ITALFARMACO

# CLINICAL STUDY PROTOCOL

**Protocol Number: DSC/14/2357/48**
**Protocol Title: Randomised, double blind, placebo controlled, multicentre study to evaluate the efficacy and safety of givinostat in ambulant patients with Duchenne Muscular Dystrophy.**

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EPIGENETIC RESCUE OF DYSTROPHIN DYSFUNCTION

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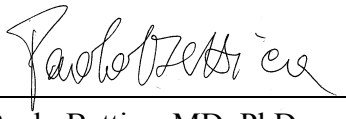
| **IND Number:** | 126598 |
| **EudraCT Number:** | 2016-000401-36 |
| **Name of Product:** | Givinostat |
| **Phase of Development:** | 3 |
| **Indication:** | Duchenne Muscular Dystrophy (DMD) |
| **Sponsor:** | Paolo Bettica, MD, PhD<br>Director of Clinical Research and Development<br>ITALFARMACO S.p.A.<br>54, Via dei Lavoratori<br>20092 Cinisello Balsamo, Italy<br>Tel: + 39 02 64431<br>Fax: + 39 02 644346 |
| **Protocol Version:** | 8.0 |
| **Protocol Date:** | 08 APR 2020 |

-CONFIDENTIAL-

**SPONSOR APPROVAL PAGE**



Date: 10 April 2020

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

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| **Title of Study:** | Randomised, double blind, placebo controlled, multicentre study to evaluate the efficacy and safety of givinostat in ambulant patients with Duchenne Muscular Dystrophy |
| **Acronym** | EPIDYS – Epigenetic Rescue of Dystrophin Dysfunction |
| **Protocol Number:** | DSC/14/2357/48 |
| **Study Design:** | Randomised, double blind, parallel group, placebo controlled study. Subjects will be randomised 2:1 to givinostat or placebo. |
| **Investigators/Study Sites:** | Approximately 50 sites worldwide |
| **Phase of Development:** | 3 |
| **Objectives:** | Primary objective:<br>• To establish the effects of givinostat versus placebo administered chronically over 18 months to slow disease progression in ambulant DMD subjects.<br>Secondary objectives:<br>• To assess the safety and tolerability of givinostat versus placebo administered chronically in DMD subjects.<br>• To evaluate the pharmacokinetic (PK) profile of givinostat administered chronically in DMD subjects;<br>• To evaluate the impact on quality of life and activities of daily living of givinostat versus placebo administered chronically.<br>Secondary exploratory objectives:<br>• To evaluate the correlation between PK profile of givinostat and pharmacodynamics (PD) data;<br>• To explore whether the effects of givinostat versus placebo administered chronically may be related to the type of DMD mutation or to the biomarkers. |
| **Study Population and Subset Definition:** | Ambulant male paediatric subjects aged ≥6 years at baseline affected by DMD will be included in this study.<br>Target population (Group A): subjects with a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range >5% and ≤30%.<br>Off-target population (Group B): subjects with a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range ≤5% or >30%.<br>MR Cohort: all subjects in the target population who will perform the magnetic resonance assessment at baseline, 12 and 18 months. |

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| **Selection of Subjects:** | Main Inclusion Criteria:<br>Subjects must meet all the following inclusion criteria:<br>1. Are ambulant males aged ≥6 years at randomisation with DMD characteristic clinical symptoms or signs (e.g., proximal muscle weakness, Gowers' maneuver, elevated serum creatinine kinase level) already present at screening;<br>2. Have DMD diagnosis confirmed by genetic testing;<br>3. Are able to give informed assent and/or consent in writing signed by the subject and/or parent/legal guardian (according to local regulations);<br>4. Are able to complete 2 Four Stairs Climb test (4SC) screening assessments; the results of these tests must be within ±1 second of each other;<br>5. Have the mean of 2 screening 4SC assessments ≤8 seconds;<br>6. Have time to rise from floor between ≥3 and <10 seconds at screening;<br>7. Have manual muscle testing (MMT) of quadriceps at screening ≥ Grade - 3;<br>8. Have used systemic corticosteroids for a minimum of 6 months immediately prior to the start of study treatment, with no significant change in corticosteroids type or dosage or dosing regimen (excluding changes related to body weight change) for a minimum of 6 months immediately prior to start of study treatment and a reasonable expectation that dosage and dosing regimen will not change significantly for the duration of the study.<br>9. Subjects must be willing to use adequate contraception.<br>Contraceptive methods must be used from Randomization Visit 3 through 3 months after the last dose of study drug, and include the following:<br>• True abstinence (absence of any sexual intercourse), when in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.<br>• 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. |

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| | Main Exclusion Criteria:<br>Subjects presenting with any of the following criteria will not be included in the study:<br>1. Have exposure to another investigational drug within 3 months prior to the start of study treatment (only exception allowed is use of Deflazacort in US as part of the Expanded Access Program and in Canada as part of the Special Access Program);<br>2. Have exposure to idebenone within 3 months prior to the start of study treatment;<br>3. Have exposure to any dystrophin restoration product (e.g., Ataluren, Exon-skipping) within 6 months prior to the start of study treatment;<br>4. Use of any pharmacologic treatment, other than corticosteroids, that might have had 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 as long as their intake has been stable for 3 months prior to the start of study treatment; Testosterone will also be allowed if it is used as a replacement therapy for the treatment of delayed puberty, and testosterone dose and regimen have been stable for at least 6 months and circulating testosterone levels are within the normal ranges for the subject's age;<br>5. Have 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;<br>6. Loss of ≥30 degrees of plantar flexion from the normal range of movement at the ankle joint due to contracture (i.e. fixed loss of more than 10 degrees of plantar flexion from plantigrade, assuming normal range of dorsiflexion of 20 degrees);<br>7. Change in contracture treatment such as serial casting, contracture control devices, night splints, stretching exercises (passive, active, self) within 3 months prior to enrollment, or expected need for such intervention during the study;<br>8. Have presence of other clinically significant disease, which, in the Investigator's opinion, could adversely affect the safety of the subject, making it unlikely that the course of treatment or follow-up would be completed, or could impair the assessment of study results;<br>9. Have a diagnosis of other uncontrolled neurological |

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| | diseases or presence of relevant uncontrolled somatic disorders that are not related to DMD;<br>10. Have platelets count, White Blood Cell and Hemoglobin at screening <Lower Limit of Normal (LLN) (for abnormal screening laboratory test results (<LLN), the platelets count, White Blood Cell and Hemoglobin will be repeated once; if the repeat test result is still <LLN, then exclusionary);<br>11. Have symptomatic cardiomyopathy or heart failure (New York Heart Association Class III or IV) or left ventricular ejection fraction <50% at screening;<br>12. Have a current or history of liver disease or impairment, including but not limited to an elevated total bilirubin (i.e. > 1.5 x ULN), unless secondary to Gilbert disease or pattern consistent with Gilbert's;<br>13. Have inadequate renal function, as defined by serum Cystatin C >2 x the upper limit of normal (ULN). If the value is >2 x ULN, the serum Cystatin C will be repeated once; if the repeated test result is still >2 x ULN, the subject should be excluded);<br>14. Have Triglycerides > 300 mg/dL (3.42 mmol/L) in fasting condition at screening visit;<br>15. Have a positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening;<br>16. Have a 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);<br>17. Have a psychiatric illness/social situations rendering the potential subject unable to understand and comply with the muscle function tests and/or with the study protocol procedures;<br>18. Have any hypersensitivity to the components of study medication;<br>19. Have a sorbitol intolerance or sorbitol malabsorption, or have the hereditary form of fructose intolerance.<br>20. Have contraindications to MRI or MRS (e.g., claustrophobia, metal implants, or seizure disorder).<br>At the discretion of the Investigator, subjects not meeting inclusion/exclusion criteria may be re-screened twice with an interval of at least 3 months between assessments. |

<table>
<tr><td>Planned Sample Size:</td><td>The primary, target population for formal statistical analysis and inference is those children with a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range >5% and ≤30% (Group A). A total of 110 male ambulant subjects in this target population will be randomised. Up to 50 subjects (about 35% of the overall population) outside of the target may also be recruited into the study (Group B). The overall subject population (Group A + Group B) will provide supportive data.</td></tr>
<tr><td>Stratification:</td><td>Subjects will be stratified for their concomitant use of steroids in 4 strata:<br>1. Deflazacort Daily regimen<br>2. Deflazacort Intermittent regimen<br>3. Other steroids Daily regimen<br>4. Other steroids Intermittent regimen</td></tr>
<tr><td>Randomisation ratio:</td><td>2:1</td></tr>
<tr><td>Study duration for participants:</td><td>The study duration is planned for 19 months.<br>The study comprises 2 phases:<br>1. Screening period: starting 4 weeks before randomisation<br>2. Treatment period: 18 months of treatment</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:<br>Placebo or Givinostat starting dose
<table>
<tr><td>Weight (kg)</td><td>≥10 and <12.5</td><td>≥12.5 and <20</td><td>≥20 and <25</td><td>≥25 and <30</td><td>≥30 and <40</td><td>≥40 and <50</td><td>≥50 and <60</td><td>≥60 and < 70</td><td>≥70</td></tr>
<tr><td>Dose (mg) bid</td><td>13.3</td><td>16.7</td><td>20</td><td>23.3</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>1.3</td><td>1.7</td><td>2.0</td><td>2.3</td><td>2.7</td><td>3.3</td><td>3.7</td><td>4</td><td>4.7</td></tr>
</table>
The subjects who were randomized under protocol version 4.0 will continue the current dose.<br>If subjects (i.e. those already in treatment or those who will be randomized under protocol version 6.0, or 7.0 or 8.0) gain weight during the study the drug dose must be kept unchanged. If subjects (i.e. those already in treatment or those who will be randomized under protocol version 6.0, or 7.0 or 8.0) lose weight during the study, the drug dose should be adjusted accordingly.</td></tr>
<tr><td>Criteria for Evaluation:</td><td><u>Efficacy:</u><br>The primary efficacy assessment in the target population is the time to climb 4 standard stairs.</td></tr>
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| | The secondary key efficacy assessments in the target population are:<br>1. Time to rise from floor;<br>2. Distance walked in 6 minutes using six-minute walking test (6MWT);<br>3. Physical function assessed by North Star Ambulatory Assessment (NSAA);<br>4. Muscle strength evaluated by knee extension, elbow flexion as measured by hand-held myometry (HHM).<br>5. Fat fraction of vastus lateralis muscles evaluated by MRS technique (MR Cohort)<br>The exploratory efficacy assessments are:<br>• Time to walk/run 10 meters;<br>• Quality of life assessed by Paediatric Outcomes Data Collection Instrument (PODCI);<br>• %-predicted 6MWT;<br>• MRI parameters (e.g., fat fraction of thigh muscles, cross sectional area (CSA) of vastus lateralis and other thigh muscles, etc.) only in the MR cohort;<br>• Time to 10% persistent worsening in 6MWT (Baseline through end of study);<br>• Proportion of subjects with ≥10% worsening in 6MWT at end of study;<br>• Time to loss of standing (Baseline through end of study);<br>• Proportion of subjects who loose ambulation during the study.<br>• Evaluation of any correlation between the effect of Givinostat on disease progression and the type of DMD mutation, LTBP4 and Osteopontin genotype;<br>• Evaluation of any possible DMD serum biomarker.<br>Pharmacokinetic:<br>The concentration of givinostat and its metabolites will be determined from the plasma samples using a validated analytical method.<br>Safety:<br>Safety and tolerability will be evaluated by monitoring hematology and blood chemistry, coagulation, urinalysis; by measurement of vital signs, physical examinations, weight, height, echocardiogram (ECHO) and electrocardiogram (ECG) recording; and by respiratory function evaluation, cognitive function evaluation, and by adverse events (AEs) recording to be performed at protocol-specified visits.<br>In addition, the evaluation of acceptability/palatability of the oral |

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| | suspension will be assessed. |
| **Study Endpoints:** | Primary efficacy endpoints:<br>• Mean change in 4SC before and after 18 months of treatment of givinostat versus placebo.<br>Key secondary efficacy endpoints:<br>Function:<br>• Mean change in time to rise from floor;<br>• Mean change in 6MWT;<br>• Mean change in NSAA;<br>• Cumulative loss of function on the NSAA<br>• Mean change in muscle strength evaluated by knee extension, elbow flexion as measured by HHM;<br>Imaging:<br>• Mean change in vastus lateralis muscles fat fraction;<br>The above endpoints will be formally assessed after 18 months of treatment<br><br>Safety endpoints:<br>• Number of subjects experiencing treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) (Baseline through end of study [EOS]);<br>• Type, incidence, and severity of TEAEs and SAEs (Baseline through EOS);<br>• Changes from baseline to end of study of:<br>• Vital signs and clinical laboratory tests (blood chemistry and hematology);<br>• Respiratory function evaluated by forced vital capacity (FVC), forced expiratory volume at 1 second (FEV1), FVC/FEV1, Peak Expiratory Flow (PEF);<br>• Cardiac function evaluated by ECG and ECHO;<br>• Cognitive function evaluated by the Raven coloured progressive matrices;<br>• Weight, height, and body mass index (BMI).<br>• Evaluation of acceptability/palatability of the oral suspension.<br>Pharmacokinetic Endpoints:<br>• Description of the PK of givinostat and its major metabolites: ITF2374 and ITF2375 in the subject population;<br>• Identification of the relevant demographic and |

