ITALFARMACO

FINAL CLINICAL STUDY PROTOCOL

Protocol Number: DSC/15/2357/53
Protocol Title: A randomised, double-blind, placebo-controlled study to evaluate the micro-macroscopic effects on muscles, the safety and tolerability, and the efficacy of givinostat in patients with Becker Muscular Dystrophy

IND Number:	NA
EudraCT Number:	2017-001629-41
Name of Product:	Givinostat
Phase of Development:	2
Indication:	Becker Muscular Dystrophy (BMD)
Sponsor:	Paolo Bettica, MD, PhD Director of Clinical Research and Development ITALFARMACO S.p.A. Via dei lavoratori 54 20092 Cinisello Balsamo, Italy Tel: + 39 02[redacted] Fax: + 39 02 644346
Amended Protocol Version:	5.0
Protocol Date:	20 June 2017
Amendment No. 1 Date:	24 January 2018
Amendment No. 2 Date:	31 July 2018
Amendment No. 3 Date:	13 December 2019
Amendment No. 4 Date:	17 June 2020

-CONFIDENTIAL-

SPONSOR APPROVAL PAGE

_____ Date: _____

Paolo Bettica, MD, PhD
Director of Clinical Research and Development

INVESTIGATOR PROTOCOL AGREEMENT PAGE

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by Italfarmaco S.p.A.
- Not to implement any changes to the protocol without written agreement from Italfarmaco S.p.A and prior review and written approval from the Institutional Review Board/Independent Ethics Committee, except where necessary to eliminate an immediate hazard to subjects.
- That I am thoroughly familiar with the appropriate use of the study drug, as described in this protocol and any other information provided by Italfarmaco S.p.A including, but not limited to, the Investigator's Brochure (IB).
- That I am aware of, and will comply with, GCP and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the Italfarmaco S.p.A study drug and of their study-related duties and functions as described in the protocol.

Signature: _____ Date: _____

Name (print): _____
Principal Investigator

Site Number: _____

1 SYNOPSIS

Title of Study:	A randomised, double-blind, placebo-controlled study to evaluate the micro-macroscopic effects on muscles, the safety and tolerability, and the efficacy of givinostat in patients with Becker Muscular Dystrophy
Protocol Number:	DSC/15/2357/53
Study Design:	This is a phase 2, randomised, double-blind, placebo-controlled study. Approximately 48 eligible patients will be randomized in a 2:1 ratio to be treated with givinostat or placebo for a period of 12 months.
Investigators/Study Sites:	The study will be conducted at 1-2 investigational site(s).
Phase of Development:	Phase 2
Objectives:	The primary objective is to establish the histological effects of givinostat versus placebo administered over 12 months. The secondary objectives of this study are the following: • To establish the macroscopic muscle effects of givinostat versus placebo administered chronically over 12 months assessed by MRI/MRS. • To establish the other histological effects of givinostat versus placebo administered over 12 months. • To establish the efficacy of givinostat versus placebo administered chronically over 12 months in slowing disease progression. • To assess the safety and tolerability of givinostat versus placebo administered chronically. • To evaluate the pharmacokinetic (PK) profile of givinostat administered chronically in the target population. • To evaluate the impact of givinostat versus placebo administered chronically on quality of life and activities of daily living. The exploratory objectives are the following: • To evaluate the correlation between the PK profile of givinostat and pharmacodynamics (PD) data. • To evaluate a possible disease-related biomarker. • To explore additional disease-related MRI biomarkers.
Study Population:	The study will enroll adult male patients with an established molecular diagnosis of Becker Muscular Dystrophy (BMD).
Selection of Subjects:	Patients must meet all of the following criteria in order to be included in the study: 1. Ambulant male patients aged ≥18 years to ≤ 65 years at

	randomization with BMD diagnosis confirmed by genetic testing. 2. Able and willing to give informed consent in writing. 3. Able to perform 6MWT at screening with a minimum distance of 200 m and maximum distance of 450 m. 4. If in treatment with systemic corticosteroids and/or ACE inhibitor, and/or β or α adrenergic receptor blocker, no significant change in dosage or dosing regimen (excluding changes related to body weight change) for a minimum of 6 months immediately prior to start of study treatment. 5. Patients must be willing to use adequate contraception. Contraceptive methods must be used from Randomization through 3 months after the last dose of study treatment. Patients meeting any of the following criteria are ineligible to participate in this study: 1. Exposure to another investigational drug within 3 months prior to the start of study treatment. 2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength or function within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and any other supplements will be allowed. 3. Surgery that might affect muscle strength or function within 3 months before study entry or planned surgery at any time during the study. 4. Presence of other clinically significant disease that in the Investigator's opinion could adversely affect the safety of the patient, making it unlikely that the course of treatment or follow-up is completed, or could impair the assessment of study results. 5. A diagnosis of other uncontrolled neurological diseases or presence of relevant somatic disorders not related to BMD that may interfere with the ability to perform the muscle function tests and/or to comply with the study protocol procedures. 6. Platelet count, WBC count and hemoglobin at screening < Lower Limit of Normal (LLN). If laboratory screening results are < LLN, platelet count, WBC count and hemoglobin are to be repeated once, and if again < LLN become exclusionary. 7. Symptomatic cardiomyopathy or heart failure (New York Heart Association Class III or IV) or left ventricular ejection fraction < 50% at screening or with heart transplant. 8. Current liver disease or impairment, including but not limited to elevated total bilirubin (> 1.5 x ULN), unless secondary to Gilbert's disease or pattern consistent with Gilbert's disease. 9. Inadequate renal function, as defined by serum Cystatin C > 2 x the upper limit of normal (ULN). If the value is > 2 x ULN,

<table>
<tr><td></td><td>serum Cystatin C will be repeated once, and if again > 2 x ULN becomes exclusionary.
10. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening.
11. Baseline corrected QT interval, Fridericia's correction (QTcF) > 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, or family history of long QT syndrome).
12. Current psychiatric illness/social situations rendering the potential patient unable to understand and comply with the muscle function tests and/or with the study protocol procedures.
13. Hypersensitivity to the components of study medication.
14. Sorbitol intolerance or sorbitol malabsorption, or the hereditary form of fructose intolerance.
15. Contraindications to muscle biopsy.
16. Contraindications to MRI/MRS (e.g., claustrophobia, metal implants, or seizure disorder).
17. Hypertriglyceridemia (> 1.5 x upper limit of normal [ULN])*

* At screening, patients with hypertriglyceridemia can be enrolled if in stable treatment and with controlled levels of triglycerides (i.e. within normal range) for at least six months.</td></tr>
<tr><td>Planned Sample Size:</td><td>Approximately 48 patients are to be randomized.</td></tr>
<tr><td>Stratification:</td><td>The randomization process will be stratified by the concomitant steroids use at baseline (yes vs. no).</td></tr>
<tr><td>Randomization ratio:</td><td>The givinostat or placebo randomization ratio is 2:1.</td></tr>
<tr><td>Study duration for participants:</td><td>The study is made up of a 4-week screening period, a 12-month treatment period and a 4-week follow-up period. The total study period for each patient is therefore of 14 months.</td></tr>
<tr><td>Reference Therapy:</td><td>Givinostat or placebo oral suspension (10 mg/mL) twice daily (bid) (in a fed state) as described below:
<table>
<tr><th>Weight (kg)</th><th>≥30 and <40</th><th>≥40 and <50</th><th>≥50 and <60</th><th>≥60 and < 70</th><th>≥70</th></tr>
<tr><td>Dose (mg) bid</td><td>26.7</td><td>33.3</td><td>36.7</td><td>40</td><td>46.7</td></tr>
<tr><td>Oral suspension (mL) bid</td><td>2.7</td><td>3.3</td><td>3.7</td><td>4.0</td><td>4.7</td></tr>
</table></td></tr>
<tr><td>Criteria for Evaluation:</td><td>The primary efficacy assessment is Total fibrosis (%) assessed through histological examination of muscle biopsies.</td></tr>
</table>

	The secondary efficacy assessments are the following:
	• Fat fraction of vastus lateralis and soleus evaluated by MRS technique
	• Fat fraction of pelvic girdle and lower limb muscles evaluated by Dixon MRI technique
	• CSA of pelvic girdle and lower limb muscles by means of Dixon MRI technique
	• Other biopsy histological parameters (Cross-Sectional Area (CSA), Muscle Fiber Area Fraction [MFAF], regenerative fibers)
	• Motor Function Measurement (MFM)
	• Time Function Tests (TFT)
	  ○ Time to rise from floor
	  ○ Run/walk 10 m
	  ○ Time to climb 4 standard steps
	• Six Minute Walk Test (6MWT)
	• Muscle strength evaluated by knee extension, elbow flexion as measured by Hand-Held Myometry (HHM)
	• Quality of life (assessed by SF36)
	
	Safety and tolerability will be evaluated by monitoring hematology and blood chemistry, coagulation, urinalysis, vital signs, physical examinations, weight, height, echocardiogram, ECG and respiratory function evaluation.
	
	A blood sample will be collected during the study for LTBP4 and Osteopontin genotyping. Patients who have already concluded the study may be asked to return to the center for a blood sample collection
	
	Blood sample collection for serum circulating proteins (by an ELISA based system) at randomization, 6 months and at the end of treatment.
	
	Blood samples will be obtained for PK analysis of givinostat and its metabolites.

Study Endpoints:	Primary endpoint: Mean change in total fibrosis (%) comparing the histology of muscle biopsies before and after 12 months of treatment with givinostat versus placebo. Secondary endpoints: • Mean change in fat fraction of vastus lateralis and soleus comparing Magnetic Resonance Spectroscopy (MRS) before and after 12 months of treatment with givinostat versus placebo. • Mean change in fat fraction of pelvic girdle and lower limb muscles comparing Magnetic Resonance Imaging (MRI) before and after 12 months of treatment with givinostat versus

	placebo. • Mean CSA of pelvic girdle and lower limb muscles comparing MRI before and after 12 months of treatment with givinostat versus placebo. • Mean change in other histology parameters (e.g. Cross-Sectional Area (CSA), MFAF, regenerative fibers) comparing the histology biopsies before and after 12 months of treatment with givinostat. • Mean change in Motor Function Measurement (MFM) before and after 12 months of treatment with givinostat versus placebo. • Mean change in Time Function Tests (time to climb four standard steps, time to rise from floor and time to walk/run 10 m) before and after 12 months of treatment with givinostat versus placebo • Mean change in 6 Minute Walking Test (6MWT) before and after 12 months of treatment with givinostat versus placebo. • Proportion of patients with < 10% worsening in 6MWT at the end of study. • Proportion of patients who lose the ability to rise from floor (Baseline through end of study). • Proportion of patients who lose ambulation during the study. • Mean change in muscle strength evaluated by knee extension, elbow flexion as measured by Hand Held Myometry (HHM), before and after 12 months of treatment with givinostat versus placebo. • Mean changes in quality of life (assessed by the 36-item Short Form survey [SF36]) before and after 12 months of treatment with givinostat as compared to placebo. Safety endpoints: • Number of patients experiencing treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) from Baseline through end of study (EOS). • Type, incidence, and severity of TEAEs and SAEs (Baseline through EOS). • Changes from baseline to end of study of: o Vital signs and clinical laboratory tests (blood chemistry and hematology). o Physical examination. o Pulmonary function evaluated by Forced Vital Capacity (FVC), Forced Expiratory Volume at 1 second (FEV1), FVC/FEV1, Peak Expiratory Flow (PEF). o Cardiac function evaluated by ECG and ECHO. o Weight, height, and body mass index (BMI).

	Pharmacokinetic endpoints • Description of the PK of givinostat and its major metabolites: ITF2374 and ITF2375. Exploratory endpoints: • PK-PD analyses of the relationship between metrics of exposure and the efficacy/safety endpoints of givinostat. • Analyses to explore whether the effects of givinostat versus placebo administered chronically may be related to the type of LTBP4 or osteopontin genotype. • Evaluation of serum circulating proteins, by an ELISA based system, as potential biomarkers for BMD. • Additional evaluations of other muscle image parameters assessed by MRI (details will be provided in a specific imaging protocol).
Rules for cessation of randomised treatment in an individual participant:	The Study treatment should be permanently discontinued if any of the following events occur: • Severe drug-related diarrhea (increase of $\geq$ 7 stools per day) • Any drug-related SAE • QTcF > 500 msec • Platelet count $\leq 50 \times 10^9$/L (see protocol for details) • White blood cell count $\leq 2.0 \times 10^9$/L (see protocol for details) • Hemoglobin $\leq$ 8.0 g/dL (see protocol for details)
Rules for temporary interruption of randomised treatment in an individual participant and dose modification	The Study treatment should be temporarily interrupted if any of the following events occur, see protocol for details: • Moderate or severe diarrhea (increase more than 4 stools/day). • Platelet count $< 75 \times 10^9$/L but $> 50 \times 10^9$/L. • White blood cell $< 3.0 \times 10^9$/L but $> 2.0 \times 10^9$/L. • Hemoglobin < 10.0 g/dL but > 8.0 g/dL • Triglycerides >300 mg/dL* Study treatment can be resumed at a level 20% smaller than the dose at which an AE leading to a temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin and/or triglycerides are normalized or when diarrhea is mild. *Treatment for high triglycerides can be prescribed by the investigator if values are above ULN.
Sample size calculation:	The primary hypothesis to be tested in this study is that total fibrosis (%) will increase less in the givinostat group than in the placebo group. The primary endpoint of the study is the mean change from baseline to 12 months in total fibrosis (assuming normal distribution).

	A sample size of 48 patients with evaluable baseline biopsies (in a 2:1 ratio, 32 and 16 respectively) will provide 80% power to test the null hypothesis of no treatment effect (givinostat – placebo) on total fibrosis vs the alternative hypothesis that the treatment effect is $\geq$ 9% using a two-sided t-test with alpha level of 5% and assuming a common SD of 10% (with the SD being based upon blinded interim data from first 20 patients with valid biopsies). Allowing for an approximate 5% of patients with not evaluable biopsies, the total number of patients to be randomized is 51 (i.e. 34 in givinostat arm and 17 in placebo arm).
Interim analysis:	No formal interim efficacy analysis is planned. After the first 20 baseline biopsies were collected, a descriptive blinded interim analysis was performed aimed at checking the real variability of the primary endpoint under observation in the previous protocol study. The histopathological features observed in this blinded interim analysis showed that the mean CSA of the biceps fibers in BMD (mean: 4871.68 μm^2; min 1933.49 μm^2; and max 9446.34 μm^2; and S.D.: 1904.86 μm^2) is similar to the age-matched CSA in male adult healthy individuals, although with a larger degree of fiber size variability due to a substantial number of both hypertrophic and hypotrophic fibers. As a consequence, the likelihood that givinostat can increase the CSA of fibers further is low and also it is doubtful that a further increase in fiber CSA would be beneficial. The histopathological features observed in these preliminary biopsies showed also significant fibro-adipose replacement, which can be considered the hallmark of the disease. The change of total fibrosis is considered a more indicative outcome measure of the possible effect of givinostat relative to the assessment of CSA and, hence, it is to be evaluated as the primary endpoint for the trial. After study enrollment is completed and baseline data are collected for all subjects, a second blinded interim analysis will be performed to obtain a preliminary overview of the baseline patient characteristics.
Statistical Methods and Planned Analyses:	**Analysis of Primary Efficacy Endpoint** The efficacy analysis will be performed on the intent to treat analysis set as primary analysis and on the per protocol set as supportive. The primary efficacy variable, the absolute change in mean total fibrosis from baseline to endpoint after 12 months of therapy, as assessed by biopsy, will be analyzed on the original scale by means of an analysis of covariance (ANCOVA) model with baseline total fibrosis value as covariate and treatment and concomitant steroid use at baseline as independent class variables. Mean estimates will be

	provided together with their corresponding two-sided 95% confidence intervals. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. The analysis for the primary variables described above will be performed using multiple imputations to account for missing values. **Analysis of Secondary Efficacy Endpoints** All secondary efficacy analyses will be performed based on the ITT and will be reported by planned treatment group. The following endpoints will be analyzed using an ANCOVA model similar to the one described for the primary endpoint: • Change in fat fraction of vastus lateralis and soleus after 12 months of therapy • Change in fat fraction of pelvic girdle and lower limb muscles after 12 months of therapy • Change in Cross Sectional Area (CSA) of pelvic girdle and lower limb muscles after 12 months of therapy • Change in other histology parameters (e.g. CSA, MFAF, , regenerative fibers) after 12 months of therapy • Change in MFM total score after 12 months of therapy • Change in Timed Function Tests (TFTs) (i.e. time to climb four standard steps, time to rise from floor and time to walk 10m) after 12 months of therapy • Change in 6MWT score after 12 months of therapy • Change in muscle strength evaluated by knee extension and elbow flexion after 12 months of therapy The following endpoints will also be analyzed: • The proportion of patients with <10% worsening in 6MWT score after 12 months of therapy, the proportion of patients who lose the ability to rise from floor during the study, and the proportion of patients who lose ambulation during the study will be compared between arms using a stratified Cochran Mantel-Haenszel (CMH) chi square test with a two-sided α=0.05 level. As for the primary endpoint, the stratification factor is concomitant steroid use at baseline. The proportion, along with its exact two-sided 95% CI, will be computed within each treatment group. A two-sided 95% CI for difference of proportion between the treatment groups will also be computed. • Change in quality of life after 12 months of therapy

	Summary descriptive statistics will be provided at each time point for the quality of life scores/domains as assessed by the SF-36 questionnaire.
Protocol Date: **Amendment No. 1 Date:** **Amendment No. 2 Date** **Amendment No 3 Date** **Amendment No 4 Date**	20 June 2017 24 January 2018 31 July 2018 Final 13 December 2019 12 May 2020

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IN-TEXT TABLES

IN-TEXT FIGURES

3 LIST OF ABBREVIATIONS

Abbreviation	Definition
4SC	4-Stairs Climb
6MWT	Six-Minute Walking Test
ADR	Adverse Drug Reaction
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
bid	twice daily
BMD	Becker Muscular Dystrophy
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CK	Creatinine Phosphokinase
CMH	Cochran Mantel-Haenszel
CMV	Cytomegalovirus
CRA	Clinical Research Associate
CRP	C-Reactive Protein
CSA	Cross Sectional Area
CSR	Clinical Study Report
DAPC	Dystrophin-Associated Protein Complex
DCM	Dilated Cardiomyopathy
DMD	Duchenne Muscular Dystrophy
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
EOS	End Of Study

FEV1	Forced Expiratory Volume at 1 second
FVC	Forced Vital Capacity
Ft3	Free T3
FUV	Follow-Up Visit
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HDAC	Histone Deacetylase
HDACi	Histone Deacetylase Inhibitors
HDL	High-Density Lipoprotein
HHM	Hand-Held Myometry
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL-1β	Interleukin-1 beta
ITT	Intention-to-Treat
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LDH	Lactate Dehydrogenase
LDL	Low-Density Lipoprotein
IFN-γ	Interferon gamma
IL-6	Interleukin-12
IL-12	Interleukin-6
LLN	Lower Limit Normal
LOCF	Last Observation Carried Forward
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Competent Activities
MFAF	Muscle Fibers Area Fraction
MFF	Muscle Fat Fraction
MFM	Motor Function Measurement
MRI	Magnetic Resonance Imaging

MRS	Magnetic Resonance Spectroscopy
NO	Nitric Oxide
PEF	Peak Expiratory Flow
PI	Principal Investigator
PK	Pharmacokinetic
PPS	Per Protocol Set
PT	Prothrombin Time
PT	Preferred Term
QT	QT interval
QTc	Corrected QT interval
QTcF	QT interval, Fridericia's correction
ROM	Range of Motion
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SF36	Short Form 36
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TFT	Timed Function Test
TNF-α	Tumor Necrosis Factor-alpha
ULN	Upper Limit Normal
WBC	White Blood Cell
WHO-DRL	WHO-Drug Reference List

AMENDMENT No. 1

The main purposes of this protocol amendment are:

1. To specify that the fat fraction and cross section evaluations by means of Dixon MRI will involve muscles of the lower leg, as well as those of the thigh and pelvic girdle as originally planned.
2. To modify the age limit stated in Inclusion Criterion 1. Age has been raised from 60 to 65 years after Investigators advised of the presence of potential patients over the age of 60 who otherwise meet all eligibility criteria.
3. To delete the repetition of the functional tests (i.e. time to climb 4 standard steps, time to rise from floor, time to walk 10 meters, motor function measure and muscle strength) at the randomization visit, since noteworthy changes are not expected in these patients within 1 month of screening. Only 6MWT will be performed and the average of the scores at screening and randomization will be used as baseline value.
4. To correct the information related to the evaluation of serum circulating proteins as potential biomarkers for BMD. These analyses will not be carried out through SomaScan® technique because the central laboratory originally chosen for this study no longer provides this service. Biomarker evaluation will be performed by a different provider using an ELISA-based system.
5. To increase the frequency of thyroid function monitoring to ensure patient safety. Monitoring will now be carried out monthly until the third month and then every 3 months until the end of the study.
6. To include information on the backup system for randomization and treatment assignment. The Interactive Voice Response System (IVRS) will be used in the unlikely case of Web Response System (IWRS) unavailability.
7. To correct the information on the urine analysis, which will be done by dipstick on site and not by the central lab.
8. To include minor changes to increase clarity and correct typographic errors.

