ± 2 weeks prior to the start of treatment).
- Obtain an MRI of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb; MRI does not need to be repeated if obtained within 4 ± 2 weeks prior to the start of treatment).
- Assess AEs

3.4.2.2 Weekly Visits (Part 1; Weeks 0 to 6)

Children will visit the study center weekly during Part 1 of the study. Children will continue to come in weekly (i.e., every 7 ± 1 days) until they are switched to Part 2 of the study when the RD is selected. At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain blood for PK assessments at Visit 2 only as noted in the schedule of assessments.
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.2.3 Part 2 Visits (Months 1 to 10.5)

Children will visit the study center as noted in the schedule of assessments during Part 2 of the study (i.e., every 1 to 1.5 months ± 1 week). At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain blood samples for miRNA (Visits 4 and 6 only).
- Obtain a urine sample for urinalysis.
- Obtain blood for a PK assessments at Visits 2, 3, 4, and 6 only as noted in the schedule of assessments.
- Obtain muscle evaluations (6MTW, NSAA, and PUL; Visits 4 and 6 only).
- Dispense IMP and perform accountability.
- Assess AEs.
- Acceptability/palatability evaluation (Visit 8 only)

3.4.3 Unscheduled Visits

A subject may return for an unscheduled visit at the discretion of Investigator to undergo additional safety evaluations (i.e., laboratory re-testing, AE assessments), or for additional medical evaluations. Platelet count may be assessed at an unscheduled visit if deemed necessary by the Investigator.

Subjects who experience a hematological toxicity will be monitored closely until resolution of the event.

3.4.4 Assessments for End of Study Part 2 (Visit 10 [Month 12])/Beginning of Extension Phase 1

Children who remain in the study will continue to take study drug through Visit 10. The end of study (EOS) visit overlaps with the 12-month visit, if the child completes treatment.

The following assessments will be performed and recorded:

- Ask for the written informed consent from the child's parent/legal guardian and assent from the child, if applicable, for the continuation in Extension 1 (12 months).
- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC, and PEF.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Perform a quality of life test.
- Obtain muscle evaluations (6MWT, NSAA, PUL).
- Perform a muscle biopsy (please refer to Section 3.4.11 for instructions).
- Obtain an MRI of dystrophic muscle.
- Obtain blood sample for miRNA.
- Obtain blood for PK assessment.
- Perform accountability.
- Assess AEs.
- Acceptability/palatability evaluation

- Dispense IMP and perform accountability for children continuing into the extension phases.

The extension phases will be followed only for children whose parent/guardian has given their written consent and the child has assented to participate in these phases.

3.4.5 Extension Phase 1 Visits (Months 12 to 22)

Extension phase 1 will start at the EOS visit (Visit 10 [Month 12]). As noted in the schedule of assessments during Extension 1, for Months 14 to 22, children will visit the study center at Visits 11 to 15 (i.e., every 2 months ± 1 week). At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MTW, NSAA, and PUL) at Visit 12 and Visit 14 only.
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.6 Assessments for End of Extension Phase 1 (Visit 16 [Month 24])/Beginning of Extension Phase 2

For children who continue in Extension 1, the end of the extension phase visit will be performed on Month 24 (Visit 16). The following assessments will be performed and recorded:

- Ask for the written informed consent from the child's parent/legal guardian and assent from the child, if applicable, for the continuation in Extension 2 (12 months).
- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.

- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC, and PEF.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MWT, NSAA, PUL).
- Obtain an MRI of dystrophic muscle.
- Perform accountability.
- Perform a quality of life test.
- Assess AEs.
- Collect LTBP4 and osteopontin genotype information.

3.4.7 Extension Phase 2 Visits (Months 24 to 34)

Extension phase 2 will start at the EOS visit (Visit 16 [Month 24]). As noted in the schedule of assessments during Extension 2, for Months 26 to 34, children will visit the study center at Visits 17 to 21 (i.e., every 2 months ± 1 week). At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MTW, NSAA, and PUL) at Visit 18 and Visit 20 only.
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.8 Assessments for End of Extension Phase 2 (Visit 22 [Month 36]/Beginning of Extension Phase 3)

For children who continue in Extension 2, the end of the extension phase visit will be performed on Month 36 (Visit 22). The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.

- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC, and PEF.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MWT, NSAA, and PUL).
- Perform accountability.
- Perform a quality of life test.
- Assess AEs.

3.4.9 Extension Phase 3 Visits (Months 40 to 48)

Extension phase 3 will start at the EOS visit (Visit 22 [Month 36]). As noted in the schedule of assessments during Extension 3, for Months 40 to 52, children will visit the study center at Visits 23 to 26 (i.e., every 4 months). The time window for Visits 23 and 24 is ± 1 week; the time window for Visits 25 and 26 is ± 1 month.

At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- Measure height (Visit 25 [Month 48] only)
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MTW, NSAA, and PUL)
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.10 Assessments for End of Extension Phase 3 (Visit 26 [Month 52])

For children who continue in Extension 3, the end of the extension phase visit will be performed on Month 52 (Visit 26) ± 1 month.
The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.

- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC, and PEF.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MWT, NSAA, and PUL).
- Perform accountability.
- Perform a quality of life test.
- Assess AEs.

3.4.11 Early Termination Visit

Children who discontinue participation prior to completion of all study drug administration (i.e., 12 months of treatment or 48 months of treatment if the children continue in the extension phases) will be asked to return to the hospital within 2 weeks after the last dose of study drug for completion of the same assessments given at the EOS visit (for details, see Section 3.4.4 or Section 3.4.6, if applicable).

3.4.12 Follow-up Visit

All children, regardless of whether they complete the study (including the extension phase) or terminate early in Part 1, Part 2, or from the extension phases (if applicable), should return to the study center within 4 weeks of the last dose of study drug for the follow-up visit.

The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Obtain a 12-lead ECG in triplicate.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Assess AEs.

3.4.13 Description of Assessments

Unless otherwise indicated, all assessments will be performed by the investigator or other regular study personnel. Assessments are to be performed according to the schedule

shown in Section 3.4.1. The assessments at the end-of-study and follow-up visits should also be performed if the child terminates the study early.

Efficacy Assessments

Muscle Biopsies: A first brachial biceps biopsy (baseline) will be taken prior to the first dose of study drug. A second brachial biceps biopsy will be taken at Visit 10 (12 months) from the opposite arm.

The muscle biopsy samples from the biceps muscle will be collected by open biopsy according to standard hospital procedures for obtaining muscle biopsies from children. The minimum amount of muscle tissue required is a small piece of muscle of at least 0.5 x 0.5 x 0.5 cm. The muscle sample, embedded with tragacanth gum on a piece of cork, must be frozen in liquid nitrogen-cooled 2-methylbutane and stored at -80°C or -70ºC until shipment.

The collection, processing, and shipment of these muscle biopsy samples to the Ospedale Pediatrico Bambino Gesù laboratory will be described in detail in the study-specific laboratory manual.

6-Minute Walk Test: A modified version of the 6MWT recommended by American Thoracic Society (2002) for use in adults will be performed.

North Star Ambulatory Assessment: The NSAA will be graded using the standard scorecard with each assessment rated as 0 – unable to achieve independently, 1 – modified method but achieves goal independent of physical assistance from another, or 2 – normal with no obvious modification of activity.

Performance of Upper Limb: The PUL was devised to assess motor performance in the upper limb for patients with Becker and Duchenne muscular dystrophy. The purpose is to assess change that occurs in motor performance of the upper limb over time from when a child is still ambulant until he loses all arm function when non-ambulant. The PUL will be administered according to the guidelines developed by the Physiotherapy Working Group (Mayhew A et al. 2012, Mercuri E et al. 2012).

MRIs: MRIs will be taken of muscle (initially on the muscles of the lower limb and, if possible, on the muscles of the upper limb) and evaluated per the specifications in the CSOM.

miRNA: miR-1, miR-133, and miR-206 may be evaluated. Additional miRNA parameters may be evaluated if warranted. Evaluation of miRNA will be documented in a separate report.

Other assessments – exploratory analysis regarding type of mutation: the DMD gene mutation will be evaluated in the study to explore whether the effects of Givinostat on disease progression may be related to the underlying type of mutation.