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| | pathophysiological covariates influencing the PK of givinostat.<br><br>Exploratory endpoints:<br>• Mean changes in:<br>• time to walk/run 10 meters;<br>• PODCI scores;<br>• %-predicted 6MWT;<br>• Only in the MR cohort: MRI parameters (e.g., fat fraction of thigh muscles, CSA of vastus lateralis and other thigh muscles).<br>• Time to 10% persistent worsening in 6MWT (Baseline through end of study);<br>• Proportion of subjects with ≥10% worsening in 6MWT at end of study;<br>• Time to loss of standing (Baseline through end of study);<br>• Proportion of subjects who loose ambulation during the study;<br>• Evaluation of any correlation between the effect of Givinostat on disease progression and the type of DMD mutation, LTBP4 and Osteopontin genotype;<br>• Evaluation of any possible DMD serum biomarker;<br>• PK-PD analyses: relationships between metrics of exposure and efficacy/safety endpoints of givinostat. |
| **Rules for cessation of randomised treatment in an individual participant:** | Individual subjects may cease taking randomised treatment at the discretion of the Investigator.<br>Study drug should be permanently stopped if any of the following occur:<br>• severe drug-related diarrhoea (i.e., increase of ≥7 stools per day);<br>• any drug-related SAE;<br>• QTcF >500 msec;<br>• platelets count ≤50 x $10^9$/L (to avoid laboratory errors and anomalous values, platelets count ≤50 x $10^9$/L 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 platelets count is still ≤50 x $10^9$/L, study drug must be permanently discontinued. If the repeated test is acceptable, the subject can resume treatment.);<br>• white blood cells ≤2.0 x $10^9$/L (to avoid laboratory errors and anomalous values, white blood cells ≤2.0 x $10^9$/L must be confirmed with a repeated test performed on the next working |

<table>
<tr><td></td><td>day. The treatment should be stopped until the retest result becomes available. If the repeated white blood cells is still ≤2.0 x $10^9$/L, study drug must be permanently discontinued. If the repeated test is acceptable, the subject can resume treatment.);<br>• hemoglobin ≤8.0 g/dL (to avoid laboratory errors and anomalous values, hemoglobin ≤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 drug must be permanently discontinued. If the repeated test is acceptable, the subject can resume treatment.).<br><br>Study drug should be <u>temporarily</u> stopped if any of the following occur:<br>• moderate or severe diarrhoea (i.e., increase more than 4 stools per day);<br>• platelets count <75 x $10^9$/L but >50 x $10^9$/L (the treatment should be temporarily stopped and a platelets count has to be performed by 1 week and re-tested until platelets will be normalized);<br>• white blood cell <3.0 x $10^9$/L but >2.0 x $10^9$/L (the treatment should be temporarily stopped and white blood cells have to be measured by 1 week and re-tested until white blood cells will be normalized);<br>• hemoglobin <10.0 g/dL but > 8.0 g/dL (the treatment should be temporarily stopped and hemoglobin has to be measured by 1 week and re-tested until hemoglobin will be normalized);<br>• Triglycerides >300 mg/dL (3.42 mmol/L) in fasting condition (the treatment should be temporarily stopped and triglycerides has to be measured every 2 weeks until triglycerides return to levels below 300mg/dL (3.42 mmol/L);</td></tr>
<tr><td>Dose adjustment:</td><td>In case the study drug is temporarily stopped, the study drug can be resumed at a level 20% smaller (see table below) than the one at which the AE leading to temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin are normalized and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L ) or diarrhoea is mild.
<table>
<tr><td colspan="10">Rules for Dose Reduction of 20%</td></tr>
<tr><td>Starting Dose (mg) bid</td><td>13.3</td><td>16.7</td><td>20</td><td>23.3</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>1.3</td><td>1.7</td><td>2.0</td><td>2.3</td><td>2.7</td><td>3.3</td><td>3.7</td><td>4</td><td>4.7</td></tr>
</table></td></tr>
</table>

| **Reduced Dose (mg) bid** | **10.6** | **13.4** | **16** | **18.6** | **21.4** | **26.6** | **29.4** | **32** | **37.4** |
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| **Oral suspension (mL) bid** | 1.1 | 1.3 | 1.6 | 1.9 | 2.1 | 2.7 | 2.9 | 3.2 | 3.7 |

In addition, in case a subject will have a consistent (e.g., at least 2 consecutive evaluations) platelets count ≤150 x $10^9$/L and not meeting the stopping criteria for platelets, the Investigator will have to reduce the dose by 20% of the current dose.

Only one dose reduction is allowed during the treatment period.

**Subjects who were randomized under protocol version 4.0,** will continue to follow the instructions of protocol version 4.0, related to starting dose and dose modifications:

In case the study drug was temporarily stopped, the study drug can be resumed at a level 1/3 smaller (see table below) than the one at which the AE leading to temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin are normalized and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L) or diarrhoea is mild.

| **Rules for Dose Reduction of 1/3** | | | | | | | | | |
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| **Starting Dose (mg) bid** | **20** | **25** | **30** | **35** | **40** | **50** | **55** | **60** | **70** |
| **Oral suspension (mL) bid** | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 5.0 | 5.5 | 6.0 | 7.0 |
| **Reduced Dose (mg) bid** | **13.3** | **16.7** | **20** | **23.3** | **26.7** | **33.3** | **36.7** | **40** | **46.7** |
| **Oral suspension (mL) bid** | 1.3 | 1.7 | 2.0 | 2.3 | 2.7 | 3.3 | 3.7 | 4 | 4.7 |

In addition, in case a subject will have a consistent (e.g., at least 2 consecutive evaluations) platelets count ≤150 x $10^9$/L and not meeting the stopping criteria for platelets, the Investigator will have to reduce the dose of 1/3 of the current dose.

Moreover, for subjects who have already reduced the dose of 1/3 an additional reduction by 20% is allowed for safety reasons.

For **all subjects**, during the first month of treatment, platelets count assessment will be performed weekly, while during the second month it will be performed every 2 weeks, in order to strictly monitor this parameter for safety reasons.

If the dose is reduced due to platelet count ≤150 x $10^9$/L, and/or white blood cell <3.0 x $10^9$/L and/or hemoglobin <10.0 mg/dL, a complete blood count (i.e.CBC) test must be performed weekly for 8 consecutive weeks.

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| | Any decision relevant to the 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 its authorized designee |
| **Other:** | As the subjects to be randomised are on stable steroids, the recommended national immunisation schedule, including varicella immunity, should be completed (i.e., as recommended in the "Diagnosis and Management of Duchenne muscular dystrophy" DMD guideline[4]), before the Screening Visit. However, in case of live or live attenuated vaccines to be administered during the study, the Investigator can discuss, but it is not mandatory, the case with the Medical Monitor of the study and must carefully monitor the subject. |
| **Independent Data Monitoring Committee:** | An independent Data Monitoring Committee (IDMC) will review, evaluate and categorise safety findings every 3 months during the study and will be responsible for oversight of the formal interim analysis (see below). The IDMC will have access to unblinded data, if necessary, and will operate according to the rules defined in the IDMC charter. |
| **Sample size calculation:** | The sample size (utilizing a 2:1 randomisation scheme) is calculated to provide 90% power and a 1-sided alpha of 2.5% to detect a true difference between givinostat and placebo in the target population, in 4SC 18 month change from baseline, of 3 seconds, assuming a common standard deviation (SD) of 6 seconds. The estimated SD is based on publicly available Phase 3 study data on ataluren and drisapersen in subjects with DMD in addition to internal Italfarmaco S.p.A data.<br><br>Vastus lateralis muscles fat fraction (VL MFF) assessed via MRS is the first key endpoint in the MR cohort and will be formally assessed in the target population at 12 months. Based on internal Italfarmaco S.p.A. data, a total of N=99 subjects will be sufficient to provide 80% power to detect a 55% reduction in the annual mean increase in MFF (of 6.6%) with givinostat as compared to placebo with a 1-sided alpha of 2.5% and assuming an SD for the change from baseline in MFF of 6%.<br><br>The pre-planned interim analysis was performed in January 2020, concluding that futility was not met and the trial should continue, and thus a pre-planned blinded sample size re-calculation was performed. The within treatment SD for the change in 4SC from baseline to 18 month was estimated to be 3.094 seconds, approximately half of the SD that as assumed in the original power calculation. With this revised SD estimate, N= 102 (on 2:1 basis) subjects is sufficient to provide 90% power to detect a true difference in 4SC of 2 seconds between givinostat and placebo in the target population at a 1-sided |

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| | alpha of 2.5%. With an estimated drop-out rate of 8%, a total of 110 subjects in the target population (Group A) will be randomized.<br><br>For the MR cohort, the blinded SD estimate was 5.941%, being in line with SD assumed in the original power calculation for VLFF, hence all the subjects in the target population will be included in the analysis of the MR cohort. |
| **Interim analysis:** | One formal interim analysis is planned and will be overseen by the IDMC in order to ensure study integrity ; this is to assess futility on VLFF and, if not futile, esecute a blinded sample size reestimation. |
| **Statistical Methods and Planned Analyses:** | An IDMC charter will be prepared to describe the role, function and operations of the IDMC and the rules to be used when reviewing interim data.<br><br>The planned interim analysis will be performed when the first N=50 subjects randomised in the target population have reached the 12 month time point. In this interim analysis, the IDMC will evaluate the effects of givinostat versus placebo on the VL MFF in terms of futility. Futility will be considered if VLMFF in the givinostat group is equal to or worse than that seen in the placebo group since the biologic plausibility of a subsequent treatment effect on 4SC time would be greatly diminished. The IDMC will only communicate to the Sponsor whether the study should proceed or it should be stopped having met the futility criteria.<br><br>If the IDMC indicates that the study should proceed, a blinded sample size re-estimation will be conducted for MFF and 4SC in the target population as follows:<br><br>• To maintain the blind on the primary endpoint, the within treatment SD for the change from baseline will be estimated as $\widehat{\boldsymbol{\sigma}}_{\boldsymbol{within}} = \sqrt{\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{overall}} - \left(\frac{\boldsymbol{\Delta}}{\boldsymbol{2}}\right)^{\boldsymbol{2}}}$ where $\widehat{\boldsymbol{\sigma}}_{\boldsymbol{overall}}$ is the overall SD for the change from baseline in 4SC based on the n=50 subjects in the interim and $\Delta$ is the hypothesized treatment effect, i.e. $\Delta = 3$ seconds. Dependent upon the blinded SD estimate, the sample size may be decreased or increased to maintain 90% power. Any increase in sample size for 4SC will be limited to 1.5 times maximum of the initial target population sample size, i.e., to a maximum of $1.5 \times 192 = 288$ subjects. For a 20% increase in the SD, this |

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| | degree of increase in sample size will maintain 90% power, and for a 40% increase in SD power will be maintained at 80%.<br>• With respect to VL MFF, a similar approach will be applied; the within treatment SD for the change from baseline will be estimated as $\hat{\sigma}_{within} = \sqrt{\hat{\sigma}^2_{overall} - \left(\frac{\Delta}{2}\right)^2}$ where $\hat{\sigma}_{overall}$ is the overall SD for the change from baseline in MFF and $\Delta$ is the hypothesized treatment effect, i.e. $\Delta = 3.63\%$ (i.e. 55% of 6.6%). Dependent upon the blinded SD estimate, the sample size may be decreased or increased to maintain 90% power. Any increase in sample size require to assess VL MFF will also be limited to 1.5 times the required target population sample size stated in the preceding power calculation, to a maximum of $1.5 \times 99 = 150$ subjects.<br>At the interim futility analysis, there will be no efficacy assessment, no unblinded analysis of the primary endpoint and, hence, no alpha spend nor early stopping of the study for efficacy.<br>The interim took place as planned on 14th January 2020 and included 50 patients with 12 month data and 37 with 18 month data. The independent data monitoring committee (IDMC) meeting to review the VL MFF data for futility took place on 23rd January 2020 and concluded that futility was not met and the trial should continue.<br>The sample size reassessment therefore took place, with estimated overall SDs of 3.094 seconds and 5.941% for the change from baseline in 4SC at 18 months and the change from baseline in VL MFF at 12 months respectively.<br><br>The revised sample size (utilizing a 2:1 randomisation scheme) N= 102 was calculated to provide 90% power and a 1-sided alpha of 2.5% to detect a true difference between givinostat and placebo in the target population, in 4SC 18 month change from baseline, of 2 seconds, with the blinded SD estimates during the interim analysis of 3.094 seconds. With an estimated drop-out rate of 8%, a total of 110 subjects in the target population (Group A) will be randomized.<br>For the MR cohort, since the blinded SD estimate was 5.941%, all the subjects in the target population will be part of the MR cohort.<br>Since the interim only assessed futility on VLFF and executed a blinded sample size reestimation, no alpha was spent and, hence the analysis of the primary endpoint conducted at the end of the trial will continue to be assessed at the 0.025 1-sided level. |
| **Statistical analysis:** | <u>General</u> |

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| | All efficacy analyses will be performed on Intent-To-Treat (ITT) population. Formal efficacy analyses will be performed in the target population; overall population analyses will be supportive and informal. Randomised subjects who have taken at least 1 dose of randomised therapy will be included in the relevant analyses. Descriptive statistics for qualitative data will include frequency tabulation and presentation of percentages. Descriptive statistics for quantitative data will include mean and SD or geometric mean, coefficient of variation, median and interquartile range.<br><br>A comprehensive SAP will be prepared prior to the planned interim analysis to provide full details of all planned analyses and methodology.<br><br><u>Primary Efficacy Endpoint</u><br><br>The change in 4-stairs climb (4SC) from baseline to 18 months will be evaluated in the target population. The data will be analyzed using an Analysis of Covariance (ANCOVA) model with the change in 4SC from baseline to 18 months as the dependent variable and with terms for baseline 4SC value as a covariate and randomised treatment, concomitant steroid use and age at baseline as independent class variables. Least squares means (Lsmeans) will be estimated for givinostat and placebo. The treatment effect, being difference in Lsmeans, will be presented along with the associated 95% confidence interval and the p-value. Significance will be achieved if the 1-sided p-value is $\leq$0.025.<br><br><u>Analysis of Key Secondary Endpoints</u><br><br>Formal analyses of functional secondary endpoints (i.e.,cumulative loss of function in the NSAA, change in 6MWT, NSAA, time to rise from floor and muscle strength by knee extension and elbow flexion) and Vastus lateralis MFF will be performed in the target population at 18 months using the same approach as described for the primary endpoint. Possible need for log transformation of these variables will be assessed prior to unblinding by assessment of ANCOVA model residuals without a treatment effect term.<br><br><u>Safety Data</u><br><br>Safety data will be evaluated descriptively. All subjects (target and off-target population combined) who took at least 1 dose of study drug will be included in the summary of safety. The following parameters will be summarized: rates of discontinuation, AEs, and laboratory abnormalities. Adverse event data will be coded using the Medical Dictionary for Competent Activities (MedDRA). Summary statistics will be presented for changes in vital signs. Laboratory test values will be summarised similarly, but will also include tabulation of the number of subjects shifting from within the reference range at |

| | |
|---|---|
| | baseline to outside of the reference range on randomised treatment. |