The changes in the protocol affect Informed Consent and therefore a revised Informed Consent that considers the changes described in this amendment will be provided and submitted for approval.

AMENDMENT No. 2

1. The main purpose of Amendment 2 is to address safety issue, namely thrombocytopenia arising following the treatment of the first 21 patients enrolled in the present study.

Thrombocytopenia is a well-documented side effect of treatment with givinostat and mitigation measures consisting of treatment interruption and/or dose reduction were fully incorporated into the original protocol. As stated in Sections 7.3.1 and 8.2.2, patients with a platelet count $< 75 \times 10^9$/L but $> 50 \times 10^9$/L were to temporarily interrupt treatment and then resume it at a dose level 1/3 smaller than the original dose (26.7-46.7 mg b.i.d according to body weight instead of 40-70 mg b.i.d) once platelets and/or other laboratory parameters were normalized. In addition, in case of two consecutive platelet counts $\leq 150 \times 10^9$/L patients were to reduce dosage as indicated above.

During study conduct, the Sponsor noticed a high incidence of patients requiring dose reduction due to thrombocytopenia (11/21 patients, with three requiring temporary interruption). Since the study is still blinded, the Sponsor does not know the exact number of patients who are on givinostat. However, as treatment assignment is randomized (ratio 2:1), the Sponsor can assume that 2/3 of the patients are taking givinostat, therefore 14/21 should be in the givinostat group and it is estimated that approximately 80% of patients in the givinostat group (11/14) required a dose reduction. In addition, 3 patients experienced a platelet count decrease and are at risk of dose reduction, which would bring the percentage of patients in the givinostat group who required or will likely require a dose reduction to 100% (14/14).

These preliminary blinded results therefore suggest that the starting dose of the current protocol would be difficult to manage outside of a clinical trial environment, and since the current dose reduction rule is adequate in keeping an acceptable level of platelets, a new starting dose corresponding to the reduced dose of the original protocol (i.e. 26.7-46.7 mg

b.i.d according to body weight) is now proposed by the Sponsor and Investigator. This new starting dose ensures a mean AUC_{0-24} similar to that achieved with 25 mg/bid based on the data from study DSC/11/2357/43 in DMD patients (i.e., approximately 674 ng*h/mL), which showed an acceptable safety profile along with demonstration of significant benefits according to histological examination (see more details in section 8.2.1).

In addition, new safety rules will be applied, allowing the study drug to be reduced by 20% from the new starting dose if the patient meets stopping criteria (see section 7.3.1).

2. A significant increase in plasma triglycerides has so far been reported in four patients under treatment although for two of them the increased levels were already present at baseline. Unlike thrombocytopenia, givinostat-related hypertriglyceridemia has not been reported in previous studies leading to believe that such condition may be a specific feature of patients with Becker muscular dystrophy. The relationship between hypertriglyceridemia, Becker muscular dystrophy and treatment with givinostat is currently under study. In the meantime, and to safeguard patient safety, a new exclusion criterion has been added to the protocol excluding patients with hypertriglyceridemia, i.e. exclusion criterion n. 17 "Hypertriglyceridemia (> 1.5 x upper limit of normal [ULN]). Moreover, for patients who develop hypertriglyceridemia during the study treatment, a safety rule was added (e.g. the Study treatment should be temporarily interrupted if Triglycerides >300 mg/dL; see section 7.3.1), and the Investigator will be allowed to prescribe any available treatment for this condition (see section 8.8).

Furthermore, to provide the highest degree of safety, the study protocol has been amended to intensify patient monitoring as follows:

3. Additional unscheduled visits with a cardiologist may be required at the discretion of study clinicians if patients present clinical or instrumental suspicion of cardiac abnormalities such as signs and symptoms of heart failure or new alterations on routine ECG (see Table 5 and section 12).

4. Additional safety assessments (blood tests) may be required at the discretion of study clinicians. To minimize inconvenience to patients, these assessments will be

performed at the patient's home by a local qualified nurse trained in the study protocol (see Table 5 and section 12).

Other minor changes have been made to the protocol as follows:

5. Patients will be given a diary to assess compliance with study treatments (see additional rows in the Table 5: "Patient diary dispensation" at visit 3 and "Patient compliance through diary" at visit 8, 9, 10 and 11 and the related visits description in section 9.1).
6. At the discretion of the Investigator, patients not meeting inclusion/exclusion criteria may be re-screened twice with an interval of at least 30 days between assessments (see section 7).
7. Eligibility criteria will be evaluated at screening only (and not also at the randomization visit, see visit description at section 9.1.2 "Review of eligibility criteria using the assessments done at screening").
8. Clarification of two exclusion criteria, i.e. exclusion criteria n. 5 "A diagnosis of other uncontrolled neurological diseases or presence of relevant somatic disorders not related to BMD that may interfere with the ability to perform the muscle function tests and/or to comply with the study protocol procedures" and n. 12 "Current psychiatric illness/social situations rendering the potential patient unable to understand and comply with the muscle function tests and/or with the study protocol procedures" .
9. In the footnote n.3 of the Table 5 and section 12.3 was specified the level of QTcF for which the site has to performed the 12-lead ECG in triplicate (e.g. 12-lead ECG is to be done in triplicate at the screening visit and during the other visits if an ECG demonstrates a prolonged QTcF interval > 450 msec obtained on 2 more ECGs over a brief period. If the averaged QTcF value meets the stopping criteria (>500 ms), the patient has to interrupt the study treatment).
10. A description of the muscle biopsy randomization procedure used to allocate the patient to one of the 2 muscle biopsy sequences by the system: V2: Right Arm - V11: Left Arm or V2: Left Arm - V11: Right Arm, was added (see section 9.1).

11. Changes have been made to increase clarity and correct typographic errors (e.g. fat fraction assessment of the soleus muscle by MRS see section 5.2.2, 10.2 and 13.6.2).

The changes in the protocol will be reflected in an amended Informed Consent that will be submitted for approval. Changes to the protocol and corresponding paragraphs of the synopsis are shown in the track changes version of the protocol using strike through red font for deletions and red underlined for additions.

AMENDMENT No. 3

The main purposes of amendment 3 are as follows:

1. The protocol has been integrated with pertinent information added to the latest version of the Investigator's Brochure (Version 20.0).

   Section 4.2.2 – *Clinical Experience with Givinostat Including Risks and Benefits* – has been updated with new information concerning hemorrhagic drug-related AEs.

   Section 8.7.2 – *Prior and concomitant medications* – now includes P-glycoproteins (P-gp) as medications whose use requires caution. The study drug is a P-glycoprotein and breast cancer resistance protein (BCRP) substrate and therefore co-administration of P-gp inhibitors may result in increased plasma concentrations of givinostat. Increased oral absorption is usually of limited clinical concern except for drugs that have a narrow therapeutic index, which is not the case of givinostat. Nevertheless, P-gp inhibitors should be properly managed in clinical studies and a new appendix (Appendix 16.3) listing P-gp inhibitors has been added to the protocol.

2. Section 9.1.10 – *End of Study Visit* – and Table 5 – *Schedule of Assessments* – now indicate that MRI/MRS and biopsy evaluations scheduled for the end of study visit may be performed on different days and that treatment is to continue up to the last assessment.

3. Section 13.10 - *Interim Analyses* – besides the interim analysis foreseen to check the sample size assumption, it was decided to plan an additional interim analysis to obtain a preliminary overview of the baseline patient characteristics after study enrollment is completed.

4. The *Interim analysis* section of the synopsis has been updated according to the protocol changes described in item 3.

AMENDMENT No. 4

1. The main purposes of amendment 4 is to amend the primary endpoint.

   The choice of the primary endpoint was based on the histological findings collected after 1 year of treatment with givinostat in the phase II clinical trial (DSC/11/2357/43) in DMD (*Bettica et al. 2016*). In that study givinostat was able to counteract the pathogenesis of DMD by increasing fiber CSA and Muscle Fiber Area Fraction (MFAF%) and by reducing necrosis, fibrosis and fatty replacement. In the absence of histomorphometrical data of muscle biopsies in BMD and since fibro-adipogenic muscle replacement is considered less prominent in BMD versus DMD, the Sponsor considered CSA the parameter that could be better translated from DMD to BMD to evaluate the histological effects of givinostat. The histopathological features observed in the analysis done on the preliminary 20 baseline biopsies showed that the mean CSA of the biceps fibers in BMD (mean: 4871.68 μm^2; min 1933.49 μm^2 ;and max 9446.34 μm^2; and S.D.: 1904.86 μm^2) is similar to the age-matched CSA in male adult healthy individuals (i.e. fiber type 1 mean 4524.62 ±1630.43 μm^2, fiber type 2a 5255.93 ± 2245.14 μm^2 and fiber type 2b mean 4077.81 ±1639 μm^2) (*Pereira et al. 2014*), although with a larger degree of fiber size variability due to a substantial number of both hypertrophic and hypotrophic fibers. As a consequence, the likelihood that givinostat can increase the CSA of fibers further is low and also it is doubtful that a further increase in fiber CSA would be beneficial. The histopathological features observed in these preliminary biopsies confirmed a significant fibro-adipose replacement. Considering that the histological results after 12 months of treatment with givinostat in DMD children showed that givinostat significantly reduced the amount of fibrotic and fat replacement (*Bettica et al. 2016*), and that the fibro-adipose replacement can be considered the hallmark of the disease, the change of total fibrosis is considered a more indicative outcome measure of the possible effect of givinostat relative to the assessment of CSA and, hence, it is to be evaluated as the primary endpoint for the trial.

2. Sections "Sample size determination" (13.1) and "Statistical Analysis" (13) were revised according to the change of the primary endpoint described above. A sample size of 48 patients with evaluable baseline biopsies (in a 2:1 ratio, 32 and 16 respectively) will provide 80% power to test the null hypothesis of no treatment effect (givinostat – placebo) on total fibrosis vs the alternative hypothesis that the treatment effect is $\geq$ 9% using a two-sided t-test with alpha level of 5% and assuming a common SD of 10% (with the SD being based upon blinded interim data from first 20 patients with valid pre and post-treatment biopsies). With a reasonable allowance of 5% of patients with unevaluable biopsies at the end of study, the total number of patients to be randomized is 51.

   Moreover, in the context of the new primary endpoint (it is expected that total fibrosis (%) will increase less in the givinostat group than in the placebo group) a *LOCF* analysis is considered not appropriate because any missing follow-up value will be replaced by that subject's previously observed value (i.e. the baseline value) and this approach can be questionable and not conservative, for this reason a multiple imputation method will be used to handle missing data.

3. Sections "Study Design" (6.1 and 6.2) and "Interim Analysis" (13) were updated including a description of conclusions on the first blinded interim analysis performed on the first 20 baseline biopsies which led the protocol amendment n. 4. as described above. The details about methodology and the results are reported in the specific SAP and Statistical Report available as stand-alone documents.

4. Section "Biomarker" (11.3) was modified to indicate that patients who have already concluded the study may be asked to return to the center for a blood sample collection necessary for LTBP4 and Osteopontin genotyping if the sample was not already collected.

4 INTRODUCTION

4.1 Background on Becker Muscular Dystrophy

Dystrophinopathies include a spectrum of muscle diseases caused by pathogenic variants in the *DMD* gene, which encodes the protein dystrophin. The mild end of the spectrum includes the phenotypes of asymptomatic increase in the serum concentration of creatine phosphokinase (CK) and muscle cramps with myoglobinuria. The severe end includes progressive muscle diseases that are classified as Duchenne/Becker muscular dystrophy when skeletal muscle is primarily affected and as DMD-associated dilated cardiomyopathy (DCM) when the heart is primarily affected (*Darras et al. 2000*). The BMD phenotype occurs when some dystrophin is produced due to deletions or duplications that juxtapose in-frame exons, some splicing variants, and most non-truncating single-base changes that result in translation of a protein product with intact N and C termini. The shorter-than-normal dystrophin protein molecule, which retains partial function, produces the milder Becker phenotype (*Deburgrave et al. 2007*). Becker muscular dystrophy (BMD) occurs in approximately 1 in 30,000 male births (*Hedge et al. 2008*).

Symptoms of Becker muscular dystrophy usually begin in the teens or late twenties. Initial symptoms may include cramping and reduced endurance during exercise. Muscle gradually deteriorates in the hips, pelvis, thighs and shoulders leading to walking on toes with the stomach forward. Shortening of muscle fibers can result in the inability to move certain muscles (contractures). The progression of BMD is slower and more variable than Duchenne Muscular Dystrophy (DMD) but usually results in the need for a wheelchair.

Subclinical or clinical cardiac involvement is present in approximately 90% of individuals with BMD and is characterized by replacement of myocardium with connective tissue or fat. The left ventricular walls are most extensively affected, sparing the right ventricle and the atrium. Typical initial manifestations of cardiac involvement are sinus tachycardia, tall R1 in V1, prominent Q in I, aVL, V6 or in II, III, and aVF, increased QT dispersion and possibly autonomic dysfunction. Initially, echocardiography is normal or shows regional wall motion abnormalities in areas of fibrosis. With spreading of fibrosis, left ventricular dysfunction and

ventricular arrhythmias additionally occur. Heart failure and sudden death may occur in the final stages of the disease (*Finsterer and Stollberger 2003*).

The diagnosis of Becker muscular dystrophy is based on physical symptoms, family history, an elevated concentration of creatine kinase (CK) in the blood indicating destruction of muscle, and molecular genetic testing. DMD is the only gene that has been associated with Becker muscular dystrophy and many different types of DMD gene mutations have been identified in individuals with this condition. Identification of a DMD gene mutation from molecular genetic testing confirms the diagnosis. If molecular genetic testing is performed and a DMD gene mutation is not found, a skeletal muscle biopsy is recommended to examine the presence of the dystrophin protein.

No specific treatment is available for Becker muscular dystrophy but quality of life and lifespan can be improved with appropriate care. Physical and occupational therapy can reduce or delay joint contractures. Surgery is sometimes recommended to treat contractures or scoliosis. Weight control can help to reduce stress on the heart and muscles. Corticosteroids are often prescribed to help slow down the loss of muscle function. Routine monitoring by a cardiologist is recommended. Only few drugs are under investigation including Ataluren, an orally small-molecule compound that interacts with the ribosome, to enable the ribosome to read through premature nonsense stop signals on mRNA and allow the cell to produce a short-length, functional dystrophin; and granulocyte colony-stimulating factor (G-CSF), which was reported to exert the proliferation of satellite cells, the regulation of myoblast proliferation, and the differentiation and promotion of muscle regeneration and repair (https://clinicaltrials.gov).

4.2 Background on Givinostat (ITF2357)

Zinc dependent histone deacetylases (HDACs) are a class of 11 isoenzymes associated with numerous nuclear repressor complexes that, once recruited to specific sites of euchromatin, maintain nucleosome histones in a state of deacetylation so that deoxyribonucleic acid (DNA) remains tightly bound and inaccessible to transcription factors for gene expression. In contrast, inhibition of HDAC results in hyperacetylation of these histones and allows the unraveling of DNA sufficient for the binding of transcription factors and the synthesis of messenger ribonucleic acid (mRNA).

Givinostat is an orally active hydroxamic acid derivative with potent HDAC (Class I and II) inhibitory activity. Consequently, givinostat shares the anti-tumor properties of the histone deacetylase inhibitor (HDACi)- class. In the clinic, the HDACi doses required to achieve efficacy in cancer patients are generally poorly tolerated *(Guha 2015*), however while reactivation of epigenetically silenced tumor suppressors apparently requires high concentrations of HDACi, some gene promoters may be affected at much lower and better tolerated doses. It has been suggested that regulators of cell fate in stem or progenitor cells are under the control of promoters that are in a poised, "bivalent" state, characterized by the simultaneous presence of both chromatin activation and silencing marks within the same regulatory region (*Azuara et al. 2006, Bernstein et al. 2006*). Exposure to low doses of HDACi may, in these cases, tip the balance and redirect differentiation programs.

Dystrophin interacts with a group of peripheral membrane and transmembrane proteins to form the dystrophin-associated protein complex (DAPC), which provides the molecular link between the cytoskeleton and the extracellular matrix of skeletal myofibers that is disrupted in dystrophic muscle (*Ervasti 2007*). As such, DAPC supports sarcolemmal integrity during muscle contraction. Neuronal nitric oxide synthase (nNOS) is an important component of the DAPC. Nitric oxide (NO) produced by nNOS was shown to specifically inactivate HDAC2 via S-nitrosylation of a cysteine residue (*Colussi et al. 2008*). This mechanism is dysfunctional in dystrophic muscle, leading to aberrantly upregulated HDAC activity. There are at least two distinct mechanisms by which this upregulation impinges pathologically on cell fate decisions during muscle regeneration (*Consalvi et al. 2011*):

1. Resident muscle interstitial fibro-adipogenic progenitors (FAPs) retain the ability to turn into fibroadipocytes in response to signals released by degenerating muscles. These cells affect differentiation of muscle satellite cells into muscle fibers through secretion of follistatin, the endogenous antagonist of the most potent inhibitor of skeletal myogenesis, myostatin. Direct inhibition of myostatin or delivery of follistatin exerted similar beneficial effects in mdx mice, the mouse model of DMD. The follistatin gene promoter is controlled by HDAC2 in muscle cells (*Minetti et al. 2006*) and direct inhibition of HDAC2 by HDACi, or inactivation by either NO donors or by reconstitution of dystrophin-NO signaling leads to derepression of follistatin, which mediates the ability of

HDACi and NO signaling to stimulate myogenesis *in vitro* and counters muscle degeneration in mdx mice.

2. Histone deacetylase inhibitors may directly stimulate the myogenic differentiation of FAPs. The HDAC inhibition induces two core components of the myogenic transcriptional machinery, MyoD and BAF60C, and up-regulates the myogenic miRs (myomiRs) (miR-1.2, miR-133, and miR-206), which target the alternative BAF60 variants BAF60A and BAF60B, ultimately promoting promyogenic differentiation while suppressing the fibro-adipogenic phenotype (*Saccone et al. 2014*). Histone deacetylase inhibitors, therefore, seem to derepress a ''latent'' myogenic program in FAPs from dystrophic muscles, at least at early stages of disease.