Other efficacy assessments – exploratory analysis of muscle biopsies: Muscle biopsies will be analyzed in 2 distinct steps, by using different complementary approaches (fluorescence-activated cell sorting [FACS]-mediated isolation of cells and functional characterization ex vivo and staining of frozen sections) that are finalized to the identification and functional/molecular characterization of specific cell types that contribute to the regeneration or fibro-adipogenic degeneration of dystrophic muscles. These cells include muscle satellite cells (MuSCs) and a heterogeneous population that is referred to as "fibro-adipogenic progenitors" (FAPs). These cell types can be isolated by FACS in mouse and human muscle samples as distinct populations, based on specific combination of surface antigens.

In the mouse system, MuSCs can be isolated from skeletal muscles by FACS, as $CD34^{pos}/\alpha7\text{-integrin}^{pos}/Sca1^{neg}$ cells (Sacco A et al. 2008), while FAPs are isolated either as $CD34^{pos}/\alpha7integrin^{neg}/Sca1^{pos}$ cells or platelet-derived growth factor receptor (PDGFR) PDGFR- α^{pos} cells (Joe AW et al. 2010; Uezumi A et al. 2010). In muscle biopsies of human patients or normal individuals, MuSCs are isolated as PDGFR- $\alpha^{neg}/\alpha7\text{-integrin}^{pos}$/N-CAM ^{pos} cells, while FAPs can be isolated as PDGFR- $\alpha^{neg}/\alpha7\text{-integrin}^{pos}$/N-CAM ^{pos} cells.

Pre-clinical studies have demonstrated that functional interactions between these cell types contributes to the disease progression in mouse models of DMD (*mdx* mice) and that HDAC inhibitors promote the FAP property of stimulating muscle regeneration at the expense of fibro-adipogenic degeneration. The sponsor has identified a novel nuclear network that regulates FAP lineage identity and ability to support regeneration or fibro-adipogenic degeneration in dystrophic muscles. This network consists of an HDAC-repressed miRNA (the myomiRs 1.2, 133a and 206) that target 2 specific sub-units (BAF60a and b) of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex, which promotes the expression of fibro-adipogenic genes. Upon treatment with HDAC inhibitors, de-repression of myomiRs 1.2, 133a and 206 causes the down regulation of BAF60a and b, and the simultaneous activation of BAF60c, leading to the formation of BAF60c-based SWI/SNF complex that promotes skeletal myogenesis in FAPs. The sponsor has found that *mdx* mice that respond to HDAC inhibitors, such as Givinostat, show increased levels of BAF60c and myomiRs 1.2, 133a and 206.

1. FACS-sorting of MuSCs and FAPs from biopsies of children before and at the end of the treatment will be used to measure: a) the relative amount of these cell populations; b) their ability to differentiate in culture, in myogenic and adipogenic

media, and their functional interactions by co-culture experiments; c) gene and miRNA expression analysis by quantitative polymerase chain reaction.

It should be emphasized that in vitro exposure to Givinostat of MuSCs and FAPs from biopsies of children before the treatment might provide a useful measure predictive of child response that can be used to better select children in a follow-up trial.

2. Histologic analysis of muscle sections from biopsies of children before and at the end of the treatment will be used to measure: a) the expression levels of BAF60 a, b, and c variants, and myomiRs 1.2, 133a and 206 in MuSCs and FAPs, which will be identified as $Pax7^{pos}$ or PDGFR- α^{pos}, respectively.

Safety Assessments

Physical Examination: Physical examination will include examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system. An AE form must be completed for all changes identified as clinically noteworthy. Height without shoes and weight will be recorded as noted in the schedule of assessments.

Vital Signs: Vital signs will include body temperature (ºC), pulse rate, and blood pressure.

Electrocardiogram: Standard 12-lead ECGs will be conducted in triplicate per the schedule of assessments and more often if clinically indicated. ECG will be acquired at the sites, then ECG data will be transmitted and a central reading will be performed. For details, see the relevant CSOM.

ECHOs: Standard ECHOs will be conducted as per the schedule of assessments and more often if clinically indicated.

Pulmonary function tests: FEV_1, FVC, FEV_1/FVC, and PEF, will be collected per the site's standard process as noted in the schedule of assessments and more often if clinically indicated.

Laboratory Parameters: The following laboratory tests are to be performed as indicated in the schedule of assessments and more often if clinically indicated:

1. Hematology: red blood cell (RBC) count, hemoglobin, hematocrit, WBC count with differential, platelets, ANC, and abnormal cells. Additional platelet counts may be assessed for subjects in Part 2, at an unscheduled visit, if deemed necessary by the Investigator (refer Section 3.4.3 for details).

2. Blood chemistry: total bilirubin, alkaline phosphatase, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, blood urea nitrogen (BUN), and creatine phosphokinase (CPK) (creatinine clearance [CrCl] will be calculated by the Cockcroft and Gault formula.)
3. Urinalysis: pH of freshly voided specimen, specific gravity, protein, glucose, ketones, cytology, and myoglobin (myoglobin not assessed during Extension 3).
4. Other: enzyme-linked immunosorbent assay (ELISA) for HIV- Ab, HCV-Ab, HbsAg, hepatitis B e antigen (HbeAg), anti-EBV, and anti-cytomegalovirus (CMV) immunoglobulin M (IgM).

Lab tests will be performed and analyzed by a local laboratory to ensure consistent interpretation of results. In the event of an unexplained clinically noteworthy abnormal laboratory test value, the test should be repeated immediately and followed up until it has returned to the normal range and/or an adequate explanation of the abnormality is found.

Adverse Events: An AE is "any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (International Conference on Harmonisation [ICH] E2A)." All medical and psychiatric conditions (except those related to the indication under study) present at screening will be documented on the Prior Illnesses eCRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE eCRF during the rest of the study. Laboratory abnormalities should be recorded as AEs only if they meet the criteria for an SAE, result in discontinuation of the study drug, require medical intervention, or are judged by the investigator to be clinically significant changes from baseline values. See Section 4, for additional information.

Pharmacokinetic Assessments

Blood samples for PK assessments will be collected as indicated in the schedule of assessments. Additional information about the PK time points will be provided in the relevant CSOM.

Quality of Life Assessment

The PedsQL Test will be performed before treatment start, at Visit 10 (12 months), and at the end of the extension phases at 24, 36 and 52 months for Extension 1, 2 and 3, respectively, if applicable.

Acceptability/Palatability Evaluation

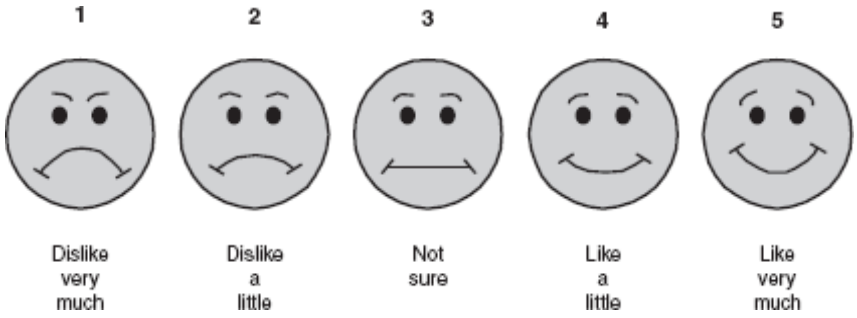
At Visits 8 and 10 of the study Part 2, the palatability of the oral suspension will be evaluated using the Five-Point Hedonic Scale palatability.

The investigator will explain to the child that he would be asked "how much did you like the taste of this medicine?" and encouraged to indicate his preference by pointing to the appropriate face that depicts five degrees of pleasure:

- 5 = like very much
- 4 = like a little
- 3 = not sure
- 2 = dislike a little
- 1 = dislike very much

The explanation will be repeated if the child did not understand.

Figure 2 Five-Point Hedonic Scale – Face Depictions



In addition, the palatability/patient acceptability will be also indirectly assessed in parents with two questions:

1. "On the basis of reaction/facial expression of your child, do you think that the medication is: pleasant = 3; not sure = 2; or unpleasant = 1?"
2. "Do you sometimes have problems in giving the medication to your child because he refuses to take it or throws it up? (Yes/No)

3.4.14 Appropriateness of Measurements

All assessments to be used in this study are commonly used, standard measurements frequently seen in DMD studies.

4 Adverse Event Reporting

Throughout the course of the study, all AEs will be monitored and reported in the AE eCRF, including the event's seriousness, severity, action taken, and relationship to the IMP. If AEs occur, the first concern will be the safety of the children. All AEs will be followed until resolved or stable and the outcome documented on the appropriate eCRF.

In order to avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology rather than the child's or parent's/legal guardian's own words.

4.1 Definitions and Criteria

4.1.1 Adverse Events

An AE is "any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (ICH E2A)".