## 2 TABLE OF CONTENTS

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 |
| ATS | American Thoracic Society |
| AUC | Area under the curve |
| bid | twice daily |
| BMI | Body mass index |
| BUN | Blood urea nitrogen |
| CBC | Complete Blood Count |
| CFR | Code of Federal Regulations |
| $C_{max}$ | Maximum observed concentration |
| CRA | Clinical research associate |
| CRF | Case Report Form |
| CSA | Cross sectional area |
| CSOM | Clinical Study Operations Manual |
| CSR | Clinical study report |
| DAPC | Dystrophin-associated protein complex |
| DMD | Duchenne muscular dystrophy |
| DNA | Deoxyribonucleic acid |
| ECG | Electrocardiogram |
| ECHO | Echocardiogram |
| eCRF | electronic Case Report Form |
| EDC | Electronic Data Capture |
| EOS | End of study |
| EU | European Union |
| FAPs | Fibro-adipogenic progenitors |
| FDA | Food and Drug Administration |

| | |
|---|---|
| FEV1 | Forced Expiratory Volume at 1 second |
| FVC | Forced Vital Capacity |
| GCP | Good Clinical Practice |
| GGT | Gamma-glutamyl transpeptidase |
| GI | Gastrointestinal |
| GSPV | Global Safety and Pharmacovigilance |
| Hct | Hematocrit |
| HDAC | Histone deacetylase |
| HDACi | Histone deacetylase inhibitors |
| HDL | High-Density Lipoprotein |
| HHM | Hand-held myometry |
| HIPPA | Health C Portability Accountability Act |
| IB | Investigator's Brochure |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonization |
| IDMC | Independent Data Monitoring Committee |
| IEC | Independent Ethics Committee |
| IL-1β | Interleukin-1 beta |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| IRT | Interactive response technology |
| ITT | Intention-to-Treat |
| LDH | Lactate dehydrogenase |
| LDL | Low-Density Lipoprotein |
| IFN-γ | Interferon gamma |
| IL-6 | Interleukin-12 |
| IL-12 | Interleukin-6 |
| LLN | Lower Limit Normal |
| Lsmeans | Least squares means |
| 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 |
| MMT | Manual muscle testing |

| | |
|---|---|
| MoA | Mechanism of action |
| MRI | Magnetic resonance imaging |
| MRS | Magnetic resonance spectroscopy |
| mRNA | Messenger ribonucleic acid |
| nNOS | Neuronal nitric oxide synthase |
| NO | Nitric oxide |
| NOAEL | No-Adverse Effect Level |
| NSAA | North Star Ambulatory Assessment |
| OCT | Organic Cation Transporter 2 |
| PEF | Peak Expiratory Flow |
| PI | Principal Investigator |
| PK | Pharmacokinetic |
| PODCI | Paediatric Outcomes Data Collection Instrument |
| PT | Prothrombin time |
| QT | QT interval |
| QTc | Corrected QT interval |
| QTcF | QT interval, Fridericia's correction |
| RBC | Red blood cell |
| ROM | Range of motion |
| SAE | Serious adverse event |
| SAP | Statistical Analysis Plan |
| SD | Standard deviation |
| TEAE | Treatment-emergent adverse event |
| TFT | Timed function test |
| TNF-α | Tumor necrosis factor-alpha |
| TSA | Trichostatin A |
| ULN | Upper Limit Normal |
| US | United States |
| VL MFF | Vastus lateralis muscle fat fraction |
| WBC | White blood cell |

# 4 INTRODUCTION

## 4.1 Background on Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is a rare degenerative, X-linked recessive genetic disorder with estimated incidence of 1 in 5000 live births (Mendell, J. R., et al.[1]), caused by mutations in the dystrophin gene. In DMD, mutations in the dystrophin gene disrupt the open-reading frame, resulting in an absence of functional dystrophin, a critically important part of the protein complex that connects the cytoskeletal actin of a muscle fiber to the extracellular matrix.

Lack of dystrophin causes repetitive muscle damage. In a normal muscle, the physiological repair process removes the damaged muscle fibers and reconstitutes normal muscle fibers. In DMD, on the other hand, the repair process leads to a combination of new muscle fibers, fatty replacement and fibrosis, with the two latter components progressively prevailing as a process called fibroadipose degeneration. This detrimental outcome typically compromises muscle function, alters the tissue environment, and probably limits the potential effectiveness of regenerative approaches. As described by Peverelli et al.[2], fibrosis is already significant at one year of age in DMD patients constituting approximately 16% of a muscle biopsy (3% in age matched controls) and remains relatively constant until 7 years of age. At this age, there is an important increase in the proportion of fibrosis that reaches approximately 30% of muscle biopsy between 7 and 10 years of age. Similarly MRI studies have shown that fat infiltration is already present at 5 years of age and constantly increases (Forbes, et al.[3]).

Duchenne muscular dystrophy is characterized by progressive symmetrical muscular weakness that affects proximal muscles more than distal muscles, often accompanied by calf muscle pseudo hypertrophy.

Significant motor deficits may be present during the first year of life, but diagnosis is usually made between the ages of 3 to 5 years when the affected patients begin to show waddling gait, toe walking, and difficulty climbing stairs. Over time, ambulation becomes increasingly abnormal, and by 8 years of age, most patients lose the ability to rise from the floor and climb stairs, and often fall while walking. By 12 to 14 years of age, most lose the ability to walk and the heart and respiratory muscles also are affected. Upper limb, cardiac, and

diaphragmatic muscles progressively weaken during adolescence. After 18 years all patients are affected by cardiomyopathy. Only few survive beyond the third decade; most patients die because of respiratory complications and heart failure due to cardiomyopathy. There are still no curative treatments for such a debilitating disease and the current management of the disease is based on prevention and management of complications (Bushby, et al.[4]).

Corticosteroids (e.g., prednisone or deflazacort) have been demonstrated to slow the rate of muscle weakness when initiated in ambulatory boys, however, the use of corticosteroids is limited by all the potential side effects, including weight gain, cataracts, osteopenia, and avascular necrosis (Manzur, A, et al.[5]). Recently, the European Medicines Agency has granted a conditional approval to Ataluren, which targets nonsense mutations as the cause of DMD, which is relevant for only 13% of DMD population, or approximately 2,000 patients in the United States (US) and 2,500 patients in the European Union (EU).

In addition, FDA has granted an accelerated approval to Eteplirsen and Golodirsen, which targets DMD gene mutations skippable with exon 51 skipping and exon 53 skipping, respectively, which are relevant for only 21% of DMD population, or approximately 3,200 patients in the United States (US).

Ataluren, Eteplirsen and Golodirsen are the first of a number of treatments that are aimed at correcting the original genetic defect in the dystrophin gene.

Genetic Duchenne therapy via AAV Micro-dystrophin may be promising.

In light of the above considerations, it becomes evident that an unmet therapeutic need exists for the treatment of this disabling and fatal condition.

### 4.2 Background on Givinostat

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).

Some HDACs may shuttle between nucleus and cytosol in a stimulus-dependent way or may have a predominantly cytosolic localization, where they affect the post-translational modification of cytosolic proteins (Choudhary C et al. 2009[6]).

Givinostat is an orally active hydroxamic acid derivative possessing a potent HDAC (Class I and II) inhibitory activity. As potent and specific inhibitor of the HDAC enzymes, givinostat shares the anti-tumor properties of the histone deacetylase inhibitor (HDACi)- class, i.e., cell-cycle inhibition, induction of apoptosis and stimulation of cell-differentiation.

In the clinic, the HDACi doses required to achieve efficacy in cancer patients are generally poorly tolerated and dose-limiting toxicities include thrombocytopenia, gastrointestinal (GI) tract adverse events (AEs), and fatigue (Guha 2015[7]). 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 decisions 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, Perry et al. 2006[8]; Bernstein, Mikkelsen et al. 2006[9]). 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 through the C-terminal domain 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[10]). 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, Mozzetta et al. 2008[11]). 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, Saccone et al. 2011[12]):

1. Resident muscle interstitial cells 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, Colussi et al. 2006[13]) and direct inhibition of HDAC2 by HDACi, or inactivation by either NO donors or by reconstitution of the 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, Consalvi et al. 2014[14]). 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, F et al. 2005[15] and Joosten, L. A., F. Leoni, et al 2011[16]). Pro-inflammatory cytokines have been shown to inhibit muscle satellite cells differentiation into muscle fibers in DMD, thus contributing to the impaired muscle regeneration in this disease (Boldrin et al, 2015[17], Tierney et al. 2014[18], Doles, Olwin, 2014[19])

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 (see Section 4.2.1).

### *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 paediatric 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) (-84% males, -78% females) and smaller effects on red blood cells (RBC) (-10% males, -5% females) and platelets (-16% males, -11% females). It has to be pointed out, that this toxicity pattern is different from what is observed clinically in humans, where thrombocytopenia and not leukopenia is dose-limiting, suggesting a different sensitivity of the hematopoietic lineage in rats versus humans.

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. All of these changes were modest (equal to or less than 50%), their extent overlapped with control animals and they were not considered of toxicological significance. Lower weight of liver, spleen and adrenals was also observed. In all cases the underlying histopathological changes were minimal. All pathologic effects trended toward reversibility or completely reversed following the recovery period.

In contrast to rats, 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. In animals at recovery, the decrease of thymus weight was minimal, whilst no histological changes were noted. The dose of 12 mg/kg/day was therefore defined as the No-Adverse Effect Level (NOAEL) under the conditions used in the present study, even if the compound was well tolerated also at the highest dose of 30 mg/kg/day. Similar effects on thymus weight were reported also for other

HDACi and are likely reflective of the known and well-characterized cytostatic mechanism of action of high doses of these compounds (Kerr, Galloway et al. 2010[20]).

Additional findings of possible toxicological relevance 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. Also this effect can be ascribed to the well-characterized cytostatic mechanism of action of high doses of the HDACi (Kerr, Galloway et al. 2010[20]). The liver showed minimal bile duct hyperplasia in two male animals out of four, receiving 30mg/kg/day, whilst males from the others dose-groups and females at all doses were unaffected. After recovery, a similar finding was still noted in a single animal.

Givinostat is eliminated for approximately 50% in faeces after IV administration of 14C-ITF2357 to rats, highlighting the quantitative importance of the biliary excretion route. 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).

Thus, the bile duct hyperplasia is 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 was recorded in approximately 450 subjects treated so far; bilirubin levels are also monitored clinically in DMD subjects with, so far, no evidence for significant alterations, providing no confirmation for 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 month the 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/14/2357/48). Only data from male animals were considered since DMD occurs in male subjects.

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; $AUC_{0-4}$ = area under the curve (over the first 4 hours)

Even though the safety margin between NOAEL in non-clinical studies/paediatric 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.

A post-weaning study in young rats treated with givinostat 20 to 180 mg/kg/day for 4 weeks was also performed and showed a toxicological profile similar to that seen in adult animals. Givinostat did not affect the behavioral and physical development and reproductive performance of pups. The NOAEL in this study was 60 mg/kg/day.

Concerning the 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 subjects both adults and paediatric 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), but givinostat did not display any genotoxicity/mutagenic potential in mammalians in both in vitro and in vivo studies.

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 month old at the beginning of the treatment) was performed (Consalvi, et. Al 2012[12]). 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) 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 fibers 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. The PK/PD analysis showed that at least a blood $AUC_{0\text{-}24}$ 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 mouse.

### *4.2.2 Clinical Experience with Givinostat Including Risks and Benefits*

Completed 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 disorder 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 paediatric subjects, doses up to 0.75 mg/kg bid have been administered for up to 6 months (with 1 additional subject having been treated for 2 years and 7 months) in patients with Juvenile Idiopathic Arthritis. 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 i.e., 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 (see Section 8.2.1) doses have been 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 in this extension phase is 60 mg bid.

The most common AEs observed were thrombocytopenia, as well as gastrointestinal toxicities. Adverse events were generally mild to moderate and reversible upon discontinuation of study drug. Moreover, dose-dependent asymptomatic and reversible platelets count reductions were observed both in healthy volunteers and subjects treated with givinostat. These decreases typically occurred within the first week after treatment initiation, reached a nadir after 2 to 3 weeks of treatment, and then remained at a plateau (see the IB, Section 7). The majority of thrombocytopenic events were mild in severity with fewer than 10% of subjects developing platelets count below 75 x $10^9$/L. All occurrences resolved completely within 2 to 3 weeks of discontinuation of treatment, suggesting a rapidly reversible effect. Overall, sixteen haemorrhagic drug-related AEs were reported by 14 patients

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

Therefore, given these known effects of HDAC inhibitors including Givinostat a thorough monitoring of haematologic parameters will be implemented in order to avoid the occurrence of severe thrombocytopenia (see Section 12.8).

In addition, during the study particular attention will be paid in monitoring possible effects on QTc (see Table 6: Schedule of Assessments for details), since some episodes (21 episodes in 15 subjects) of Corrected QT interval (QTc) prolongation have been recorded during the clinical studies performed so far. It is worth noting that 90% of the recorded events were reported in oncological studies in subjects treated at highest doses (e.g., ≥100 mg/daily) and no events were recorded during the previous study in DMD subjects.

Concerning the clinical experience in DMD, the aforementioned histological results observed in the mdx mouse model were replicated in a Phase 2 study of givinostat (Study DSC/11/2357/43, EudraCT n. 2012-002566-12) in 20 ambulant DMD subjects (from 7 to 10 years of age at study start, on stable steroid treatment) where, after one year of treatment, the muscle biopsy analysis showed a significant increase in muscle fibers area fraction (MFAF) and a significant reduction of muscle necrosis, fatty replacement and fibrosis. Analysis of the effect on CSA of the muscle fibers showed that givinostat significantly increases CSA of all type of fibers (small, medium, large) in a similar manner and that such effect on CSA predicts the increase in MFAF and the reduction of fibrosis. In addition, an increase in the number of regenerating fibers and satellite cells was observed. Moreover, descriptive analysis conducted on the secondary efficacy endpoints on muscle function (i.e., six-minute walking test [6MWT], North Star Ambulatory Assessment [NSAA], time function tests and pulmonary function tests) showed an overall stability after 1 year of treatment in this population.