By inhibiting several pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha [TNF-α], interleukin-1 beta [IL-1β], interferon gamma [IFN-γ], Interleukin-6 [IL-6], and Interleukin 12 [IL-12]), givinostat also reduces the production and activity of pro-inflammatory cytokines (*Leoni et al. 2005, Joosten et al. 2011*), which have been shown to inhibit muscle satellite cell differentiation into muscle fibers (*Boldrin et al. 2015, Tierney et al. 2014, Doles and Olwin 2014*).

Non-clinical data in a corneal fibrosis rabbit model and in a mouse model of liver fibrosis suggest also anti-fibrotic properties of givinostat (*Lim et al, 2016; Wang et al, 2015*).

Experimentally, the preclinical effectiveness of givinostat was demonstrated in mouse models with muscular dystrophy, such as the mdx mice. Givinostat produced beneficial functional and morphological effects, such as increased cross-sectional area of myofibers, decreased inflammatory infiltrate, and prevention of fibrotic scars, which contribute to counter the muscle loss and the functional decline that are typically observed in mdx mice.

4.2.1 Nonclinical Studies

A standard nonclinical program composed of safety pharmacology, pharmacokinetics, single and repeat dose toxicity, mutagenicity/genotoxicity, reprotoxicity and juvenile toxicity studies has already been carried out to support the oral administration of givinostat to adult and pediatric subjects. Details of each study are available in the current Investigator's Brochure (IB).

In summary, in rats the predominant toxicities at the highest doses were hematologic, with larger decreases in white blood cells (WBC) and smaller effects on red blood cells (RBC) and platelets. This toxicity pattern is different from the one observed in humans, where thrombocytopenia and not leukopenia is dose-limiting.

Non-hematologic toxicity in rats included changes in blood chemistry: decrease in total proteins, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and increase in aspartate aminotransferase (AST), bilirubin, urea, creatinine and triglycerides. These changes were modest (equal to or less than 50%), their extent overlapped with that of control animals and they were not considered of toxicological significance. Lower weight of liver, spleen and adrenals was also observed. The underlying histopathological changes were minimal in all cases and all pathologic effects trended toward reversibility following the recovery period.

In the 39-week study in monkeys, hematologic toxicities were of minor significance, with a minimal decrease in RBC counts seen during and at the end of the treatment period at the high dose only. A dose-related decrease in weight of the thymus was observed in both sexes at all doses. Histologically, an increased incidence of minimal involution/atrophy was observed in high-dose animals. The dose of 12 mg/kg/day was defined as the No-Adverse Effect Level (NOAEL) under the conditions used in this study, even if the compound was well tolerated also at the highest dose of 30 mg/kg. Additional findings included minimal effects on hepatic enzymes (ALT, AST), triglycerides, and bilirubin. Some of these effects were observed at intermediate examination (Week 20) and partially recovered at the end of the treatment period. Treatment-related effects were observed at the high dose also in the bone marrow and liver. The bone marrow showed slightly reduced cellularity in two animals given 30 mg/kg/day. The liver showed minimal bile duct hyperplasia in two male animals out of four receiving 30mg/kg/day.

Givinostat is eliminated for approximately 50% in feces after IV administration to rats, highlighting the quantitative importance of biliary excretion. In addition, a more intensive biotransformation occurs in animal species with respect to humans as shown in *in vitro* metabolic stability tests with hepatocytes of different species (Intrinsic Clearance (µL/min*106 cells: humans=4.0; monkey=25.4; dog=32.4; rat=33.8; mouse=42.0). Bile duct hyperplasia is thus likely to be the result of a pronounced metabolism and excretion of

givinostat in the preclinical species, which is also associated with increased plasma bilirubin levels. Therefore, this finding can be easily monitored in humans. Of note, no increase of hepatic enzyme or bilirubin has been recorded in subjects treated so far; bilirubin levels are also monitored clinically in DMD subjects with, so far, no evidence of significant alterations, providing no confirmation of the occurrence of analogous toxicities in humans.

The maximum observed concentration (C_{max}) and area under the curve (AUC) safety margins were calculated using the NOAEL of 10 mg/kg/day obtained in rats after 6 months of administration, the NOAEL of 12 mg/kg/day obtained after exposing monkeys for 39 consecutive weeks, and the actual human data obtained in the clinical study in DMD subjects (Study DSC/11/2357/43) after 12 months administration of 37.5 mg twice daily (bid) (i.e., C_{max} approximately 104 ng/mL and AUC approximately 1144 ng*h/mL). The human exposure achieved at this dose level is the exposure to be used in this study (DSC/15/2357/53).

Safety ratios calculated using data from both the animal species used in toxicology studies differed quite markedly one from the other. The rat to human exposure margins (Table 1) were 0.16 (C_{max}) and 0.04 (AUC) at the human exposure considered (i.e., C_{max} approximately 100 ng/mL and AUC approximately 1150 ng*h/mL), while the exposure margins C_{max} and AUC were larger for monkeys than those seen in rats. The C_{max} ratio was about 2.4 fold and the mean AUC ratio was 0.34. A more intensive biotransformation is observed in rats and monkeys as evidenced by the larger intrinsic clearance and the shorter half-life of givinostat in rats and monkeys compared to humans.

Table 1: Exposure margins for C_{max} and AUC: rat vs. DMD subjects and monkey vs. DMD subjects

Ratios NOAEL rats/paediatric DMD exposure			**Ratios NOAEL monkey/paediatric DMD exposure**		
	C_{max}	**AUC_{0-t}**		**C_{max}**	**AUC_{0-t}**
Week 26	0.16	0.04	**Week 39**	2.40	0.34

NOAEL= no-observed-adverse-effect-level; DMD = Duchenne muscular dystrophy; C_{max}= maximum observed concentration; AUC_{0-t} = area under the curve (over the first 4 hours)

Even though the safety margins between NOAEL in non-clinical studies/pediatric DMD exposure are <1 (except for C_{max} monkey), the main findings observed in non-clinical toxicology studies (e.g., reduction in white blood cells, cellularity reduction in bone marrow, reduced thymus weight, and bile duct hyperplasia) were observed at high doses of givinostat

(e.g., rats, 90-160-250 mg/kg/day depending on the treatment duration; dogs, 50 mg/kg/day; and monkeys, 30-90 mg/kg/day); all of them, as described above, can and will be carefully monitored during this clinical study.

Concerning safety pharmacology, givinostat has a favorable profile. The IC50 of givinostat in the *in vitro* hERG assay was 1.4 µM. At 3 and 10 µM, givinostat increased the action potential duration at 50% and 90% repolarization during bradycardia, although not in a statistically significant manner. Givinostat did not induce any marked changes in heart rate, electrocardiogram (ECG) intervals, or waveform at any dose in *in vivo* in a cardiovascular study in dogs. The combined evidence from these *in vitro* and *in vivo* studies plus the lack of accumulation of drug in the heart as seen in tissue distribution studies, suggest that the drug is unlikely to exert any cardiovascular side effect at therapeutic doses. The maximal circulating concentrations seen in both adult and pediatric subjects receiving the highest doses of givinostat were generally below 100-150 ng/mL (200-300 nM) of total givinostat, corresponding to 5-7.5 ng/mL (10-15 nM) of free givinostat, approximately 90-140 fold less than the IC50 on hERG.

Finally, givinostat was positive for frameshift mutations in the bacterial mutagenesis assay (2 out of 5 strains), negative in two *in vitro* mammalian cell assays and in two *in vivo* genotoxicity studies in rats resulting not genotoxic to mammalian organisms.

Nonclinical evidence supports a potential therapeutic role of givinostat in DMD. A dose-finding study using doses of givinostat ranging from 1 to 10 mg/kg/day orally delivered daily for 3 months to mdx (i.e., murine model of DMD) mice (2 months old at the beginning of the treatment) was conducted (*Consalvi et al. 2011*). The effect of these doses was compared to that of vehicle alone (oral delivered) or the HDACi TSA (0.6 mg/kg, i.p.) and assessed by monitoring total body weight during the time of exposure, the weight of single muscles at the end of the treatment, and by measuring muscle function (with treadmill test at specific time points during the treatment) and Cross Sectional Area (CSA) at the end of the treatment. The results showed an increase of the weight of a single muscle analysed, a dose-dependent reduction of intramuscular fibrosis and inflammatory infiltrate (by myeloperoxidase activity) and a dose-related increase of muscle mass and fiber CSA. All these histological effects translated into an improvement in function as assessed by monitoring the performance of

mdx mice on a treadmill test at defined time points during the treatment. To further elucidate the relationship between givinostat exposure and effects on muscle tissue and on performance, a pharmacokinetic/pharmacodynamic (PK/PD) analysis was conducted, which showed that at least a blood AUC_{0-24} of 600 nmol*h/L (i.e., corresponding to 300 ng*h/mL) was estimated to be needed to exert the beneficial histological and functional effects in mice.

4.2.2 Clinical Experience with Givinostat Including Risks and Benefits

Complete and updated data that describe the clinical experience with givinostat are reported in Section 6 "Effects in Humans" of the current Investigator Brochure Dossier related to ITF2357.

Givinostat has been tested in a number of clinical studies in adults and paediatric populations.

Three major indications have been explored with Givinostat: chronic inflammatory diseases, neuromuscular disorders and oncology. 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 in adults. In subjects with DMD enrolled in study DSC/11/2357/43 (i.e., subjects between 7 and 10 years of age at study start), the maximum dose administered in the dose escalation phase was 50 mg bid for up to 2 weeks, while the maximum dose administered for up to 1 year in the second part of the study was 37.5 mg bid. Since clearance increases with increased weight, doses were increased in the extension phase of study DSC/11/2357/43 due to the increase in weight of the enrolled children; as a result, the maximum dose administered was 60 mg bid.

In patients suffering from chronic inflammatory diseases, or DMD or healthy volunteers, the most commonly occurring adverse events were mild-to-moderate, dose-related platelet count reductions, and non-specific gastrointestinal symptoms including nausea, vomiting, abdominal pain and diarrhea. Patients with chronic inflammatory diseases reported also mild upper respiratory tract infections. Worsening of disease under study (e.g. psoriasis or Crohn's disease) was also reported in patients. The effects on platelets in Crohn's disease and psoriasis patients were mainly observed at the 100 mg b.i.d. dose, which due to the unfavorable risk-benefit ratio was discontinued prematurely in the study and has never been applied again in further studies in chronic inflammatory conditions; conversely, at doses such

as 50 mg o.d. or 50 mg b.i.d. the decreases in platelet counts were well confined within the range of normality. The majority of reductions in platelets were mild and occurred usually within the first week after treatment initiation and reached a nadir point after 2 to 3 weeks of treatment and then a 'plateau." All occurrences resolved fully within 2 to 3 weeks following discontinuation of therapy, suggesting a rapidly reversible effect. The majority of thrombocytopenic events were mild in severity with fewer than 10% of subjects developing platelets count below 75 x 10^9/L. In order to prevent any severe thrombocytopenia, close monitoring of hematology values will be implemented during the study.

Overall, sixteen hemorrhagic drug-related AEs were reported by 14 patients in the completed clinical trials with givinostat. However, only in seven of these cases were the hemorrhagic drug-related AEs in patients with concomitant thrombocytopenia; these were all oncology patients and, in some thrombocytopenia, up to grade 4 was already present before givinostat treatment started. In all the remaining cases (nine hemorrhagic drug-related AEs occurring in seven subjects), platelet counts at the time of the hemorrhagic AE were either within normal range or above the upper limit of normal. None of the drug-related hemorrhagic episodes were observed in DMD subjects.

Most premature discontinuations seen in completed or discontinued studies were associated with a decrease in platelet counts. Discontinuations due to this reason were mainly confined to the 100 mg b.i.d. dose. Other causes of premature discontinuations were mostly related to worsening of disease under study.

Cases of mild upper respiratory tract infections were reported in subjects treated with ITF2357. Five patients with multiple myeloma experienced severe systemic infections.

Overall, treatment with ITF2357 of both healthy volunteers and patients did not show clinically meaningful changes for any of the measured chemistry parameters, with the exception of a transient and slight increase in serum creatinine levels observed in only one study in Crohn's disease patients.

There was no evidence of general systemic toxicity reflected by changes in vital signs in the studied populations. Finally, although some episodes of QTc prolongation were reported (90% of those reported in oncological studies), no events of clinical concern or dose related trend were seen.

In conclusion, ITF2357 appeared to be reasonably well tolerated at the tested doses in healthy volunteers or patients, where dose-related platelets count reductions and diarrhea were the most frequently reported AEs.

Concerning the clinical experience in dystrophinopathies, a phase II clinical trial (DSC/11/2357/43) has been conducted to evaluate the safety and the potential of ITF2357 as a treatment for DMD (*Bettica et al. 2016*). DSC/11/2357/43 was an open label 2-part, Phase II study in ambulatory children aged ≥ 7 to 10. Part 1 was a dose escalation study to identify the Maximum Tolerated Dose (MTD) and recommend the dose for Part II (12 months). The primary objective of the study was to confirm in humans that ITF2357 can counteract the histological signs of the disease by evaluating the drug's effects comparing baseline and end of treatment muscle biopsies (brachial biceps). The primary endpoint was the change of Muscle Fiber Area Fraction (MFAF) after 12 months of treatment. Additional histological parameters assessed as secondary endpoints were cross-sectional area (CSA), necrosis, hypercontracted fibers, fatty replacement and fibrosis. Secondary endpoints were change in muscular function after 12 months of treatment based on the 6 Minute Walking Test (6MWT), North Star Ambulatory Assessment (NSAA) and Performance Upper Limb (PUL).

MFAF increased in all children with a mean relative increase of 29.1% ($p<0.0001$). Endomysial and perimysial fibrosis decreased in all children with a mean relative decrease in total fibrosis of 27.4% ($p<0.0001$). Moreover, significant improvements of histological features were observed with reduction of necrotic and hypercontracted fiber number, as well as decrease of fat tissue replacement. MFAF increased due to a homogeneous increment of the CSA value of all fibers with a mean change of 865.27±555.35 μm^2. Change in 6MWT distance, NSAA total score, time to stand from floor and PUL were -24.6±36.11 meters (mean±SD), -2.5±2.85 points, 0.72±1.36 seconds and -0.2±2.69, respectively.

The study protocol was amended and an extension phase is ongoing. The extension will allow the evaluation of the safety and tolerability of long-term administration of ITF2357 (for up to 4 years of treatment) and to evaluate the effect of treatment on muscle function.

The risk/benefit ratio of the proposed study is postulated to be favorable for both the results of the clinical safety and pre-clinical toxicology studies and for the efficacy results in the previous Phase 2 DMD study.

4.2.3 Rationale of the study

The concept of disease modification in DMD/BMD is characterized by slowing down or stopping the accumulation and the progression of disability. This includes the delay of symptoms of weakness, or loss of function, in certain muscle groups, the delay of general loss of energy, as well as the delay in time to milestone events (e.g. time to wheelchair, time to loss of upper limb use, time to assisted ventilation).

Nonclinical studies have shown that givinostat has a potent anti-inflammatory effect (see (current IB) and that HDACi regulate the transcription of key factors in muscle regeneration such as follistatin. ITF2357 significantly counteracts histological disease progression in children with Duchenne Muscle dystrophy aged 7 to 10 years, increasing muscle fibers, reducing necrosis, fatty replacement and fibrosis and decreasing the number of hypercontracted fibers. Although underpowered, the function tests (6MWT, NSAA and PUL) and the pulmonary function tests show an overall stability.

These results indicate that the induction of an active regeneration program, the reduction of adipose replacement and the enlargement of pre-existing muscle fibers are all key elements of givinostat's mechanism of action (MoA), supporting the potential therapeutic role of givinostat in counteracting muscle tissue degeneration.

On the basis of its putative MoA as described above, givinostat is expected to act at all stages of the disease and to counter the disease pathogenetic events in all muscular districts. As the pathogenetic mechanism of BMD is similar to DMD (i.e. same gene mutated, but different mutation), givinostat is expected to counter the disease pathogenetic events also in BMD.

The rational of this study was to replicate the histologic findings collected during the phase II clinical trial (DSC/11/2357/43) in DMD (*Bettica et al. 2016*). The primary endpoint was selected on the basis of the results of study DSC/11/2357/43, where fiber CSA enlargement appeared key in givinostat's pharmacological effect. However, as described in the section "amendment 4", the histopathological features observed in the analysis done on the preliminary 20 baseline biopsies showed a similar age-matched male adult healthy individuals mean CSA of the biceps fiber, with a very large degree of fiber size variability being accounted for a substantial number of hypertrophic and also by hypotrophic fibers. As a consequence, the likelihood that givinostat can increase the CSA of fibers further is low

and also it is doubtful that a further increase in fiber CSA would be beneficial. The histopathological features observed in these preliminary biopsies showed also significant fibro-adipose replacement, probably due to the chronicity of the disease. Considering that the histologic results after 12 months of treatment with givinostat in DMD children showed that givinostat significantly reduced the amount of fibrotic tissue and fat replacement (*Bettica et al. 2016*), and that the fibro-adipose replacement can be considered the hallmark of the disease, the change of total fibrosis is considered a more indicative outcome measure of the possible effect of givinostat relative to the assessment of CSA and, hence, it is to be evaluated as the primary endpoint for the trial. Considering the recent recommendations outlined in the EMA "Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy" (December 2015) and in the Federal Drug Administration (FDA) draft guidance for industry "Duchenne Muscular Dystrophy and related dystrophinopathies: developing drugs for treatment" (June 2015), some functional tests have been chosen to evaluate the efficacy of givinostat in slowing down disease progression. Unlike DMD, there is scarce data in BMD about standardized functional measures such as the Six Minute Walk Test (6MWT), the North Star Ambulatory Assessment (NSAA), and Timed Function Tests (TFTs: run/walk 10 m, rise from the floor, climb four standard steps), therefore the functional endpoints were selected on the basis of few literature data (*Bello et al, 2016; Mendell et al, 2015; Fischer et al, 2016*) and clinical practice.

The functional secondary endpoints included in this trial are MFM, 6MWT, and TFTs; as described by Fisher *(Fischer et al, 2016)*, they are reliable tests to assess disability in an ambulant BMD population, showing a high inter-correlation.

Even if no data are available on muscle strength in BMD patients, it has been included as secondary endpoint in light of givinostat's MoA (e.g., increase fibers CSA) and is expected to be appropriate for evaluating the efficacy of givinostat.

Concerning the imaging endpoints, previous studies have shown that MRI can visualize structural alterations of muscle in muscular dystrophies (*Tasca et al, 2012, Willcocks et al, 2016, Triplett et al, 2014, Bonati et al, 2015*) and that fat fraction measured by MRI or magnetic resonance spectroscopy (MRS) highly correlates with lower limb function.

Although longitudinal data on MRI/MRS particularly from randomised clinical trials are still limited, fatty degeneration of the muscle, in particular Muscle Fat Fraction (MFF) evaluated by MRI Dixon technique of the thigh muscles showed excellent correlation with clinical function in BMD patients (*Fischer et al, 2016*), assessed by MFM, 6MWT and TFTs and might be a promising surrogate outcome marker in clinical trials. For the reason described above, MFF evaluated by MRI with Dixon technique as well as MRS are secondary endpoints of the study.

Finally, safety and tolerability and pharmacokinetics will be also assessed in the study for a proper risk-benefit evaluation of givinostat.

5 STUDY OBJECTIVES AND ENDPOINTS

5.1 Study Objectives

5.1.1 Primary Objective

The primary objective of the present study is to establish the histological effects of givinostat versus placebo administered over 12 months.

5.1.2 Secondary Objectives

The secondary objectives of this study are the following:

- To establish the macroscopic muscle effects of givinostat versus placebo administered chronically over 12 months assessed by MRI/MRS.
- To establish the other histological effects of givinostat versus placebo administered over 12 months.
- To establish the efficacy of givinostat versus placebo administered chronically over 12 months in slowing disease progression.
- To assess the safety and tolerability of givinostat versus placebo administered chronically.
- To evaluate the pharmacokinetic (PK) profile of givinostat administered chronically in the target population.
- To evaluate the impact of givinostat versus placebo administered chronically on quality of life and activities of daily living.