AEs include:

1. Onset of any clinical sign or symptom
2. Worsening (change in nature, severity or frequency) of conditions present at the start of the trial
3. Subject deterioration due to the primary illness
4. Intercurrent illness(es)
5. Drug interactions
6. Events related or possibly related to concomitant medications
7. Abnormal laboratory values, as well as significant shifts from baseline within the range of normal that the investigator considers to be clinically significant

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

4.1.2 Adverse Drug Reactions

In the pre-approval clinical experience with a new medicinal product: "all noxious and unintended responses to a medicinal product related to any dose should be considered Adverse Drug Reaction."

4.1.3 Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction is an event in which the nature or severity is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved IMP).

4.1.4 Serious Adverse Events

An SAE (experience) or reaction is any untoward medical occurrence that at any dose:

- Is fatal (results in the outcome death)
- Is life-threatening*
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above

*The term life-threatening refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the child or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An SAE is not necessarily severe; e.g., an overnight hospitalization for a diagnostic procedure must be reported as an SAE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: nausea of several hours' duration may be rated as severe but may not be considered serious.

4.1.5 Assessing Intensity and Relationship

All AEs will be assessed on 2 descriptive parameters: intensity and relationship to study drug:

Intensity refers to the "severity" of an event and references impact on a child's functioning.

Relationship refers to the likelihood that the event being assessed was caused by the study drug.

Intensity

Each AE will be classified according to the following criteria:

Mild:	The AE does not interfere in a significant manner with the child's normal functioning level.
Moderate:	The AE produces some impairment of functioning, but is not hazardous to health.
Severe:	The AE produces significant impairment of functioning or incapacitation and is a definite hazard to the child's health.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience should be noted. If the intensity category changes over a number of days, those changes should be recorded separately (with distinct onset dates).

Relationship

Each AE will be assessed as to its relationship to study drug based on the following criteria. Although investigator attribution will be collected for reported events, for analytic purposes a temporal association with the use of study drug will be assumed sufficient for at least plausible association.

Not related:	No causal relationship exists between the investigational product and the AE, but an obvious alternative cause exists, e.g., the child's underlying medical condition or concomitant therapy.
Related:	There is a reasonable/plausible possibility that the AE may have been caused by the investigational product.

When assessing the relationship to study drug, the follow criteria will be considered:

- Positive rechallenge
- Positive dechallenge (resolution upon stopping suspect product, in absence of other intervention or treatment)
- Known class effect
- Biological plausibility

- Lack of alternative explanation—concomitant drug or disease

Factors to be considered in assessing the relationship of the AE to study drug include:

- The temporal sequence from IMP administration
- The recovery on discontinuation and recurrence on reintroduction
- The concomitant diseases
- The evolution of the treated disease
- The concomitant medication(s)
- The pharmacology and pharmacokinetics of the IMP

4.2 Reporting Procedures and Requirements

4.2.1 Adverse Events

The investigator or his/her designees are requested to collect and assess any spontaneous AE reported by the child and to question the child about AEs and intercurrent illnesses at each visit during the treatment period and follow-up. The questioning of children regarding AEs is generalized such as “How have you been feeling since your last visit?” Any AE occurring after the informed consent form/assent has been signed and up to the follow-up study visit, whether volunteered by the child; discovered during general questioning by the investigators; or detected through physical examination, laboratory test, or other means will be recorded on the specific section of the eCRF. Each AE will be described by:

- Seriousness
- Duration (start and end dates)
- Severity
- Relationship to the IMP
- Action taken
- Outcome

The severity of AE should be assessed and graded according to the most recently published National Cancer Institute Common Terminology Criteria for AEs (v. 4.0).

The relationship to the investigational drug should be assessed as:

- Related to IMP
- Not related to IMP
- Unknown

The assessment of the relationship of an AE to the administration of IMP is a clinical decision based on all available information at the time of the completion of the eCRF.

An assessment of 'not related' would include the existence of a clear alternative explanation, or non-plausibility.

An assessment of 'related' indicates that there is a reasonable suspicion that the AE is associated with the use of the IMP.

4.2.1.1 Abnormal Laboratory Findings and Other Objective Measurements

Abnormal laboratory findings and other objective measurements should not be routinely captured and reported as AEs in the eCRF as they will be collected and analyzed separately. However, abnormal laboratory findings and other objective measurements that meet the criteria for an SAE, result in discontinuation of the IMP, require medical intervention, or are judged by the investigator to be clinically significant changes from baseline values should be captured and reported as AEs in the eCRF.

When reporting an abnormal laboratory finding as an AE in the eCRF, a clinical diagnosis should be recorded in addition to the abnormal value itself, if this is available (for example "anemia" in addition to "hemoglobin = 10.5 g/dL").

4.2.1.2 Baseline Medical Conditions

Medical conditions present at the screening visit that do not worsen in severity or frequency during the study are defined as baseline medical conditions and are not AEs. These medical conditions should be adequately documented on the appropriate page of the eCRF (i.e., the medical history page). However, medical conditions present at the initial study visit that worsen in severity or frequency during the study should be recorded and reported as AEs.

4.2.2 Serious Adverse Events

Any SAE, including death from any cause that occurs after a child has signed the informed consent /assent and up to the final follow-up visit (regardless of relationship to study drug) must be reported by the investigators to the sponsor within 24 hours of learning of its occurrence.

Related SAEs ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

The investigators are required to complete the SAE form provided by the sponsor. Sufficient details must be provided to allow for a complete medical assessment of the AE and independent determination of possible causality. The investigators are obliged to

pursue and provide additional information as requested by the sponsor's drug safety manager, or study director, or designee.

The investigator must send notification of the SAE to the sponsor's Drug Safety Unit by faxing the SAE form, within 24 hours of a SAE, at the number specified below; then, the investigator must confirm any SAE notifications by mailing to the mail address or phoning to the phone number specified below:

Drug Safety Unit, Italfarmaco S.p.A.
Via dei Lavoratori
54 20092 Cinisello Balsamo (MI), Italy
Fax: 02 6610 6538 (or Fax 02 6443 2955 as backup)
phone: +39 02 6443 2510, mobile +39 333 9262611
e-mail: drug-safety@italfarmaco.com

The same procedure must be applied to the SAE follow-up information.

The sponsor's drug safety manager will report all serious and unexpected AE that are related to the use of the study drug to the competent authority within the required time and following procedures required by applicable laws. It is imperative that the sponsor be informed as soon as possible, so that reporting can be done within the required time frame.

The SAEs will also be recorded in the AE section of the eCRF.

Overdose and Other Situations Putting the Child at Risk of an Adverse Reaction

Any instance of overdose (suspected or confirmed) must be reported to the sponsor within 24 hours and be fully documented as a SAE. Details of any signs or symptoms and their management should be recorded including details of any antidote(s) or systematic treatment administered. Any signs or symptoms of overdose will be treated symptomatically.

Any other situations putting the child at risk of adverse reaction, such as misuse and abuse, medication errors, suspicion of transmission of infective agents must be reported to the sponsor within 24 hours and be fully documented as a SAE.

5 Data Management and Statistical Analysis

5.1 Data Management Considerations

Electronic CRFs will be employed for this study. Completed eCRFs for this study will be forwarded to the sponsor or its representative where editing and construction of a

quality-assured database will occur. The statistical analysis of these data will be performed by the sponsor or its representative.

Data Analysis of the Biopsies

All images will be digitally captured, using both light microscopy (hematoxylin and eosin [H&E] and Gomori trichrome stain) and fluorescence microscopy coupled to the Olympus Fluoview FV1000 confocal microscope. Fields for fluorescent imaging will be randomly selected while viewing the laminin-dystrophin signal.

In the only step involving operator discretion, all image parameters including pinhole size, detector gain, amplifier offset, amplifier gain, and laser intensity will be first set for the dystrophin and laminin channels using normal control tissue, and the same setting used for all samples imaged on a given day. Frame size, scan speed, and averaging will be the same for all images. For each sample 4 non-overlapping images for each channel will be acquired and stored as 12-bit fluorescent images (.TIFF) for analysis. A single technician will perform all sectioning, staining, and morphometry steps, while another operator will execute the confocal imaging.

Image processing and quantitative analyses will be done using Metamorph (Molecular Devices, Inc.) software program using a custom script. Additional details are provided in the relevant CSOM.

5.2 Statistical Considerations

The statistical analysis will be undertaken by the contract research organization Worldwide Clinical Trials (WCT) in collaboration with the sponsor.