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*

Nonclinical studies have shown that givinostat has a potent anti-inflammatory effect (see (current IB) and that HDACi regulate the transcription of a number of factors that are key in muscle regeneration, e.g., follistatin. The combination of these effects is expected to re-balance the repair process in DMD muscle towards increased muscle regeneration and reduced fatty infiltration and fibrosis. Indeed, in a study in mdx mice and in a phase 2 study in DMD children, givinostat was shown to reduce muscle inflammation, necrosis and fibrosis and to increase muscle regeneration.

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 putative mechanism of action (MoA), supporting the potential therapeutic role of givinostat in counteracting muscle tissue degeneration, which occurs downstream of the genetic defect in DMD.

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. However, as the fibers CSA enlarging effect appears key in givinostat's pharmacological effect, the functional benefit obtained will likely depend on the amount of muscle that is still present when therapy is started.

In Study DSC/11/2357/43, in ambulant DMD subjects ≥7 years of age on steroid for more than 6 months, only 50% of the muscle biopsy was occupied by muscle fibers at baseline with the remainder being represented mainly by fibrotic tissue. Although after one year of treatment with givinostat, the histological results showed a significant increase in muscle fibers CSA and a significant improvement in all the histological parameters analyzed, the fact that already 50% of muscle was lost in these children suggests that givinostat should be started at an earlier age if the objective is to achieve a clinical benefit in terms of ambulation within a reasonable timeframe. Several studies, however, have shown that ambulatory function naturally improves in children with DMD up to the age of 7 years (Goemans,

Klingels et al. 2013[22]; McDonald, Henricson et al. 2013[23]) suggesting that studies in DMD children <7 years of age may require larger numbers and a longer treatment duration to show a significant difference from placebo. As a result all the pivotal studies in DMD ambulant children reported so far (e.g., Ataluren, Drisapersen, Tadalafil phase III trial ) have selected children who were expected to have a sufficient decline in ambulatory function after 1 year on placebo based on age and 6 Minute Walk Test at baseline. However, all these studies were not able to show a functional benefit of the tested drug versus placebo.

Although a lack of efficacy cannot be ruled out, the poor therapeutic response observed in these studies could be the result of selecting a patient population characterized by a degree of functional impairment that may be less likely to benefit from treatment. This is also suggested by the fact that while ambulatory function improves up to the age of 7 years and then declines, the underlying muscle morphology constantly declines from the age of 5 years (Forbes, et al.3). A post-hoc analysis on study DSC/11/2357/43 suggests that children with a more preserved Vastus Lateralis (VL) muscle at baseline MRI (score 0 to 2 on a 0 to 5 semi-quantitative MRI score, modified Mercuri's score[24]) have a lesser degree of functional decline 2 years after start of givinostat treatment. This is in line with data from the Imaging DMD group suggesting that changes from baselite at 1 year in VL Fat Fraction (FF) assessed by magnetic resonance spectroscopy (MRS) are correlated with Timed Function Tests (TFTs) and 6MWT (Data on file). These analyses, however, have also shown that children with a baseline MVL MFF <10% show very little change in any parameter (MRI/MRS or function) within 1 year. A more recent (i.e. July 2018) and detailed analysis of Imaging DMD data showed that patients with a baseline MRS VL FF up to 5% have little change in clinically relevant functional parameters such as 4 stairs climb after 1 and 2 years. Based on these data, the current protocol will select a population of subjects with a VL MFF >5% but ≤30% (corresponding approximately to a score >2 in the aforementioned 0 to 5 semi-quantitative MRI score Mercuri, et al. 2002[24]) as this population is expected to still have enough muscle to build on and to have enough function decline in 18 months if on placebo. Since the analyses of the Imaging DMD data (Data on file) also suggested that TFTs predict VL MFF, an algorhythm has been developed to identify at screening those children with a VL MFF within the required range. However, the screening data colleted up to 18th June 2018 showed that the accuracy of the algorithm was less predictive since it did not allow the selection of a

population with the predefined range of VL FF that identified a population most likely to benefit from treatment. As a result, the algorhythm is removed and the selection of eligible subjects will be based on age and the functional tests at screening (see below). The assessment of VL FF at screening (range >5% to ≤30%), on the other end, will be used to identify the target population on which to perform the primary and key secondary analyses. The inclusion of an additional off-target population (up to 50 subjects) outside of the VL FF range of >5% to ≤30% will provide supportive data. 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), the time to climb 4 standard-sized stairs has been chosen to evaluate the efficacy of givinostat in slow down disease progression. Timed function tests, such as time to climb 4-stairs (4SC), to walk 10 m, and to stand up, are reliable and feasible in the majority of the DMD population from age 4 and older (McDonald, C. M., et al. 2013[25]) and have a documented response to therapeutic intervention with steroids (Manzur AY, K. T., 2009[26]). In particular, time to climb 4 standard-sized stairs is a TFT that represents stair climbing ability, a clinically meaningful function in and of itself. It has been used as an endpoint in DMD studies for decades and the test is reproducible and simple to administer. It is predictive of loss of stair climbing ability, loss of ambulation and time to 10% decline in ambulatory capacity (Bushby, K. 2011[27]). Furthermore, climbing stairs requires a greater range of motion (ROM) from the joints of the lower limb and greater muscle strength than 6MWT, which is a functional test more related to endurance. Therefore, in light of givinostat MoA (e.g., increase fibers CSA) the time to 4SC test is expected to be more appropriate to evaluate the efficacy of givinostat in DMD subjects.

The key secondary endpoints (cumulative loss of function in the NSAA, change inTime to Rise from Floor, in NSAA, in 6MWT and in strength) include those parameters which are considered relevant in this population to corroborate the results obtained on the primary endpoint.

Changes in MRS parameters will be also key endpoints. Previous studies have shown that MRI can visualize structural alterations of muscle in muscular dystrophies (Willcocks, 2014[28], 2016[29], Wren 2008[30], Bonati 2015[31]) and have shown 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, a number of randomized clinical trials currently ongoing include magnetic resonance evaluations and both the FDA and EMA guidelines identify MRI/MRS as a potential biomarker.

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*

To establish the effects of givinostat versus placebo administered chronically over 18 months to slow disease progression in ambulant DMD subjects.

### *5.1.2 Secondary Objectives*

The secondary objectives of this study are:

- To assess the safety and tolerability of givinostat versus placebo administered chronically in DMD subjects.
- To evaluate the PK profile of givinostat administered chronically in DMD subjects
- To evaluate the impact on quality of life and activities of daily living of givinostat versus placebo administered chronically.

### *5.1.3 Secondary Exploratory Objectives*

- To evaluate the correlation between PK profile of givinostat and pharmacodynamics (PD) data;
- To explore whether the effects of givinostat versus placebo administered chronically may be related to the type of DMD mutation (Pane M et al. 2014[32]) or to the biomarkers.

## 5.2 Study Endpoints

### *5.2.1 Primary Endpoint*

The primary endpoint of this study is the mean change in 4SC before and after 18 months of treatment of givinostat versus placebo.

### *5.2.2 Key Secondary Endpoints*

The key secondary efficacy endpoints for this study are as follows:

Function :

- Mean change in time to rise from floor
- Mean change in 6MWT
- Mean change in NSAA
- Cumulative loss of function on the NSAA
- Mean change of muscle strength evaluated by knee extension, elbow flexion as measured by hand-held myometry (HHM)

Imaging:

- Mean change in vastus lateralis muscles fat fraction

The above endpoints will be formally assessed after 18 months of treatment.

### *5.2.3 Safety Endpoints*

The safety endpoints of this study are as follows:

- Number of subjects experiencing treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) (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);
  - Respiratory 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 echocardiogram (ECHO);
  - Cognitive function evaluated by the Raven coloured progressive matrices;
  - Weight, height, and body mass index (BMI);
- Evaluation of acceptability/palatability of the oral suspension.

### *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 in the subject population;
- Identification of the relevant demographic and pathophysiological covariates influencing the PK of givinostat;

### *5.2.5 Exploratory Endpoints*

The exploratory endpoints of this study are as follows:

- Mean changes in:
  - time to walk/run 10 meters;
  - PODCI scores.
  - %-predicted 6MWT (the predicted value is against healthy subjects and is calculated using the equation of Geiger [Henricson, et.al.[33]]);
  - Only in the MR cohort: MRI parameters (e.g., fat fraction of thigh muscles, CSA of vastus lateralis and other thigh muscles);
- Time to 10% persistent worsening in 6MWT (Baseline through end of study);
- Proportion of subjects with ≥10% worsening in 6MWT at end of study;
- Time to loss of standing (Baseline through end of study);
- Proportion of subjects who loose ambulation during the study;
- Evaluation of any correlation between the effect of Givinostat on disease progression and the type of DMD mutation, LTBP4 and Osteopontin genotype;
- Evaluation of any possible DMD serum biomarker;
- PK-PD analyses: relationships between metrics of exposure and efficacy/safety endpoints of givinostat.

# 6 INVESTIGATIONAL PLAN

## 6.1 Description of Overall Study Design and Plan

This is a Phase 3, randomised, double blind, placebo controlled, multicentre study to evaluate the efficacy and safety of givinostat in ambulant subjects with DMD. This study will include ambulant male paediatric subjects aged ≥6 years at baseline affected by DMD.

A total of 110 subjects in the target population (Group A: subjects with a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range >5% and ≤30%) will be randomised. Up to 50 subjects (about 35% of the overall population) outside of the target may also be recruited into the study (Group B: subjects with a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range ≤5% or >30%). Subjects who assent to participate in this study (if capable of doing so) and whose parent/legal guardian sign the Informed Consent Form (ICF) to participate will undergo pre-study screening assessments up to 4 weeks before the first scheduled dose of study drug.

At the randomisation visit, in addition to the continued standard of care corticosteroids regimen, DMD subjects will be randomised (2:1 ratio) to receive givinostat oral suspension 10 mg/mL or placebo oral suspension bid (in a fed state) as described in Section 8.3.

At randomisation, subjects will be stratified for their concomitant use of steroids in 4 strata:

1. Deflazacort Daily regimen
2. Deflazacort Intermittent regimen
3. Other steroids Daily regimen
4. Other steroids Intermittent regimen

The study duration is planned for 19 months and comprises 2 phases:

1. Screening period: starting 4 weeks (± 2 weeks) before randomisation
2. Treatment period: 18 months of treatment

An overview of the study is provided in

Figure **6-1**.

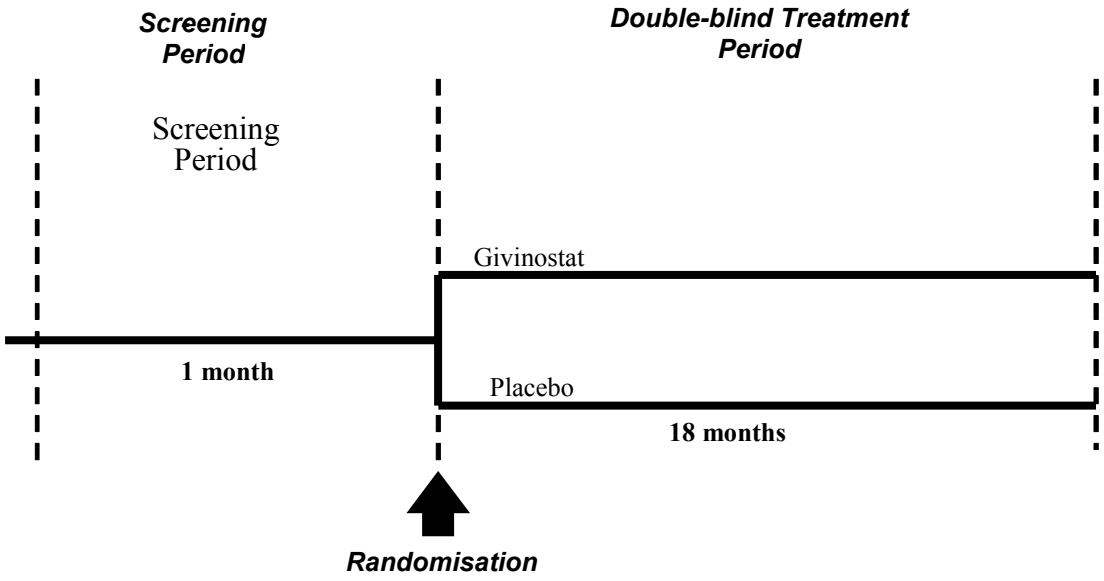



**Figure 6-1: Study Design**

There will be a total of 15 visits, including the Screening and the Randomisation Visits and excluding the Follow up Visit. Subjects may be evaluated more often if necessary for safety reasons. Subjects who discontinue the study drug early will be asked to come in for an Early

Withdrawal Visit within 2 weeks after the last dose of study drug. Subjects who have ongoing AEs at discontinuation will be followed until resolution or stabilization.

In order to guarantee the continuation of treatment with givinostat, the Sponsor has planned a long-term study which will start when the first subject enrolled in this study has attended his last visit. As a consequence, at the end of the treatment, parent/ legal guardian will be asked to consent and subject will be asked to assent/consent to his participation to the long-term study. In case the subject will not assent/consent and/or the parent/ legal guardian will not consent it, a final follow up visit will be performed 4 weeks after last dose administration.

## 6.2 Discussion of Study Design

This study is designed as a conventional parallel group design. Since the study will be conducted in a paediatric population with a serious and life-threatening disease, the unequal randomisation ratio (i.e., 2:1 to givinostat and placebo) will be applied to reduce the exposure to placebo to only one third of subjects. A 2:1 randomisation also provides for an increased number of subjects who are exposed to givinostat.