5.1.3 Secondary Exploratory Objectives

- To evaluate the correlation between the PK profile of givinostat and pharmacodynamics (PD) data.

- To evaluate a possible disease-related biomarker.
- To explore additional disease-related MRI biomarkers.

5.2 Study Endpoints

5.2.1 Primary Endpoint

The primary endpoint of this study is the mean change in mean total fibrosis (%) comparing the histology of muscle biopsies before and after 12 months of treatment with givinostat versus placebo.

5.2.2 Secondary Endpoints

The secondary endpoints for this study are as follows:

- Mean change in fat fraction of vastus lateralis and soleus comparing Magnetic Resonance Spectroscopy (MRS) before and after 12 months of treatment with givinostat versus placebo.
- Mean change in fat fraction of pelvic girdle and lower limb muscles comparing Magnetic Resonance Imaging (MRI) before and after 12 months of treatment with givinostat versus placebo.
- Mean change in Cross Sectional Area (CSA) of pelvic girdle and lower limb muscles comparing MRI before and after 12 months of treatment with givinostat versus placebo.
- Mean change in other histology parameters (e.g. CSA, MFAF, regenerative fibers) comparing the histology biopsies before and after 12 months of treatment with givinostat.
- Mean change in Motor Function Measurement (MFM) before and after 12 months of treatment with givinostat versus placebo.
- Mean change in Time Function Tests (time to climb four standard steps, time to rise from floor and time to walk/run 10 m) before and after 12 months of treatment with givinostat versus placebo
- Mean change in 6 Minute Walking Test (6MWT) before and after 12 months of treatment with givinostat versus placebo.
- Proportion of patients with < 10% worsening in 6MWT at the end of study.
- Proportion of patients who lose the ability to rise from floor (Baseline through end of study).
- Proportion of patients who lose ambulation during the study.

- Mean change in muscle strength evaluated by knee extension, elbow flexion as measured by Hand Held Myometry (HHM), before and after 12 months of treatment with givinostat versus placebo.
- Mean changes in quality of life (assessed by the 36-item Short Form survey [SF36]) before and after 12 months of treatment with givinostat as compared to placebo.

5.2.3 Safety Endpoints

The safety endpoints of this study are as follows:

- Number of patients experiencing treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) from Baseline through end of study (EOS).
- Type, incidence, and severity of TEAEs and SAEs (Baseline through EOS).
- Changes from baseline to end of study of:
  - Vital signs and clinical laboratory tests (blood chemistry and hematology).
  - Physical examination.
  - Pulmonary function evaluated by Forced Vital Capacity (FVC), Forced Expiratory Volume at 1 second (FEV1), FVC/FEV1, Peak Expiratory Flow (PEF).
  - Cardiac function evaluated by ECG and ECHO.
  - Weight, height, and body mass index (BMI).

5.2.4 Pharmacokinetic Endpoints

The PK endpoints of this study are as follows:

- Description of the PK of givinostat and its major metabolites: ITF2374 and ITF2375.

5.2.5 Exploratory Endpoints

The exploratory endpoints of this study are the following:

- PK-PD analyses of the relationship between metrics of exposure and the efficacy/safety endpoints of givinostat.
- Analyses to explore whether the effects of givinostat versus placebo administered chronically may be related to the type of LTBP4 or Osteopontin genotype.
- Evaluation of serum circulating proteins, by an ELISA based system, as potential biomarkers for BMD.
- Additional evaluations of other muscle image parameters assessed by MRI (details will be provided in a specific imaging protocol).

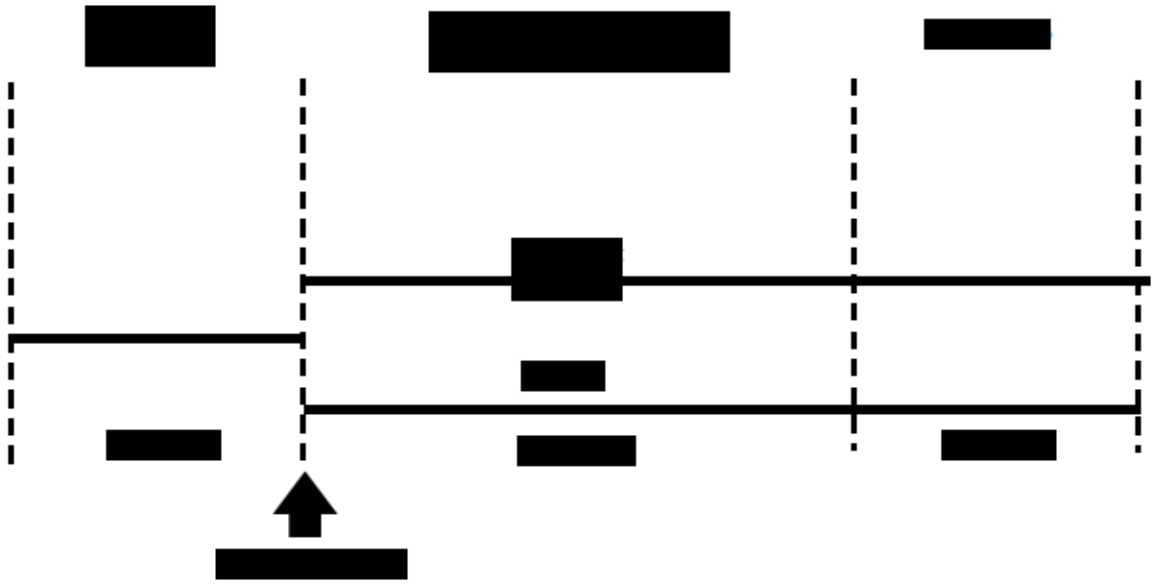
6 INVESTIGATIONAL PLAN

6.1 Description of Overall Study Design and Plan

This is a Phase 2, randomised, double-blind, placebo-controlled study. Ambulant patients who have provided written informed consent will undergo a 4-week screening period to determine eligibility for the study. Approximately 48 eligible patients will then be randomized in a 2:1 ratio to be treated with givinostat or placebo for a period of 12 months. The randomization process will be stratified by the factor related to concomitant steroid use at baseline (yes vs. no). Patients who complete the study (i.e. 12 months of treatment) will be asked to return for a follow-up visit 4 weeks after the end of treatment.

An overview of the study is provided in Figure 1.

Figure 1: Study Design



A total of 12 visits will take place during the study: Screening (V1, V2), Randomization (V3), Treatment (V4-V10), End of Study (V11) and Follow-up (V12). Visits during the treatment period will take place every 12 weeks except for the first 2 months, when they will occur every 2 weeks to allow close monitoring of hematological safety parameters. Patients may be evaluated more often if necessary, for safety reasons. Patients who discontinue the study treatment early will be asked to come in for an Early Withdrawal Visit within 4 weeks

after the last dose of study treatment. Patients who have ongoing AEs at discontinuation will be followed until resolution or stabilization.

6.2 Discussion of Study Design

This study has been designed as a conventional parallel-group design. Since the study will be conducted in a population with a serious and life-threatening disease, the unequal randomization ratio (2:1 givinostat vs. placebo) will be applied to reduce the exposure to placebo. A 2:1 randomization also provides for an increased number of patients who are exposed to givinostat. The randomization process will be stratified by the factor related to concomitant steroid use at baseline (yes vs. no).

Considering a 5% of unevaluable baseline biopsies, 51 patients will be randomized in the study to have 48 fully evaluable patients.

After the first 20 baseline muscle biopsies were collected, a blinded interim analysis was planned, aimed at checking the variability of the original primary endpoint (i.e. CSA) . The observed variability of the original primary endpoint was very large, leading to a revision of the primary endpoint to % total fibrosis. The sample size was amended accordingly to require a total of 51 patient to be randomized on a 2:1 basis.

7 SELECTION AND WITHDRAWAL OF PATIENTS

The study will enroll adult male patients with an established molecular diagnosis of BMD.

Specific entry criteria are detailed in Section 7.1 and in Section 7.2.

At the discretion of the Investigator, patients not meeting inclusion/exclusion criteria may be re-screened twice with an interval of at least 30 days between assessments.

Patients dropping out before the end of treatment will not be replaced.

7.1 Inclusion Criteria

Patients must meet all of the following criteria in order to be included in the study:

1. Ambulant male patients aged ≥18 years to ≤ 65 years at randomization with BMD diagnosis confirmed by genetic testing.
2. Able and willing to give informed consent in writing.

3. Able to perform 6MWT at screening with a minimum distance of 200 m and maximum distance of 450 m.

4. If in treatment with systemic corticosteroids and/or ACE inhibitor, and/or β or α adrenergic receptor blocker, no significant change in dosage or dosing regimen (excluding changes related to body weight change) for a minimum of 6 months immediately prior to start of study treatment.

5. Patients must be willing to use adequate contraception. Contraceptive methods must be used from Randomization through 3 months after the last dose of study treatment, and include the following:

   - True abstinence (absence of any sexual intercourse), when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g. calendar ovulation, symptothermal, post ovulation methods) and withdrawal are not acceptable methods of contraception.
   - Condom with spermicide and the female partner must use an acceptable method of contraception, such as an oral, transdermal, injectable or implanted steroid-based contraceptive, or a diaphragm or a barrier method of contraception in conjunction with spermicidal jelly such as for example cervical cap with spermicide jelly.

7.2 Exclusion Criteria

Patients meeting any of the following criteria are ineligible to participate in this study:

1. Exposure to another investigational drug within 3 months prior to the start of study treatment.

2. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength or function within 3 months prior to the start of study treatment (e.g., growth hormone). Vitamin D, calcium, and any other supplements 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. Presence of other clinically significant disease that in the Investigator's opinion could adversely affect the safety of the patient, making it unlikely that the course of treatment or follow-up is completed, or could impair the assessment of study results.

5. A diagnosis of other uncontrolled neurological diseases or presence of relevant somatic disorders not related to BMD that may interfere with the ability to perform the muscle function tests and/or to comply with the study protocol procedures.

6. Platelet count, White Blood Cell (WBC) count and hemoglobin at screening < Lower Limit of Normal (LLN). If laboratory screening results are < LLN, platelet count, WBC count and hemoglobin are to be repeated once, and if again < LLN become exclusionary.
7. Symptomatic cardiomyopathy or heart failure (New York Heart Association Class III or IV) or left ventricular ejection fraction < 50% at screening or with heart transplant.
8. Current liver disease or impairment, including but not limited to elevated total bilirubin (> 1.5 x ULN), unless secondary to Gilbert's disease or pattern consistent with Gilbert's disease.
9. Inadequate renal function, as defined by serum Cystatin C > 2 x the upper limit of normal (ULN). If the value is > 2 x ULN, serum Cystatin C will be repeated once, and if again > 2 x ULN becomes exclusionary.
10. Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening.
11. Baseline corrected QT interval, Fredericia's correction (QTcF) > 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, or family history of long QT syndrome).
12. Current psychiatric illness/social situations rendering the potential patient unable to understand and comply with the muscle function tests and/or with the study protocol procedures.
13. Hypersensitivity to the components of study medication.
14. Sorbitol intolerance or sorbitol malabsorption, or the hereditary form of fructose intolerance.
15. Contraindications to muscle biopsy.
16. Contraindications to MRI/MRS (e.g., claustrophobia, metal implants, or seizure disorder).
17. Hypertriglyceridemia (> 1.5 x upper limit of normal [ULN])*

    *At screening, patients with hypertriglyceridemia can be enrolled if in stable treatment and with controlled levels of triglycerides (i.e. within normal range) for at least six months.

7.3 Withdrawal and Removal of Patients

Withdrawal from study participation and study medication may occur under the following circumstances:

- Withdrawal of consent: The patient desires to withdraw from further participation in the study in the absence of the Investigator determining a medical need to withdraw. If the patient gives a reason for withdrawing, it should be recorded in the electronic Case Report Form (eCRF).
- Protocol Violation: The patient's findings or conduct fails to meet the protocol entry criteria
- Lost to Follow-Up: The patient stopped coming for visits, and study personnel were unable to contact the patient (at least 3 contact attempts should be documented).
- Other reason: The patient was discontinued for a reason other than those listed above, such as at the discretion of the Investigator (i.e., any other condition that, in the opinion of the Investigator, may jeopardize the study conduct according to the protocol, or when the Investigator feels that it is in the best interest of the patient to discontinue) or termination of study by the Sponsor.

Withdrawal from study medication may occur under the following circumstance:

- Adverse event: Clinical or laboratory events occur that, in the medical judgment of the Investigator for the best interest of the patient, are grounds for discontinuation. Moreover, if the patient meets one of the safety stopping criteria described in Section 7.3.1, he will be withdrawn from the study medication. In case of withdrawal due to AEs/SAEs, the Investigator will follow up the patient until resolution or acceptable stabilization of the event and document all the relevant information, as applicable.

Before withdrawing a subject, each case can be discussed with the study medical monitor, but the final decision remains with the Investigator only or authorized designee. If the safety of the subject can be reasonably assured, the subject will not be discontinued.

In case of withdrawal from study participation and /or study medication, the following general rules apply:

- If a patient is discontinued from study medication and is withdrawn from the study for any reason, the study site must immediately notify the medical monitor.
- When a patient withdraws from the study, the date and reasons for withdrawal shall be recorded by the Investigator on the patient's medical file and on the relevant page of the eCRF. In case of multiple reasons, AEs should be indicated as the primary reason whenever applicable. All relevant information related to the reason for

treatment discontinuation including contributory factors must be included on the eCRF.

- All patients prematurely discontinuing the study must be seen for a final evaluation performed within 4 weeks after the last drug intake. Final evaluation is defined as completion of the assessments scheduled for the Early Withdrawal Visit (as detailed in Section 9.1.10). Data collected during the final evaluation are crucial to the integrity of the final study analysis because early withdrawal may be related to the safety profile of the study drug. The Investigator will make every effort to see patients who fail to return for a final visit.
- If a patient is discontinued prematurely from the study due to a treatment-related TEAE or serious TEAE, the TEAE or serious TEAE will be followed until it resolves (returns to normal or baseline values) or stabilizes, or until it is judged by the Investigator to be no longer clinically significant.

  Once a patient is withdrawn from the study, he may not re-enter it.

7.3.1 Safety Stopping Rules

The study treatment should be **permanently** discontinued if any of the following events occur:

- Severe drug-related diarrhea (increase of ≥ 7 stools per day).
- Any drug-related SAE.
- QTcF > 500 msec*.
- Platelet count $\leq 50 \times 10^9$/L. To avoid laboratory errors and anomalous values, a platelet count $\leq 50 \times 10^9$/L must be confirmed with a repeated test performed on the next working day. Treatment should be stopped until the retest result becomes available. If the repeated platelet count is still $\leq 50 \times 10^9$/L, study treatment must be permanently discontinued. If the repeated test is acceptable, the patient can resume treatment.
- White blood cell count $\leq 2.0 \times 10^9$/L. To avoid laboratory errors and anomalous values, white blood cells $\leq 2.0 \times 10^9$/L must be confirmed with a repeated test performed on the next working day. Treatment should be stopped until the retest result becomes available. If the repeated white blood cells count is still $\leq 2.0 \times 10^9$/L, study treatment must be permanently discontinued. If the repeated test is acceptable, the patient can resume treatment.
- Hemoglobin $\leq$ 8.0 g/dL. To avoid laboratory errors and anomalous values, hemoglobin $\leq$ 8.0 g/dL must be confirmed with a repeated test performed on the next

working day. The treatment should be stopped until the retest result becomes available. If the repeated hemoglobin is still ≤ 8.0 g/dL, study treatment must be permanently discontinued. If the repeated test is acceptable, the patient can resume treatment.

The Investigator will follow up until resolution or acceptable stabilization of the event and document all the relevant information, as applicable. After the resolution/stabilization of the event, the patient will be withdrawn from the study and the EOS Visit (see Section 9.1.10) will be performed.

* Based on average QTc value of triplicate ECGs. For example, if an ECG demonstrates a prolonged QT interval, 2 more ECGs should be obtained over a brief period, and then the averaged QTcF values of the 3 ECGs should be used to determine whether the patient should be discontinued from the study. If the mean QTcF value of the 3 replicates still meets the stopping criteria, the patient has to interrupt the study treatment, the medical monitor has to be informed and the value must be confirmed by central reading before withdrawing the patient from the study. The QTc has to be calculated with the Fridericia formula $QTc = QT/RR^{1/3}$.

The Study treatment should be **temporarily** interrupted if any of the following events occur:

- Moderate or severe diarrhea (increase more than 4 stools per day).
- Platelet count < 75 x 10^9/L but > 50 x 10^9/L (treatment should be temporarily stopped, and the platelet count repeated within 1 week and retested until platelets are normal).
- White blood cell < 3.0 x 10^9/L but > 2.0 x 10^9/L (treatment should be temporarily stopped, and white blood cells re-measured within 1 week and retested until white blood cells are normal).
- Hemoglobin < 10.0 g/dL but > 8.0 g/dL (treatment should be temporarily stopped, and hemoglobin re-measured within 1 week and retested until hemoglobin is normal).
- Triglycerides > 300mg/dL (see also section 8.8)

The study treatment can be reduced by 20% of the current dose at which the AE leading to temporary interruption occurred, once platelets and/or white blood cell and/or hemoglobin and/or triglycerides are normalized, or diarrhea is mild. See Section 8.2.2 for more details.

The patient may have unscheduled visits if needed until the AE resolves, and will then continue the study as per scheduled visits. Further procedures (blood sample collection) may also be performed by a qualified trained nurse at the patient's home.

In addition, if a patient has a consistent (at least two consecutive evaluations) platelet count ≤ 150 x 10^9/L that does not meet stopping criteria for platelets, the Investigator will have to reduce the dose by 20% of the current dose. See Section 8.2.2 for more details.

Hematology parameters will be evaluated every 2 weeks during the first 2 months of treatment (Visits 4-7) to closely monitor these safety parameters.

Any decision concerning dose adjustment and/or modification of schedule of assessments can be discussed with the Medical Monitor, but the final decision remains with the Investigator only or authorized designee.

If a patient has a medical event not necessarily drug related that requires interruption of study treatment dosing for > 4 weeks, the Investigator can discuss the case with the medical monitor to decide whether the patient may resume study treatment, however the final decision remains with the Investigator only or authorized designee.

8 TREATMENTS

8.1 Details of Study Treatments

The investigational study drug to be used in this study is givinostat (ITF2357) and a placebo. For the duration of the study, givinostat and placebo will be supplied by Italfarmaco S.p.A.

The characteristics of the study treatment are listed in the Table below:

Table 2: Investigational Medicinal Products

	Investigational Medicinal Drug	
	Test treatment	**Comparator**
Product name:	ITF2357/givinostat*	Placebo
Dosage form:	Oral suspension	Oral suspension
Unit dose strength:	Bottle containing 140 mL of givinostat 10 mg/mL	Bottle containing 140 mL of placebo, a combination of 0.1% w/v Titanium (opacifier, to resemble suspension appearance) and 0.1% w/v of an additional flavoring agent (to resemble the slightly bitterish aftertaste typical of the drug substance)
Route of Administration	Oral administration under fed conditions	Oral administration under fed conditions
Dosing instructions:	See Table 3: Givinostat-Placebo Starting Dose	See Table 3: Givinostat-Placebo Starting Dose.
Physical description:	White to off-white or faintly pink, homogenous suspension when mixed	White to off-white or faintly pink, homogenous suspension when mixed
Device included in the packaging:	Two graduated dosing syringes for oral use: One 5 mL syringe and One 1 mL syringe	Two graduated dosing syringes for oral use: One 5 mL syringe and One 1 mL syringe

*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.

8.2 Dosage

8.2.1 Rational for Dose Selection

The dose for this study has been selected based on the dose ranging evaluation performed in the mdx mouse model, the results of the Phase 2 study in pediatric patients with Duchenne Muscular Dystrophy (DMD) (DSC/11/2357/43), and the evaluation of PK and efficacy results obtained in other inflammatory diseases.