Any deviations from the analyses described below will be included in the Statistical Analysis Plan (SAP).

5.2.1 General Considerations

Data will be provided in data listings sorted by treatment group and child number. Tabular summaries will be presented by treatment group for Part 1. Categorical data will be summarized by the number and percentage of children in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided, unless otherwise stated. Details of endpoint analyses will be described in the SAP.

For analyses of change from baseline, baseline will generally be defined as the value collected before any Givinostat treatment is administered. If this value is unavailable, the last non-missing value prior to dosing will be used. Otherwise, missing observations will

be treated as missing at random, and no data imputation will be performed. All data from the CRFs, as well as any derived variables, will be presented in data listings.

All hypothesis tests will be two-sided with a 5% significance level, and 95% CIs will be used, unless stated otherwise. As this is a Phase 2 exploratory study, no adjustments for multiplicity will made.

5.2.2 Sample Size Justification

A minimum of 20 evaluable children will be enrolled in this study.

5.2.2.1 Part 1

Approximately 20 children will be enrolled in Part 1 (dose-finding) of this study.

5.2.2.2 Part 2

Muscle fibers: a sample size of 20 children from part 1 completing the treatment period of part 2 should provide 90% power (at a 2-sided alpha level of 5%) to detect at least a 12.5% increase in MFA% between pre- and post-treatment (which corresponds approximately to a 26% relative increase given the observed mean in the Desguerre publication of 48% [Desguerre I et al. 2009]), the "worst case" standard deviation of 16%, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment. This sample size is based on a paired t-test and the assumption of normal distribution of MFA%.

After the first 20 baseline biopsies are collected, an interim analysis aimed at checking the real variability observed in the study will be implemented: the within-subject standard deviation of MFA% will be calculated, the actual distribution of MFA% will be checked and the final sample size will be adjusted based on the observed standard deviation and actual distribution of MFA%. Results by Al-Sunduqchi and Guenther (1990) indicate that power calculations for the Wilcoxon test may be made using the standard t-test formulations with a simple adjustment to the sample size. The size of the adjustment depends upon the actual distribution of the data. They give sample size adjustment factors for four distributions. These are 1 for the uniform distribution, 2/3 for the double exponential distribution, $9/\pi^2$ for the logistic distribution, and $\pi/3$ for the normal distribution. So depending on the actual distribution of MFA%, sample size re-calculation will be based on a Wilcoxon Signed Rank test with the corresponding adjustment if the observed distribution of MFA% is not normal.

5.2.3 Analysis Populations

The intent-to-treat (ITT) population includes all children who are enrolled in the Part 1 portion or entered the Part 2 portion of the study. The efficacy analysis will be conducted based on the ITT population.

The evaluable population will include all children who are in the Part 2 of the study, receive Givinostat of at least 80% dose, have at least one baseline and one post-baseline assessment of biopsies, and have no major protocol violations. The evaluable population will be identified prior to database lock. A sensitivity analysis may be conducted based on the evaluable population.

The safety population will include all children who receive any investigational product. The safety analysis will be conducted based on the safety population.

The PK population will include all children with at least one quantifiable post-dose concentration datum available. All statistical analyses of PK data will be performed using the PK population.

5.2.4 Primary Endpoint

The primary endpoint of this study is change in the value of MFA% comparing the histology biopsies before and after 12 months of treatment with Givinostat.

The primary endpoints of the extension phases are:

- Number of children experiencing treatment-emergent AEs and SAEs
- Type, incidence, and severity of treatment-emergent AEs and SAEs correlated with dose.

5.2.5 Secondary Efficacy Endpoints

The secondary efficacy endpoints are the following:

- Change in additional histological endpoints (i.e., cross-sectional area, inflammation, necrosis, fibrosis, and muscle regeneration) after 12 months of treatment with Givinostat at the selected daily dose
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the NSAA
- Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based on the PUL

The secondary efficacy endpoints of the extension phases are the change of muscular function (including loss of ambulation) after 24, 36, and 52 months of treatment (Extension 1, Extension 2, and Extension 3, respectively) with Givinostat as assessed by 6MWT, NSAA, and PUL. For the children who are not able to complete the 6MWT, the time to wheelchair and how much time the children spend in wheelchair were assessed.

Additional details on secondary efficacy endpoints will be provided in the SAP.

5.2.6 Safety Endpoints

The safety endpoints will include the following:

- The number of children experiencing AEs
- The type, incidence, and severity of AEs correlated with dose
- ECG, ECHO, vital sign, physical examination, PFTs, and clinical laboratory parameter findings

5.2.7 Secondary Pharmacokinetic Endpoints

Individual Givinostat concentrations will be tabulated by dose cohort along with descriptive statistics for Part 1 and tabulated along with descriptive statistic for Part 2. Noncompartmental PK data analysis will be performed for data obtained from each dose cohort with scheduled PK sample collection. If data allow, descriptive statistics of noncompartmental PK parameters (area under the plasma–concentration time curve, maximum plasma concentration, clearance, terminal elimination half-life) will be provided.

5.2.8 Exploratory Endpoints

The exploratory endpoints will include the following:

- Changes in muscle, fat, and fibrosis content after treatment with Givinostat as measured by MRI (also evaluated during Extension 1)
- Change in muscle biomarkers (e.g., miRNA) following treatment with Givinostat
- Evaluation of LTBP4 and osteopontin genotype
- PK–PD correlations
- Evaluation of acceptability/palatability of the oral suspension
- Evaluation of any correlation between the effects of Givinostat on disease progression and the type of DMD mutation patients may have in relation to their disease.

5.2.8.1 Magnetic Resonance Imaging

MRI will be collected via T1w imaging and quantitative Dixon imaging (the fat content of the image as a percentage of the total signal per voxel). Additional details will be provided in the SAP.

5.2.8.2 miRNA

miR-1, miR-133, and miR-206 will be evaluated. Other miRNA may be evaluated as appropriate. Additional details will be provided in the SAP.

5.2.8.3 LTBP4 and Osteopontin Genotype

The genotype of LFTP4 and osteopontin will be assessed. Additional details will be provided in the SAP.

5.2.8.4 DMD Gene

DMD gene in relation to the disease will be evaluated. Additional details will be provided in the SAP.

5.2.8.5 Acceptability/Palatability of the Oral Suspension

Summary statistics of palatability score assessed with Five-Point Hedonic Scale palatability and patient acceptability will be reported. Additional details will be provided in the SAP.

5.2.9 Efficacy Analyses

Efficacy analyses will be conducted on the evaluable population.

All values will be expressed as means ± standard deviation or standard error of the mean.

Each of all efficacy endpoints will be subject to normality test and formal statistical test performed will be based on whether it is normally distributed. If normal, statistical differences will be calculated using the paired t test; if not normal, Wilcoxon signed rank test will be used to assess the statistical significance of differences between groups. $P<0.05$ will be set as significant.

5.2.10 Safety Analyses

Safety analyses will be conducted on the safety population.

The safety of Givinostat will be assessed primarily by summarizing treatment-emergent AEs and SAEs. Other safety data (e.g., laboratory, ECG, PFTs, physical examination, and vital sign findings) will be summarized. Treatment-emergent AEs and SAEs that occur after administration of Givinostat will be summarized by system organ class and preferred terms, by severity, and by relationship to investigational product. Change from

baseline in laboratory values, ECG findings, PFTs, physical examination findings, and vital signs measurements will be summarized.

5.2.11 Pharmacokinetic Analyses

The PK analysis will be conducted on the PK population.

Plasma concentrations from Part 1 will be listed and tabulated by dose and time point for all children and time points with at least 1 PK assessment. Plasma concentrations from Part 2 will be listed and tabulated by time point for all children and time points with at least 1 PK assessment.

Descriptive statistics for all PK parameters for Part 1 will be calculated by treatment. Descriptive statistics for all PK parameters for Part 2 will also be calculated. These tables will include number of observations, mean, standard deviation, median, minimum and maximum and additionally the geometric mean and coefficient of variation (not for time to maximum plasma concentration).

5.2.12 Interim Analyses

The following interim analyses will be conducted:

- After the first 20 baseline biopsies are collected, the between-subject standard deviation of MFA% fraction will be calculated, the actual distribution of MFA% will be checked. The within-subject standard deviation will be estimated by between-subject standard deviation under the “worst case” scenario, which assumes that there is no correlation within a subject in the muscle area fiber percentage between pre- and post-treatment.
- Based on the standard deviation obtained above, the final sample size will be adjusted. A conservative approach will be adopted, where the sample size may be increased but not decreased.

