[redacted]	Statistical Analysis Plan
	Sponsor: ITALFARMACO Protocol: DSC/15/2357/53

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

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Document History

Status and Version	Release Date	Change Description	Reason/Comment
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Authorization

The signatures on this page indicate review and approval of the Statistical Analysis Plan, version 1.0, dated April 27, 2021.

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LIST OF ABBREVIATIONS

6MWT	6 Minute Walking Test
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANCOVA	Analysis of covariance
aPPT	activated Partial Thromboplastin Time
AST	Aspartate Amino Transferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CMH	Cochran Mantel-Haenszel
CRP	C-Reactive Protein
CSA	Cross Sectional Area
DMD	Duchenne Muscular Dystrophy
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	electronic Case Report Form
eGFR	estimate Glomerular Filtration Rate
EOS	End Of Study
FEV1	Forced expiratory volume in 1 second
fT3	Free T3
fT4	Free T4
FVC	Forced Vital Capacity
GGT	Gamma-Glutamyl Transferase
HCT	Hematocrit
HDL	High-Density Lipoprotein
HHM	Hand-Held Myometry
Hb	Haemoglobin
ITT	Intention-to-Treat
LDH	Lactate Dehydrogenase

LDL	Low-Density Lipoprotein
LOCF	Last Observation Carried Forward
LSmeans	Least Square means
LVED	left ventricular end-diastolic
LVEDD	left ventricular end-diastolic internal dimension
LVEF	left ventricular ejection fraction calculation
LVES	left ventricular end-systolic
LVESD	left ventricular end-systolic internal dimension
LVOT	left ventricular outflow tract
LVS	left ventricular septal wall thickness
MCH	Mean corpuscolar hemoglobin
MCHC	Mean corpuscolar hemoglobin concentration
MedDRA	Medical Dictionary for Competent Activities
MFAF	Muscle Fibers Area Fraction
MFM	Motor Function Measurement
MMRM	Mixed Model Repeated Measures
MMT	manual muscle testing
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
PDHD	Protocol Deviation Handling Document
PEF	Peak Expiratory Flow
PK	Pharmacokinetic
PPS	Per Protocol Set
PR	Pulse Rate
PT	Preferred Term
PW	posterior wall
QT	QT interval
QTcF	QT interval, Fridericia's correction
RBC	Red Blood Cells
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error
SF-36	Short Form 36
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TFT	Timed Function Test
TSH	thyroid-stimulating hormone
WB	Western blot
WBC	White Blood Cell
WHO-DRL	WHO-Drug Reference List

1. INTRODUCTION

This document describes the statistical methods and outputs required for the Final analysis for the phase II clinical trial DSC/15/2357/53. Details of study conduct, and data collection can be found in the study protocol (version 5.0 amendment 4, dated June 17, 2020).

1.1. Changes from The Study Protocol

The following changes have been made to the statistical methodology planned in the study protocol:

- Total fibrosis (primary endpoint): the main analysis will be based on log-trasformed data. Sensitivity analyses will be performed on the original scale and also applying different multiple imputation approaches (both MAR and MNAR)
- MRI (key secondary endpoint): same analysis planned for the primary endpoint
- Other secondary endpoints: statistical analyses will be performed by means of Mixed Model for Repeated Measures instead of ANCOVA
- Total WB dystrophin 1 and Total WB dystrophin 2 will be analyzed
- Difference between biopsy sites at baseline will be evaluated for Total fibrosis, Total CSA and MFA%

2. STUDY OBJECTIVES AND ENDPOINTS

OBJECTIVES	VARIABLES	ENDPOINTS
Primary objective(s): Establish the histological effects of givinostat versus placebo administered over 12 months.	**Primary variable(s):** Fibrosis (%) assessed through histological examination of muscle biopsies	**Primary endpoint(s):** Mean change in mean total fibrosis (%) comparing the histology of muscle biopsies before and after 12 months of treatment with givinostat versus placebo.
Secondary objective(s): To establish the macroscopic muscle effects of givinostat versus placebo administered chronically over 12 months assessed by MRI/MRS.	**Secondary variable(s):** MRS variables: Fat Fraction in the vastus lateralis Fat Fraction in the soleus	**Secondary endpoint(s):** 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.

OBJECTIVES	VARIABLES	ENDPOINTS
To establish the other histological effects of givinostat versus placebo administered over 12 months.	MRI variables: Fat Fraction of the lower limb muscles (i.e. whole thigh, quadriceps, medial thigh, hamstrings, triceps surae and Pelvis Girdle) measured by Dixon technique	Mean change in fat fraction of lower limb muscles (i.e. whole thigh, quadriceps, medial thigh, hamstrings, triceps surae and Pelvis Girdle) comparing Magnetic Resonance Imaging (MRI) before and after 12 months of treatment with givinostat versus placebo.
	Cross-Sectional Area (CSA) and contractile CSA of lower limb muscles (i.e. whole thigh, quadriceps, medial Thigh, Hamstrings, Triceps surae muscles and Pelvis Girdle)	Mean change in CSA and contractile CSA of lower limb muscles comparing MRI before and after 12 months of treatment with givinostat versus placebo.
	Other histological parameters: - SLIDE I 1- MFA (%) 2- Adipose tissue (%) 3- Other histological structure (%) 4- Number of fibers with centralized nuclei 5- Total number of fibers counted in slide I - SLIDE II 1- Number of Regenerative fibers (MYH3) 2- Total number of fibers counted in slide II - SLIDE III 1- Cross-Sectional Area Type I fibers (CSAI) 2- Cross-Sectional Area Type II fibers (CSAI)	Mean change in other histology parameters comparing the histology biopsies before and after 12 months of treatment with givinostat.

OBJECTIVES	VARIABLES	ENDPOINTS
To establish the efficacy of givinostat versus placebo administered chronically over 12 months in slowing disease progression.	3- Total Cross-Sectional Area (CSAI and CSAII) 4- Total number of fibers counted in slide III 5- Fiber size variability - Dystrophin 1- Total WB Dystrophin 1 and Total WB Dystrophin 2 Motor Function Measurement (MFM): 1- Standing and transfers (D1) 2- Axial and proximal motor function (D2) 3- Distal motor function (D3) 4- Total score (Total mark) Time Function Tests (TFT): 1- 4-Stair ascent duration 2- Rising from floor duration 3- 10-Metre Walk/Run test duration Functional Tests: 1- Distance walked after 6 minutes (6MWT) 2- Patients with < 10% worsening in 6MWT	Mean change in 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 6MWT distance before and after 12 months of treatment with givinostat versus placebo. Number and proportion of patients with < 10% worsening in 6MWT at the end of study will be compared between treatment groups.