The PK/PD analysis done with the data collected during the non-clinical studies in the mdx model showed that a blood AUC_{0-24} of at least 300 ng*h/mL was estimated to be needed to exert the beneficial histological and functional effects in mice and thus was considered the target exposure for the exploratory Phase 2 study in the DMD population.

Study DSC/11/2357/43 was designed to first verify that doses of givinostat leading to an AUC_{0-24} of at least 300 ng*h/mL in most DMD patients were tolerated and then to verify that

such exposures would translate into a significant histological benefit. Results confirmed that doses of 25/37.5 mg bid are tolerated, result in an AUC_{0-24} of at least 300 ng*h/mL in the majority of treated DMD patients and lead to a significant histological benefit. In study DSC/11/2357/43, the effects of givinostat on the Muscle Fiber Area Fraction (primary endpoint of the study) and on the other key histological parameters (Total Fibrosis, Total Necrosis, Fatty Replacement) were comparable when patients who maintained the 37.5 mg b.i.d. dose throughout the study (n=7) were compared to those who reduced the dose to 25mg b.i.d. (n=12). In a post-hoc analysis, the Cross Sectional Area (CSA) of the muscle fibers in patients who maintained the 37.5mg b.i.d. dose throughout the study (n=7) was larger than that in patients who reduced the dose to 25mg b.i.d. (n=12); however a highly significant increase in CSA was seen in both dose groups.

On the other hand, clinical experience in chronic inflammatory diseases in adult and pediatric populations (i.e., Crohn's Disease, Psoriasis, Systemic Onset Juvenile Idiopathic Arthritis and Polyarticular Course Juvenile Idiopathic Arthritis) have shown that givinostat was overall not effective in controlling symptoms and biological features of these diseases at exposures (AUC_{0-24}) comparable or lower to those obtained in study DSC/11/2357/43.

Based on these considerations, the Sponsor believes that doses leading to daily exposures lower than those obtained in study DSC/11/2357/43 are very unlikely to be effective and therefore chose an initial study dose to ensure a mean AUC_{0-24} similar to that achieved with 37.5 mg/bid during the DSC/11/2357/43 study in DMD patients (i.e., approximately 1150 ng*h/mL).

Unfortunately, the majority of the patients treated with the starting dose used in protocol version 2.0 (i.e. dose leading to daily exposures obtained with 37.5 mg/bid in study DSC/11/2357/43) experienced a platelet count decrease that required dose reduction. Even if a strict regimen of platelet count monitoring and the implementation of a set of safety rules were adequate to ensure patient safety, these preliminary blinded results suggest that the starting dose indicated in protocol version 2.0 would be difficult to manage outside of a clinical trial environment. As a consequence, a new starting dose corresponding to the reduced dose of protocol version 2.0 (i.e. 26.7-46.7 mg b.i.d according to body weight) is proposed by the Sponsor in protocol version 3.0. This new starting dose ensures a mean

AUC_{0-24} similar to that achieved with 25 mg/bid during the DSC/11/2357/43 study in DMD patients (i.e., approximately 674 ng*h/mL), which was safe and showed significant histological benefit.

Lastly, since weight was shown to significantly affect givinostat clearance, the initial dose will be adjusted to the body weight of the patient (see Section 8.2.2).

8.2.2 Dosage Schedule

Givinostat oral suspension (10 mg/mL) and placebo oral suspension is to be administered orally as 2 oral doses daily in a fed condition (e.g. in the morning after breakfast and in the evening after dinner). Before its use, the suspension must be shaken for at least 30 seconds by rotating the bottle 180°. The suspension is administered by means of a graduated dosing syringe. The dosage to be administered is based on patient weight as described below:

Table 3: Givinostat-Placebo Starting Dose

Weight (kg)	**≥30 and <40**	**≥40 and <50**	**≥50 and <60**	**≥60 and < 70**	**≥70**
Dose (mg) bid	**26.7**	**33.3**	**36.7**	**40**	**46.7**
Oral suspension (mL) bid	**2.7**	**3.3**	**3.7**	**4**	**4.7**

Dose Modifications:

After randomization, patients will be asked to have hematology assessments every two weeks during the first two months of treatment to closely monitor these parameters for safety purposes.

Study treatment dose adjustment including safety rules for permanently or temporarily stopping study treatment are described in Section 7.3.1.

As mentioned in Section 7.3.1, if a patient has consistent (at least 2 consecutive evaluations) platelet counts $\leq 150 \times 10^9$/L but does not meet the stopping criteria due to platelet reduction, the Investigator will have to reduce the dose by 20% of the current dose (See Table 4).

Study treatment can be resumed at a level 20% smaller than the dose at which an AE leading to a temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin and/or triglycerides are normalized or when diarrhea is mild (See Table 4).

Table 4: Givinostat-Placebo Dose Modification

Starting Dose (mg) bid	**26.7**	**33.3**	**36.7**	**40**	**46.7**
Oral suspension (mL) bid	2.7	3.3	3.7	4	4.7
Reduced Dose (mg) bid	**21.4**	**26.6**	**29.4**	**32**	**37.4**
Oral suspension (mL) bid	2.1	2.7	2.9	3.2	3.7

8.3 Study Treatment Assignment

Patients will be dispensed the study treatment on an outpatient basis. All study treatment supplies are to be used only for this protocol and for no other purpose.

Patients will be assigned to one of the two treatment arms (givinostat vs. placebo) in a 2:1 ratio. The randomization process will be stratified by the factor related to concomitant steroid use at baseline (yes vs. no).

The randomization numbers will be generated using procedures that ensure that treatment assignment is unbiased. A patient randomization list will be produced using a validated system that automates the random assignment of patient numbers to randomization numbers.

At Visit 3, all patients who fulfill all inclusion criteria and none of the exclusion criteria will be randomized via an Interactive Web Response System (IWRS) to one of the treatment arms. The investigator or his/her delegate will log on to the IWRS, confirm that the patient fulfills all the inclusion/exclusion criteria and enter information relevant to the stratification factor. The IWRS will assign a randomization number to the patient which will be used to link the patient to a treatment arm.

During the study, the Investigator will supply the patient with the appropriate number of suspension bottles sufficient to cover the period between two visits (3 months of treatment).

The Investigator is to use the IWRS at each dispensing visit to obtain the packaging number of the study treatment to be dispensed to the patient. Study treatment should be dispensed by authorized personnel only.

The Investigator will provide the patient with written instructions on the dosage and corresponding volume in milliliters of suspension to be taken at each administration.

In the unlikely case of system unavailability, the Interactive Voice Response System (IVRS) will be used as backup for randomization and treatment assignment.

8.4 Blinding

This is a double-blind, placebo-controlled study, so patients receiving givinostat or placebo will receive medication indistinguishable in appearance. Personnel involved in the study (Investigators, nurses, all other site personnel, clinical research associate [CRA], Medical Monitors, Project Managers, Data Managers and Statisticians) will remain blind at all times, unless knowledge of the study treatment is relevant to the safety of the patient. If the Investigator or authorized designee decides to break the code of a patient, it is suggested that the Monitoring Team (Medical Monitor or CRA) be consulted if possible before breaking the code, however this is not mandatory since the decision of the Investigator cannot be influenced nor does it require the approval of the Monitoring Team.

Code breaking can be performed by the Investigator or authorized designee at any time by using the proper module of the IWRS. In the unlikely case of system unavailability, the Interactive Voice Response System (IVRS) will be used as backup for code breaking.

If the code is broken the Investigator must record the date, time, and reason for breaking the code in the eCRF, and must notify the Monitoring Team as soon as possible.

Before withdrawing a patient, each case can be discussed with the study medical monitor, but the final decision remains with the Investigator only or authorized designee. If the safety of the subject can be reasonably assured, the subject will not be discontinued.

As platelet count reductions are observed after treatment with givinostat, study site personnel who perform the histology analysis, the functional tests and the MRI/MRS analysis must be different from the personnel who review safety results and these must not be shared with the personnel responsible for the functional tests, histology and MRI/MRS analyses.

Regarding the PK results, all the personnel involved in the study will remain blind at all times and Sponsor may review the PK results only in scrambled format.

8.5 Drug Supply

The investigational site will be supplied with a number of suspension bottles sufficient to treat the patients enrolled in the study.

8.5.1 Packaging

Primary packaging will consist of an amber plastic bottle containing the suspension. Givinostat/placebo oral suspension is supplied in a 150 mL capacity amber PET bottle closed by means of a red-white HDPE child-proof screw cap with a LDPE shutter/syringe-adapter. Each bottle contains 140 mL of givinostat 10 mg/mL or placebo. The secondary packaging will be a carton box containing one amber bottle and two syringes as dosing systems for dispensing the suspension.

8.5.2 Labelling

The primary and secondary labels will show all the information requested in accordance with Annex 13 of Good Manufacturing Practice and local legislation.

Medication labels will be in the local language.

The labels will include at least:

- Name of the medicinal product
- Pharmaceutical dosage form
- Route of administration
- Quantity of dosage units
- Strength/potency
- Batch number/Packaging number
- Sponsor's study code (*)
- EudraCT number (*)
- Investigator name (*)
- Site and patient number (*)
- Treatment/Randomization number (*)
- Name, address, and telephone number of the main contact (the sponsor or the contract research organization or the investigator) (*) (**)
- Period of use (*)
- Storage conditions (*)
- Directions for use (*) (**)
- For clinical trial use only (*)

- Keep out of reach of children (*)

Notes:

(*) According to Annex 13 and EU law in force, when using a centralized electronic randomization system, some of the listed information may be omitted from the label.

(**) According to Annex 13, such information might not need to appear on the label if separately provided in a leaflet or card.

8.5.3 Storage

The investigational site will store the study treatment under the conditions specified here below, ensuring that it is not accessible to unauthorized persons until it is dispensed to the patient.

Givinostat/placebo suspension is to be stored in a refrigerator at 5°C ± 3°C. Once the container is opened, the suspension can be administered for a maximum of 30 days.

8.6 Treatment Accountability and Compliance

The study treatment packaging will be provided with tear-off labels; before treatment dispensation the tear-off label should be removed from the patient's pack by the Investigator and stuck on the appropriate drug accountability form.

At each dispensing visit, the patient will return all bottles previously received (used, partially used and unused) and receive a new supply of the study treatment.

Patient compliance will be evaluated through a patient diary indicating how many doses were effectively administered by the patient.

Drug accountability will be checked by Italfarmaco S.p.A. or its designee based on an accountability log completed by site staff and monitored by Italfarmaco S.p.A. or its designee.

The used, partially used, and unused bottles will be collected and sent back to Italfarmaco S.p.A. or their designee periodically or at the end of the study.

8.7 Prior and Concomitant Illnesses and Medications

8.7.1 Prior and Concomitant Illnesses

Investigators should document all significant prior illnesses that the patient has experienced within 6 months of screening. Additional illnesses present at the time when informed consent is given and up to the time of first dosing 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 on the eCRF.

8.7.2 Prior and Concomitant Medications

All medications and other treatments taken by the patient during the study, including those treatments initiated within 6 months prior to the start of the study, must be recorded on the eCRF.

Concomitant treatments are defined as treatments taken after study treatment administration. Any medication or therapy that is taken by or administered to the patient during the study must be recorded in the eCRF. The entry must include dose, regimen, route, indication, and dates of use.

After the Screening visit, medication to treat minor treatment-emergent illnesses are generally permitted; supportive treatments such as antiemetics, antidiarrheals, antipyretics, anti-allergics, analgesics and antibiotics are allowed.

Use of Vitamin D, calcium and any other supplements if clinically indicated before enrollment and for the duration of the study is allowed

The following therapies are expressly **prohibited** throughout the study:

- Any other investigational drug while on this study.
- Any dystrophin restoration product.
- Any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength or function (e.g., growth hormone).

The following are concomitant therapy **requiring caution** throughout the study:

- Drugs known to increase the QT interval (see Appendix 16.1 for a list of such compounds).

- Live or live attenuated vaccines: the patient must be carefully monitored. While the decision of the Investigator cannot be influenced, rejected nor approved by the Medical Monitor, it is advised but not mandatory that the Investigator discuss the case with the Medical Monitor before using these vaccines.
- Drugs known to be a substrate of Organic Cation Transporter 2 (see Appendix 16.2 for a list of such compounds).
- Antiplatelet, thrombolytic or anticoagulant drugs must be carefully monitored.
- Drugs known to be P-glycoprotein inhibitors (see Appendix 16.3 for a list of such compounds).

8.8 Treatment for hypertriglyceridemia

If a subject experiences an increase of triglycerides >ULN during the study, the investigator is allowed to prescribe adequate treatment including dietary supplements. These treatments will be reimbursed by the Sponsor.

9 STUDY PROCEDURES

Table 5 outlines the timing of procedures and assessments to be performed throughout the study. See Section 9.1 for additional details of study procedures.

Table 5: Schedule of Assessments

Month	**-1**		**0**	**1**		**2**		**3**	**6**	**9**	**12**	**13**
Week	-4		0	2	4	6	8	12	24	36	48	52
Visit window	+ 2 weeks		0	± 3 days	± 3 days	± 3 days	± 3 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days
Visit*	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
	Screening		**Rand**								**EOS/Early Withdrawal[1]**	**FUV[2]**
Informed consent	X											
Eligibility criteria	X											
Medical history and demographic data	X											
Prior and 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
Weight	X		X	X	X		X	X	X	X	X	
Height	X										X	
ECG Test[3]	X		X	X	X		X	X	X	X	X	X
ECHO	X										X	
Pulmonary function tests[4]	X										X	
Serology[5]	X											
Hematology[6]	X		X	X	X	X	X	X	X	X	X	X
Blood chemistry[7]	X						X	X	X	X	X	X
Thyroid function tests (TSH, fT3, fT4)	X				X		X	X	X	X	X	X
Coagulation tests (PT and aPTT)	X						X	X	X	X	X	X
Urine analysis[8]	X						X	X	X	X	X	X
SF-36	X										X	
Time function tests (time to climb 4 standard steps, time to rise from floor, time to walk 10 m)	X							X	X	X	X	

Month	**-1**		**0**	**1**		**2**		**3**	**6**	**9**	**12**	**13**
Week	-4		0	2	4	6	8	12	24	36	48	52
Visit window	+ 2 weeks		0	± 3 days	± 3 days	± 3 days	± 3 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days
Visit*	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
	Screening		**Rand**								**EOS/Early Withdrawal[1]**	**FUV[2]**
6MWT	X		X					X	X	X	X	
MFM	X							X	X	X	X	
Muscle strength[9]	X							X	X	X	X	
PK blood sample collection[10]					X			X	X		X	
Biomarkers blood collection[12]			X						X		X	
Study treatment dispensation and accountability			X					X	X	X		
Patient diary dispensation			X									
Patient compliance through diary								X	X	X	X	
AE assessment	X	X	X	X	X	X	X	X	X	X	X	X
MR + Muscular biopsy		X[11]									X[13]	

6MWT=6-minute walking test; AE=adverse event; MFM= Motor Function measure; SF36= Short Form (36) Health Survey; PT=Prothrombin time; aPPT= activated partial thromboplastin time; ECG= electrocardiogram; ECHO= echocardiogram; PK= pharmacokinetics; MRI= magnetic resonance imaging; FEV1= forced expiratory volume at 1 second; FVC=forced vital capacity; PEF= peak expiratory flow; RBC-red blood cell; WBC=white blood cell; ALT=alanine aminotransferase; AST=aspartate aminotransferase; LDH= lactate dehydrogenase; CRP=C-reactive protein; GGT=gamma-glutamyl transpeptidase; BUN=blood urea nitrogen; fT3= free T3 (the active part of triiodothyronine); fT4=Free T4 (the active part of thyroxine); TSH=thyroid-stimulating hormone; eGFR=estimate Glomerular Filtration Rate; HHM=hand-held myometry; MMT=manual muscle testing

* Unscheduled visits may take place at the discretion of the Investigator such as additional cardiology visits at the study center or visits at the patient's home by a qualified trained nurse for blood sample collection.

1. If the patient is discontinued from the study treatment, the patient will be asked to return for the early withdrawal visit to be performed within 4 weeks of the last dose of study treatment. In this case, the SF-36 evaluation and MRI may not be performed.
2. The patients who complete the study (i.e. 12 months of treatment) will be asked to return to the centre for the Follow-up visit to be performed after 4 weeks from the last dose of study treatment (i.e. 4 weeks ±1).
3. A 12-lead ECG is to be done in triplicate at the screening visit and during the other visits if an ECG demonstrates a prolonged QTcF interval > 450 msec obtained on 2 more ECGs over a brief period. The averaged QTcF values of the 3 ECGs is to be used to determine whether the patients should be discontinued from the study.
4. Pulmonary function tests: FEV1, FVC and PEF.
5. Serology CMV IgM, HBsAg/HCV/HIV, EBV IgG.

6. Hematology test: RBC, WBC (including differential count), hemoglobin, MCH, MCHC, MCV, hematocrit, and platelets. Test will be done according to the schedule (i.e., every two weeks the first 2 months of treatment) and are to be performed more frequently if clinically indicated. Note: Repeat visits for the platelets count will be performed at the request of the Investigator.
7. Serum blood chemistry: total bilirubin, direct bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, cystatin C, CRP, GGT, creatine kinase, total protein, albumin, uric acid, triglycerides, phosphorus, total cholesterol, LDL, HDL, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, bicarbonate), NT-proBNP.
8. Urinalysis: appearance, pH, specific gravity, protein, glucose, ketone bodies, blood and WBCs, and urobilinogen.
9. Muscle strength tests: knee extension, elbow flexion by HHM.
10. Pharmacokinetic sample collection: sampling (2 pre-dose during the treatment and 4 post-dose) will be collected as described in the protocol during the following visits: 5 (4 weeks), 8 (12 weeks), 9 (24 weeks), and 11 (Week 48). At these visits, the patient will be asked to take the morning dose at site.
11. The first MRI (by Dixon technique), MRS tests and muscular biopsy have to be performed when all inclusion/exclusion criteria have been already evaluated and the patient is eligible. MRI/MRS and muscular biopsy can be performed in different days.

12 LTBP4 and osteopontin genotype after randomization and blood sample collection for serum circulating proteins (by an ELISA based system) at randomization, 6 months and at the end of treatment.

13 MRI/MRS and biopsy may be performed on different days. Treatment is to continue up to the last assessment.

9.1 Procedures by Study Visit

Twelve (12) study visits, including the Screening and Randomization Visits, are planned. Assessments will be performed as outlined in the following by-visit subsections. As platelet count reductions are observed after treatment with givinostat, study site personnel who perform functional tests and MRI/MRS must be different from the personnel who review safety results related to laboratory tests, and these must not be shared with the personnel responsible for the functional tests and MRI/MRS.

9.1.1 Visit 1 and Visit 2 - Screening (Week -4 + 2 weeks)

The Screening visits (Visit 1 and Visit 2) will occur within 4 weeks (± 2 weeks) prior to the Randomization Visit. The following assessments will be performed at Visit 1:

- Written Informed Consent
- Eligibility criteria: review of inclusion and exclusion criteria
- Medical history and demographic data
- Prior medications (taken in the past 6 months)
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight and height
- 12-lead ECG (in triplicate)
- Echocardiogram
- Pulmonary function tests: FEV1, FVC, FEV1/FVC, and PEF
- Blood collection for:
  - serology
  - hematology
  - blood chemistry
  - coagulation tests (prothrombin time [PT] and activated partial thromboplastin time [aPTT])
  - thyroid function tests (TSH, fT3, fT4)
- Urine analysis
- Quality of Life test (SF-36)
- 6MWT + MFM
- Muscle strength test (knee extension and elbow flexion by HHM)
- Time function tests
- AE assessments

If all inclusion and exclusion criteria have been met and the patient is eligible, an MRI, MRS and muscle biopsy are to be performed before the first IMP dose. At the discretion of the Investigator, patients not meeting inclusion/exclusion criteria may be re-screened twice with an interval of at least 30 days between assessments.