The sample size was originally calculated to provide 90% power and a 1-sided alpha of 2.5% to detect a true difference between givinostat and placebo in the target population in the 4SC 18 month change from baseline of 3 seconds, assuming a common standard deviation (SD) of 6 seconds (being based on publicly available phase 3 study data on ataluren and drisapersen in DMD subjects in addition to internal Italfarmaco S.p.A data.), giving rise to an estimated sample size of N=192 subjects. However, the planned interim analysis took place in January 2020 based upon the first 50 randomised patients reaching 12 months of treatment. This interim provided a revised, blinded within treatment SD estimate of 3.094 seconds, approximately half of that assumed in the original power calculation. Based on this revised SD estimate, the revised sample size is N=102 which provides 90% power for a 2 second difference between givinostat and placebo for the change in 4SC at 18 months.

In a recent analyses performed by C-Tap (Wong B, et al. Action Duchenne International Conference, November 9–11, 2018, Birmingham, UK), the minimally clinically important difference (MCID) for timed function tests (TFTs) in DMD was estimated at ~1.0 to 1.6 seconds. These results were confirmed by additional analyses performed on the CINRG

natural history database (Data on File), which showed that a change of 1 to 1.5 seconds in TFTs has clinical meaningfulness based on health-related QoL and participation measures in DMD and that a 1.6 to 1.7 second change over 12-months in TFTs translates to a 3-year prolongation of ambulation in DMD. Together, these new data suggest that 2 seconds is an appropriate conservative estimate of a clinically meaningful effect size for TFTs in DMD, which is less than the 3 seconds originally hypothesized in the study protocol(MCID; McDonald CM et al. 2013[22]); however, with the blinded SD estimate made at the planned interim, the revised total number of subjects, N=102 has 90% power to detect this smaller, yet clinically relevant treatment effect. The treatment duration of 18 months has been chosen because DMD children with more preserved muscle mass will be enrolled in this study, and despite the underlying progression of muscle tissue deterioration, a decline of function is expected to be slower in the selected patient population compared to DMD subjects in "decline phase" (e.g., DMD subjects >7 years with 6MWT < 350 meter, McDonald, Henricson et al. 2013[23]); thus, a longer observation period is needed to demonstrate functional benefits compared givinostat to placebo controls.

At randomisation, the population will be stratified according to steroid type and regimens (i.e., daily regimen or intermittent regimen) because some evidence suggests Deflazacort use was associated with later loss of ambulation and increased frequency of side effects (Bello et al. 2015[36]) and daily administration of steroids to be more effective than treatment on alternate days (Bushby, Finkel et al. 2010[34]; Ricotti, Baranello et al. 2013[35]). Thus, to help to ensure balance between the randomised treatment arms stratification by steroid type and regimens will be implemented.

Daily regimen means every daily intake of steroids, while intermittent regimens comprise steroids used every other day, high dose weekend and 10 days on alternate to 10 or 20 days off. Different schedule can be discussed with the Medical Monitor before the subject randomisation, however the decision remains with the Investigator only or its authorized designee.

An Independent Data Monitoring Committee (IDMC) will review, evaluate, and categorise safety findings every three months during the study and will be responsible for oversight of the interim analysis (see below) at which futility will be assessed and a blinded sample size

reestimation will be made. The IDMC will have access to unblinded data, if necessary, and will operate according to the rules defined in the IDMC charter.

A single interim analysis has been planned in this study which will be overseen by the IDMC in order to ensure the study integrity:

- The first interim analysis will be performed when the first N=50 subjects randomised in the target population have reached the 12 month time point and the effects of givinostat versus placebo on the mean vastus lateralis muscles fat fraction (VL MFF) assessed by MRS will be evaluated. The purpose of this interim is to assess futility. **There will be no formal assessment of efficacy and no unblinded evaluation of the primary endpoint, even by the IDMC**.
  Based on prior data, a 5% annual mean increase in VL MFF in the placebo group is expected and a corresponding 3.5% annual mean increase in the givinostat group is desired. Futility will be considered if the mean change in VL MFF in the givinostat group is equal to or worse than that seen in the placebo group since the biologic plausibility of a subsequent treatment effect on 4SC time would be greatly diminished. The IDMC will only communicate to the Sponsor, whether the study should proceed or be stopped, based on whether the study has met the futility criteria. If the IDMC indicates that the study should proceed, a blinded sample size re-estimation will also be conducted for VL MFF and 4SC in the target population.

More details about the interim analysis are described in Section 13.10.

# 7 SELECTION AND WITHDRAWAL OF SUBJECTS

The study will enroll male subjects with an established molecular diagnosis of DMD who are at least 6 years of age at randomisation.

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

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

## 7.1 Inclusion Criteria

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

1. Are an ambulant male aged ≥6 years at randomisation with DMD characteristic clinical symptoms or signs (e.g., proximal muscle weakness, Gowers' maneuver, elevated serum creatinine kinase level) already present at screening;
2. Have DMD diagnosis confirmed by genetic testing;
3. Are able to give informed assent and/or consent in writing signed by the subject and/or parent/legal guardian (according to local regulations);

4. Are able to complete 2 Four Stairs Climb test (4SC) screening assessments; the results of these tests must be within ±1 second of each other;
5. Have the mean of 2 screening 4SC assessments ≤8 seconds;
6. Have time to rise from floor between ≥3 and <10 seconds at screening;
7. Have manual muscle testing (MMT) of quadriceps at screening ≥ Grade - 3;
8. Have used systemic corticosteroids for a minimum of 6 months immediately prior to the start of study treatment, with no significant change in corticosteroids type or dosage or dosing regimen (excluding changes related to body weight change) for a minimum of 6 months immediately prior to start of study treatment and a reasonable expectation that dosage and dosing regimen will not change significantly for the duration of the study.
9. Subjects must be willing to use adequate contraception.

   Contraceptive methods must be used from Randomization Visit 3 through 3 months after the last dose of study drug, and include the following:

   - True abstinence (absence of any sexual intercourse), when in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, postovulation 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

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

1. Have exposure to another investigational drug within 3 months prior to the start of study treatment (only exception allowed is use of Deflazacort in US as part of the Expanded Access Program and in Canada as part of the Special Access Program;
2. Have exposure to idebenone within 3 months prior to the start of study treatment;
3. Have exposure to any dystrophin restoration product (e.g., Ataluren, Exon-skipping) within 6 months prior to the start of study treatment;
4. Use of any pharmacologic treatment, other than corticosteroids, that might have had 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 as long as their intake has been stable for 3 months prior to the start of study treatment; Testosterone will also be allowed if it is used as a replacement therapy for the treatment of delayed puberty, and testosterone dose and regimen have been stable for at least 6 months and circulating testosterone levels are within the normal ranges for the subject's age;
5. Have 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;

6. Loss of ≥30 degrees of plantar flexion from the normal range of movement at the ankle joint due to contracture (i.e. fixed loss of more than 10 degrees of plantar flexion from plantigrade, assuming normal range of dorsiflexion of 20 degrees);
7. Change in contracture treatment such as serial casting, contracture control devices, night splints, stretching exercises (passive, active, self) within 3 months prior to enrollment, or expected need for such intervention during the study;
8. Have presence of other clinically significant disease, which, in the Investigator's opinion, could adversely affect the safety of the subject, making it unlikely that the course of treatment or follow-up would be completed, or could impair the assessment of study results;
9. Have a diagnosis of other uncontrolled neurological diseases or presence of relevant uncontrolled somatic disorders that are not related to DMD;
10. Have platelets count, White Blood Cell and Hemoglobin at screening < Lower Limit of Normal (LLN) (for abnormal screening laboratory test results (<LLN), the platelets count, White Blood Cell and Hemoglobin will be repeated once; if the repeat test result is still <LLN, then exclusionary);
11. Have symptomatic cardiomyopathy or heart failure (New York Heart Association Class III or IV) or left ventricular ejection fraction <50% at screening;
12. Have a current or history of liver disease or impairment, including but not limited to an elevated total bilirubin (i.e. > 1.5 x ULN), unless secondary to Gilbert disease or pattern consistent with Gilbert's;
13. Have inadequate renal function, as defined by serum Cystatin C >2 x the upper limit of normal (ULN). If the value is >2 x ULN, the serum Cystatin C will be repeated once; if the repeated test result is still >2 x ULN, the subject should be excluded);
14. Have Triglycerides > 300 mg/dL (3.42 mmol/L) in fasting condition at screening visit;
15. Have a positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus at screening;
16. Have a 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);
17. Have a psychiatric illness/social situations rendering the potential subject unable to understand and comply with the muscle function tests and/or with the study protocol procedures;
18. Have any hypersensitivity to the components of study medication;
19. Have a sorbitol intolerance or sorbitol malabsorption, or have the hereditary form of fructose intolerance;
20. Have contraindications to MRI or MRS (e.g., claustrophobia, metal implants, or seizure disorder).

At the discretion of the Investigator, subjects not meeting inclusion/exclusion criteria may be re-screened twice with an interval of at least 3 months between assessments.

## 7.3 Withdrawal, Removal, and Replacement of Subjects

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

- Withdrawal of consent. The subject or his parent/guardian desire to withdraw from further participation in the study in the absence of the Investigator determining a medical need to withdraw. If the subject or parent/guardian give a reason for withdrawing, it should be recorded in the electronic Case Report Form (eCRF).
- Protocol Violation. The subject's findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g., failure to return for defined number of visits). Before withdrawing a subject each case will be discussed with the study medical monitor and if the safety of the subject can be reasonably assured, the subject will not be discontinued.
- Lost to Follow-Up. The subject stopped coming for visits, and study personnel were unable to contact the subject.
- Other reason. The subject was terminated 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 subject 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 subject, are grounds for discontinuation. Moreover, if the subject 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 with the subject until resolution or acceptable stabilization of the event and document all the relevant information, as applicable.

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

- If a subject 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 subject withdraws from the study, the date and reasons for withdrawal shall be recorded by the Investigator on the Subject'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 subjects prematurely discontinuing the study must be seen for a final evaluation performed within 2 weeks after the last drug intake. Final evaluation is defined as completion of the assessments scheduled for the Early Termination Visit (as detailed in Section 9.1.8). Data collected during the final evaluation are crucial to the integrity of the final study analysis because early withdrawal could be related to the safety profile of the study drug. The Investigator will make every effort to see subjects who fail to return for a final visit.
- In the event that a subject 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 subject is withdrawn from the study, he may not re-enter the study.

### *7.3.1 Safety Stopping Rules*

Study drug should be <u>permanently</u> interrupted if any of the following occurs:

- severe drug-related diarrhoea (i.e., increase of $\geq$7 stools per day);
- any drug-related SAE;
- QTcF >500 msec*;
- platelets count $\leq$50 x $10^9$/L (to avoid laboratory errors and anomalous values, platelets count $\leq$50 x $10^9$/L 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 platelets count is still $\leq$50 x $10^9$/L, study drug must be permanently discontinued. If the repeated test is acceptable, the subject can resume treatment.);
- white blood cells $\leq$2.0 x $10^9$/L (to avoid laboratory errors and anomalous values, white blood cells $\leq$2.0 x $10^9$/L 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 white blood cells is still $\leq$2.0 x $10^9$/L, study drug must be permanently discontinued. If the repeated test is acceptable, the subject 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 $\leq$8.0 g/dL, study drug must be permanently discontinued. If the repeated test is acceptable, the subject can resume treatment.).

The Investigator will follow up on him until resolution or acceptable stabilization of the event occurs and document all the relevant information, as applicable. After the resolution/stabilization of the event, the subject will be withdrawn from the study and the EOS Visit (see Section 9.1.8) will be performed.

* based on average QTc value of triplicate ECGs. For example, if an ECG demonstrates a prolonged QTcF interval >450msec, 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 subjects should be discontinued from the study. If the mean QTcF value of the 3 replicates still meets the stopping criteria the subject has to interrupt the study drug, the medical monitor has to be informed and the value must be confirmed by the central reading before withdraw the subject from the study. The QTc has to be calculated with the Fridericia formula $QTc = QT/RR^{1/3}$.

Study drug should be <u>temporarily</u> stopped if any of the following occur:

- moderate or severe diarrhoea (i.e., increase more than 4 stools per day).
- platelets count <75 x $10^9$/L but >50 x $10^9$/L (the treatment should be temporarily stopped and platelets count has to be performed by 1 week and re-tested until platelets will be normalized);
- white blood cell <3.0 x $10^9$/L but >2.0 x $10^9$/L (the treatment should be temporarily stopped and white blood cells have to be measured by 1 week and re-tested until white blood cells will be normalized);
- hemoglobin <10.0 g/dL but > 8.0 g/dL (the treatment should be temporarily stopped and hemoglobin has to be measured by 1 week and re-tested until hemoglobin will be normalized);
- Triglycerides >300 mg/dL (3.42 mmol/L) in fasting condition (the treatment should be temporarily stopped and triglycerides has to be measured every 2 weeks until triglycerides return to levels below 300 mg/dL (3.42 mmol/L);

**<u>For subjects randomized under protocol version 6.0, or 7.0 or 8.0:</u>**

Study drug can be reduced by 20% of the current dose at which the AE leading to temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin are normalized, and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L), or diarrhoea is mild. The subject may have more than the usual number of scheduled visits if needed, until the AE resolves, and then will continue the study as per scheduled visits.

In addition, in case a subject will have a consistent (e.g., at least two consecutive evaluation) platelets count ≤150 x $10^9$/L and not meeting the 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).

For subjects randomized under protocol version 6.0 or 7.0 or 8.0, only one dose reduction is allowed during the treatment period.

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

**For subjects randomized under protocol version 4.0:**

Study drug can be reduced by 1/3 of the starting dose at which the AE leading to temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin are normalized and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L), or diarrhoea is mild. The subject may have more than the usual number of scheduled visits if needed, until the AE resolves, and then will continue the study as per scheduled visits.
If a subject was randomized with baseline triglycerides above 300 mg/dL (3.42 mmol/L) the Investigator can discuss how to manage the case with the medical monitor, however the final decision remains with the Investigator only or its authorized designee.