OBJECTIVES	VARIABLES	ENDPOINTS
	3- Patients who lose ambulation during the study (6MWT not done because patient unable to physically perform the test)	Number and proportion of patients who lose ambulation during the study will be compared between treatment groups.
	4- Patients who lose the ability to rise from floor (Rise From Floor grading = 0)	Number and proportion of patients who lose the ability to rise from floor (from baseline through end of study) will be compared between treatment groups.
	Muscle strength measured by Hand Held Myometry (HHM): 1- Left knee extension measurements 2- Right knee extension measurements 3- Left elbow flexion measurements 4- Right elbow flexion measurements.	Mean change in muscle strength (right/left knee extension and right/left elbow flexion) before and after 12 months of treatment with givinostat versus placebo.
To evaluate the impact of givinostat versus placebo administered chronically on quality of life and activities of daily living.	Quality of life test (SF-36): 1- Items 2- Domains: 1- Physical Functioning (PF) 2- Role-Physical (RP) 3- Bodily Pain (BP) 4- General Health (GH) 5- Vitality (VT) 6- Social Functioning (SF) 7- Role-Emotional (RE) 8- Mental Health (MH) 9- Total score	Mean changes in quality of life (SF-36) before and after 12 months of treatment with givinostat as compared to placebo.
	Adverse events	

OBJECTIVES	VARIABLES	ENDPOINTS
To assess the safety and tolerability of givinostat versus placebo administered chronically.		Number and proportion of patients experiencing treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) from Baseline through end of study (EOS). Summary of type, incidence, and severity of TEAEs and SAEs (Baseline through EOS).
	Vital Signs: 1- Height 2- Weight 3- Body mass index (BMI) 4- Sitting blood pressure systolic 5- Sitting blood pressure diastolic 6- Heart rate 7- Temperature	Changes from baseline to end of study of vital signs.
	Laboratory tests: 1- Biochemistry 2- Hematology 3- Coagulation 4- Urinalysis	Changes from baseline to end of study of clinical laboratory tests.
	Physical examination	Changes from baseline to end of study of physical examination.
	Pulmonary function: 1- Forced Vital Capacity (FVC) 2- Forced Expiratory - Volume at 1 second (FEV1) 3- FVC/FEV1 4- Peak Expiratory Flow (PEF)	Changes from baseline to end of study of pulmonary function.
	Cardiac function parameters	

OBJECTIVES	VARIABLES	ENDPOINTS
To evaluate the pharmacokinetic (PK) profile of givinostat administered chronically in the target population	1- ECG 2- ECHO Details will be provided in a specific PK protocol.	Changes from baseline to end of study of Cardiac function parameters. Summary of the PK of givinostat and its major metabolites.
Secondary Exploratory objective(s): To evaluate the correlation between the PK profile of givinostat and pharmacodynamics (PD) data.	**Exploratory variable(s):** Details will be provided in a specific Statistical Analysis Plan.	**Exploratory endpoint(s):** PK-PD correlation analyses between metrics of exposure and the efficacy/safety endpoints of givinostat.
To evaluate a possible disease-related biomarker - Genetic:	LTBP4 and osteopontin genotype	Analyses to explore whether the effects of givinostat versus placebo administered chronically may be related to the type of LTBP4 or osteopontin genotype.
- Serum	Serum Biomarkers. Details will be provided in a specific Statistical Analysis Plan.	Evaluation of serum circulating proteins as potential biomarkers for BMD.
To explore additional disease-related MRI biomarkers.	Details will be provided in a specific imaging protocol. Details will be provided in a specific Statistical Analysis Plan.	Additional evaluations of other muscle image parameters assessed by MRI.

OBJECTIVES	VARIABLES	ENDPOINTS
To evaluate the functional tests velocity of givinostat versus placebo.	Velocity for each functional test will be computed from the following variables: 1- 4-Stair ascent 2- Rising from floor 3- 10-Metre Walk/Run test	Mean change in Time Function Tests' velocities before and after 12 months of treatment with givinostat versus placebo.

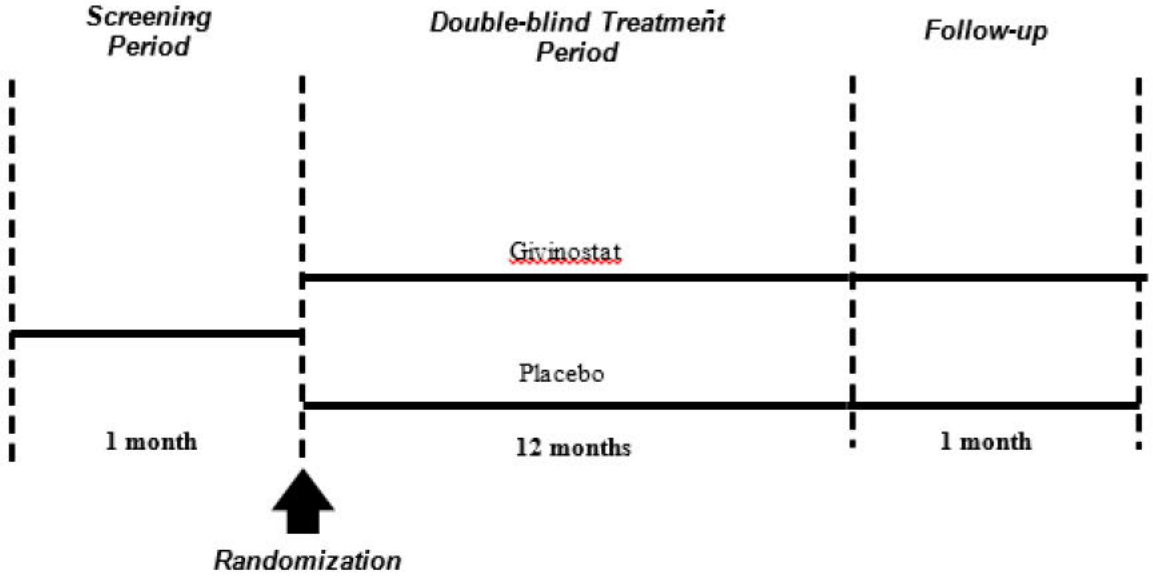
3. BACKGROUND AND RATIONALE

3.1. Overall Study Design and Plan Description

This is a phase 2, randomized, 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 51 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 the concomitant steroids 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 Figur.

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.

3.2. Selection of Study Population

The study will enroll adult male patients with an established genetic diagnosis of Becker Muscular Dystrophy. A complete list of all inclusion and exclusion criteria is provided in Sections 7.1 and 7.2 of the study protocol. Stopping rules and withdrawal reasons are described in Section 7.3.1 of the same protocol.

3.3. Treatment

3.3.1. Treatment Administered

Givinostat or placebo oral suspension (10 mg/mL) is administered twice daily (bid) (in a fed state) as indicated in the table below:

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	4.0	4.7

The dosage to be administered is based on patient weight because the weight significantly affects the givinostat clearance.

Additional information on "Dose Modifications" can be found in the study protocol section 8.2.2.

3.3.2. Method of Assigning Patients to Treatment Group

TREATMENT RANDOMIZATION

Approximately 51 eligible patients will be randomized in a 2:1 ratio to be treated with givinostat or placebo for a period of 12 months. The randomization process has been stratified by the concomitant steroids use at baseline (yes vs. no).

BIOPSY SITES RANDOMIZATION

At Visit 1, after having filled in the "Eligibility criteria" eCRF page, eligible patients will be randomized to one of the two following sequences to assign to the patient:

- V2: Right Arm - V11: Left Arm
- V2: Left Arm - V11: Right Arm

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

PHARMACOKINETIC (PK) SAMPLING RANDOMIZATION

Blood samples for PK analysis of Givinostat and its metabolites should be collected at 4 visits: 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.

Eight sequences of combinations will be randomly selected from all the possible ones and each sequence of combinations will be randomly allocated to randomized patients in a ratio of 2:1 (Givinostat vs. Placebo).
PK randomization will be stratified according to the treatment group assigned at randomization.