At Visit 1, after having filled in the "Eligibility criteria" section of the eCRF, eligible patients will be randomized to one of the two following muscle biopsy sequences by the system:

V2: Right Arm - V11: Left Arm

V2: Left Arm - V11: Right Arm

The site will receive the muscle biopsy sequence by email.

Randomization will be balanced in a ratio of 1:1 and will not be stratified.

If needed for logistic reasons, Visit 2 can be completed on two different days included in the window period between Visit 1 and Visit 3. Patients will be instructed to avoid physical activity that would exceed their normal activity for 3 days before each visit.

9.1.2 Visit 3 - Randomization (Week 0)

Data collected at Visit 3 will represent baseline values for study endpoints.
At Visit 3, the following assessments will be performed:

- Review of eligibility criteria using the assessments done at screening.
- Prior and concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight
- 12-lead ECG
- Blood collection for hematology and biomarker evaluation
- 6MWT
- Randomization and dispensation of study treatment
- AE assessments
- Patient diary dispensation

9.1.3 Visit 4 (Week 2 ± 3 days)

The following assessments will be performed at Visit 4, after 2 weeks (± 3 days) of treatment:

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight
- 12-lead ECG
- Blood collection for hematology
- AEs assessments

9.1.4 Visits 5 (Week 4 ± 3 days)

The following assessments will be performed at Visit 5, after 4 weeks (± 3 days) of treatment. Patients will be instructed to take the morning dose at the site in case of pre-dose PK analysis.

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight
- 12-lead ECG
- Blood collection for hematology, thyroid function tests (TSH, fT3, fT4) and PK analysis
  AEs assessments

9.1.5 Visit 6 (Week 6 ± 3 days)

Visit 6 is required if the platelet values at any visit during the first month of treatment decreased more than 10% from the PLT value measured at the Randomization Visit (i.e. Visit 3-week 0) or at the discretion of the Investigator.

Visits 6 will be performed 6 weeks after the Randomization Visit (± 3 days) essentially for safety purposes, and the following assessments will be performed:

- Blood collection for hematology
- AE assessments.

9.1.6 Visit 7 (Week 8 ± 3 days)

At Visit 7, after 2 months of treatment (± 3 days), the assessments listed below will be performed.

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)

- Weight
- 12-lead ECG
- Blood collection for:
  - hematology
  - blood chemistry
  - coagulation tests (prothrombin time [PT] and activated partial thromboplastin time [aPTT])
  - thyroid function tests (TSH, fT3, fT4)
- Urine analysis
- AE assessments

9.1.7 Visit 8 (Week 12 ± 7 days)

The following assessments will be performed at Visit 8 (± 3 days), after 3 months of treatment. Patients will be instructed to take the morning dose at the site in case of pre-dose PK analysis.

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight
- 12-lead ECG
- Blood collection for:
  - hematology
  - blood chemistry
  - coagulation tests (PT and aPTT)
  - thyroid function tests (TSH, fT3, fT4)
  - PK analysis
- Urine analysis
- 6MWT + MFM
- Muscle strength test (knee extension and elbow flexion by HHM)
- Time function tests
- Dispensation and accountability of study treatment
- Diary return and patient compliance
- AE assessments

9.1.8 Visit 9 (Week 24 ± 7 days)

Visits 9 will take place 24 weeks after the Randomization visit, with a window of ± 7 days. Subjects will be instructed to take the morning dose at the site in case of pre-dose PK analysis.

The following assessments will be performed:

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight
- 12-lead ECG
- Blood collection for:
  - hematology
  - blood chemistry
  - coagulation tests (PT and aPTT)
  - thyroid function tests (TSH, fT3, fT4)
  - PK analysis
  - Biomarker evaluation
- Urine analysis
- 6MWT + MFM
- Muscle strength test (knee extension and elbow flexion by HHM)
- Time function tests
- Dispensation and accountability of study treatment
- AE assessments
- Diary return and patient compliance

9.1.9 Visit 10 (Week 36 ± 7 days)

Visits 10 will take place 36 weeks after the Randomization visit, with a window of ± 7 days.

The following assessments will be performed:

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weigh
- 12-lead ECG
- Blood collection for:
  - hematology
  - blood chemistry
  - coagulation tests (PT and aPTT)
  - thyroid function tests (TSH, fT3, fT4)
- Urine analysis
- 6MWT + MFM
- Muscle strength test (knee extension and elbow flexion by HHM)

- Timed function tests
- Dispensation and accountability of study treatment
- AE assessments
- Diary return and patient compliance

9.1.10 Visit 11: End of Study Visit (EOS, Week 48 ± 7 days) and early withdrawal visit

The EOS (Visit 11) will be scheduled at the end of treatment (i.e., 48 weeks after the Randomization Visit, ± 7 days). Patients will be instructed to take the morning dose at the site in case of pre-dose PK analysis.

Patients who prematurely withdraw from the study for any reason will undergo an Early Withdrawal Visit within four weeks after the last dose of study treatment. The Early Withdrawal Visit requirements are the same as those of the EOS Visit described in this section.

The following assessments will be performed during this visit:

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- Weight
- Height
- 12-lead ECG
- Echocardiogram
- Pulmonary function tests: FEV1, FVC, FEV1/FVC, and PEF
- Blood collection for
  - hematology
  - blood chemistry
  - coagulation tests (PT and aPTT)
  - thyroid function tests (TSH, fT3, fT4)
  - PK analysis
  - Biomarker analysis
- Urine analysis;
- 6MWT + MFM
- Muscle strength test (knee extension and elbow flexion by HHM)
- Timed function tests
- Quality of Life test (assessed by SF-36)
- AE assessments

- Diary return and patient compliance
- MRI/MRS and muscle biopsy evaluations, which may be performed on different days (in case of early withdrawal visit the MRI/MRS and Quality of Life test do not have to be performed). Treatment is to continue up to the last assessment.

9.1.11 Follow-up Visit (Week 52) (± 7 days)

The follow-up visit will be performed 4 weeks after the last dose is administered (± 7 days).

The following assessments will be performed:

- Concomitant medications
- Physical examination
- Vital signs (blood pressure, heart rate and body temperature)
- 12-lead ECG
- Blood collection for
  - hematology
  - blood chemistry
  - coagulation tests (PT and aPTT)
  - thyroid function tests (TSH, fT3, fT4)
- Urine analysis
- AE assessments

9.1.12 Unscheduled Visits

The Investigator may, at his/her discretion, arrange for a patient to have an unscheduled assessment, especially in case of AEs or abnormal cardiac/blood test results that require follow-up or an AE considered by the Investigator to be possibly related to the use of the study treatment. Additional blood draws for safety may also be performed by a study nurse at the patient's home. The unscheduled visit page in the eCRF must be completed in these cases.

9.2 Study Conclusion

The end of the trial is defined as the date of the last visit of the last patient undergoing the trial.

10 EFFICACY ASSESSMENTS

10.1 Primary Efficacy Assessment

The primary efficacy assessment for this study is the total fibrosis (%) assessed through histological examination of muscle biopsies.

10.2 Secondary Efficacy Assessments

The secondary efficacy assessments are the following:

- Fat fraction of vastus lateralis and soleus evaluated by MRS technique
- Fat fraction of pelvic girdle and lower limb muscles evaluated by Dixon MRI technique
- Cross Sectional Area (CSA) of pelvic girdle and lower limb muscles by means of Dixon MRI technique.
- Other biopsy histological parameters (CSA, MFAF, regenerative fibers).
- Motor Function Measurement (MFM)

The Motor Function Measure (MFM) is a tool designed for neuromuscular diseases and is applicable to all degrees of disease severity. It was validated in terms of reproducibility, construct validity, and concurrent validity. The MFM consists of 32 items (tasks) classified into the following three dimensions: D1, standing and transfers; D2, axial and proximal motor capacity; and D3, distal motor capacity. Each item is scored on a four-point Likert scale. The generic grading is measured as follows: 0, cannot initiate the task or cannot maintain the starting position; 1, partially performs the task; 2, performs the task with compensatory movements (position maintained for an insufficient period of time, slowness, uncontrolled movements, etc.); and 3, performs the task fully and 'normally', the movement being controlled, mastered, directed, and performed at a constant speed (vuillerot et al. 2010).

- Time Function Tests (TFT)
  - Time to rise from floor
  - Run/walk 10 m
  - Time to climb 4 standard steps

The TFTs will be performed in a standardized manner described in a specific site manual.

- Six Minute Walk Test (6MWT)

The 6MWT will be performed in a standardized manner described in a specific site manual.

- Muscle strength evaluated by knee extension, elbow flexion as measured by Hand-Held Myometry (HHM).

Using HHM, the muscle strength of the knee extensor and elbow flexor will be measured following standardized procedures; bilateral assessments will be done, and 3 measurements will be recorded from each muscle group on each side.

- Quality of life (assessed by SF36)

The SF-36 is a set of generic, coherent, and easily administered quality-of-life measures. These measures rely upon patient self-reporting and are now widely utilized by managed care organizations for routine monitoring and assessment of care outcomes in adult patients.

All the functional and strength assessments will be evaluated by qualified functional evaluators (i.e., physiotherapists) who will be different from the site personnel who review safety results. The safety results must not be shared with physiotherapists.

The MRI/MRS personnel involved will be different from the site personnel. They will not review safety results; safety results must not be shared with them. The exams will be performed in a standardized manner described in a specific site manual.

11 PHARMACOKINETICS AND BIOMARKERS

11.1 Pharmacokinetic Sampling

11.1.1 Blood Samples

Blood samples for PK analysis of givinostat and its metabolites will be collected as indicated in the Schedule of Assessments (Table 5). There are 4 visits where the PK samples can be taken: Visits 5 (Week 4), 8 (Week 12), 9 (Week 24) and 11 (Week 48).

All patients will have a total of 6 PK blood specimens drawn during the study:

- Two samples drawn pre-dose, these 2 specimens must be drawn at 2 different visits;
- One sample drawn between 0 and 2 hours post-dose;
- One sample drawn between 2 and 4 hours post-dose;
- One sample drawn between 4 and 6 hours post-dose;
- One sample drawn between 6 and 10 hours post-dose.

During Visits 5 (week 4), 8 (week 12), 9 (week 24) and 11 (week 48), at least one PK sample is collected for each patient. After randomization, site personnel will be informed at which time the PK samples will be drawn during these visits.

On the day when the pre-dose PK blood is drawn, patients should be instructed to take the morning dose at the site and provide the date and time of the last evening dose from the day before the visit, which will be recorded in the source documents and eCRF together with the date and time of the blood draws prior to the morning intake of the compound.

On the days that post-dose PK samples are obtained, the morning dose may be taken at home, but patients should be reminded to remember the time the dose was taken. For the post-dose blood draws, date and time of the dose prior to the blood draws and the date and time of blood draws will be recorded in the source documents and eCRF. The date and time of the first dose will be recorded in the source documents and eCRF for each patient.

If the patient is withdrawn from the study prematurely, if not all PK samples were obtained prior to early withdrawal, the remaining PK samples may be drawn during the Early Withdrawal Visit to obtain all required PK samples.

Specific details regarding collection, handling, processing, storage, and shipment of PK samples can be found in a separate laboratory manual (found in the relevant section of the Investigator's Folder).

11.2 Pharmacokinetic Analytical Methodology

The concentration of givinostat and its main metabolites will be determined from the plasma samples using a validated analytical method. Details of the method validation and sample analysis will be included in the final Clinical Study Report (CSR).

11.3 Biomarkers

A blood sample will be collected during the study for LTBP4 and Osteopontin genotyping. Patients who have already concluded the study may be asked to return to the center for a blood sample collection

Blood sample collection for serum circulating proteins (by an ELISA based system) will be collected at randomization, 6 months and at the end of treatment.

12 SAFETY ASSESSMENTS

Safety and tolerability will be evaluated by monitoring hematology and blood chemistry, coagulation, urinalysis, vital signs, physical examinations, weight, height, echocardiogram, ECG and respiratory function evaluation to be performed at protocol-specified visits, as specified in the Schedule of Assessments, Table 5, and by recording AEs occurring throughout the study.

Additional unscheduled visits with a cardiologist may be required at the discretion of study clinicians for patients presenting clinical or instrumental suspicion of cardiac abnormalities such as signs and symptoms of heart failure or new alterations on routine ECG. Moreover, additional safety assessments (blood tests) may be required at the discretion of study clinicians and will be performed at the patient's home by a qualified nurse trained on the study protocol.

12.1 Vital Signs, weight and height

Vital signs (body temperature, heart rate, systolic and diastolic blood pressure measurements, will be measured after the patient has been resting in a sitting position for at least 5 minutes. Blood pressure measurements are to be taken in the same arm for the duration of the study. Body weight (without shoes) and height (without shoes) will be recorded at the visits indicated in the Schedule of Assessments, Table 5.

Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out-of-range blood pressure or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements must be recorded as an AE.

12.2 Physical Examination

A complete physical examination (head, eyes, ears, nose, and throat, heart, lungs, abdomen, skin, lymph nodes, extremities and nervous system) will be performed at Screening (Visit 1) by a physician. In addition, medical history will be recorded at screening.

A limited physical examination to verify continued patient eligibility and to follow up on any change in medical history will be performed at the visits indicated in the Schedule of

Assessments, Table 5. All changes not present at baseline or described in the medical history and identified as clinically noteworthy must be recorded as AEs.

12.3 Electrocardiogram

A 12-lead resting ECG will be obtained at the visits indicated in the Schedule of Assessments, Table 5.

At the Screening Visit, the Investigator will examine the 3 ECG traces for signs of cardiac disease that could exclude the patient from the study.

An assessment of normal or abnormal will be recorded. If the ECG is considered abnormal, the abnormality will be documented on the eCRF. If an ECG demonstrates a prolonged QTcF interval (≥450 ms), the Investigator will obtain 2 more ECGs over a brief period (5 minutes between recordings), and then use the averaged QTcF value of the 3 ECGs. If the averaged QTcF value meets the stopping criteria (>500 ms), the patient has to interrupt the study treatment, the Medical Monitor must be informed and the mean QTcF value must be confirmed by central reading.

The QTc has to be calculated with the Fridericia formula $QTc = QT/RR^{1/3}$.

Electrocardiograms will be repeated if clinically significant abnormalities are observed or artifacts are present.

12.4 Echocardiogram

Standard Echocardiograms will be conducted at the visits indicated in the Schedule of Assessments, Table 5, and more often if clinically indicated.

12.5 Pulmonary Function Test

Forced expiratory volume at 1 second (FEV_1), FVC, FEV_1/FVC, and PEF, will be collected as per the site's standard process at the visits indicated in the Schedule of Assessments, Table 5 and more often if clinically indicated.

At each assessment, three attempts with maximal effort will be evaluated for each patient, and an experienced technician performing the testing will determine if the effort is acceptable. All the study results will be recorded in the eCRF.

12.6 Laboratory Assessments

Laboratory assessment samples, Table 6, will be obtained at designated visits as detailed in the Schedule of Assessments, Table 5.

Table 6: Laboratory Assessment Samples

Hematology	Serum chemistry	Urine analysis (dipstick)	Coagulation	Serology
• Hematocrit (Hct) • Hemoglobin (Hb) • Mean corpuscular hemoglobin (MCH) • Mean corpuscular hemoglobin concentration (MCHC) • Mean corpuscular volume (MCV) • Platelet count • Red blood cell (RBC) count • White blood cell (WBC) count with differential	• Albumin • Alanine aminotransferase (ALT) • Alkaline phosphatase (ALP) • Amylase • Aspartate aminotransferase (AST) • Bicarbonate • Blood Urea Nitrogen (BUN) • Calcium • Chloride • Cystatin C • Creatinine • Creatine kinase • C-reactive protein (CRP) • Gamma-glutamyl transpeptidase (GGT) • Glucose • Lactate dehydrogenase (LDH) • NT-proBNP • Potassium • Phosphorus • Sodium • Total bilirubin • Direct bilirubin • Total protein • Thyroid-stimulating hormone (TSH) • Triglycerides • Total cholesterol • Low-Density Lipoprotein (LDL) • High-Density Lipoprotein (HDL) • fT3 (free T3 – the active part of triiodothyronine) • fT4 (free T4 – the active part of thyroxine) • Uric acid	• Appearance • pH • Protein • Glucose • Ketone bodies • Indicators of blood and WBCs • Specific gravity • Urobilinogen	• Prothrombin time (PT) • Activated partial thromboplastin time (PTT)	• CMV IgM • HBsAg • HCV • HIV • EBV IgG

Blood samples will be analyzed in a central laboratory, urinalysis by dipstick will be evaluated at site. All laboratory reports must be reviewed, signed and dated by the Investigator. A legible copy of all reports must be filed with the medical record (source document) for that visit. Any laboratory test result considered by the Investigator to be clinically significant should be considered an AE. Clinically significant abnormal values occurring during the study will be followed until repeat test results return to normal, stabilize, or are no longer clinically significant.

12.7 Adverse Events

12.7.1 Definitions

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)." Study treatment includes both the investigational drug under evaluation and placebo. Medical conditions that were present before starting study treatment are only considered AEs if they worsen after the subject has started the study treatment. Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, or require treatment. All medical and psychiatric conditions (except those related to the indication under study) present at screening will be documented in the medical history eCRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE eCRF page during the rest of the study. Clinically significant laboratory abnormalities should also be recorded as AEs. Surgical procedures that were planned before the subject enrolled in the study are not considered AEs if the conditions were known before study inclusion; the medical condition should be reported in the subject's medical history.

Adverse Drug Reaction:

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 an Adverse Drug Reaction (ADR)." The phrase "responses to a medical product" means that a

causal relationship between a medical product and an AE is at least a reasonable possibility, i.e., the relations cannot be ruled out.

Regarding marketed medicinal products, a well-accepted definition of an adverse drug reaction in the post-marketing setting is found in WHO Technical Report 498 [1972] and reads as follows: "A response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function."

Unexpected Adverse Drug Reaction - An unexpected ADR is an ADR, the nature or severity of which is not consistent with the applicable product information (i.e., Sections 7 and 8 of the current IB for ITF2357 Givinostat Hydrochloride Monohydrate).

Serious Adverse Event:

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 in-patient hospitalization or prolongation of 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 is at risk of death at the time of the event; it does not refer to an event that hypothetically may cause death if it is more severe.

In addition, any suspect of transmission of infective agents through study drug must be reported to the Sponsor as a SAE as medically significant event.

Other important medical events that may not be immediately life-threatening or result in death or hospitalization, based upon appropriate medical judgment, are considered SAEs if they are thought to jeopardize the subject and/or require medical or surgical intervention to prevent one of the outcomes defining a SAE.

The event does not qualify as an SAE if the hospitalization:

- was for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition e.g. seizure monitoring

- involved treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen
- was for general care, not associated with any deterioration in condition
- involved only treatment on an emergency, outpatient basis for an event not fulfilling any of the other definitions of serious and not resulting in hospital admission.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is referred to an ADR that complies with both the definitions of "serious" and "unexpected."

12.7.2 Adverse Event Reporting

The Investigators or their designees are requested to collect and assess any spontaneous AE reported by the patient and to question the patient about AEs and under current illnesses at each visit during the treatment period and any follow-up visit performed to monitor any drug-related AE that is still ongoing beyond the last scheduled visit until recovery. The questioning of the patient regarding AEs is generalized such as: "*How have you been feeling since your last visit?*"

Any AE occurring from the Informed Consent signature up to the first study drug intake will be recorded on the medical history section of the eCRF as baseline condition, while any AE occurring after a patient has taken the first study treatment up to the follow-up study visit, whether volunteered by the patient, 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 with the study drug
- action taken

The severity of an AE should be assessed and graded according to the most recently published National Cancer Institute Common Terminology Criteria for AE (CTCAE v. 4.03, 14 June 2010).