5.2.13 Analysis at Month 12

At 12 months, efficacy and safety analysis will be conducted, on the primary and secondary endpoints. If results show a positive effect on patients, and no safety concern is raised, then patients will continue study treatment for up to an additional 40 months (Extension 1 [12 months], Extension 2 [12 months], and Extension 3 [16 months]) to evaluate the safety and tolerability of long-term administration of Givinostat and to evaluate the effect of treatment on muscle function. If, on the other hand, the final analysis of the results of the first 12 months of treatment does not support a beneficial effect of Givinostat in DMD, the study will be considered complete.

6 Study Management

6.1 Approval and Consent

6.1.1 Regulatory Guidelines

The study will be performed in accordance with local national laws (as applicable), the guidelines of the ICH, and the guidelines of the Declaration of Helsinki adopted by the 18th World Medical Assembly in Helsinki, Finland in 1964 and amended by subsequent assemblies in Tokyo, Japan in 1975; Venice, Italy in 1983; Hong Kong in 1989; Somerset West, South Africa in 1996, and in Edinburgh, Scotland in October 2000. These guidelines are on file at WCT.

This clinical study has been designed to comply with the Good Clinical Practice guidelines.

6.1.2 Institutional Review Board/Independent Ethics Committees

This study will be undertaken only after approval of the protocol has been obtained from the appropriate Independent Ethics Committee (IEC), and a copy of the approval has been received by Italfarmaco S.p.A.

The IEC must be informed of all subsequent protocol amendments and should be asked whether a re-evaluation of the ethical aspects of the study is necessary.

If applicable, interim reports on the study and reviews of its progress will be submitted to the IEC by the investigator at intervals stipulated in their guidelines.

6.1.3 Informed Consent

For each trial subject, written informed consent from the legally accepted representative will be obtained prior to any protocol-related activities. Informed assent may be obtained from children who are capable of providing assent. As part of this procedure, the principal investigator or a designated representative must explain orally and in writing the nature, duration, and purpose of the study, and the action of the drug in such a manner that the child and (if applicable) appointed guardian are aware of the potential risks, inconveniences, or adverse effects that may occur. Children and their legally accepted representatives should be given ample time and opportunity to inquire about the details of the study prior to deciding whether to participate in the study. It is the responsibility of the investigator to ensure that all questions about the study are answered to the satisfaction of the children and their legally accepted representatives.

Children and their legally accepted representatives should be informed that children may withdraw from the study at any time. They will receive all information that is required by local regulations and ICH guidelines. The principal investigator or a designated representative will provide the sponsor or its representative with a copy of the IEC-approved informed consent form prior to the start of the study.

The informed consent form should be signed and dated by the child's legally accepted representative and the investigator on the same day. If the child and/or legally accepted representative are not able to read, an impartial witness should be present during the informed consent discussion, and the witness must co-sign and date the informed consent form. The child's legally accepted representative and/or impartial witness should receive a copy of the signed documents.

For details of the information provided, refer to the informed consent form.

6.2 Discontinuation of the Study by the Sponsor

The sponsor reserves the right to discontinue the study at this site or at multiple sites for safety or administrative reasons at any time. In particular, a site that does not recruit at a reasonable rate may be discontinued. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to the sponsor or its representative.

6.3 Study Documentation

The investigator will supply the sponsor with:

- Curricula vitae for all investigators
- Signed protocol signature page
- List of IEC members and their occupations/affiliations or multiple assurance number
- Letter indicating IEC approval to conduct the protocol
- Copy of IEC-approved informed consent form
- Laboratory certification records and reference ranges

The main documents that will be supplied by the Sponsor to investigator are:

- Clinical study protocol
- Investigational drug brochure
- Sample informed consent form
- eCRFs/instruction manual
- Insurance letter

6.4 Study Monitoring and Auditing

This study will be monitored at all stages of its development by the clinical research personnel employed by the sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to protocol and in order to comply with guidelines of Good Clinical Practice. On-site review of eCRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each child. Note that a variety of original documents, data, and records will be considered as source documents in this trial.

The eCRF itself is not to be used as a source document under any circumstances.

Medical advisors and clinical research associates or assistants may request to witness child evaluations occurring as part of this protocol. The investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the sponsor to assure acceptable protocol execution. The study may be subject to audit by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required child records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in eCRF generation, where clinically appropriate.

6.5 Retention of Records

The investigator must arrange for retention of study records at the site. The nature of the records and the duration of the retention period must meet the requirements of the relevant regulatory authority. In addition, because this is an international study, the retention period must meet the requirements of the most stringent authority. The investigator should take measures to prevent accidental or premature destruction of these documents.

6.6 Use of Study Findings

By signing the study protocol, the investigator agrees to the use of results of the study for the purposes of national and international registration. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement. Reports covering clinical and biometric aspects of the study will be prepared by the sponsor or its representative.

6.7 Publications

The sponsor assures that the key design elements of this protocol will be posted in a publicly accessible database, such as clinicaltrials.gov. In addition, upon study completion and finalization of the study report the results of this study will be either

submitted for publication and/or posted in a publicly accessible database of clinical study results.

7 References

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8 Appendices

Appendix 1: Drugs Known to Prolong the QTc Interval

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Albuterol (Proventil®;Ventolin®)	ß2-receptor agonist/Asthma		Congenital QT Avoid
Alfuzosin (Uroxatral®)	Alpha1-blocker/Benign prostatic hyperplasia		Possible Risk of TdP
Amantadine (Symmetrel®)	Dopaminergic/Anti-viral/Anti-infective/ Parkinson's Disease		Possible Risk of TdP
Amiodarone (Cordarone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	Risk of TdP
Amiodarone (Pacerone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	Risk of TdP
Amitriptyline (Elavil®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	Conditional TdP Risk
Amphetamine (Dexedrine®)	CNS stimulant/ADHD	This includes dextroamphetamine and amphetamine / dextroamphetamine combinations.	Congenital QT Avoid
Amphetamine (Adderall®)	CNS stimulant/ADHD	This includes dextroamphetamine and amphetamine / dextroamphetamine combinations.	Congenital QT Avoid
Arsenic trioxide (Trisenox®)	Anti-cancer/Leukemia		Risk of TdP
Astemizole (Hismanal®)	Antihistamine/Allergic rhinitis	No Longer available in U.S.	Risk of TdP
Atazanavir (Reyataz®)	Protease inhibitor/HIV		Possible Risk of TdP
Atomoxetine (Strattera®)	norepinephrine reuptake inhibitor /ADHD		Congenital QT Avoid
Azithromycin (Zithromax®)	Antibiotic/bacterial infection		Risk of TdP
Bepridil (Vascor®)	Anti-anginal/heart pain	Females>Males	Risk of TdP
Chloral hydrate (Noctec®)	Sedative/sedation/ insomnia		Possible Risk of TdP
Chloroquine (Aralen®)	Anti-malarial/malaria infection		Risk of TdP
Chlorpromazine (Thorazine®)	Anti-psychotic/ Anti-emetic/schizophrenia/ nausea		Risk of TdP
Ciprofloxacin (Cipro®)	Antibiotic/bacterial infection	Drug metabolism inhibitor- Risk for drug interactions	Conditional TdP Risk