3.3.3. Prior and Concomitant Therapy

A complete list of all prohibited medication or concomitant therapy requiring caution is provided in section 8.7.2 of the study protocol.

3.4. Schedule of Time and Events

The detailed Schedule of Assessments (SoA) is provided in sections 9 of the study protocol.

3.5. Sample Size and Power Estimation

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.

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 so the primary efficacy variable of the study is the mean change from baseline to 12 months in total fibrosis (%).

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 ≥ 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).

After the first 20 baseline muscle biopsies was collected, an interim blinded sample size reassessment was undertaken to check the SD assumption used in sizing the trial. Details are capture in the specific SAP for the Interim Analysis.

After study enrollment was completed and baseline data were collected for all patients, a second blinded interim analysis was performed to obtain a preliminary overview of the baseline patient characteristics.

4. DEFINITIONS AND GENERAL METHODOLOGY

4.1. General Methodology

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.

4.2. Definitions

Definition of Screening Failures
Screening failures are defined as patients who consent to participate in the clinical trial but are not subsequently randomly assigned to the study treatments.

Definition of Baseline

For all evaluations, the last available assessment collected during the screening phase or before the first study treatment dose, is considered as baseline assessment, with the exception of 6 Minute Walking Test whereby, if more than one value is recorded for the baseline, the mean value will be taken.

First/Last Administration of Study Treatment
The date of first administration of study treatment is the earliest start date when study treatment was administered and recorded on the "IMP Dosage" eCRF page.

The date of last administration of study treatment is the last end date when study treatment was administered and recorded on the "IMP Dosage" eCRF page.

Study day

Study day is defined as the number of days from the study reference date to the visit/event date.

It is calculated as:

Study day = Visit/event date - Reference date + 1 day

If the visit/event date is before the reference date, then the study day is calculated as follows:

Study day = Visit/event date - Reference date

The reference day is used to calculate the "Study day" variable it is defined as the date of the first drug administration. If this date is missing or not done, then the reference date will be null, and the study day will be not calculated.

4.3. Coding of Therapies and Medical Terms

Medical history and Adverse Events will be coded using the last available version of MedDRA; prior and concomitant medications will be coded using the last available version of WHO Drug Dictionary.

4.4. Handling of Drop-Outs or Missing Data

4.4.1. Missing or Partial Dates

The following imputation rules will be applied in case of partially missing information:

- Day missing => day will be replaced with 15
- Day and month missing => day and month will be replaced with July 1st
- Day, month and year missing => no imputation will be done.

Additional imputation rules for partial dates:

Prior, concomitant and steroid medications

- Start date
  When only the start day is missing, the last day of the month is imputed.
  When both day and month are missing, January 1st will be imputed.
  If day, month and year are missing no imputation will be done but medication will be assumed to be started before first intake.

- End date
  When only the end day is missing, the last day of the month is imputed.
  When both day and month are missing, December 31st will be imputed.

If day, month and year missing no imputation will be done and medication will be assumed as concomitant medications.

Diagnosis date

When only the day is missing, the first day of the month is imputed.
When both day and month are missing, January 1st will be imputed.

No imputation will be done if day, month and year are missing.

4.4.2. Handling of Missing Data/Imputation/Censoring Rules

For the primary efficacy endpoint, defined as mean fibrosis % from baseline to 12 months of therapy, as well as for the key secondary endpoint (MRI) different multiple imputation approaches will be applied to handling missing data. Further details on the missing imputation strategies are provided in Section 6.3.1 of the present document for the primary efficacy endpoints, and in Section 6.3.2.2 and 6.3.2.3 for the key secondary endpoints.

Results for the other efficacy and safety endpoints will be based on non-missing data only (i.e. missing observations will not be replaced).

4.4.3. Handling of Drop-Out Patients

Patients will be included in each analysis based on available assessments. Sensitivity analyses will be performed in order to evaluate the influence of missing data: further details on the missing imputation strategies are provided in Section 6.3.1 of the present document for the primary efficacy endpoints, and in Section 6.3.2.2 and 6.3.2.3 for the key secondary endpoints.

5. ANALYSIS POPULATIONS

On the basis of the study protocol, the following analysis populations will be defined:

- **Intent-To-Treat Analysis (ITT) Set:** it 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.
- **Per Protocol (PP) Set:** it 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 PP set will serve as supportive analysis of the primary endpoint.

- **Safety (SAF) Set:** it 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.
- **Pharmacokinetic (PK) Set:** it consists of all randomized patients who have received at least one dose of study treatment and have at least post-baseline PK sample.

6. STATISTICAL METHODOLOGY

6.1. Study Patients

A complete description of screening disposition will be performed overall on screened patients (i.e. patients who provided a valid Informed Consent Form) summarizing the number of patients who complete the screening phase and the primary reason for screening failure.

A description of patients' disposition will be also provided on enrolled patients (i.e. screened patients who completed the screening phase), specifying the number of randomized patients, the number of patients at each visit, the number of completed and discontinued patients and the reason for the discontinuation.

The analysis populations will be described and the reasons for excluding a patient from any particular population will be provided with the number of protocol deviators per each criterion. Protocol deviations, including COVID-related protocol deviations, will be summarized by treatment for the randomized patients.

Non-protocol deviations will be also summarized on randomized patients. The list of Non-protocol deviations for this study is listed in Table 6.1-1:

Table 6.1-1: List of non-protocol deviations

Deviation code	Description	Exclusion from analysis population
NOPD01	Patient who did not take at least one dose of the study treatment	ITT/PP/SAF/PK

NOPD = Non-protocol deviations.

6.2. Background and Demographic Characteristics

All data about patient demographics and baseline characteristics will be summarized on the ITT and PP set overall and by treatment group by means of summary descriptive statistics.

Demographic characteristics will include sex, age, ethnicity and race. The patient's age will be computed as the time in years between the date of birth to the date of the informed consent signature.

Baseline characteristics will include time from diagnosis, DMD mutation (deletion, duplication, point mutation) and Mutated exon(s) data (Exon 45, Prior to exon 45, Post-exon). Time from diagnosis to informed consent will be computed as years elapsed from diagnosis to informed consent, plus one day.

Medical history data will be presented as the number and percentages of patients with at least one pathology. Condition will be presented by System Organ Class (SOC) and Preferred Term (PT) according to the MedDRA dictionary.

6.3. Efficacy Evaluation

The efficacy analyses will be performed on patients valid for the ITT population and will be presented by planned treatment group. The primary efficacy endpoint will be also analyzed on the PP population for supportive purpose.

6.3.1. Primary efficacy analysis

Definition of primary endpoint

The primary endpoint is the mean change in total fibrosis comparing the histology of muscle biopsies before and after 12 months of treatment with givinostat versus placebo. The primary efficacy assessment for this study is the Total Fibrosis assessed through histological examination of muscle biopsies. A muscle biopsy was collected at the beginning and at the end of treatment to calculate the mean change from baseline to 12 months.

For each patient, the percentage of total fibrosis will be calculated as a mean of available fields at each evaluation.

Analysis methodology

The primary hypothesis to be tested is that total fibrosis (%) will increase less in the givinostat group than in the placebo group.