The relationship with the study drug should be assessed as:

- related to study drug;
- not related to study drug;
- unknown.

The assessment of the relationship of an adverse event with the administration of study drug is a clinical decision based on all available information at the time of the completion of the eCRF.

An assessment of "*Related*" indicates that there is a reasonable suspicion that the AE is associated with the use of the study drug.

An assessment of "*Not related*" would include the existence of a clear alternative explanation, or non-plausibility.

An assessment "*Unknown*" indicates there is not a reasonable suspicion that the AE is associated with the use of the study drug and at the same time there is not the existence of a clear alternative explanation or non-plausibility. In this case, the Investigator has to collect all possible information in order to assess the relationship with the study drug, particularly in case of SAEs.

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

- temporal sequence from study drug administration
- recovery on discontinuation and recurrence on reintroduction
- concomitant diseases
- evolution of the treated disease
- concomitant medication
- pharmacology and PK of the study drug
- presence of an alternative explanation

<u>Abnormal Laboratory Findings and Other Objective Measurements:</u>

Abnormal laboratory findings and other objective measurements should not be routinely captured and reported as AEs as they will be collected and analyzed separately in the eCRF. However, abnormal laboratory findings and other objective measurements that meet the criteria for an SAE, result in discontinuation of the study drug or require medical

intervention, or are judged by the Investigator to be clinically significant changes from baselines values should be captured and reported on the AE pages of the eCRF.

When reporting an abnormal laboratory finding on the AE pages of 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").

Baseline Medical Condition:

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 visits that worsen in severity or frequency during the study should be recorded and reported as AEs.

12.7.3 Serious Adverse Event Reporting

Any SAE, including death from any cause[1]t that occurs after patient/parent/legal guardian have signed the Informed Consent and up to the follow-up visit (regardless of relationship to study drug/comparator) must be reported by the Investigators to the Sponsor within 24 hours of learning of its occurrence.

Serious adverse event reports must be made whether or not the Investigator considers the event to be related to the investigational drug.

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.

As soon as an AE becomes serious, it will be recorded in the AE section of the eCRF and the electronic SAE tool will send the Investigator a warning to access the tool, which will send an email containing an SAE form in attachment. The Investigator is to complete the form and send it to the Pharmacovigilance Unit of the CRO via email using the contact details provided below. A paper SAE Form will be also available as backup and may be sent by fax.

[1] Death is an outcome and should not be an event unless the death is sudden and cannot be clearly attributed to any cause. The cause of a fatal SAE should be the condition that led to the subject's death.

Initial completion and follow-up reporting updates of the SAE are to be carried out by sending the SAE report directly to the CRO PHV via email.

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 [redacted] of the study or Clinical Research and Development Director, or his designee.

The Investigator must notify the CRO Pharmacovigilance of the SAE by completing the SAE reporting form within 24 hours of a SAE; then, only in case of SAE notification by fax (backup system) the Investigator must confirm any SAE notifications by mailing to the mail address or phoning to the phone number specified below:

[redacted]

If notification cannot be made via these means due to technical delivery problems, initial notification may be made by telephone, using the SAE [redacted]

The same procedure must be applied to the SAE follow-up information. Preliminary reports of SAEs must be followed by detailed descriptions, including clear and anonymized photocopies of hospital case reports, consultant reports, autopsy reports, and other documents when requested and applicable.

Appropriate remedial measures should be taken to treat the SAE and the response should be recorded. Clinical, laboratory and diagnostic measures should be employed as needed to determine the etiology of the problem. All SAEs will be followed until the Investigator and Sponsor agree the event is satisfactorily resolved.

Any SAE that is not resolved by the end of the study or upon discontinuation of the patient's participation in the study is to be followed until it either resolves, stabilizes, returns to baseline values (if a baseline value is available), or is shown to not be attributable to the study drug or procedures.

All serious and unexpected AEs that are associated with the use of the study drug (SUSARs) will be notified by the [redacted] Pharmacovigilance Unit to the Ethic Committees or Institutional Review Board and 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.

12.7.4 Overdose

In general, a drug overdose in a clinical study is defined as the accidental or intentional use of a drug or medicine in an amount exceeding the protocol defined dose. The Investigator must immediately notify the Sponsor of any occurrence of overdose with study drug. In this study, if an AE is associated with ("results from") the overdose of givinostat/placebo, the AE is reported as a SAE, even if no other criteria for seriousness are met.

If a dose of givinostat/placebo meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest, using the terminology "accidental or intentional overdose without adverse effect."

Any instance of overdose (suspected or confirmed, with and without an AE) must be reported to the Sponsor within 24 hours and, only in case of AEs, must be fully documented as a SAE. Details of any signs or symptoms and their management should be recorded in the SAE Form including details of any antidote or systematic treatment administered. Any signs or symptoms of over-dosage will be treated symptomatically.

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

12.7.5 Pregnancy reporting

Since it is possible to randomize fertile trial male patients in this trial, the patient must be informed by the investigator that if it is suspected that his partner becomes pregnant during the study treatment or within 3 months after the study treatment, he should inform the site personnel immediately of this pregnancy.

If the Investigator is made aware that the partner of a patient who is participating in the study becomes pregnant, he/she is required to report within 24 hours the pregnancy, using the Pregnancy Notification Form available in the Investigator Study File, to CRO Pharmacovigilance by mailing to the mail address or faxing numbers specified below:

[redacted]

If notification cannot be made via these means due to technical delivery problems, initial notification may be made by telephone, using the [redacted]

Whenever possible, such pregnancy should be followed until termination, any premature termination should be reported, and the status of the mother and child should be reported to the sponsor after delivery.

13 STATISTICAL ANALYSIS

A Statistical Analysis Plan (SAP) will be prepared prior to the planned interim analysis to provide full details on the methods described here and provide a complete description of the data presentations required for this study. Changes to the statistical analysis planned in this protocol will be described in the SAP along with the rationale for the changes.

Data collected in this study will be listed and summarized as described below.

Continuous data will be summarized by mean, standard deviation (SD), median, first and third quartiles, minimum and maximum. Categorical data will be presented by absolute and relative frequencies (n and %) or contingency tables.

All statistical tables, listings, figures and analyses will be performed by means of SAS® release 9.4 or later (SAS Institute, Inc., Cary, NC, USA).

Two-sided alpha level 0.05 will be considered. No alpha level adjustment will be carried out for primary and secondary outcome variables.

13.1 Determination of Sample Size

The hypothesis to be tested in this study is as follows:

$$H_0: \mu_G - \mu_P = 0 \quad vs \quad H_1: \mu_G - \mu_P \neq 0$$

where μ_G and μ_P the mean change from baseline to 12 months in total fibrosis (%) for the givinostat and placebo groups respectively.

A sample size of 48 patients with evaluable baseline biopsies (in a 2:1 ratio, 32 and 16 respectively) will provide 80% power to test the null hypothesis of no treatment effect (givinostat – placebo) on total fibrosis vs the alternative hypothesis that the treatment effect is $\geq$ 9% using a two-sided t-test with alpha level of 5% and assuming a common SD of 10% (with the SD being based upon blinded interim data from first 20 patients with valid pre and post-treatment biopsies).

Allowing for an approximate 5% of patients with unevaluable biopsies, the total number of patients to be randomized is 51 (i.e. 34 in givinostat arm and 17 in placebo arm).

13.2 Analysis Populations

13.2.1 Intent-To-Treat Analysis Set

The Intent-to-Treat (ITT) Analysis Set comprises all patients to whom study treatment has been assigned by randomization and who received at least one dose of study medication. According to the intent to treat principle, patients will be analyzed according to the treatment they have been assigned to during the randomization procedure. The ITT set will serve as the basis for the analysis of efficacy.

13.2.2 Per Protocol Set

The Per-Protocol Set (PPS) consists of a subset of patients in the ITT who had no major protocol deviation. Protocol deviations leading to exclusion from the PPS will be defined in a separate document. The PPS will serve as supportive analysis of the primary endpoint.

13.2.3 Safety Set

The Safety Analysis Set consists of all patients who received at least one dose of study medication. Patients who have been randomized and did not take at least one dose of study medication will not be included in the safety set. Patients will be analyzed according to the study treatment they actually received. This analysis set will be used for the analysis of safety.

13.2.4 Pharmacokinetic Set

Pharmacokinetic analyses will be performed on the Pharmacokinetic Set (PK), defined as all randomized patients who have received at least one dose of study treatment and have at least post-baseline PK sample.

13.3 Demographic and Baseline Characteristics

Patient demographics and baseline characteristics will be summarized on the ITT, overall and by treatment group, by means of summary descriptive statistics.

A complete description of patient disposition will be provided, overall and by treatment group specifying the number of randomized patients, number of patients at each visit, and completed and discontinued patients and the reason for the discontinuation.

The analysis populations will be described and the reasons for excluding the patient from any analysis set will be provided with the number of protocol violators per each criterion.

Medical history data will be presented by MedDRA System Organ Class and Preferred Term.

13.4 Investigational Medicinal Products

Duration of exposure to study treatment, defined as the time elapsed from the date of the first treatment intake to the date of the last treatment intake, and daily dosage (mg) will be summarized. The number of patients with dose changes/temporary interruptions/permanent discontinuation will be presented along with the reasons for the dose change/interruptions/discontinuation. Analysis will be based on the safety set.

13.5 Prior and Concomitant Therapy

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

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

Prior and concomitant medications will be classified using the WHO-DRL drug dictionary (using the most recent version) and summarized overall by WHO Anatomical Therapeutic Chemical /ATC) Class and Preferred Term. All analyses will be based on the safety population.

13.6 Efficacy Analysis

13.6.1 Analysis of Primary Efficacy Endpoint

The primary efficacy analysis will be performed on the ITT set as primary analysis and on the PPS as supportive analysis and will be reported by planned treatment group.

The primary efficacy variable, the absolute change in mean total fibrosis (%) from baseline to endpoint after 12 months of therapy, as assessed by biopsy, will be analyzed on the original scale by means of an analysis of covariance (ANCOVA) model with baseline total fibrosis (%) value as covariate and treatment and concomitant steroid use at baseline as

independent class variables. Mean estimates will be provided together with their corresponding two-sided 95% confidence intervals. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals.

Multiple imputation methods will be applied for handling missing data. Further details will be provided in the Statistical Analysis Plan (SAP).

13.6.2 Analysis of Secondary Efficacy Endpoints

All secondary efficacy analyses will be performed based on the ITT set and will be reported by planned treatment group.

- Change in fat fraction of vastus lateralis and soleus after 12 months of therapy

The absolute change in fat fraction of vastus lateralis and soleus after 12 months of therapy, as assessed by Magnetic Resonance Spectroscopy (MRS), will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals

- Change in fat fraction of pelvic girdle and lower limb muscles after 12 months of therapy

The absolute change in fat fraction of pelvic girdle and lower limb muscles after 12 months of therapy, as assessed by Magnetic Resonance Imaging (MRI), will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of these variable will be assessed by check of ANCOVA model residuals

- Change in Cross Sectional Area (CSA) of pelvic girdle and lower limb muscles after 12 months of therapy

The absolute change in CSA of pelvic girdle and lower limb muscles after 12 months of therapy, as assessed by MRI, will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals

- Change in other histology parameters (i.e. Cross-Sectional Area (CSA), MFAF, regenerative fibers) after 12 months of therapy

The absolute change in other histology parameters after 12 months of therapy, as assessed by biopsy, will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of these variables will be assessed by check of ANCOVA model residuals.

- Change in MFM total score after 12 months of therapy

The absolute change in MFM total score after 12 months of therapy will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals.

- Change in Timed Function Tests (TFTs) (i.e. time to climb four standard steps, time to rise from floor and time to walk 10m) after 12 months of therapy

The absolute change in functional parameters after 12 months of therapy, as assessed by Timed Function Tests (TFTs), will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of these variables will be assessed by check of ANCOVA model residuals.

- Change in 6MWT score after 12 months of therapy

The absolute change in 6MWT score after 12 months of therapy will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals.

- Proportion of patients with <10% worsening in 6MWT score after 12 months of therapy

Worsening is defined as a reduction in 6MWT score after 12 months of therapy compared to baseline score. The average of the scores at screening and randomization will be used as baseline score. The proportion of patients with <10% worsening in 6MWT after 12 months of therapy will be compared between arms using a stratified Cochran Mantel-Haenszel (CMH) chi square test with a two-sided α=0.05 level. As for the primary endpoint, the stratification factor is concomitant steroid use at baseline. The proportion, along with its exact two-sided 95% CI, will be computed within each treatment group. A two-sided 95% CI for difference of proportion between the treatment groups will also be computed. In case of

patients with no 6MWT assessment at 12 months/EOS, the last available score will be used for the analysis.

- Proportion of patients who lose ability to rise from floor during the study

The proportion of patients who lose ability to rise from floor during the study, as assessed by TFT, will be compared between arms using a stratified Cochran Mantel-Haenszel (CMH) chi square test with a two-sided α=0.05 level. As for the primary endpoint, the stratification factor is concomitant steroid use at baseline. The proportion, along with its exact two-sided 95% CI, will be computed within each treatment group. A two-sided 95% CI for difference of proportion between the treatment groups will also be computed. In case of patients with no 6MWT assessment at 12 months/EOS, the last available score will be used for the analysis

- Proportion of patients who lose ambulation during the study

The proportion of patients who lose ambulation during the study, as assessed by 6MWT, will be compared between arms using a stratified Cochran Mantel-Haenszel (CMH) chi square test with a two-sided α=0.05 level. As for the primary endpoint, the stratification factor is concomitant steroid use at baseline. The proportion, along with its exact two-sided 95% CI, will be computed within each treatment group. A two-sided 95% CI for difference of proportion between the treatment groups will also be computed. In case of patients with no 6MWT assessment at 12 months/EOS, the last available score will be used for the analysis

- Change in muscle strength evaluated by knee extension and elbow flexion after 12 months of therapy

The absolute change in muscle strength evaluated by knee extension and elbow flexion after 12 months of therapy, as assessed by HHM, will be analyzed using an ANCOVA model similar to the one described for the primary endpoint. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals.

- Change in quality of life after 12 months of therapy

Summary descriptive statistics will be provided at each time point for the quality of life scores/domains as assessed by the SF-36 questionnaire.

13.7 Pharmacokinetic Analysis

PK analyses will be performed on the Pharmacokinetic Set to:

- describe the pharmacokinetics of givinostat and its major metabolites: ITF2374 and ITF2375 in the patient population;
- explore the relationships between metrics of exposure and efficacy/safety endpoints of givinostat.

Further details on the exploratory analyses will be provided in the pharmacokinetic analysis plan.

13.8 Safety Analysis

Safety analyses will be conducted on the Safety Set and will be reported by actual treatment group. No methodology for missing data handling will be applied for safety parameters.

- Adverse events

According to the onset date of the event, AEs will be defined as follows:

  - Treatment-emergent AEs, those events with an onset date on or after treatment initiation.
  - Non-treatment-emergent AEs, those events with an onset date between informed consent and treatment initiation.

Non-treatment-emergent AEs will only be listed for all screened patients.

The incidence of treatment-emergent AEs will be tabulated by MedDRA System Organ Class (SOC) and Preferred Term (PT). The incidence of TEAEs will also be summarized by system organ class, preferred term and severity (based on investigator's judgment).

The analysis by SOC and PT will be repeated for SAEs regardless of drug relationship, for drug-related SAEs, AEs with an outcome of death and AEs leading to permanent discontinuation of treatment. AEs for which relationship to study drug is not specified will be considered treatment related.

Deaths, other SAEs and AEs leading to permanent treatment discontinuation will also be listed.

- Laboratory parameters

The absolute value and the change from baseline will be analyzed for each laboratory parameter (separately for hematology and blood chemistry) by means of summary descriptive statistics at each time point. In addition, shift tables using the low/normal/high classification to compare baseline to the worst on-treatment value will be provided.

Listings of all laboratory data with values flagged to show the classifications relative to the laboratory normal ranges will also be generated.

- Other safety parameters

Summary descriptive statistics will be provided for vital signs, pulmonary function (including FVC, FEV1, FVC/FEV1, PEF), cardiac parameters evaluated by ECG and ECHO, physical examination (including weight and BMI).

13.9 Exploratory Analysis

Biomarkers

The genotype of LTBP4 and osteopontin and the serum circulating proteins, using an ELISA based system, will be assessed as potential biomarkers for BMD. Analyses to explore whether the effects of givinostat versus placebo administered chronically may be related to the type of LTBP4 or Osteopontin genotype will be done. Additional details will be provided in the SAP.

MRI biomarker

In order to explore disease-related MRI biomarkers, evaluations of additional muscle images parameters, as assessed by MRI, will be performed. Further details will be provided in a specific imaging protocol.

13.10 Interim Analyses

A blinded interim on the first 20 patients with valid biopsies was planned, with the goal to assess the variability assumed in the original power calculation. No formal interim efficacy analysis was (or is) planned in this study.

As planned, after the first 20 baseline biopsies were collected, a descriptive blinded interim analysis was performed to assess the variability of the original primary endpoint, i.e. CSA. The interim data showed that the mean and variability of the Cross Sectional Area (CSA)(mean: 4871.68 μm^2; min 1933.49 μm^2 ; max 9446.34 μm^2; and S.D.: 1904.86 μm^2) were approximately 4 fold (300%) higher than the Duchenne data that was used in the original calculation of the sample size (mean: 1191.09 μm^2, S.D.: 400.98 μm^2 in DSC/11/2357/43 study). As consequence of this interim analysis the protocol was amended (AMENDMENT No. 4) with % total fibrosis replacing CSA as the primary endpoint. The sample size was adjusted accordingly to ensure adequate power for the new primary endpoint proposed.

Additional details about methodology and the results are reported in the specific SAP and Statistical Report available as stand- alone documents.

Moreover, after study enrollment is completed and baseline data are collected for all subjects, a second blinded interim analysis will be performed to obtain a preliminary overview of the baseline patient characteristics. The results of this analysis will not be used to change the sample size and/or any endpoints.

14 STUDY MANAGEMENT

14.1 Approval and Consent

14.1.1 Regulatory Guidelines

The Investigator will ensure that this study is conducted in full conformity with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords greater protection to the individual.

The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline or with local law if it affords greater protection to the subject.

This study will be performed in accordance with EU and country regulation in force.

This study will also be carried out in accordance with SOPs of Italfarmaco S.p.A. and/or its designee.

The Investigator agrees, when signing the protocol, to adhere to the instructions and procedures described therein and thereby to adhere to the principles of GCP to which it conforms.

14.1.2 Independent Ethics Committees

The protocol, informed consent form, recruitment materials, and all participant materials will be submitted to the Independent Ethics Committee (IEC) for review and approval. This study will be undertaken only after approval of the protocol, informed consent form and all other materials described above has been obtained from the appropriate IEC and a copy of the signed and dated approval has been received by Italfarmaco S.p.A. The name and occupation of the chairman and the members of the IEC must be supplied to Italfarmaco S.p.A. or its designee. 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. At the completion or termination of the study, the Investigator must submit a close-out letter to the IEC and to Italfarmaco S.p.A.

14.1.3 Patient Informed Consent

Prior to the beginning of the study, the Principal Investigator (PI) must have IEC written approval/favorable opinion of the written Informed Consent Form and any other written information to be provided to the patient. The approved patient information letter/Informed

Consent Form must be filed in the study files (clinical Trial Master File [TMF] and Investigator File [IF]). For each study patient, a written Informed Consent Form will be obtained prior to any protocol related activities. Informed consent may be obtained from patients who are capable of providing consent. As part of this procedure, the PI 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 patient is aware of the potential risks, inconveniences, or adverse effects that may occur. Patients 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 patients.