In addition, in case a subject will have a consistent (e.g., at least two consecutive evaluations) platelets count $\leq 150 \times 10^9$/L and not meeting the stopping criteria for platelets, the Investigator will have to reduce the dose by 1/3 vs the starting dose (see Section 8.2.2 for more details).

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

Moreover, for subjects who have already reduced the dose of 1/3 an additional reduction of 20% is allowed for safety reasons following the inscruction in section "*For subjects randomized under protocol version 6.0, or 7.0 or 8.0".*

Any decision relevant to the dose interruption and/or modification of the schedule of assessments can be discussed with the Medical Monitor, but the final decision remains with the Investigator only or its 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.

| | **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 |
| **Route of Administration** | Oral administration under fed conditions | Oral administration under fed conditions |
| **Dosing instructions:** | See Table 2 | See Table 2 |
| **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:<br>One 5 mL syringe and<br>One 1 mL syringe | Two graduated dosing syringes for oral use:<br>One 5 mL syringe and<br>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.

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

## 8.2 Dosage

### *8.2.1 Rational for Dose Selection*

The dose for this study has been selected based on: the dose ranging evaluation done in the mdx model; the results of the Phase 2 study in DMD paediatric subjects (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 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 mouse and thus was considered the target exposure for the exploratory Phase 2 study in the DMD population (DSC/11/2357/43 study).

The 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 the majority of DMD subjects were tolerated and then to verify that such exposures would translate into a significant histological benefit. Results have confirmed that doses of 25/37.5 mg bid are tolerated, do result in an $AUC_{0-24}$ of at least 300 ng*h/mL in the majority of treated DMD subjects, and result in a significant histological benefit.

On the other hand, previous clinical experience in chronic inflammatory diseases in adult and paediatric 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 efficacious in controlling symptoms and biological features of these diseases at exposures ($AUC_{0-24}$) lower or comparable to those obtained in study DSC/11/2357/43.

Lack of efficacy of doses lower than those tested in study DSC/11/2357/43 were also suggested by the post-hoc analysis of study DSC/11/2357/43 results which showed that a 37.5 mg bid dose was associated with a larger increase in CSA of fibers compared to 25 mg bid.

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 efficacious and therefore chose an initial study dose to ensure a mean $AUC_{0-24}$ similar to that achieved with 37.5 mg/bid (i.e., approximately 1150 ng*h/mL). As of 18$^{th}$ of June 2018, based on blinded safety haematologic data, the Sponsor has evaluated that, a vast majority of randomized subjects who had completed at least 8 weeks of treatment (approximately 50 to 60%) had to reduce the starting dose due to a decrease in platelet levels. It is, therefore, expected that a relevant amount of subjects that will be enrolled into the study will need to require the same dose adjustment. Therefore, a new starting dose corresponding to the reduced dose (i.e. 1/3 lower than the starting dose) of protocol version 4.0 (i.e. 13.3-46.7 mg/bid according to body weight) is proposed by the Sponsor in protocol version 6.0. 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.

As weight was shown to significantly affect givinostat clearance, and the study will enroll paediatric DMD subjects with a broad range of weight, the initial dose will be adjusted to the current body weight of the subject (see Table 2). A strict monitoring of platelets count has been planned, and it will be followed with dose adjustment instruction as described in the safety rules as described in Section 7.3.1 in order to avoid the risk of platelet reduction <75 x $10^9$/L.

### *8.2.2 Dosage Schedule*

The following is a description of the dosage, treatment regimen, and route of administration. Givinostat oral suspension (10 mg/mL) and placebo oral suspension have to be administered orally as 2 oral doses daily while the subject is in a fed state. Before its use, the suspension must be shaken for at least 30 seconds by rotating the bottle by 180° and the homogeneity of the obtained suspension verified. The suspension will be administered by means of a graduated dosing syringe. The dosage to be administered is based on subject weight and the starting doses are described below:

**Table 2: Placebo or Givinostat Starting Dose**

| **Weight (kg)** | **≥10 and < 12.5** | **≥12.5 and < 20** | **≥20 and < 25** | **≥25 and < 30** | **≥30 and < 40** | **≥40 and < 50** | **≥50 and < 60** | **≥60 and < 70** | **≥70** |
|---|---|---|---|---|---|---|---|---|---|
| **Dose (mg) bid** | 13.3 | 16.7 | 20 | 23.3 | 26.7 | 33.3 | 36.7 | 40 | 46.7 |
| **Oral suspension (mL) bid** | 1.3 | 1.7 | 2.0 | 2.3 | 2.7 | 3.3 | 3.7 | 4 | 4.7 |

The subjects who were randomized under protocol version 4.0 will continue the current dose (see Table 4).

**Table 3: Placebo or Givinostat Starting Dose for subjects enrolled under Protocol version 4.0**

| **Weight (kg)** | **≥10 and <12.5** | **≥12.5 and <20** | **≥20 and <25** | **≥25 and <30** | **≥30 and <40** | **≥40 and <50** | **≥50 and <60** | **≥60 and < 70** | **≥70** |
|---|---|---|---|---|---|---|---|---|---|
| **Dose (mg) bid** | **20** | **25** | **30** | **35** | **40** | **50** | **55** | **60** | **70** |
| **Oral suspension (mL) bid** | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 5.0 | 5.5 | 6.0 | 7.0 |

**Dose Modifications for subjects randomized under protocol version 6.0 or 7.0 or 8.0**:

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

As mentioned in Section 7.3.1, in case a subject has a consistent (e.g., at least 2 consecutive evaluations) platelets count ≤150 x $10^9$/L and 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 (first example: e.g., starting dose = 20 mg bid → 20% dose reduction → new dose = 16 mg bid; second example: starting dose = 26.7 mg bid → 20% dose reduction → new dose = 21.4 mg bid) (see Table 4).

**Table 4: Placebo or Givinostat Dose Modification for subjects randomized under study protocol version 6.0 or 7.0 or 8.0.**

| **Weight (kg)** | **≥10 and <12.5** | **≥12.5 and <20** | **≥20 and <25** | **≥25 and <30** | **≥30 and <40** | **≥40 and <50** | **≥50 and <60** | **≥60 and < 70** | **≥70** |
|---|---|---|---|---|---|---|---|---|---|
| **Starting Dose (mg) bid** | **13.3** | **16.7** | **20** | **23.3** | **26.7** | **33.3** | **36.7** | **40** | **46.7** |
| **Oral suspension (mL) bid** | 1.3 | 1.7 | 2.0 | 2.3 | 2.7 | 3.3 | 3.7 | 4 | 4.7 |
| **Reduced Dose (mg) bid** | **10.6** | **13.4** | **16** | **18.6** | **21.4** | **26.6** | **29.4** | **32** | **37.4** |
| **Oral suspension (mL) bid** | 1.1 | 1.3 | 1.6 | 1.9 | 2.1 | 2.7 | 2.9 | 3.2 | 3.7 |

Study drug can be resumed at a level 20% smaller than the dose at which the AE leading to temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin are normalized and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L) or when diarrhoea is mild.

For subjects randomized under protocol version 6.0 or 7.0 or 8.0, only one dose reduction is allowed during the treatment period.

**Dose Modifications for subjects randomized under protocol version 4.0**:

The subjects who were randomized under protocol version 4.0, will follow the dose modification instruction as per protocol version 4.0, as described below in Table 5.

**Table 5: Placebo or Givinostat Dose Modification for subject randomized under study prococol version 4.0**

| **Weight (kg)** | **≥10 and <12.5** | **≥12.5 and <20** | **≥20 and <25** | **≥25 and <30** | **≥30 and <40** | **≥40 and <50** | **≥50 and <60** | **≥60 and < 70** | **≥70** |
|---|---|---|---|---|---|---|---|---|---|
| **Starting Dose (mg) bid** | **20** | **25** | **30** | **35** | **40** | **50** | **55** | **60** | **70** |
| **Oral suspension (mL) bid** | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 5.0 | 5.5 | 6.0 | 7.0 |
| **Reduced Dose (mg) bid** | **13.3** | **16.7** | **20** | **23.3** | **26.7** | **33.3** | **36.7** | **40** | **46.7** |
| **Oral suspension (mL) bid** | 1.3 | 1.7 | 2.0 | 2.3 | 2.7 | 3.3 | 3.7 | 4 | 4.7 |

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

As mentioned in Section 7.3.1, in case a subject has a consistent (e.g., at least 2 consecutive evaluations) platelets count ≤150 x $10^9$/L and does not meet the stopping criteria due to platelet reduction, the Investigator will have to reduce the dose by 1/3 of the starting dose (see Table 5).

Study drug can be resumed at a level 1/3 smaller than the dose at which the AE leading to temporary stop occurred, once platelets and/or white blood cell and/or hemoglobin are normalized and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L) or when diarrhoea is mild.

If a subject was randomized with baseline triglycerides above 300 mg/dL (3.42 mmol/L), the Investigator can discuss how to manage the case with the medical monitor, however the final decision remain with the Investigator or its authorized designee only.

Moreover, for subjects who have already reduced the dose of 1/3 an additional reduction by 20% is allowed for safety reasons following the instruction reported in Table 4.

**Dose Modification (all subjects):**

If subjects (i.e. those already in treatment or those who will be randomized under protocol version 6.0 or 7.0 or 8.0) gain weight during the study the drug dose must be kept unchanged.

If subjects (i.e. those already in treatment or those who will be randomized under protocol version 6.0 or 7.0 or 8.0) lose weight during the study, the drug dose should be adjusted

according to Table 2 or Table 3 if the subject is still on treatment at starting dose, or according to Table 4 or Table 5 if the subject has already reduced the starting dose.

All subjects will be asked to have platelets count assessments weekly during the first month of treatment and performed every two weeks during the second month of treatment, in order to strictly monitor this parameter for safety reasons.

If the dose is reduced due to platelet count ≤150 x $10^9$/L, and/or white blood cell <3.0 x $10^9$/L and/or hemoglobin <10.0 mg/dL a complete blood count (i.e.CBC) test must be performed weekly for 8 consecutive weeks.

If a dose adjustment and/or modification of schedule of assessments is decided by the Investigator or authorized designee, it is advised to discuss this decision with the Medical Monitor, however this is not mandatory as the Investigator is the only responsible for the medical care of the subject and the Investigator's decision should not be influenced, deleted nor approved by the Medical Monitor.

### 8.3 Study Treatment Assignment

The Investigator will be responsible for the delivery of study drug to the subject's parent/legal guardian according to the protocol. Subjects will be administered the study drug on an outpatient basis. All study drug supplies are to be used only for this protocol and for no other purpose.

At Visit 3, subjects fulfilling all inclusion criteria and none of the exclusion criteria will be randomised via interactive response technology (IRT). During the randomization procedure, the Investigator will be asked to record the type of steroid used by the subject (i.e. Deflazacort or other type of steroids) and to record the regimen of intake (i.e. daily regimen or intermittent regimen) following the instruction provided in the IRT manual. The IRT will assign the subject to one stratum according to the collected information (i.e. Deflazacort Daily regimen, Deflazacort Intermittent regimen, Other steroids Daily regimen, Other steroids Intermittent regimen) and them to a treatment arm in a 2:1 ratio to receive givinostat or placebo.

During the first 3 months, the Investigator will supply the child's parent/legal guardian with the appropriate number of suspension bottles sufficient to cover one month of treatment at scheduled visits (i.e., at Visit 3, 7 and 9). Then, for the remaining part of the study (i.e., at

Visit 10, 11, 12, 13, and 14, the Investigator will supply the child's parent/legal guardian with the appropriate number of suspension bottles sufficient to cover the period between two visits (i.e., 3 months of treatment).

The Investigator is to call using IRT at each dispensing visit in order to obtain the packaging number of the study treatment to be dispensed to the subject by the subject's parent/legal guardian.

Study treatment should be dispensed to each subject's parent/legal guardian by authorized personnel only.

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

## 8.4 Blinding

This is a double-blind, placebo controlled study, so subjects 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, and personnel involved in data management) will remain blind at all times, unless under exceptional circumstances when knowledge of the study drug is essential for treating the subject, such as in case of an AE. If the Investigator or authorized designee decides to break the code of a subject, it is suggested that the Monitoring Team (Medical Monitor or CRA) would be consulted if possible before breaking the code, however this is not mandatory because the decision of the Investigator cannot be influenced, deleted nor approved by the Monitoring Team.

Code breaking can be performed by the Investigator or authorized designee at any time by using the proper module of the IRT system.

If the code is broken, treatment with the study drug must be stopped.

In any case, after breaking the code, the Investigator must record the date, time, and reason for breaking the code in the CRF, and must notify the Monitoring Team as soon as possible.

As platelets count reductions are observed after treatment with givinostat, study site personnel who will perform functional tests and MRI/MRS must be different from the personnel who will review subjects' safety results and safety results must not be shared with the personnel responsible for the functional tests and MRI/MRS.

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 initially with a congruous number of suspension bottles, in order to have sufficient supply of study drug to treat the children who are enrolled in the study.

### *8.5.1 Packaging*

The primary packaging will consist of an amber plastic bottle containing the suspension. Givinostat/Placebo oral paediatric 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 a two syringe for oral use as dosing systems for dispensing the suspension.

### *8.5.2 Labelling*

The primary and secondary labels will show all the information requested according to the Annex 13 of the Good Manufacturing Practice and comply with the legal Investigation use requirements of each country.

Medication labels will be in the local language.

The labels for European countries 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 subject 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.

The labels for United State and Canadian sites will include at least:

- Sponsor's study code
- IND number
- Packaging number
- Name of the medicinal product
- Pharmaceutical dosage form
- Route of administration
- Quantity of dosage units
- Strength/potency
- Batch number
- Expiry date
- Administer as per Protocol
- Caution: Investigational Drug – Limited by Federal (United States) Law to Investigational Use
- Storage conditions
- Keep out of the reach of children
- Sponsor.

### *8.5.3 Storage*

The investigational site will store the study drug under the conditions specified here below, ensuring that it is not accessible to unauthorized persons until it is dispensed to the subject's parent/legal guardian.

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

## 8.6 Treatment Accountability and Compliance

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

At each dispensing visit, the subject's parent/legal guardian will bring back to the study site all bottles previously received (used, partially used and unused) and receive a new supply of the study drug.