Since the blinded data at the time of the sample size re-estimation indicated the primary efficacy variable to be non-Normally distributed, the change in mean fibrosis from baseline to endpoint after 12 months of therapy will be analyzed on the log scale. Therefore, mean fibrosis at baseline and Visit 11 will be log transformed prior to analysis. An analysis of covariance (ANCOVA) model will be fitted to the data with the difference between log visit 11 and log baseline values as the dependent variable; log baseline value will be included as covariate and treatment and concomitant steroid use at baseline as independent class variables. LSmean values for the log change from baseline to visit 11 will be extracted, together with the associated log scale SEs; the difference in LSmean values will also be extracted as the measure of treatment effect along with corresponding log scale SE and two-

sided 95% Confidence Interval (CI). LSmean values, the difference in LSmean values and the associated CI will be back transformed to the ratio scale for the purpose of presentation.

Summary descriptive statistics (N, geometric mean, %CV, mean, median, SD, min and max) for total fibrosis value and change versus baseline, at each time point, will be provided.

A Mixed Model will be fitted to the data with the difference between log visit 11 and log baseline values as the dependent variable; log baseline as a covariate, treatment will be included as independent class variables and treatment by sequence interaction. The sequence of biopsy site (Right-Left, Left-Right) will be included as a fixed effect and subject as a random effect to evaluate the difference in Total Fibrosis between biopsy sites.

If the treatment by sequence interaction is p>0.10, then the model without the interaction term will be presented.

Data handling rules

Primary analysis will be based on observed data only.

Sensitivity analysis for the primary endpoint will be performed as follow:

1. Multiple imputation for missing data:
    a. Missing baseline primary endpoint data will be imputed as the geometric mean of all non-missing baseline primary endpoint values across the randomized treatment groups. Visit 11 missing data will be imputed (on the log scale) under a Missing At Random (MAR) assumption using SAS procedure MI within each treatment group using distribution implied by the non-missing patient data for that treatment group. A total of 20 imputation runs will be performed. Post imputation, each imputed dataset will be analysed separately exactly as described for primary efficacy analysis. The SAS procedure MIANALYZE will then be used to combine the 20 datasets of estimates by Rubin's rules. The resulting multiply imputed means and difference in means will be presented, along with the associated SEs, 2-sided 95% CIs and 2-sided p-values.

    b. Missing baseline primary endpoint data will be imputed as the geometric mean of all non-missing baseline primary endpoint values across the randomized treatment groups. Visit 11 missing data will be imputed (on the log scale) under a Missing Not At Random (MNAR) assumption using SAS procedure MI for both randomized treatment groups using the distribution implied by the non-missing patient data within the placebo group. A total of 20 imputation runs will be performed. Post imputation, each imputed dataset will be analysed separately exactly as described for primary efficacy analysis. The SAS procedure MIANALYZE will then be used to combine the 20 sets of estimates by Rubin's rules. The

resulting multiply imputed means and difference in means will be presented, along with the associated SEs, 2-sided 95% CIs and 2-sided p-values.

2. An analysis will be conducted as per the primary analysis (including multiple imputations), but on the non-log transformed data.
3. Both the log scale and non-log scale analyses of the primary endpoint (excluding the multiple imputations) will be repeated using the PP Set.

6.3.2. Secondary efficacy analysis

6.3.2.1. Magnetic Resonance Spectroscopic (MRS)

Definition

MRS variables will include:

- Fat Fraction in the vastus lateralis muscles
- Fat Fraction in the soleus

Analysis methodology

The absolute value of fat fraction of vastus lateralis and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics.

The absolute change in fat fraction of vastus lateralis after 12 months of therapy, as assessed by MRS, will be analyzed using an ANCOVA model with baseline fat fraction of vastus lateralis value as covariate and treatment and concomitant steroid use at baseline as independent class variables.

Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. If considered non-Normal, a supportive analysis will be performed on the log scale.

The same analyses will be done for the fat fraction of soleus.

Data handling rules

The analysis will be based on observed data only.

6.3.2.2. Magnetic Resonance Imaging (MRI)

Definition

MRI variables will include:

- Fat Fraction (%) in the lower limb muscles (i.e. whole thigh, quadriceps, medial Thigh, Hamstrings, Triceps surae muscles and Pelvis Girdle)
- Cross-sectional area and contractile area in the lower limb muscles (i.e. whole thigh, quadriceps, medial Thigh, Hamstrings,Triceps surae muscles and Pelvis Girdle)

Analysis methodology

The absolute value of fat fraction of lower limb muscles and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics.

The absolute change in fat fraction of lower limb muscles after 12 months of therapy, as assessed by MRI, will be analyzed using an ANCOVA model with baseline fat fraction of lower limb muscles value as covariate and treatment and concomitant steroid use at baseline as independent class variables. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residual. If considered non-Normal, a supportive analysis will be performed on the log scale.

The absolute value of Cross-sectional Area (CSA) and contractile area of lower limb muscles and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics.
The absolute change in CSA and contractile area of lower limb muscles after 12 months of therapy, as assessed by MRI, will be also analyzed using an ANCOVA model with baseline CSA and contractile area of lower limb muscles value as covariate and treatment and concomitant steroid use at baseline as independent class variables. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residual. If considered non-Normal, a supportive analysis will be performed on the log scale.

Data handling rules

Primary analysis will be based on observed data only.

Sensitivity analyses for the MRI endpoints will be performed as follow:

1. Multiple imputation for missing data:
    a. Missing baseline primary endpoint data will be imputed as the mean of all non-missing baseline primary endpoint values across the randomized treatment groups. Visit 11 missing data will be imputed under a Missing At Random (MAR) assumption using SAS procedure MI within each treatment group using distribution implied by the non-missing patient data for that treatment group. A total of 20 imputation runs will be performed. Post imputation, each imputed dataset will be analysed separately exactly

as described for primary efficacy analysis. The SAS procedure MIANALYZE will then be used to combine the 20 datasets of estimates by Rubin's rules. The resulting multiply imputed means and difference in means will be presented, along with the associated SEs, 2-sided 95% CIs and 2-sided p-values.

b. Missing baseline primary endpoint data will be imputed as the mean of all non-missing baseline primary endpoint values across the randomized treatment groups. Visit 11 missing data will be imputed under a Missing Not At Random (MNAR) assumption using SAS procedure MI for both randomized treatment groups using the distribution implied by the non-missing patient data within the placebo group. A total of 20 imputation runs will be performed. Post imputation, each imputed dataset will be analysed separately exactly as described for primary efficacy analysis. The SAS procedure MIANALYZE will then be used to combine the 20 sets of estimates by Rubin's rules. The resulting multiply imputed means and difference in means will be presented, along with the associated SEs, 2-sided 95% CIs and 2-sided p-values.

6.3.2.3. Other biopsy histological parameters

Definition

- SLIDE I
  - MFA (%) will be calculated as a mean of available fields at each evaluation
  - Adipose tissue (%) will be calculated as a mean of available fields at each evaluation
  - Other histological structure (%) will be calculated as a mean of available fields at each evaluation
  - Fibers with nuclear centralizations will be calculated at baseline and at Visit 11 according to the following formula:

$$Fibres\ with\ centralizations\ \%_j = \frac{\sum_{i=1}^{4} \frac{Fibres\ with\ \text{nuclear}\ centralizations_{ji} * 100}{Total\ fibers\ number_{ji}}}{4}$$

where j = patient 1, 2... and i= field 1, 2, 3 or 4.