Patients should be informed that they may withdraw from the study at any time. They will receive all information that is required by local regulations and ICH guidelines. The PI or a designated representative will provide the sponsor or its representative with a copy of the IEC approved ICF prior to the start of the study.

The ICF should be signed and dated by the patient and the Investigator on the same day. If the patient is 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 patient must receive a copy of the signed documents.

14.1.4 Discontinuation of the study by the Sponsor

The Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include the following:

- unsatisfactory patient enrolment
- inaccurate or incomplete quality or quantity of data recording
- incidence or severity of adverse drug reactions in this or other studies with study drug indicating a potential health hazard to patients
- poor adherence to protocol and regulatory requirements
- plans to modify or discontinue the development of the study drug

14.1.5 Data Handling

Data on patients collected on eCRFs during the trial will be documented in an anonymous fashion and the patient will only be identified by the patient number. All the information required by the protocol should be provided and any omissions require explanation. All eCRFs must be completed expeditiously after the patient's visit.

The Investigator must maintain source documents for each subject in the study. Data reported on the eCRF that are derived from source documents should be consistent with the source documents, or the discrepancies must be explained.

Clinical data will be entered on eCRFs, a 21 CRF Part 11 compliant, for transmission to the Sponsor. Data on eCRFs transmitted via the web-based data system must correspond to and be supported by source documentation maintained at the study site. All study forms and records transmitted to the Sponsor must carry only coded identifiers such that personally identifying information is not transmitted. The primary method of data transmittal is via the secure, internet-based EDC system. Access to the EDC system is available to authorized users via the study's Internet web site, where an assigned username and password are required for access.

Case Report Forms will be considered complete when all missing and/or incorrect data have been resolved.

14.2 Source Documents

Source documents contain all information in original records and certified copies of original records of clinical findings, observations, data or other activities in a clinical study necessary for the reconstruction and evaluation of the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

14.3 Record Retention

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 regulatory authorities.

The Sponsor and the Investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical study. However, the medical files of patients shall be archived in accordance with national law.

The content of the clinical trial master file shall be archived in a way that ensures that it is readily available and accessible, upon request, to the competent authorities.

Any transfer of ownership of the content of the clinical trial master file shall be documented.

Any alteration to the content of the Investigator file shall be traceable and the Investigator should take measures to prevent accidental or premature destruction of these documents.

14.4 Monitoring

The study will be monitored to ensure that it is conducted and documented properly according to the protocol, GCP, and all applicable regulatory requirements.

A site visit will be held prior to initiation of patient enrolment. The protocol, eCRFs, study drug supplies and relevant procedures will be explained to the Investigators and his/her staff in detail at the site visit. On-site monitoring visits will be made at appropriate times during the study.

Clinical monitors must have direct access to source documentation to check the completeness, clarity, consistency of the data recorded in the eCRFs for each patient and the adherence to the protocol and to GCP. The clinical monitors will also check the progress of enrolment and the handling of study medication to ensure that study medication is being stored, dispensed, and accounted for according to specifications.

The Investigator will make available to the clinical monitor source documents and medical records necessary to check the eCRFs. No information in these records about the identity of the patients will leave the study center. In addition, the Investigator and key trial personnel will work closely with the clinical monitor and, as needed, provide them appropriate evidence that the conduct of the study is being done in accordance with applicable regulations and GCP guidelines.

Monitoring standard procedures require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the

recording of primary efficacy and safety variables. The Investigator is responsible for completing the eCRFs expeditiously to capture all the relevant information, while the monitor is responsible for reviewing them and clarifying any data queries.

14.5 Quality Control and Quality Assurance

The Sponsor or its designee will perform the quality assurance and quality control activities of this study; however, responsibility for the accuracy, completeness, and reliability of the study data presented to the Sponsor lies with the Investigator generating the data.

The Sponsor will arrange audits as part of the implementation of quality assurance to ensure that the study is being conducted in compliance with the protocol, Standard Operating Procedures, GCP, and all applicable regulatory requirements. Audits will be independent of and separate from the routine monitoring and quality control functions. Quality assurance procedures will be performed at study sites and during data management to assure that safety and efficacy data are adequate and well documented.

A Regulatory Authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a Regulatory Authority, the Investigator must inform Italfarmaco S.p.A. immediately that this request has been made.

For laboratories handling clinical laboratory samples, the accreditation certificate and laboratory normal units and ranges must be provided to the Sponsor and/or CRO and must be updated as needed by each trial centre.

14.6 Protocol Amendment and Protocol Deviation

14.6.1 Protocol Amendment

Any change or addition to this protocol requires a written protocol amendment that must be approved by Italfarmaco S.p.A.

Amendments can be classified as substantial when they impact one of the following criteria:

- The safety or physical or mental integrity of the patient
- The scientific value of the study
- The conduct or management of the study
- The quality or safety of any IMP used in the study

Substantial amendments require the authorization to the Competent Authority and the positive opinion of the relevant IEC before implementation.

In case of urgent safety measures to protect the patient against any immediate hazard, these measures may be taken without prior authorization from the Competent Authority or favorable opinion of the IEC. In this case, the Competent Authority and IEC will be informed as soon as possible using the fastest means of communication followed by a written report.

Amendments classified as non-substantial require only notification to the IEC involved.

14.6.2 Protocol Deviations

Should a protocol deviation occur, the Sponsor must be informed as soon as possible. Protocol deviations and/or violations and the reasons they occurred will be included in the CSR. Reporting of protocol deviations to the IEC and in accordance with applicable Regulatory Authority mandates is an Investigator responsibility.

14.7 Ethical Considerations

This study will be conducted in accordance with the accepted version of the Declaration of Helsinki and/or all relevant federal regulations, as set forth in Parts 50, 56, 312, Subpart D, of Title 21 of the CFR, in accordance with European and country regulations in force and in compliance with GCP guidelines.

The IEC will review and approve this protocol, the informed consent form, the recruitment materials, and all participant materials.

14.8 Financing and Insurance

Prior to the study commencing, the Sponsor (or its designee) and the Investigator (or the institution, as applicable) will agree on costs necessary to perform the study. This agreement will be documented in a financial agreement that will be signed by the Investigator (or the institution signatory) and the Sponsor (or its designee).

The Investigator is required to have adequate current insurance to cover claims for negligence and/or malpractice. The Sponsor will provide insurance coverage for the clinical study as required by national regulations.

14.9 Publication Policy/Disclosure of Data and Confidentiality

The Investigator must assure that patients' anonymity will be maintained and that their identities will be protected from unauthorized parties. On eCRFs or other documents submitted to the Sponsor, patients should not be identified by their names, but by an identification code. The Investigator should keep an enrolment log showing codes, names and addresses.

By signing the protocol, the Investigator agrees to keep all information provided by Italfarmaco S.p.A. in strict confidence and to request similar confidentiality from his/her staff and the IEC. Study documents provided by Italfarmaco S.p.A. (protocols, IBs, eCRFs, and other material) will be stored appropriately to ensure their confidentiality. The information provided by Italfarmaco S.p.A. to the Investigator may not be disclosed to others without direct written authorization from Italfarmaco S.p.A., except to the extent necessary to obtain informed consent from patients who wish to participate in the study.

Both the use of data and the publication policy are detailed within the clinical study agreement. Intellectual property rights (and related matters) generated by the Investigator and others performing the clinical study will be subject to the terms of a clinical study agreement that will be agreed upon between the Institution and the Sponsor or their designee. With respect to such rights, the Sponsor or its designee will solely own all rights and interests in any materials, data, and intellectual property rights developed by Investigators and others performing the clinical study described in this protocol, subject to the terms of any such agreement. In order to facilitate such ownership, Investigators will be required to assign all such inventions either to their Institution or directly to the Sponsor or its designee, as will be set forth in the clinical study agreement.

Italfarmaco S.p.A assures that the key design items of the Protocol will be published in a publicly accessible database such as "Clinicaltrials.gov." Moreover, upon completion of the study and finalization of the study report, the results of this study will be submitted for publication or posted in a publicly accessible data base.

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16 APPENDICES

16.1 Combined list of drugs that prolong QT and/or cause torsades de Pointes (TDP)

Source: Arizona Center for Education and Research on Therapeutics. Link: http://www.crediblemeds.org/everyone/composite-list-all-qtdrugs/. List last revised: 14 March 2017. Accessed 27 March 2017.

CredibleMeds® has reviewed available evidence for the drugs on the following list and placed them in one of three designated categories: Known Risk of TdP (KR), Possible Risk of TdP (PR) or Conditional Risk of TdP (CR). The full description of these categories can be found on the CredibleMeds.org website.

Generic Name	Brand Name
Alfuzosin (PR)	Uroxatral
Amantadine (CR)	Symmetrel and others
Amiodarone (KR)	Cordarone and others
Amisulpride (CR)	Solian and others
Amitriptyline (CR)	Elavil (Discontinued 6/13) and others
Amphotericin B (CR)	Fungilin and others
Anagrelide (KR)	Agrylin and others
Apomorphine (PR)	Apokyn and others
Aripiprazole (PR)	Abilify and others
Arsenic trioxide (KR)	Trisenox
Artenimol+piperaquine (PR)	Eurartesim
Asenapine (PR)	Saphris and others
Astemizole (KR)	Hismanal
Atazanavir (CR)	Reyataz and others
Atomoxetine (PR)	Strattera
Azithromycin (KR)	Zithromax and others
Bedaquiline (PR)	Sirturo
Bendamustine (PR)	Treanda and others
Bendroflumethiazide or bendrofluazide (CR)	Aprinox
Bepridil (KR)	Vascor
Bortezomib (PR)	Velcade and others
Bosutinib (PR)	Bosulif
Buprenorphine (PR)	Butrans and others
Capecitabine (PR)	Xeloda
Ceritinib (PR)	Zykadia
Chloral hydrate (CR)	Aquachloral and others
Chloroquine (KR)	Aralen
Chlorpromazine (KR)	Thorazine and others
Cilostazol (KR)	Pletal

Generic Name	Brand Name
Ciprofloxacin (KR)	Cipro and others
Cisapride (KR)	Propulsid
Citalopram (KR)	Celexa and others
Clarithromycin (KR)	Biaxin and others
Clomipramine (PR)	Anafranil
Clozapine (PR)	Clozaril and others
Cocaine (KR)	Cocaine
Crizotinib (PR)	Xalkori
Cyamemazine (cyamepromazine) (PR)	Tercian
Dabrafenib (PR)	Tafinlar
Dasatinib (PR)	Sprycel
Degarelix (PR)	Firmagon
Delamanid (PR)	Deltyba
Desipramine (PR)	Pertofrane and others
Dexmedetomidine (PR)	Precedex and others
Diphenhydramine (CR)	Benadryl and others
Disopyramide (KR)	Norpace
Dofetilide (KR)	Tikosyn
Dolasetron (PR)	Anzemet
Domperidone (KR)	Motilium and others
Donepezil (KR)	Aricept
Doxepin (CR)	Sinequan and others
Dronedarone (KR)	Multaq
Droperidol (KR)	Inapsine and others
Efavirenz (PR)	Sustiva and others
Eribulin mesylate (PR)	Halaven
Erythromycin (KR)	E.E.S. and others
Escitalopram (KR)	Cipralex and others
Esomeprazole (CR)	Nexium and others

Generic Name	Brand Name
Ezogabine (Retigabine) (PR)	Potiga and others
Famotidine (PR)	Pepcid and others
Felbamate (PR)	Felbatol
Fingolimod (PR)	Gilenya
Flecainide (KR)	Tambocor and others
Fluconazole (KR)	Diflucan and others
Fluoxetine (CR)	Prozac and others
Flupentixol (PR)	Depixol and others
Fluvoxamine (CR)	Faverin and others
Foscarnet (PR)	Foscavir
Furosemide (frusemide) (CR)	Lasix and others
Galantamine (CR)	Reminyl and others
Garenoxacin (CR)	Geninax
Gatifloxacin (KR)	Tequin
Gemifloxacin (PR)	Factive
Granisetron (PR)	Kytril and others
Grepafloxacin (KR)	Raxar
Halofantrine (KR)	Halfan
Haloperidol (KR)	Haldol (US & UK) and others
Hydrochlorothiazide (CR)	Apo-Hydro and others
Hydrocodone - ER (PR)	Hysinglaâ„¢ ER and others
Hydroxychloroquine (CR)	Plaquenil and others
Hydroxyzine (CR)	Atarax and others
Ibogaine (KR)	None
Ibutilide (KR)	Corvert
Iloperidone (PR)	Fanapt and others
Imipramine (melipramine) (PR)	Tofranil
Indapamide (CR)	Lozol and others
Isradipine (PR)	Dynacirc

Generic Name	Brand Name
Itraconazole (CR)	Sporanox and others
Ivabradine (CR)	Procoralan and others
Ketanserin (PR)	Sufrexal
Ketoconazole (CR)	Nizoral and others
Lansoprazole (CR)	Prevacid
Lapatinib (PR)	Tykerb and others
Lenvatinib (PR)	Lenvima
Leuprolide (PR)	Lupron and others
Levofloxacin (KR)	Levaquin and others
Levomepromazine (KR)	Nosinan and others
Levomethadyl acetate (KR)	Orlaam
Levosulpiride (KR)	Lesuride and others
Lithium (PR)	Eskalith and others
Loperamide (CR)	Imodium and many other OTC and Rx brands
Melperone (PR)	Bunil and others
Mesoridazine (KR)	Serentil
Methadone (KR)	Dolophine and others
Metoclopramide (CR)	Reglan and others
Metronidazole (CR)	Flagyl and many others
Mifepristone (PR)	Korlym and others
Mirabegron (PR)	Myrbetriq
Mirtazapine (PR)	Remeron
Moexipril/HCTZ (PR)	Uniretic and others
Moxifloxacin (KR)	Avelox and others
Nelfinavir (CR)	Viracept
Nicardipine (PR)	Cardene
Nilotinib (PR)	Tasigna
Norfloxacin (PR)	Noroxin and others
Nortriptyline (PR)	Pamelor and others
Ofloxacin (PR)	Floxin
Olanzapine (CR)	Zyprexa and others
Omeprazole (CR)	Losec and others
Ondansetron (KR)	Zofran and others
Osimertinib (PR)	Tagrisso

Generic Name	Brand Name
Oxaliplatin (KR)	Eloxatin
Oxytocin (PR)	Pitocin and others
Paliperidone (PR)	Invega and others
Panobinostat (PR)	Farydak
Pantoprazole (CR)	Protonix and others
Papaverine HCl (Intra-coronary) (KR)	none
Paroxetine (CR)	Paxil and others
Pasireotide (PR)	Signifor
Pazopanib (PR)	Votrient
Pentamidine (KR)	Pentam
Perflutren lipid microspheres (PR)	Definity
Perphenazine (PR)	Trilafon and others
Pilsicainide (PR)	Sunrythm
Pimozide (KR)	Orap
Pipamperone (PR)	Dipiperon (E.U) and others
Posaconazole (CR)	Noxafil and others
Probucol (KR)	Lorelco
Procainamide (KR)	Pronestyl and others
Promethazine (PR)	Phenergan
Propofol (KR)	Diprivan and others
Prothipendyl (PR)	Dominal and others
Quetiapine (CR)	Seroquel
Quinidine (KR)	Quinaglute and others
Quinine sulfate (CR)	Qualaquin
Ranolazine (CR)	Ranexa and others
Rilpivirine (PR)	Edurant and others
Risperidone (PR)	Risperdal
Ritonavir (CR)	Norvir
Romidepsin (PR)	Istodax
Roxithromycin (KR)	Rulide and others
Saquinavir (PR)	Invirase(combo)
Sertindole (PR)	Serdolect and others
Sertraline (CR)	Zoloft and others
Sevoflurane (KR)	Ulane and others

Generic Name	Brand Name
Solifenacin (CR)	Vesicare
Sorafenib (PR)	Nexavar
Sotalol (KR)	Betapace and others
Sparfloxacin (KR)	Zagam
Sulpiride (KR)	Dogmatil and others
Sultopride (KR)	Barnetil and others
Sunitinib (PR)	Sutent
Tacrolimus (PR)	Prograf and others
Tamoxifen (PR)	Nolvadex(discontinued 6/13) and others
Telaprevir (CR)	Incivo and others
Telavancin (PR)	Vibativ
Telithromycin (PR)	Ketek
Terfenadine (KR)	Seldane
Terlipressin (KR)	Teripress and others
Terodiline (KR)	Micturin and others
Tetrabenazine (PR)	Nitoman and others
Thioridazine (KR)	Mellaril and others
Tiapride (PR)	Tiapridal and others
Tizanidine (PR)	Zanaflex and others
Tolterodine (PR)	Detrol and others
Torasemide (CR)	Demadex and others
Toremifene (PR)	Fareston
Trazodone (CR)	Desyrel (discontinued 6/13) and others
Trimipramine (PR)	Surmontil and others
Tropisetron (PR)	Navoban and others
Vandetanib (KR)	Caprelsa
Vardenafil (PR)	Levitra
Vemurafenib (PR)	Zelboraf
Venlafaxine (PR)	Effexor and others
Voriconazole (CR)	VFend
Vorinostat (PR)	Zolinza
Ziprasidone (CR)	Geodon and others
Zotepine (PR)	Losizopilon and others

16.2 Drugs known to be a substrate of OCT2 transporter

OCT2 transporter = Organic Cation Transporter 2

Source: http://www.straighthealthcare.com/organic-cation-transporter-2.html

Accessed March 27th, 2017.

Generic Name	Brand Names (Partial List)	OCT2 interaction
Amantadine		Substrate
Amiloride	Midamor®	Substrate
Cimetidine	Tagamet®	Substrate
Creatinine		Substrate
Dofetilide	Tikosyn®	Substrate
Dopamine		Substrate
Famotidine	Pepcid®	Substrate
Memantine	Namenda®	Substrate
Metformin	Glucophage®	Substrate
Oxaliplatin	Eloxatin®	Substrate
Pindolol	Visken®	Substrate
Procainamide		Substrate
Ranitidine	Zantac®	Substrate
Trimethoprim	Bactrim®	Substrate
Varenicline	Chantix®	Substrate

16.3 Drugs known to be P-glycoprotein inhibitors

Source: https://www.straighthealthcare.com/p-glycoprotein.html

Accessed July 26th, 2019.

Generic name	Brand names (partial list)
Amiodarone	Cordarone®
Atorvastatin	Lipitor®
Azithromycin	Zithromax®
Boceprevir	Victrelis®
Bromocriptine	Cycloset®, Parlodel®, etc.
Captopril	Capoten®
Carvedilol	Coreg®
Clarithromycin	Biaxin®
Cobicistat	part of Stribild®
Conivaptan	Vaprisol®
Cyclosporine	Neoral®, Gengraf®, Sandimmune®
Daclatasvir	Daklinza™
Diltiazem	Cardizem®, Cartia®, Dilacor®, Diltia®
Doxazosin	Cardura®
Dronedarone	Multaq®
Erythromycin	E.E.S®, Ery-tab®
Felodipine	Plendil®
Fluvastatin	Lescol®
Glecaprevir	Mavyret™
Indinavir	Crixivan®

Itraconazole	Sporanox®
Ketoconazole	Nizoral®
Ledipasvir	Harvoni
Linagliptin	Tradjenta®
Lopinavir and ritonavir	Kaletra®
Lovastatin	Mevacor®
Meperidine	Demerol®
Methadone	
Nelfinavir	Viracept®
Nicardipine	Cardene®
Paritaprevir	Viekira Pak™, Technivie™
Pentazocine	Talwin®
Pibrentasvir	Mavyret™
Progesterone	
Quercetin	supplement
Quinidine	
Ranolazine	Ranexa®
Reserpine	
Ritonavir	Norvir®
Saquinavir	Invirase®
Sarecycline	Seysara™
Simeprevir	Olysio®
Simvastatin	Zocor®
Suvorexant	Belsomra® - in vitro data

Tacrolimus	Prograf®
Tamoxifen	