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Cisapride (Propulsid®)	GI stimulant/heartburn	No longer available in the U.S.; available in Mexico	Risk of TdP
Citalopram (Celexa®)	Anti-depressant/depression		Risk of TdP
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection		Risk of TdP
Clomipramine (Anafranil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Clozapine (Clozaril®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Cocaine (Cocaine)	Local anesthetic/	Cardiac stimulant	Congenital QT Avoid
Desipramine (Pertofrane®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	Conditional TdP Risk
Dexmethylphenidate (Focalin®)	CNS stimulant/ADHD		Congenital QT Avoid
Diphenhydramine (Benadryl®)	Antihistamine/Allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	Conditional TdP Risk
Diphenhydramine (Nytol®)	Antihistamine/Allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	Conditional TdP Risk
Disopyramide (Norpace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Dobutamine (Dobutrex®)	Catecholamine/heart failure and shock		Congenital QT Avoid
Dofetilide (Tikosyn®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Dolasetron (Anzemet®)	Anti-nausea/nausea, vomiting		Possible Risk of TdP
Domperidone (Motilium®)	Anti-nausea/nausea	Not available in the U.S.	Risk of TdP
Dopamine (Intropine®)	Inotropic agent/heart failure; hypotension; shock		Congenital QT Avoid
Doxepin (Sinequan®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Dronedarone (Multaq®)	Anti-arrhythmic/Atrial Fibrillation		Possible Risk of TdP
Droperidol (Inapsine®)	Sedative;Anti-nausea/anesthesia adjunct, nausea		Risk of TdP
Ephedrine (Broncholate®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma		Congenital QT Avoid
Ephedrine (Rynatuss®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma		Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Epinephrine (Primatene®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	Cardiac stimulant	Congenital QT Avoid
Epinephrine (Bronkaid®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	Cardiac stimulant	Congenital QT Avoid
Eribulin (Halaven®)	Anti-cancer/metastatic breast neoplasias		Possible Risk of TdP
Erythromycin (E.E.S.®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	Risk of TdP
Erythromycin (Erythrocin®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	Risk of TdP
Escitalopram (Cipralex®)	Anti-depressant/Major depression/ Anxiety disorders		Possible Risk of TdP
Escitalopram (Lexapro®)	Anti-depressant/Major depression/ Anxiety disorders		Possible Risk of TdP
Famotidine (Pepcid®)	H2-receptor antagonist/Peptic ulcer/ GERD		Possible Risk of TdP
Felbamate (Felbatrol®)	Anti-convulsant/seizure		Possible Risk of TdP
Fenfluramine (Pondimin®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Fingolimod (Gilenya®)	Immunosuppressant/Multiple Sclerosis		Possible Risk of TdP
Flecainide (Tambocor®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Fluconazole (Diflucan®)	Anti-fungal/fungal infection	Drug metabolism inhibitor- Risk for drug interactions	Conditional TdP Risk
Fluoxetine (Prozac®)	Anti-depressant/depression		Conditional TdP Risk
Fluoxetine (Sarafem®)	Anti-depressant/depression		Conditional TdP Risk
Foscarnet (Foscavir®)	Anti-viral/HIV infection		Possible Risk of TdP
Fosphenytoin (Cerebyx®)	Anti-convulsant/seizure		Possible Risk of TdP
Galantamine (Reminyl®)	Cholinesterase inhibitor/ Dementia, Alzheimer's		Conditional TdP Risk
Gatifloxacin (Tequin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Gemifloxacin (Factive®)	Antibiotic/bacterial infection		Possible Risk of TdP
Granisetron (Kytril®)	Anti-nausea/nausea and vomiting		Possible Risk of TdP
Halofantrine (Halfan®)	Anti-malarial/malaria infection	Females>Males	Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Haloperidol (Haldol®)	Anti-psychotic/schizophrenia, agitation	When given intravenously or at higher-than- recommended doses, risk of sudden death, QT prolongation and torsades increases.	Risk of TdP
Ibutilide (Corvert®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Iloperidone (Fanapt®)	Antipsychotic, atypical/Schizophrenia		Possible Risk of TdP
Imipramine (Norfranil®)	Tricyclic Antidepressant/depression	Risk of TdP in overdosage	Conditional TdP Risk
Indapamide (Lozol®)	Diuretic/stimulate urine & salt loss		Possible Risk of TdP
Isoproterenol (Isupres®)	Catecholamine/allergic reaction		Congenital QT Avoid
Isoproterenol (Medihaler-Iso®)	Catecholamine/allergic reaction		Congenital QT Avoid
Isradipine (Dynacirc®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Itraconazole (Sporanox®)	Anti-fungal/fungal infection	Drug metabolism inhibitor- Risk for drug interactions	Conditional TdP Risk
Ketoconazole (Nizoral®)	Anti-fungal/fungal infection	Drug metabolism inhibitor	Conditional TdP Risk
Lapatinib (Tykerb®)	Anti-cancer/breast cancer, metastatic		Possible Risk of TdP
Lapatinib (Tyverb®)	Anti-cancer/breast cancer, metastatic		Possible Risk of TdP
Levalbuterol (Xopenex®)	Bronchodilator/asthma		Congenital QT Avoid
Levofloxacin (Levaquin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	Not available in the U.S.	Risk of TdP
Lisdexamfetamine (Vyvanse®)	CNS stimulant/ADHD		Congenital QT Avoid
Lithium (Eskalith®)	Anti-mania/bipolar disorder		Possible Risk of TdP
Lithium (Lithobid®)	Anti-mania/bipolar disorder		Possible Risk of TdP
Mesoridazine (Serentil®)	Anti-psychotic/schizophrenia		Risk of TdP
Metaproterenol (Alupent®)	Bronchodilator/asthma		Congenital QT Avoid
Metaproterenol (Metaprel®)	Bronchodilator/asthma		Congenital QT Avoid
Methadone (Dolophine®)	Opiate agonist/pain control, narcotic dependence	Females>Males	Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Methadone (Methadose®)	Opiate agonist/pain control, narcotic dependence	Females>Males	Risk of TdP
Methylphenidate (Ritalin®)	CNS stimulant/ADHD		Congenital QT Avoid
Methylphenidate (Concerta®)	CNS stimulant/ADHD		Congenital QT Avoid
Midodrine (ProAmatine®)	Vasoconstrictor/low blood pressure, fainting		Congenital QT Avoid
Moexipril/HCTZ (Uniretic®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection		Risk of TdP
Nicardipine (Cardene®)	Anti-hypertensive/high blood pressure		Possible Risk of TdP
Nilotinib (Tasigna®)	Anti-cancer/Leukemia		Possible Risk of TdP
Norepinephrine (Levophed®)	Vasoconstrictor, Inotrope/shock, low blood pressure		Congenital QT Avoid
Nortriptyline (Pamelor®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Octreotide (Sandostatin®)	Endocrine/acromegaly, carcinoid diarrhea		Possible Risk of TdP
Ofloxacin (Floxin®)	Antibiotic/bacterial infection		Possible Risk of TdP
Ondansetron (Zofran®)	Anti-emetic/nausea and vomiting		Possible Risk of TdP
Oxytocin (Pitocin®)	Oxytocic/Labor stimulation		Possible Risk of TdP
Paliperidone (Invega®)	Antipsychotic, atypical/Schizophrenia		Possible Risk of TdP
Paroxetine (Paxil®)	Anti-depressant/depression		Conditional TdP Risk
Pentamidine (NebuPent®)	Anti-infective/pneumocystis pneumonia	Females>Males	Risk of TdP
Pentamidine (Pentam®)	Anti-infective/pneumocystis pneumonia	Females>Males	Risk of TdP
Perflutren lipid microspheres (Definity®)	Imaging contrast agent/Echocardiography		Possible Risk of TdP
Phentermine (Fastin®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Phentermine (Adipex®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Phenylephrine (Neosynephrine®)	Vasoconstrictor, decongestant/low blood pressure, allergies, sinusitis, asthma		Congenital QT Avoid
Phenylpropanolamine (Acutrim®)	Decongestant/allergies, sinusitis, asthma	No longer available in the U.S.	Congenital QT Avoid
Phenylpropanolamine (Dexatrim®)	Decongestant/allergies, sinusitis, asthma	No longer available in the U.S.	Congenital QT Avoid

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Pimozide (Orap®)	Anti-psychotic/Tourette's tics	Females>Males	Risk of TdP
Probucol (Lorelco®)	Antilipemic/Hypercholesterolemia	No longer available in U.S.	Risk of TdP
Procainamide (Pronestyl®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Procainamide (Procan®)	Anti-arrhythmic/abnormal heart rhythm		Risk of TdP
Protriptyline (Vivactil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Pseudoephedrine (PediaCare®)	Decongestant/allergies, sinusitis, asthma		Congenital QT Avoid
Pseudoephedrine (Sudafed®)	Decongestant/allergies, sinusitis, asthma		Congenital QT Avoid
Quetiapine (Seroquel®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Quinidine (Quinaglute®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Quinidine (Cardioquin®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Ranolazine (Ranexa®)	Anti-anginal/chronic angina		Possible Risk of TdP
Risperidone (Risperdal®)	Anti-psychotic/schizophrenia		Possible Risk of TdP
Ritodrine (Yutopar®)	Uterine relaxant/prevent premature labor		Congenital QT Avoid
Ritonavir (Norvir®)	Protease inhibitor/HIV		Conditional TdP Risk
Roxithromycin* (Rulide®)	Antibiotic/bacterial infection	*not available in the United States	Possible Risk of TdP
Salmeterol (Serevent®)	Sympathomimetic/asthma, COPD		Congenital QT Avoid
Sertindole (Serdolect®)	Antipsychotic, atypical/Anxiety, Schizophrenia		Possible Risk of TdP
Sertindole (Serlect®)	Antipsychotic, atypical/Anxiety, Schizophrenia		Possible Risk of TdP
Sertraline (Zoloft®)	Anti-depressant/depression		Conditional TdP Risk
Sibutramine (Meridia®)	Appetite suppressant/dieting, weight loss		Congenital QT Avoid
Solifenacin (VESIcare®)	muscarinic receptor antagonist/treatment of overactive bladder		Conditional TdP Risk
Sotalol (Betapace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	Risk of TdP
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection		Risk of TdP
Sunitinib (Sutent®)	Anti-cancer/RCC, GIST		Possible Risk of TdP
Tacrolimus (Prograf®)	Immunosuppressant/Immune suppression		Possible Risk of TdP
Tamoxifen (Nolvadex®)	Anti-cancer/breast cancer		Possible Risk of TdP