  - Total number of fibers counted in slide I will be calculated as sum of the number of fibers of available fields at each evaluation
- SLIDE II

- Regenerative fibers (MYH3) will be calculated at baseline and at Visit 11 according to the following formula:

$$Regenerative\ fibers\ \%_j = \frac{\sum_{i=1}^{4} \frac{(Regenerative\ fibers\ (MYH3)_{ji}) * 100}{Total\ fibers\ number_{ji}}}{4}$$

where j = patient 1, 2… and i= field 1, 2, 3 or 4.

- Total number of fibers counted in slide II will be calculated as sum of the number of fibers of available fields at each evaluation

- SLIDE III
  - Cross Sectional Area (CSA) type I will be calculated at baseline and Visit 11 according to the following formula:

$$CSA_j = \frac{\sum_{i=1}^{4} field_{ji}^{CSA\ I}}{\sum_{i=1}^{4} number\ of\ fields_{ji}^{CSA\ I}}$$

where j = patients 1, 2… and i= field 1, 2, 3 or 4.

  - Cross Sectional Area (CSA) type II will be calculated at baseline and Visit 11 according to the following formula:

$$CSA_j = \frac{\sum_{i=1}^{4} field_{ji}^{CSA\ II}}{\sum_{i=1}^{4} number\ of\ fields_{ji}^{CSA\ II}}$$

where j = patient 1, 2… and i= field 1, 2, 3 or 4.

  - Total Cross Sectional Area (CSA) will be calculated at baseline and Visit 11 according to the following formula:

$$CSA_j = \frac{\sum_{i=1}^{4} field_{ji}^{CSA\ I} + field_{ji}^{CSA\ II}}{\sum_{i=1}^{4} number\ of\ fields_{ji}^{CSA\ I} + number\ of\ fields_{ji}^{CSA\ II}}$$

where j = patient 1, 2… and i= field 1, 2, 3 or 4.

  - Total number of fibers counted in slide III will be calculated as sum of the number of fibers of available fields at each evaluation
  - Fiber size variability will be calculated at baseline and Visit 11 as the median of the interquartile range (IQR) of total CSA according to the following formula:

$$Fiber\ size\ variability_j = Q_{3j} - Q_{1j}$$

where j = patient 1, 2... and Q_1 and Q_3 are the first and third quartile for each patient (calculated among the 4 fields).

- Dystrophin
  - Total WB Dystrophin 1
  - Total WB Dystrophin 2

Analysis methodology

The absolute value of percentage of MFA and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics.

The absolute change in percentage of MFA after 12 months of therapy will be calculated as the difference between percentage of MFA at Visit 11 and its baseline value. This variable will be analyzed using an ANCOVA model with baseline percentage of MFA as covariate and treatment and concomitant steroid use at baseline as independent class variables. Results will be reported as Least-Square Means as well as difference between treatments together with their corresponding two-sided 95% CI. Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. If considered non-Normal, a supportive analysis will be performed on the log scale.
The same analyses will be done for the adipose tissue (%), other histological structure, fibers with nuclear centralizations, regenerative fibers, CSA I, CSA II, Total CSA, Fiber size variability and percentage of dystrophin.

Total number of fibers counted in Slide I, II and III will be summarized as a continuous variable at each time point as well as change vs baseline using descriptive statistics.

A Mixed Model will be fitted to the data with the difference between visit 11 and baseline values as the dependent variable; baseline as a covariate, treatment will be included as independent class variables and treatment by sequence interaction. The sequence of biopsy site (Right-Left, Left-Right) will be included as a fixed effect and subject as a random effect to evaluate the difference in Total CSA between biopsy sites. The same evaluation will be done also for MFA %.

If the treatment by sequence interaction is p>0.10, then the model without the interaction term will be presented.

Data handling rules Primary analysis will be based on observed data only.

Sensitivity analyses for the histological endpoints will be performed as follow:

1. Multiple imputation for missing data:

a. Missing baseline primary endpoint data will be imputed as the mean of all non-missing baseline primary endpoint values across the randomized treatment groups. Visit 11 missing data will be imputed under a Missing At Random (MAR) assumption using SAS procedure MI within each treatment group using distribution implied by the non-missing patient data for that treatment group. A total of 20 imputation runs will be performed. Post imputation, each imputed dataset will be analysed separately exactly as described for primary efficacy analysis. The SAS procedure MIANALYZE will then be used to combine the 20 datasets of estimates by Rubin's rules. The resulting multiply imputed means and difference in means will be presented, along with the associated SEs, 2-sided 95% CIs and 2-sided p-values.

b. Missing baseline primary endpoint data will be imputed as the mean of all non-missing baseline primary endpoint values across the randomized treatment groups. Visit 11 missing data will be imputed under a Missing Not At Random (MNAR) assumption using SAS procedure MI for both randomized treatment groups using the distribution implied by the non-missing patient data within the placebo group. A total of 20 imputation runs will be performed. Post imputation, each imputed dataset will be analysed separately exactly as described for primary efficacy analysis. The SAS procedure MIANALYZE will then be used to combine the 20 sets of estimates by Rubin's rules. The resulting multiply imputed means and difference in means will be presented, along with the associated SEs, 2-sided 95% CIs and 2-sided p-values.

6.3.2.4. Motor Function Measurement (MFM 32)

Definition

Motor Function Measurement (MFM 32) scores will be expressed as a percentage in relation to the maximum score. The score for each domain will correspond to the sum of the scores obtained by the patient for the items in that domain divided by the maximum score for the domain and multiplied by 100. The total score will be the sum of all the scores (in all the domains) divided by 96 and multiplied by 100.

MFM 32 scores include:

- Standing and transfers (D1): items 6, 8, 11, 12, 24-32
- Axial and proximal motor function (D2): items 1-3, 5, 7, 9, 10, 13-16, 23

- Distal motor function (D3): items 4, 17-22
- Total score (Total mark)

Analysis methodology

The absolute value of each score and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics.

To make best use of repeated intra-subject evaluations of MFM, the absolute change from baseline to visits 8, 9 10 and 11 (months 3, 6, 9 and 12) will be computed. These data will be analyzed using mixed model repeated measures (MMRM) analysis. Fixed effect class terms will be included for treatment, visit, visit x treatment interaction and concomitant steroid use at baseline; baseline value will be included as a covariate. An unstructured covariance matrix will be used to model the within-subject error. LSmeans and SEs and also the difference in LSmeans between treatments will be extracted by visit; SEs, 2-sided 95% CIs and 2-sided p-values will also be extracted for the difference in LSmeans. The principal focus of inference will be the results at visit 11 (month 12).

Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. If considered non-Normal, a supportive analysis will be performed on the log scale.

The analysis will be done separately for each score.

Moreover, original values collected in eCRF as well as derived values calculated for the analysis will be listed by patient.

Data handling rules

As the MMRM approach implicitly imputes missing data as missing at random, there will be no separate analysis imputing missing values, the analysis will be based on the observed data at visits 8, 9 10 and 11.