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

Compliance will be checked by Italfarmaco S.p.A. or its designee based on the drug accountability documented by the 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.

Accountability and compliance is detailed in the relevant CSOM.

## 8.7 Prior and Concomitant Illnesses and Medications

### *8.7.1 Prior and Concomitant Illnesses*

Investigators should document all prior significant illnesses that the subject has experienced within 6 months of screening. The starting date of corticosteroids use will be also collected. Additional illnesses present at the time when informed consent is given and up to the time of first dosing (Visit 3) 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 subject during the study, including those treatments initiated within 6 months prior to the start of the study, must be recorded on the eCRF.

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

As the subjects to be randomised are on stable steroids, the recommended national immunisation schedule, including varicella immunity, should be completed (i.e., as recommended in the "Diagnosis and Management of Duchenne Muscular Dystrophy" guideline4), before the Screening Visit.

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

Any medication or therapy that is taken by or administered to the subject during the course of the study must be recorded in the eCRF. The entry must include the 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 anti-emetics, anti- diarrhoeals, anti-pyretics, anti-allergics, analgesics, and antibiotics are allowed.

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

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

- Any other investigational drug while on this study,
- any dystrophin restoration product
- use of 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).
- Drugs known to be P-glycoprotein inhibitors (see Appendix 16.3 for a list of such compounds).
- Live or live attenuated vaccines: the subject must be carefully monitored. While the decision of the Investigator cannot be influenced, deleted 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 knows to be a substrate of Organic Cation Transporter 2 transporter (see Appendix 16.2 for a list of such compounds).
- Antiplatelets, thrombolytic and anticoaugulants drugs: subject must be carefully monitored (i.e. increase the frequency of hematology testing, evaluate any possible sign of bleeding).

# 9 STUDY PROCEDURES

Table 6 outlines the timing of procedures and assessments to be performed throughout the study. See Section 9.1 for additional details of study procedures.

**Table 6: Schedule of Assessments**

| **Month** | **-1** | | **0** | **1** | | | | **2** | | **3** | **6** | **9** | **12** | **15** | **18** | **19** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | -4[1] | From 1 day after V1 to -2 | 0 | 1 | 2 | 3 | 4 | 6 | 8 | 12 | 24 | 36 | 48 | 60 | 72 | 76 |
| Visit window | ± 2 weeks | + 3 days | 0 | ± 3 days | ± 3 days | ± 3 days | ± 3 days | ± 3 days | ± 3 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days from last dose |
| **Visit** | **V1** | **V2[1]** | **V3** | **V4** | **V5[2]** | **V6[2]** | **V7** | **V8[2]** | **V9** | **V10** | **V11** | **V12** | **V13** | **V14** | **V15** | **V16** |
| | **Screening** | | **Rand** | | | | | | | | | | | | **EOS/Early Withdrawal[3]** | **FUV[4]** |
| Informed consent | X | | | | | | | | | | | | | | | |
| Eligibility criteria | X | | X | | | | | | | | | | | | | |
| Medical history and demographic data | X | | | | | | | | | | | | | | | |
| Prior and concomitant medications | X | | X | X | | | X | | X | X | X | X | X | X | X | X |
| Physical examination | X[5] | | X | X | | | X | | X | X | X | X | X | X | X[5] | X |
| Vital Signs | X | | X | X | | | X | | X | X | X | X | X | X | X | X |
| Weight | X | | X | X | | | X | | X | X | X | X | X | X | X | |
| Height | X | | | | | | | | | | X | | X | | X | |
| ECG Test[6] | X | | X | X | | | X | | X | X | X | X | X | X | X | X |
| ECHO | X | | | | | | | | | | | | | | X | |
| Pulmonary function tests[7] | X[7] | | | | | | | | | | | | X[7] | | X[7] | |
| Serology[8] | X | | | | | | | | | | | | | | | |
| Hematology[9] | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Blood chemistry[10] | X | | | | | | X | | X | X | X | X | X | X | X | X |
| Coagulation tests (PT and aPTT) | X | | | | | | | | X | X | X | X | X | X | X | X |
| Urine analysis[11] | X | | | | | | | | X | X | X | X | X | X | X | X |
| LTBP4 and osteopontin genotype | | | X | | | | | | | | | | | | | |
| Exploratory serum Biomarkers | | | X | | | | | | | | | | X | | X | |
| Quality of life test (assessed by PODCI) | X | | | | | | | | | | | | X | | X | |
| 4 -stairs Climb[12] | X | X | X | | | | | | | X | X | X | X | X | X | |
| NSAA + 6MWT | X | | X | | | | | | | X | X | X | X | X | X | |

| **Month** | **-1** | | **0** | **1** | | | | **2** | | **3** | **6** | **9** | **12** | **15** | **18** | **19** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | -4[1] | From 1 day after V1 to -2 | 0 | 1 | 2 | 3 | 4 | 6 | 8 | 12 | 24 | 36 | 48 | 60 | 72 | 76 |
| Visit window | ± 2 weeks | + 3 days | 0 | ± 3 days | ± 3 days | ± 3 days | ± 3 days | ± 3 days | ± 3 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days | ± 7 days from last dose |
| **Visit** | **V1** | **V2[1]** | **V3** | **V4** | **V5[2]** | **V6[2]** | **V7** | **V8[2]** | **V9** | **V10** | **V11** | **V12** | **V13** | **V14** | **V15** | **V16** |
| | **Screening** | | **Rand** | | | | | | | | | | | | **EOS/Early Withdrawal[3]** | **FUV[4]** |
| Muscle strength[13] | X | | X | | | | | | | X | X | X | X | X | X | |
| Cognitive function evaluation | X | | | | | | | | | | | | | | X | |
| PK blood sample collection[14] | | | | | | | X | | | X | X | | X | | X | |
| Study drug dispensation and accountability | | | X | | | | X | | X | X | X | X | X | X | | |
| Subject diary dispensation and evaluation of drug compliance through diary | | | X | | | | X | | X | X | X | X | X | X | X | |
| Acceptability/ Palatability evaluation | | | | | | | X | | | | | | | | X | |
| AE assessment | X | | X | X | | | X | | X | X | X | X | X | X | X | X |
| MRI/MRS[15] | | X | | | | | | | | | | | X | | X | |
| Genetic Test[16] | X | | | | | | | | | | | | | | | |

6MWT=6-minute walking test; AE=adverse event; NSAA= North Star Ambulatory Assessment; PODCI= Paediatric Outcomes Data Collection Instrument; PFTs= pulmonary function tests; PT=Prothrombin time; aPPT= activated partial thromboplastin time; ECG= electrocardiogram; ECHO= echocardiogram; PK= pharmacokinetics; MRI= magnetic resonance imaging; MRS= magnetic resonance spectroscopy; 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

1. During screening visit V2, the second Time to 4-stair climb test (4SC) will be performed. The second 4SC can be performed one day after V1 up to a maximum of 2 weeks (+3 days).
   The cognitive function evaluation by Raven coloured progressive matrices could be performed during this visit if not done during the previous one (i.e. V1). Test should be performed at the same time at the beginning and at the end of the study
   MRI/MRS could be performed in a different day (i.e. not necessarily during the same day of Time to 4-stair climb test, due to the fact that the MRI site can be performed in a different site).

2. These visits may be performed by Investigator's authorized personnel outside the site location (e.g., at the subject's home or at the subject's General Practitioner's practice. See conditions in Section 9.1.4)
3. If the subject is discontinued from the study treatment, the subject will be asked to return for the early withdrawal visit to be performed within 2 weeks of the last dose of study drug. In this case, the cognitive function evaluation and MRI/MRS may not be performed.
4. The subjects who complete the study (i.e. 18 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 drug (i.e. 4 weeks ± 7 days).
5. During the screening visit V1 and at the end of study (i.e. V15) assessment for ankle range of motion (ROM) should be performed.
6. 12-lead ECG has to be done in triplicate at the screening visit and during the other visits if an ECG demonstrates a prolonged QTcF interval > 450 msec, obtain 2 more ECGs over a brief period, and then use the averaged QTcF values of the 3 ECGs to determine whether the subjects should be discontinued from the study (see Section 7.3.1). 12-lead ECG should be performed at 2,5 hours (i.e 150 minutes) ±30 minutes after drug intake.
7. Pulmonary function tests FEV1, FVC, FEV1/FVC and PEF. In case the test does not achieve ATS criteria and the evaluator does not feel a valid attempt has been made, the subject should repeat the test at randomization if the test is failed at screening visit. If the test is failed at 12 months and/or at 18 months the subject has to be asked to come back to perform the test.
8. Serology: immunoassay methodology for CMV IgM; in-house methodology of chemiluminescence for HBsAg/HCV/HIV; and ECLIA for EBV IgG.
9. Hematology test: CBC:RBC, WBC (including differential count), hemoglobin, MCH, MCHC, MCV, hematocrit, and platelets. Test will be done according to the schedule (i.e., weekly for the first month of treatment, every 2 weeks for the second month of treatment) and are to be performed more frequently if clinically indicated. Note: Repeat visits for the platelets count will be assumed at the request of the Investigator. If the dose is reduced due to platelet count $\leq 150 \times 10^9$/L, and/or white blood cell $< 3.0 \times 10^9$/L and/or hemoglobin <10.0 mg/dL, a complete blood count (i.e.CBC) test must be performed weekly for 8 consecutive weeks.
10. The following blood tests will be assessed: 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, TSH, fT3, fT4, bicarbonate). Note that TSH, fT3, and fT4 will be taken at Visits 7, 9, 10, 11, 12, 13, 14, 15 and 16.
11. Urinalysis: appearance, pH, specific gravity, protein, glucose, ketone bodies, blood and WBCs, and urobilinogen.
12. During the screening period (i.e., 4 weeks ±2), time to 4-stair climb test (4SC) should be performed at V1 and V2. The 4SC at V2 can be performed one day after V1 up to a maximum of 2 weeks (+3 days). For this reason the subjects should go to the centre 2 times (i.e., Visit 1 and Visit 2). The test should be video recorded during Visit 1, Visit 3, Visit 13 and Visit 15.
13. Muscle strength tests: <u>at screening</u>: knee extension, elbow flexion by HHM and knee extension by MMT. <u>During the study and at the end</u>: knee extension, elbow flexion by HHM.
14. 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: 7 (4 weeks), 10 (12 weeks), 11 (24 weeks), 13 (Week 48) and 15 (Week 72). At these visits, the subject will be asked to take the morning dose at site.
15. The baseline MRI/MRS test has to be performed when all inclusion/exclusion criteria have been already evaluated and the subject is eligible. At screening all subjects will perform the MRI of thigh and MRS of vastus lateralis; then the MRI of thigh and MRS of vastus lateralis will be performed also at 12 and 18 months in a subgroup of subjects: the first 99 - 150 randomised subjects in the target population (i.e. with MRS VL MFF between> 5% and ≤30%). The MRI evaluation using Dixon technique and the MRS will be performed at specialized sites.
16. Genetic test should be performed only in case it is not already available.

## 9.1 Procedures by Study Visit

There will be at least 15 study visits, including the Screening and Randomisation Visits.

Assessments will be performed as outlined in the following by-visit subsections. As platelets count reductions are observed after treatment with givinostat, study site personnel who will perform functional tests and MRI/MRS must be different from the personnel who will review subjects' safety results and safety results must not be shared with the personnel responsible for the functional tests and MRI/MRS.

At the discretion of the Investigator, subjects not meeting inclusion/exclusion criteria may be re-screened twice with an interval of at least 3 months between assessments.

If needed, safety assessments (e.g. blood test, unscheduled visits) may be performed at the subject's home by a local qualified nurse trained in the study protocol.

### *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 Randomisation Visit. The following assessments will be performed at Visit 1 unless noted otherwise:

- Obtain written Informed Consent from the subject's parent/legal guardian and assent or consent from the subject, if applicable;
- Eligibility criteria: review inclusion and exclusion criteria;
- Genetic test *(only if it is not already available)*;
- Collect medical history and demographic data;
- Collect and record prior medications (taken in the past 6 months);
- Perform a physical examination (at Visit 1 assessment for ankle ROM should be done);
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Measure height;
- Perform a 12-lead ECG (in triplicate);
- Perform an ECHO;
- Perform pulmonary function tests: FEV1, FVC, FEV1/FVC, and PEF;
- Collect blood samples for:
    - serology tests;

  - hematology;
  - blood chemistry;
  - coagulation tests (prothrombin time [PT] and activated partial thromboplastin time [aPTT]);
- Obtain a urine sample for urine analysis;
- Perform Quality of Life tests (using Paediatric Outcomes Data Collection Instrument [PODCI] scores);
- Perform time to 4SC (the test should be performed at Visit 1 and at Visit 2; the 4SC at Visit 2 can be performed one day after V1 up to a maximum of 2 weeks +3 days);
- Perform NSAA and 6MWT;
- Perform muscle strength test (both by HHM and MMT);
- Perform cognitive function evaluation: Raven coloured progressive matrices (at Visit 1 or at Visit 2. Test should be performed at the beginning and at the end of the study at the same time of the day);
- Assess AEs;
- If all inclusion and exclusion criteria have been met and the subject is eligible, the following assessments must be performed at Visit 2 to conclude the screening period before the randomisation:
  - MRI/MRS

At Visit 1, functional tests will be video recorded.

Subjects will be instructed to avoid physical activity that would exceed their normal activity for 3 days before each visit.

NB: As the subjects to be randomised are on stable steroids, the recommended national immunisation schedule, including varicella immunity, should be completed (i.e., as recommended in the "Diagnosis and Management of Duchenne Muscular Dystrophy" guideline4), before the Screening Visit. However, in the case of live or live attenuated vaccines to be administered during the study, the Investigator must carefully monitor the subject, and while the decision of the Investigator cannot be influenced, deleted 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.