Generic Name (Brand Name)	**Drug Class / Clinical Usage**	**Comments**	**Risk List**
Telithromycin (Ketek®)	Antibiotic/bacterial infection		Possible Risk of TdP
Terbutaline (Brethine®)	Bronchodilator/asthma		Congenital QT Avoid
Terfenadine (Seldane®)	Antihistamine/Allergic rhinitis	No longer available in U.S.	Risk of TdP
Thioridazine (Mellaril®)	Anti-psychotic/schizophrenia		Risk of TdP
Tizanidine (Zanaflex®)	Muscle relaxant/		Possible Risk of TdP
Tolterodine (Detrol®)	Bladder Antispasmodic/		Congenital QT Avoid
Tolterodine (Detrol LA®)	Bladder Antispasmodic/		Congenital QT Avoid
Trazodone (Desyrel®)	Anti-depressant/Depression, insomnia		Conditional TdP Risk
Trimethoprim-Sulfa (Bactrim®)	Antibiotic/bacterial infection		Conditional TdP Risk
Trimethoprim-Sulfa (Sulfa®)	Antibiotic/bacterial infection		Conditional TdP Risk
Trimipramine (Surmontil®)	Tricyclic Antidepressant/depression		Conditional TdP Risk
Vandetanib (Caprelsa®)	Anti-cancer/Thyroid cancer		Risk of TdP
Vardenafil (Levitra®)	phosphodiesterase inhibitor/vasodilator		Possible Risk of TdP
Venlafaxine (Effexor®)	Anti-depressant/depression		Possible Risk of TdP
Voriconazole (VFend®)	Anti-fungal/anti-fungal		Possible Risk of TdP
Ziprasidone (Geodon®)	Anti-psychotic/schizophrenia		Possible Risk of TdP

Source: Arizona Center for Education and Research on Therapeutics. Link: http://www.crediblemeds.org/everyone/composite-list-all-qtdrugs/. The last revision date: 22 Sep 2013. Accessed 02 Oct 2013.

Protocol Amendment 7
Summary of Changes

Italfarmaco S.p.A

A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy

Protocol No.: DSC/11/2357/43

Rationale for Amendment

1. The amendment is planned to allow the patients on treatment in the third extension phase of the study to continue for additional 4 months until the activation of the Long Term Safety study (Study N. DSC/14/2357/51 - EUDRACT: 2017-000397-10), that will be submitted to the Ethic Committees and for which the Sponsor estimates to obtain the approval within October 2017. In this way the patients who accept to be included in the Long Term Safety study will continue the treatment with Givinostat, without any interruptions.

   The Long Term Safety Study will be submitted on April 2017 to AIFA and to the participating sites in study DSC/11/2357/43 and it will be activated as soon as the approval is available.

2. Minor editorial changes were made throughout the document for maintaining accuracy, consistency, and clarity.

Summary of Changes

The following changes were made to the protocol. Additions to the protocol are denoted in **bold**. Deletions are in strikethrough. Order of paragraphs and section numbering reflect the amended protocol.

SYNOPSIS

Design:

[…] EXTENSION 3

A third extension phase is foreseen in order to allow the patients to continue a study treatment that supports a beneficial effect of Givinostat in DMD. The children on treatment in the Extension 2 will be asked to continue in the Extension 3 and will receive Givinostat at the same ongoing dose for a maximum of an additional 12 months. During Extension 3, the dose will be adjusted based on the weight of the children.

This part of the study will be additionally extended until the activation of the Long Term Safety Study (Study N. DSC/14/2357/51 - EUDRACT: 2017-000397-10).

Treatments:

[…] Moreover, children who will be enrolled in the extension phases will receive Givinostat at the same ongoing dose (during the last visit planned at 12 months of treatment) for a maximum of an additional 36 **40** months (12 months for Extension 1, 12 months for Extension 2, and 12 **16** months for Extension 3).

Additional children may be enrolled as needed during Part 2 to reach the sample size calculated after the interim analysis. Children who enroll during Part 2 will receive up to 12 or 48 months (if they will be enrolled in the extension phases) of treatment with Givinostat at the RD. During Extensions 2 and 3, the dose will be adjusted based on the weight of the children. The total duration of the study is anticipated to be 15 months, and an additional 36 **40** months for Extension 1 (12 months), Extension 2 (12 months), and Extension 3 (12 **16** months).

Main Parameters of Efficacy:

Extension Phases:

As secondary endpoints, the change in muscular function after 24 months, 36 months, and 48 **52** months (Extension 1, Extension 2, and Extension 3, respectively) of treatment with Givinostat as assessed by 6MWT, NSAA, and PUL will be investigated. For the children that are not able to complete the 6MWT, the time to wheelchair and how much time the children spend in wheelchair were assessed.

2. Study Objectives

Secondary Efficacy Endpoints

The secondary efficacy endpoints of the extension phases are as follows:

- Change of muscular function (including loss of ambulation) after 24, 36, and 48 **52** months of treatment with Givinostat at the selected daily dose based on the 6MWT
- Change in muscular function after 24, 36, and 48 **52** months of treatment with Givinostat at the selected daily dose based on the NSAA
- Change in muscular function after 24, 36, and 48 **52** months of treatment with Givinostat at the selected daily dose based on the PUL

3. Investigational Plan

3.1 Description of Overall Study Design and Plan

[…] The additional children (if any) will be enrolled during Part 2 of the study and will receive the RD of Givinostat up to 36 **40** months.

Table 1 Treatment Table – Part 1 and Part 2

	PART 1						PART 2									
	Treatment Visit[c]						Treatment Visit[c]									
Subject	**1**	**2[a]**	**3**	**4[a]**	**5**	**6[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[b]**
1–4																
5–12																
13–20																
Additional children (if any)																

[a] At the end of Week 2 (Visit 2) at every dose level, a safety check is foreseen.
[b] At the end of Month 12, an efficacy analysis on biopsy results and functional tests is foreseen, and a decision to consider the study complete or to continue the study for up to another 36 **40** months (extension phases [Extension 1, 12 months and Extension 2, 12 months and Extension 3, 12 **16** months]) will be taken.
[c] The visits during Part 1 will be performed every 7 days (±1 day); the visits during Part 2 will be performed periodically every 1 – 1.5 months (±7 days).

Legend:

	25 – 37.5 mg BID – low dose level
	Intermediate dose level
	High dose level
	Recommended dose level

3.1.1 Extension Phases

EXTENSION 2

[…] The patient will receive Givinostat at the same ongoing dose, during the last visit planned at 12 months, and will be treated for a maximum of an additional 24 **28** months (Month 36 **40**). The dose can be reduced or increased according to the dose modification criteria, specified below (Section 3.3.5).

EXTENSION 3

In February 2015, the results were available and they have shown that the treatment with Givinostat significantly increases the amount of muscle in the biopsies and significantly reduces the amount of fibrotic tissue. Moreover treatment with Givinostat significantly reduces tissue necrosis and fatty replacement, 2 other parameters related to disease progression. Function tests have shown overall

stability, although the sample size of the study is too small to draw definitive conclusions. Finally, the drug was well tolerated.

Based on these results, a third extension phase is foreseen in order to allow the patients to continue a study treatment that supports a beneficial effect of Givinostat in DMD. The children on treatment in the Extension 2 will be asked to continue in the Extension 3 and will receive Givinostat at the same ongoing dose for a maximum of an additional 12 **16** months.

The extension 3 will be additionally extended by 4 months, until the activation of the Long Term Safety Study (Study N. DSC/14/2357/51 - EUDRACT: 2017-000397-10).

The patient will receive Givinostat at the same ongoing dose, during the last visit planned at 12 months **(at PART 2)**, and will be treated for a maximum of an additional 36 **40** months (Month 48 **52**). The dose can be reduced or increased according to the dose modification criteria, specified below (Section 3.3.5).