6.3.2.5. Time function test (TFT)

Definition

Time Function Tests (TFT) evaluation will include:

- Time to climb four standard steps (4-Stair Climb)
- Time to rise from floor
- Time to walk/run 10m

Analysis methodology

The absolute value of time to 4-Stair Climb and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics. A box-

plot of the average time to 4-Stair Climb at each visit will also be produced by treatment group.
To make best use of repeated intra-subject evaluations of 4-Stair Climb, the absolute change in time to 4-Stair Climb from baseline to visits 8, 9 10 and 11 (months 3, 6, 9 and 12) will analyzed as described for MFM using an MMRM analysis. Fixed effect class terms will be included for treatment, visit, visit x treatment interaction and concomitant steroid use at baseline; baseline value will be included as a covariate. An unstructured covariance matrix will be used to model the within-subject error. LSmeans and SEs and also the difference in LSmeans between treatments will be extracted by visit; SEs, 2-sided 95% CIs and 2-sided p-values will also be extracted for the difference in LSmeans. The principal focus of inference will be the results at visit 11 (month 12).

Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. If considered non-Normal, a supportive analysis will be performed on the log scale.

The same analyses will be done for time to rise from floor and time to walk/run 10 m. A box-plot of the average time to rise from floor and time to walk/run 10 m at each visit will also be produced by treatment group.

Number of patients who declined to perform the test or didn't complete it (e.g. for Rise From Floor and 10-Metre Walk/Run Test) will also be provided.

Finally, the proportion of patients who lose the ability to rise from floor during the study (defined as patients with Rise From Floor grading = 0) will be summarized at each time point after baseline as a discrete variable using descriptive statistics. The rising from floor grading will also be summarized as discrete variable, at each time point, using descriptive statistics. The proportion of patients who lose ability to rise from floor after 12 months of therapy will be also compared between arms using a stratified Cochran Mantel-Haenszel (CMH) chi square test with a two-sided α=0.05 level. 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. Patients where there are missing data such that retention or loss of the ability to rise from floor cannot be determined will be included in the analysis as having lost the ability to rise from floor.

Data handling rules

In case of patients with no TFT assessments, as the MMRM approach implicitly imputes missing data as missing at random, there will be no separate analysis imputing missing values, the analysis will be based on the observed data at visits 8, 9 10 and 11.

6.3.2.6. 6-Minute Walking Test (6MWT)

Definition

6 Minute Walking Test (6MWT) evaluation will include:

- Distance walked after 6 minutes
- Proportions of patients with <10% worsening in the distance walked after 6 minutes
- Proportions of patients who lose ambulation during the study

For patients who do not complete the test, the max distance walked will be considered as distance walked after 6 minutes.

For patients who were not able to do the test, due to lack of ambulation, "zero" will be imputed as maximum distance walked.

The average of the distance at screening and randomization will be used as baseline value.

Analysis methodology

The absolute value of the maximum distance walked and change versus baseline, at each time point, by treatment group will be summarized as a continuous variable using descriptive statistics. A box-plot of the average maximum distance walked at each visit will be produced by treatment group.

The absolute values of distance walked after 1, 2, 3, 4, 5, 6 minutes, number of falls and their changes versus baseline will be summarized as continues variables too. In addition, a box-plot of the mean 6MWT at each time-point will be produced for each visit by treatment group.

To make best use of repeated intra-subject evaluations of 6MWT, the absolute change from baseline to visits 8, 9 10 and 11 (months 3, 6, 9 and 12) will computed. These data will be analyzed using mixed model repeated measures (MMRM) analysis. Fixed effect class terms will be included for treatment, visit, visit x treatment interaction and concomitant steroid use at baseline; baseline value will be included as a covariate. An unstructured covariance matrix will be used to model the within-subject error. LSmeans and SEs and also the difference in LSmeans between treatments will be extracted by visit; SEs, 2-sided 95% CIs and 2-sided p-values will also be extracted for the difference in LSmeans. The principal focus of inference will be the results at visit 11 (month 12). Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. If considered non-Normal, a supportive analysis will be performed on the log scale.

Worsening is defined as a reduction in the distance walked after 6 minutes, as assessed by 6MWT, after 12 months of therapy compared to baseline.

The proportion of patients with <10% worsening, at each time point after baseline, will be summarized as a discrete variable using descriptive statistics.

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. 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. Patients where there are missing data such that <10% worsening in 6MWT after 12 months cannot be determined will be included in the analysis as having worsened.

The proportion of patients who lose ambulation during the study (6MWT not done because patient unable to physically perform the test), at each time point after baseline, will be summarized as a discrete variable using descriptive statistics.

The proportion of patients who lose ambulation after 12 months of therapy (6MWT not done because patient unable to physically perform the test) will be also compared between arms using a stratified Cochran Mantel-Haenszel (CMH) chi square test with a two-sided α=0.05 level. 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. Patients where there are missing data such that loss of ambulation cannot be determined will be included in the analysis as having lost ambulation.

The number of patients who did not attempt the test and the reasons (i.e. unable to physically perform the test, decline to perform the test or other) will be provided.

The number and proportion of patients who falls will be summarized at each time point: the mean number of falls during 6 MWT will be also summarized at each time point after baseline as continuous variable using descriptive statistics.

Data handling rules

For the MMRM analysis of 6MWT values, since the MMRM approach implicitly imputes missing data as missing at random, there will be no separate analysis imputing missing values, the analysis will be based on the observed data at visits 8, 9 10 and 11.

6.3.2.7. Hand Held Myometry (HMM)

Definition

Muscle strength measured by Hand Held Myometry (HHM) evaluation will include:

- left knee extension measurements
- right knee extension measurements
- left elbow flexion measurements
- right elbow flexion measurements

For these variables the highest values among the three measurements at each evaluation will be considered for the present analysis.

Analysis methodology

The absolute value of right/left knee extension and change versus baseline, at each time point, will be summarized as a continuous variable using descriptive statistics.

The absolute change in right/left knee extension from baseline to visits 8, 9 10 and 11 (months 3, 6, 9 and 12) will be calculated as the difference between the mean of right/left knee extension measurements at each visit and baseline. These data will be analyzed using mixed model repeated measures (MMRM) analysis. Fixed effect class terms will be included for treatment, visit, visit x treatment interaction and concomitant steroid use at baseline; baseline value will be included as a covariate. An unstructured covariance matrix will be used to model the within-subject error. LSmeans and SEs and also the difference in LSmeans between treatments will be extracted by visit; SEs, 2-sided 95% CIs and 2-sided p-values will also be extracted for the difference in LSmeans. The principal focus of inference will be the results at visit 11 (month 12).

Possible need for log transformation of this variable will be assessed by check of ANCOVA model residuals. If considered non-Normal, a supportive analysis will be performed on the log scale.

The same analyses will be done for right/left elbow flexion.

Data handling rules

As the MMRM approach implicitly imputes missing data as missing at random, there will be no separate analysis imputing missing values, the analysis will be based on the observed data at visits 8, 9 10 and 11.

6.3.2.8. Quality of Life (SF-36)

Definition

SF-36 will include:

- Single items
- 8 Domains
- Total Score

SF-36 questionnaires will be analysed using PRO CoRE software.

Analysis methodology

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

Data handling rules

The PRO CoRE Software applies a value to a scale item rendered missing if at least one of the items in that scale has valid data. A scale receives a "missing" score (".") only if all the items in that scale are missing. Physical Component Summary (PCS) and Mental Component Summary (MCS) are calculated when at least seven of the eight profile scales have valid data, either actual or estimated.

However, to calculate PCS, the Physical Functioning (PF) scale must be one of the seven scales having valid data. Also, to calculate MCS, the Mental Health scale must be one of the seven scales having valid data.

6.3.3. Exploratory efficacy analysis

Evaluation of LTBP4 and osteopontin genotype

In order to determine whether the effects of givinostat versus placebo are related to the type of Mutation Exon or the LTBP4 and Osteopontin (i.e. SPP1) genotypes, three further subgroups of subjects will be defined: Mutation Exon (Exon 45, Prior to exon 45, Post-exon 45), LTBP4 (IAMM, VTTT, Other, VTTT+Other) and SPP1 (TT, GT, GG, GG+GT).

The number and proportion of patients in each subgroup will be summarized as appropriate. Further analyses are described in Section 6.5.

Evaluation of the functional test velocity

Velocity for each functional test will be computed at each visit from the following variables:

- 4-Stair ascent duration
- Rising from floor duration
- 10-Metre Walk/Run test duration

For patients with missing data TFT data due to lack of ability to perform the test, their velocity will be set equal to zero.

Velocity to 4-Stair Climb will be calculated according to the following formulas:

$$Velocity_{4-Stair\ Climb} = \frac{4}{time\ to\ climb\ 4\ stair}$$

The velocity will be set to zero for patients who don't attempt the test with reason "Unable to physically perform the test" or for patients with the lowest grade (1) for the 4 Stair Climb test.
The absolute value of velocity to 4-Stair Climb and change versus baseline, at each time point, will be summarized as continuous variables using descriptive statistics.

To make best use of repeated intra-subject evaluations of 4-Stair Climb velocity, the absolute change from baseline to visits 8, 9 10 and 11 (months 3, 6, 9 and 12) will be analyzed using mixed model repeated measures (MMRM) analysis. Fixed effect class terms will be included for treatment, visit, visit x treatment interaction and concomitant steroid use at baseline; baseline value will be included as a covariate. An unstructured covariance matrix will be used to model the within-subject error. LSmeans and SEs and also the difference in LSmeans between treatments will be extracted by visit; SEs, 2-sided 95% CIs and 2-sided p-values will also be extracted for the difference in LSmeans. The principal focus of inference will be the results at visit 11 (month 12).

Velocity to walk/run 10 m will be calculated according to the following formula:

$$Velocity_{walk/run\ 10\ m} = \frac{10}{time\ to\ walk/run\ 10\ m}$$

Velocity to rise from floor will be calculated according to the following formula:

$$Velocity_{Rise\ from\ floor} = \frac{1}{time\ to\ rise\ from\ floor}$$

Velocity to rise from floor and Velocity to walk/run 10 m will be analysed in the same manner as Velocity to 4-Stair Climb.

Moreover, to aid interpretation, if there will be patients unable to perform the test (or with the lowest grade) the variance-covariance matrix for LSmean estimates will be extracted by visit. The LSmeans will be reciprocated by visit to present TFT results back on the original scale. The difference in the reciprocated LSmeans (ie. $\Delta = LSmean_d^{-1} - LSmean_p^{-1}$) will be presented along with the associated 95% CI; for the latter the variance of Δ is required. This will be estimated using the variance-covariance matrix computed vis Taylor's expansion and the Delta rule as

$$Var(\Delta) = \frac{Var(LSmean_d)}{(LSmean_d)^4} - 2\frac{Cov\left(LSmean_d, LSmean_p\right)}{(LSmean_d)^2\left(LSmean_p\right)^2} + \frac{Var(LSmean_p)}{\left(LSmean_p\right)^4}$$

The CI for the difference in the reciprocated LSmeans is then given by

$$\Delta \pm t_{0.975,df} \cdot \sqrt{Var(\Delta)}$$

there $t_{0.975,df}$ is the 97.5th percentile of the t-distribution with df degrees of freedom where this is taken from the MMRM analysis.

6.3.4. Measurement of Treatment Compliance

For each patient, the compliance will be calculated according to the following formula:

$$Compliance_{ji} = \frac{((number\ of\ days\ from\ last\ evaluation_{ji} * 2) - number\ of\ doses\ not\ taken_{ji})}{number\ of\ days\ from\ the\ last\ visit_{ji} * 2}$$

where *j* = patient 1, 2... and *i*= visit 8, 9, 10 and 11.

The total compliance for each patient will be computed as the sum of the compliance at visit 8, 9, 10 and 11.

Total compliance will be summarized as <80% and 80-100%. The compliance will be also summarized as a continuous variable using descriptive statistics.

Treatment compliance will be calculated on ITT set using the information about doses not taken reported in the study diary or in the eCRF if no diary available.

6.4. Safety Evaluation

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

6.4.1. Extent of Exposure

The exposure to the study treatment will be evaluated by means of the variables defined below.

The following definitions will be applied in the variable calculation:

- First drug administration: it is the earliest date when a non-zero dose of study treatment was administered and recorded on the eCRF.
- Last drug administration: it is the latest date when a non-zero dose of study treatment was administered and recorded on the eCRF.

The duration of exposure to study treatment is calculated as:

Duration of exposure (days) = (Date of Last drug administration - Date of First drug administration + 1).

The duration of exposure to study treatment includes the periods of temporary interruption of the study treatment for any reason.

The duration of actual exposure to study treatment will be calculated excluding the periods of temporary interruption of the study treatment for any reason.

The total daily dose in mg (i.e. mL X 10 = mg) will be calculated as the total amount of drug administered during the study divided by the duration of actual exposure.

The duration of exposure to study treatment, the duration of actual exposure to study treatment and the total daily dose will be summarized by means of the usual descriptive statistics on the Safety Analysis Set overall and by treatment group.

The number and percentage of subjects with and without any IMP modification, as well as the number and percentage of subjects with each type of IMP modification (dose increased, dose reduced, dose permanently stopped, dose temporarily stopped) and the reason for the modification (Adverse event/Lab or ECG abnormality, dosing error, other) will be summarized by treatment group.

6.4.2. Concomitant medications

Prior (i.e. medications ended before first intake), concomitant (i.e. medications started before first intake and ongoing at the baseline) and steroid medications (used at baseline) will be presented as the number and percentages of patients with at least one prior/ concomitant/steroid medication. Prior concomitant and steroid medication will be also 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.

6.4.3. Adverse Events

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

- Treatment-emergent AEs (TEAEs), those events with an onset date after study treatment initiation.
- Non-treatment-emergent AEs (non-TEAEs), those events with an onset date between informed consent and study treatment initiation.

An overview of TEAEs will be provided for the number of patients with any TEAEs, numbers of TEAEs, number of patients with serious TEAEs, number of serious TEAEs, number of patients with TEAEs leading to interruption of study treatment, number of patients with TEAEs leading to withdrawal of study treatment, number of patients with fatal TEAEs, number of patients with mild/moderate/severe TEAEs, number of patients with TEAEs related/not related to study treatment.

AEs for which relationship to study drug is not specified or unknown will be considered treatment related.

The incidence of TEAEs will be tabulated by System Organ Class (SOC) and Preferred Term (PT), and by SOC and PT and severity by treatment group. A subject with multiple occurrences of an AE will be counted only once in the AE category. These analysis will be repeated for all the above categories.

TEAE will be summarized under the worst severity recorded for the event.

Deaths, SAEs and AEs leading to withdrawal of study treatment will also be listed.

Non-treatment emergent AEs will only be listed.