During the extension phases 1 and 2 of the study, children will visit the center every 2 months, for a total of 6 visits during Extension 1 (from Visit 11 to Visit 16) and a further 6 visits for Extension 2 (Visit 17 to Visit 22), and every 4 months during Extension 3 for 3 **4** visits (Visit 23 to Visit 25 **26**).

3.2.3 Removal of Children from Therapy or Assessment

[…] The children included in the extension phases will be considered to have completed the study when they complete the 12-month end-of-extension study visit (Visit 16) for Extension phase 1, the 36-month end-of-extension study visit (Visit 22) for Extension phase 2, and the 48**52**-month-end-of-extension study visit (Visit 25 **26**) for Extension phase 3. If the extension phases are stopped due to negative results of Part 2 or the child is discontinued from the study treatment, the child will be asked to attend the early termination and follow-up assessments as shown in Section 3.4.6 and Section 3.4.10, respectively.

3.3.3 Treatment Assignment

All children will receive study drug. Children enrolled in Part 1 of the study will start to take either the low, intermediate, or high dose, depending on the needs of the study at the time of enrollment and they will switch to the RD when the review team decide that this dose is safe (see Table 8).

When the safety review team has determined the RD, all children currently on study drug will be switched to that dose (the RD), and Part 2 of the study will commence. All children who enroll during Part 2 (if applicable) of the study will be given the RD of Givinostat.

Children who will be included in the extension phases will be treated at the same ongoing dose during the last visit planned at 12 months and will be treated for a maximum of an additional 36 **40** months

(Month 48 **52**). During Extension 2 and Extension 3, the dose will be adjusted based on the weight of the children.

3.3.4 Treatment Assignment

[…] For the extension phases (Extension 1 [12 months], Extension 2 [12 months], and Extension 3 [12 **16** months]), at each scheduled visit, the investigator will supply the children with the appropriate number of suspension bottles sufficient to cover the treatment for 2 months during Extensions 1 and 2 and every 4 months during Extension 3.

Table 2 Schedule of Assessments: Extension Phases

Assessment	Extension Phase 1							Extension Phase 2							Extension Phase 3				
Months	14	16	18	20	22	24/ EOS[c]	FU*	26	28	30	32	34	36/ EOS[c]	FU*	40	44	**48/** EOS	**52/ EOS[c]**	FU
Visit N. (1-week window for V11-V24; 1 month window for V25 and V26)	11	12	13	14	15	16	NA	17**	18	19	20	21	22	NA	23	24	**25**	**25 26**	NA
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**X**	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**X**	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**X**	X	X
Height						X							X				**X**	X	
Weight	X	X	X	X	X	X		X	X	X	X	X	X		X	X	**X**	X	
12-lead ECG[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**X**	X	X
ECHO[a]						X							X				**X**	X	
PFTs (FEV_1, FVC, FEV_1/FVC, PEF)[a]						X							X				**X**	X	
Clinical laboratory tests[a,b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**X**	X	X
Urinalysis[a,b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[d]	X[d]	**X[d]**	X[d]	X[d]
Quality of life test						X							X				**X**	X	
Muscle evaluations (6MWT, NSAA, PUL)		X		X		X			X		X		X		X	X	**X**	X	
MRI						X													
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**X**	X	X
Study drug administration	X	X	X	X	X			X	X	X	X	X			X	X	**X**		

AE=adverse event; 6MWT=6-Minute Walk Test; ECG=electrocardiogram; EOS=end of study; FEV_1= forced expiratory volume at 1 second; FU=follow up; FVC= forced vital capacity; MRI=magnetic resonance imaging; NA=Not applicable; NSAA=North Star Ambulatory assessment; PEF=peak expiratory flow; PFT=pulmonary function test; PUL= performance of upper limb

* To be performed only if the child does not continue the treatment in Extension 2 after Extension 1 and Extension 3 after Extension 2.

** LTBP4 and osteopontin genotype information Note: A subject may return for an unscheduled visit at the discretion of Investigator to undergo additional safety

evaluations (i.e., laboratory re-testing, AE assessments), or for additional medical evaluations. Platelet count may be assessed at an unscheduled visit if deemed necessary by the Investigator.

[a] To be performed more frequently, if clinically indicated.

[b] The following laboratory parameters will be assessed: Hematology: RBC, hemoglobin, hematocrit, WBC with differential, platelets, ANC, and abnormal cells; Blood chemistry: total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, CRP, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CrCl (CrCl will be calculated by the Cockcroft and Gault formula). Urinalysis: pH, specific gravity, protein, glucose, ketones, cytology, and myoglobin.

[c] If the child is discontinued from the study treatment, the child will be asked to return for the early termination procedures. For the assessments to be performed, see "EOS visit". Discontinued children will also have follow-up procedures performed within 4 weeks of the last dose of study drug.

[d] Urinalysis: pH, specific gravity, protein, glucose, ketones and cytology.

3.4.9 Extension Phase 3 Visits (Months 40 to 44 48)

Extension phase 3 will start at the EOS visit (Visit 22 [Month 36]). As noted in the schedule of assessments during Extension 3, for Months 40 to 48 **52**, children will visit the study center at Visits 23 to 25 **26** (i.e., every 4 months). **The time window for Visits 23 and 24 is ± 1 week; the time window for Visits 25 and 26 is ± 1 month.**

At each visit (unless otherwise indicated), the following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure weight.
- **Measure height (Visit 25 [Month 48] only)**
- Obtain a 12-lead ECG in triplicate.
- Obtain blood sample for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MTW, NSAA, and PUL) at Visit 40 and Visit 44 only
- Dispense IMP and perform accountability.
- Assess AEs.

3.4.10 Assessments for End of Extension Phase 3 (Visit 25 26 [Month 48 52])

For children who continue in Extension 3, the end of the extension phase visit will be performed on Month 48 **52** (Visit 25 **26**) **± 1 month**.
The following assessments will be performed and recorded:

- Obtain and record concomitant medications.
- Perform a physical examination.
- Measure vital signs: blood pressure, pulse rate, body temperature.
- Measure height and weight.
- Obtain a 12-lead ECG in triplicate.
- Obtain an ECHO.
- Obtain PFT measurements: FEV_1, FVC, FEV_1/FVC, and PEF.
- Obtain blood samples for hematology and blood chemistry.
- Obtain a urine sample for urinalysis.
- Obtain muscle evaluations (6MWT, NSAA, and PUL).
- Perform accountability.

- Perform a quality of life test.
- Assess AEs.

3.4.11 Early Termination Visit

Children who discontinue participation prior to completion of all study drug administration (i.e., 12 months of treatment or 36 months **52 months** of treatment if the children continue in the extension phases) will be asked to return to the hospital within 2 weeks after the last dose of study drug for completion of the same assessments given at the EOS visit (for details, see Section 3.4.4 or Section 3.4.6, if applicable).

3.4.13 Description of Assessments

[…]

Safety Assessments

Adverse Events: An AE is any **is** "any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (International Conference on Harmonisation [ICH] E2A)."

Quality of Life Assessment

The PedsQL Test will be performed before treatment start, at Visit 10 (12 months), and at the end of the extension phases at 24, 36 **and 52** months for Extension 1, **2 and 3** and Extension 2, respectively, if applicable.

5.2.5 Secondary Efficacy Endpoints

The secondary efficacy endpoints are the following:

[…]

Change in muscular function after 12 months of treatment with Givinostat at the selected daily dose based **on** the PUL.

The secondary efficacy endpoints of the extension phases are the change of muscular function (including loss of ambulation) after 24, 36, and 48 **52** months of treatment (Extension 1, Extension 2, and Extension 3, respectively) with Givinostat as assessed by 6MWT, NSAA, and PUL. For the children who are not able to complete the 6MWT, the time to wheelchair and how much time the children spend in wheelchair were assessed.

5.2.13. Analysis at Month 12

At 12 months, efficacy and safety analysis will be conducted, on the primary and secondary endpoints. If results show a positive effect on patients, and no safety concern is raised, then patients will continue study treatment for up to an additional 36 **40** months (Extension 1 [12 months], Extension 2 [12 months], and Extension 3 [12 **16** months]) to evaluate the safety and tolerability of long-term administration of Givinostat and to evaluate the effect of treatment on muscle function. If, on the other hand, the final analysis of the results of the first 12 months of treatment does not support a beneficial effect of Givinostat in DMD, the study will be considered complete.