6.4.4. Laboratory parameters

The absolute value and change from baseline will be analyzed for each laboratory parameters separately for hematology, biochemistry, coagulation, serology and urinalysis by means of summary descriptive statistics at each time point, by treatment group. 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 out of range will also be provided.

In addition, the following summaries in relation to post-baseline platelet counts will be presented by treatment group: minimum values, time to nadir (days) and time to recovery (days). These will be defined as follows:

Minimum value = minimum post-baseline platelet count for the subject.

Time to nadir (days) = time from the date of first drug administration to first minimum post-baseline value regardless if this is within the normal range.

Time to recovery (days) = for subjects whose minimum post-baseline value is below the lower limit of normal, the time to recovery is calculated as the time from the minimum value below lower limit of normal to the first value within the normal range.

Furthermore, the above summaries will be repeated for patients who reduced dose at least once due to platelet count decrease. Moreover, for this subgroup of patients, the following variables will be described as well:

- time on treatment after recovery (days), additional platelet decrease after recovery, time on treatment without recovery (days), where:
- time on treatment after recovery = for subjects who record recovery from the minimum value below the lower limit of normal, the time on treatment after recovery is calculated as the time from the date of recovery to the last treatment date or date of data cut-off.
- additional platelet decrease after recovery = number of subjects who record recovery from the minimum value below the lower limit of normal and then record a subsequent value below the lower limit of normal.
- time on treatment without recovery = for subjects who do not record recovery from the minimum value below the lower limit of normal, the time on treatment without recovery is calculated from the date of the minimum value below the lower limit of normal to the last treatment date.

A box-plot will be produced for the following parameters over time by treatment group: platelets, hemoglobin, neutrophils, leukocytes, triglycerides and total cholesterol.

Pharmacokinetics

Finally, pharmacokinetic parameters (ITF2374 and ITF2375) for patients treated with givinostat will be listed by patient together with the IMP dosage. Further details will be provided in a separate SAP.

6.4.5. Vital signs/Physical examination

The absolute value and change versus baseline will be provided for vital signs (including BMI) by treatment group. Vital signs evaluation will include height [cm], weight [Kg], sitting blood pressure systolic/diastolic [mmHg], heart rate [beats/min] and temperature [°C] and body mass index (BMI). Body Mass Index (BMI) will be computed by dividing weight in kilograms by the square of height in meters. Body Mass Index [Kg/m2] will be also classified in the subsequent classes: "Underweight" if BMI lower than 18.5, "Normal" if BMI between 18.5 Kg/m2 and 24.9 Kg/m2, "Overweight" if BMI between 25 Kg/m2 and 29.9 Kg/m2 and "Obesity" if BMI greater than or equal to 30 Kg/m2.

Physical examination will be summarized as percentage of Normal and Abnormal body system evaluations at each time point by treatment group.

Physical Examination evaluation will include the result (reported as "Normal", "Abnormal" and "Not Done") of general appearance, eyes, ears, nose and throat, cardiovascular, respiratory, abdomen, urogenital, neurological, musculoskeletal, lymph nodes, dermatology and other system.

6.4.6. Other safety parameters

The absolute value and change versus baseline will be provided for pulmonary function (including FVC, FEV1, FVC/FEV1, PEF) and cardiac parameters evaluated by ECG and ECHO.

Pulmonary Function Test will include forced expiratory volume in 1 second [L], forced vital capacity [L], peak expiratory flow [L/sec], fev1/fvc [%], percent predicted fev1 [%], percent predicted fvc [%], forced expiratory flow 25-75% [L/sec], percent predicted fev1/fvc [%] and percent predicted fef25-75 [%].

12-leads ECG evaluation will include RR Interval Aggregate [msec], ECG Mean Ventricular Rate [beats/min], PR Interval Single Beat [msec], QRS Duration Single Beat [msec], QT Interval Single Beat [msec], QTcF Interval Single Beat [msec] and Interpretation.

The incidence of outliers in QTcF intervals (>450, >480, and >500 msec), and the change from baseline in QTcF intervals (>30 and >60 msec) will be summarized by visit and overall for each treatment group as defined in ICH E14 guideline (ICH_E14, 2005). Where triplicate ECG assessments have been performed, the mean of the non-missing values will be used to

determine outliers. A subject will be counted once for each outlier in the overall summary if they experienced at least one occurrence of that outlier

Echocardiography evaluation will include mitrial insufficiency, severity (indicated as "Mild", "Moderate" or "Severe"), left ventricular end-diastolic internal dimension (LVEDD) [mm], left ventricular end-systolic internal dimension (LVESD) [mm], left ventricular septal wall thickness (LVS) [mm], left ventricular posterior wall thickness (PW) [mm], left ventricular end-diastolic (LVED) volume (4-ch) [ml], left ventricular end-diastolic (LVED) volume (2-ch) [ml], left ventricular end-diastolic (LVED) volume (Biplane) [ml], left ventricular end-systolic (LVES) volume (4-ch) [ml], left ventricular end-systolic (LVES) volume (2-ch) [ml], left ventricular end-systolic (LVES) volume (Biplane) [ml], left ventricular outflow tract (LVOT) diameter [mm], left ventricular ejection fraction (LVEF) [%], method of left ventricular ejection fraction calculation (LVEF) (indicated as "2-ch", "4-ch" or "Biplane"), mitral E max velocity [cm/s], mitral A max velocity [cm/s], LVOT TVI [cm], tricuspid regurgitation v max [ms], isovolumic relaxation time (Left Ventricle) [m/s] and mitral E' (lateral) [cm/s].

6.5. Subgroup Analyses

The subgroups are defined as below:

Subgroup	Definition
LTBP4	IAMM, VTTT+Other
SPP1	TT, GG+GT
Mutated exon(s) data	Exon 45, Prior to exon 45, Post-exon 45

The following analyses will be repeated for each subgroup defined above:

- Change from baseline to 12 months of treatment with givinostat or placebo in total fibrosis (%)
- Change from baseline to 12 months of treatment with givinostat or placebo in MRS Fat Fraction in the vastus lateralis muscles
- Change from baseline to 12 months of treatment with givinostat or placebo in MRS Fat Fraction in the soleus muscles
- Change from baseline to 12 months of treatment with givinostat or placebo in MFM total score
- Change from baseline to 12 months of treatment with givinostat or placebo in 6MWT (Distance walked after 6 minutes)
- Change from baseline to 12 months of treatment with givinostat or placebo in functional test velocity (i.e. 4-Stair ascent duration, Rising from floor duration, 10-Metre Walk/Run test duration)

6.6. Interim Analysis and Data Monitoring

An interim analysis was performed, on the first 20 baseline biopsies collected, for checking the variability of the primary endpoint under observation and the results confirmed the sample size

estimate. The histopathological features observed in this blinded interim analysis showed that the mean CSA of the biceps fibers in BMD (mean: 4871.68 µm2; min 1933.49 µm2; and max 9446.34 µm2; and S.D.: 1904.86 µm2) 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. (for more details see the Statistical Analysis Plan for Interim Analysis Final version 1.0 and Statistical Report for Interim Analysis version 1.0).

After study enrollment was completed and baseline data were collected for all patients, a second blinded interim analysis was performed to obtain a preliminary overview of the baseline patient characteristics (for more details see the Statistical Analysis Plan for Interim Analysis II Final version 1.0 and Statistical Report for Interim Analysis II version 1.0).

7. REFERENCES

Not applicable.

8. APPENDIX

Not applicable.