### *9.1.2 Visit 3 - Randomisation (Week 0)*

At Visit 3, the following assessments will be performed:

- Review eligibility criteria;

- Record prior medications;
- Perform a physical examination;
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Perform a 12-lead ECG;
- Collect blood samples for hematology;
- Perform LTBP4 and Osteopontin genotype tests and collect DMD serum biomarkers;
- Perform time to 4SC;
- Perform NSAA and 6MWT;
- Perform muscle strength test;
- Randomise the child and dispense and account for study drug;
- Subject diary dispensation
- Assess AEs.

Functional tests will be video recorded.

Pulmonary function as assessed by spirometry at screening should be repeated if the test does not meet ATS/ERS criteria and/or the evaluator deems that a valid attempt to perform a correct maneuver has not been made by the subject.

### *9.1.3 Visits 4 (Week 1, ±3 days)*

At Visit 4, after 1 week (± 3 days) of treatment, the following assessments will be performed:

- Record concomitant medications;
- Perform a physical examination;
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Perform a 12-lead ECG (at 2,5 hour ±30 minutes after drug intake);
- Collect blood samples for hematology;
- Assess AEs.

### *9.1.4 Visits 5, 6 and 8 (Weeks 2, 3 and 6, ±3 days)*

Note: Visits 5, 6, and 8 may be performed by Investigator's authorized personnel outside the site location (e.g., at the subject's home by a local qualified nurse trained in the study protocol or at the subject's General Practitioner's practice) if the following conditions are met:

- the Investigator is confident that there is no medical concern to do so;

- all study procedures will be followed as per protocol and as per Good Clinical Practices (GCP), and with the same quality standard as if being at the site;
- all required local regulation, insurance and contracting obligation are respected.

The Visits 5, 6, and 8 will performed respectively 2, 3 and 6 weeks after the Randomization Visit (± 3 days), and the following assessment will be performed:

- Collect blood samples for hematology (the sample will be shipped to the central laboratory);
- Assess AEs; in case of the visit will be performed by the subject's General Practitioner's, the Investigator will call the subject's parent/legal guardian to inquire if any AE is occurred.

### *9.1.5 Visit 7 (Week 4, ±3 days)*

At Visit 7, after 1 month of treatment (± 3 days), the following assessments will be performed unless otherwise noted:

- Record concomitant medications;
- Perform a physical examination;
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Perform a 12-lead ECG (at 2,5 hours ±30 minutes after drug intake);
- Collect blood samples for hematology and biochemistry (only TSH, fT3, fT4);
- Obtain PK blood sample;
- Dispense and account for study drug ;
- Subject diary dispensation and evaluation of drug compliance through diary;
- Perform acceptability/palatability evaluation;
- Assess AEs.

### *9.1.6 Visits 9 (Week 8, ± 3 days)*

At Visit 9, after 2 months of treatment (± 3 days), the following assessments will be performed:

- Record concomitant medications;
- Perform a physical examination;
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Perform a 12-lead ECG ( at 2,5 hours ± 30 minutes after drug intake);
- Collect blood samples for:

  - hematology;
  - blood chemistry;
  - coagulation tests (PT and aPTT);
- Obtain a urine sample for urine analysis;
- Dispense and account for study drug;
- Subject diary dispensation and evaluation of drug compliance through diary;
- Assess AEs.

### *9.1.7 Visits 10 – 14 (Weeks 12-60, ± 7 days)*

The Visits 10, 11, 12, 13 and 14 will respectively happen after 12, 24, 36, 48 and 60 weeks after the Randomization Visit, with a visit window of ± 7 days.

At Visit 10 – 14 (i.e., every 3 months), the following assessments will be performed unless otherwise noted:

- Collect and record concomitant medications;
- Perform a physical examination;
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Measure height (only at Visits 11 and 13; i.e., after 6 and 12 months of treatment) ;
- Perform a 12-lead ECG (at 2,5 hours ±30 minutes after drug intake);
- Perform pulmonary function tests: FEV1, FVC, FEV1/FVC, and PEF (only at Visit 13);

Pulmonary function as assessed by spirometry at visit 13 should be repeated if the test does not meet ATS/ERS criteria and/or the evaluator deems that a valid attempt to perform a correct maneuver has not been made by the subject.

- Collect blood samples for:
  - hematology;
  - blood chemistry;
  - coagulation tests (PT and aPTT);
- Collect DMD serum biomarkers (only at Visit 13);
- Obtain a urine sample for urine analysis;
- Perform Quality of Life tests (assessed by PODCI) (only at Visit 13; i.e., after 12 months of treatment);
- Perform time to 4SC
- Perform NSAA and 6MWT;
- Perform muscle strength tests;
- Obtain PK blood sample (only at Visits 10, 11, and 13; i.e., after 3, 6, and 12 months of treatment, respectively);

- Dispense and account for study drug;
- Subject diary dispensation and evaluation of drug compliance through diary;
- Assess AEs;
- Perform MRI/MRS evaluation (MR cohort only) (only at Visit 13; i.e., after 12 months of treatment).

At Visit 13, functional tests will be video recorded.

### *9.1.8 Visit 15: End of Study Visit (also named "EOS Visit", Week 72 ± 7 days), and early withdrawal visit*

At the end of the treatment (i.e., 72 weeks after the Randomization Visit, ± 7 days) the EOS Visit (Visit 15) will be scheduled. At EOS Visit the subject should take the morning dose at the site.

Subjects who prematurely withdraw from the study for any reason will perform an Early Withdrawal Visit within two weeks after the last study drug dose intake. The Early Withdrawal Visit requirements are the same as the EOS Visit (Visit 15) described in this section.

If the Early Withdrawal Visit or the EOS Visit is not done, the reason will be recorded in the CRF.

At this Visit 15, the following assessments will be performed unless otherwise noted:

- Collect and record concomitant medications;
- Perform a physical examination (at V15 assessment for ankle ROM should be done)
- Measure vital signs (blood pressure, pulse rate, and body temperature);
- Measure weight;
- Measure height;
- Perform a 12-lead ECG (at 2,5 hours ±30 minutes after drug intake);
- Perform an ECHO;
- Perform pulmonary function tests: FEV1, FVC, FEV1/FVC, and PEF;
- Collect blood samples for:
    - hematology;
    - blood chemistry:
    - coagulation tests (PT and aPTT);
- Collect DMD serum biomarkers;
- Obtain a urine sample for urine analysis;

- Obtain PK blood sample. The remaining PK samples may be drawn during this visit in order to obtain all required PK samples;
- Perform Quality of Life test (assessed by PODCI);
- Perform time to 4SC
- Perform NSAA and 6MWT;
- Perform muscle strength tests
- Perform cognitive function evaluation (test should be performed at the beginning and at the end of the study, at the same time of the day);
- Perform acceptability/palatability evaluation;
- Evaluation of drug compliance through diary;
- Assess AEs;
- Perform MRI/MRS evaluation (MR cohort only). (If the subject is discontinued from the study treatment, the subject will be asked to return for the early withdrawal visit to be performed within 2 weeks of the last dose of study drug. In this case, the cognitive function evaluation and MRI/MRS may not be performed).

Functional tests will be video recorded.

The subject has to be asked to attend an unscheduled visit to repeat measurement of pulmonary function as assessed by spirometry if the test performed at visit 15 has not met ATS/ERS criteria and/or the evaluator has deemed that a valid attempt to perform a correct maneuver was not made by the subject.

At visit 15, the subject's parent/legal guardian will be asked to consent in writing and subject to assent and/or consent in writing for his participation in the long-term study. In case the subject or parent/legal guardian do not provide assent/consent, a final Follow-up visit will be performed.

### *9.1.9 Follow up Visit (Week 76)( ± 7 days)*

The follow up visit will be performed 4 weeks after the last dose is administered (± 7 days) and only in case the subject or parent/legal guardian do not provide assent/consent to participate in long-term study.

The following assessments will be performed:

- Collect and record concomitant medications;
- Perform a physical examination;
- Measure vital signs (blood pressure, pulse rate, and body temperature);

- Perform a 12-lead ECG;
- Collect blood samples for:
  - hematology;
  - blood chemistry;
  - coagulation tests (PT and aPTT);
- Obtain a urine sample for urine analysis;
- Assess AEs.

#### *9.1.10 Unscheduled Visits*

The Investigator may, at his/her discretion, arrange for a subject to have an unscheduled assessment, especially in the case of AEs that require follow-up or an AE considered by the Investigator to be possibly related to the use of study drug. The unscheduled visit page in the eCRF must be completed. If needed, these assessments may be performed at the subject's home by a local qualified personell trained in the study protocol.

### 9.2 Study Conclusion

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

## 10 EFFICACY ASSESSMENTS

### 10.1 Primary Efficacy Assessment

The primary efficacy assessment for this study is the time to climb 4 standard stairs (at 18 months).

The time to climb 4 standard-sized stairs is a TFT that represents stair climbing ability. The test will be evaluated by qualified functional evaluators (i.e., physiotherapists) who will be different from the site personnel who will review subjects' safety results; safety results must not be shared with site personnel. The test will be performed in a standardized manner described in a specific site manual.

In addition to the time to climb 4 standard-sized stairs recorded in seconds, functional adaptation employed by the subject during the test will be evaluated and graded by functional evaluators according to standardized scales as follows:

1. Unable to climb up 4 standard stairs;
2. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step), using both arms on one or both handrails;
3. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step), using one arm on one handrail;
4. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step), not needing handrail;
5. Climbs 4 standard stairs alternating feet, needs handrail for support;
6. Climbs 4 standard stairs alternating feet, not needing handrail support.

### 10.2 Secondary Efficacy Assessments (All Subjects)

The secondary key efficacy assessments (all subjects) in the target population are:

- Time to rise from floor;
- Distance done in 6 walking minutes by 6MWT;
- Physical function assessed by NSAA;
- Muscle strength evaluated by knee extension, elbow flexion as measured by HHM.
- Fat fraction of vastus lateralis muscles evaluated by MRS technique (MR cohort)

The exploratory efficacy assessments are:

- Time to walk/run 10 meters;
- Quality of life (assessed by PODCI);
- %-predicted 6MWT;
- MRI parameters (e.g., fat fraction of thigh muscles, CSA of vastus lateralis and other thigh muscles, etc) only in the MR cohort;
- Time to 10% persistent worsening in 6MWT (Baseline through end of study);
- Proportion of subjects with ≥10% worsening in 6MWT at end of study;
- Time to loss of standing (Baseline through end of study);
- Proportion of subjects who loose ambulation during the study;
- Evaluation of any correlation between the effect of Givinostat on disease progression and the type of DMD mutation, LTBP4 and Osteopontin genotype;
- Evaluation of any possible DMD serum biomarker.

As for the primary efficacy assessment, 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 will review subjects' safety results. The safety results must not be shared with physiotherapists.

To limit variability associated with functional evaluators 'drift' in performing trial assessment across the course of the study, to ensure compliance with functional evaluator manual procedures, and valid conduct of assessments, some screening, baseline, 12 month and final study visits will be videoed and reviewed for quality assurance across all subjects.

The videos: will be uploaded to a central secure repository and reviewed only by an expert and independent team engaged to train and qualify the site functional evaluators.

Time taken to stand from a supine position and time to walk/run 10 meters are TFTs. Timed function tests provide a measure of functional capability in ambulatory subjects, which is complementary to the 6MWT. The TFTs will be performed in a standardized manner described in a specific site manual.

Six-minute walking test: a modified version of the 6MWT recommended by American Thoracic Society (2002) for use in adults will be performed.

North Star Ambulatory Assessment: the 17 items of the NSAA, ranging from standing to running 10 meters, will be graded using the standard score card with each assessment rated as 0 – unable to achieve independently, 1 – modified method but achieves goal independent of physical assistance from another, or 2 – normal with no obvious modification of activity.

Muscle strength: using HHM, the muscle strength of 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 (QoL): the QoL will be measured via the American Academy of Orthopaedic Surgeons Pediatric Musculoskeletal Function Instrument, also referred to as PODCI. The PODCI will be administered to the parent/legal guardian of subjects. The PODCI is comprised of several dimensions that assess upper extremity function, transfers and mobility, physical function and sports, comfort/pain (pain-free), and happiness. There is also a scale for global functioning, which is a combination of the 3 function subscales and comfort.

**MR Cohort**

The MRI and MRS will be centralized in a referred qualified site. Therefore, some subjects will be asked to perform the examination at a site that is different from the study site.

The MRI/MRS personnel involved will be different from the site personnel. They will not review subjects' safety results; safety results must not be shared with them. Moreover, the MRI/MRS images will be centrally read by an Independent Central Review team. A qualification process will be implemented in order to improve data quality and decrease inter-site variability and the test will be performed in a standardized manner described in a specific site manual.

All subjects will perform the baseline MRI/MRS tests. –All 110 randomised subjects in the target population (i.e. Group A: subjects with a baseline vastus lateralis muscle fat fraction assessed by MRS in the range >5 and ≤30%), will be included in the MR cohort.

The subjects will perform the Dixon MRI and MRS on the thigh muscles without receiving general anesthesia. The MRI/MRS images will be read to evaluate the fat fraction of each muscle. A cross sectional area of muscles will be evaluated as well.

# 11 PHARMACOKINETICS

## 11.1 Pharmacokinetic Sampling

### *11.1.1 Blood Samples*

Blood samples for PK analysis of givinostat and its metabolites will be collected. All of the PK sampling visits are indicated in the Schedule of Assessments (Table 4). There are 5 visits where the PK samples can be taken: Visits 7 (Week 4), 10 (Week 12), 11 (Week 24), 13 (Week 48), and 15 (Week 72).

All subjects 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 7 (week 4), 10 (week 12), 11 (week 24), 13 (week 48) and 15 (week 72) at least one PK sample is collected for each subject. After the randomization, the 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, subjects should be instructed to take the morning dose at the site.

For the PK draws, the parent/legal guardian must be reminded to 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.

For the post-dose blood draws, the 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 subject.

In case the subject 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 in order 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 File).

### 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 with the final Clinical Study Report (CSR).

## 12 SAFETY ASSESSMENTS

Safety and tolerability will be evaluated by monitoring hematology and blood chemistry, coagulation, urinalysis; by measurement of vital signs, physical examinations, weight, height, ECHO and ECG recording; and by respiratory function evaluation, cognitive function and

acceptability/palatability evaluation and by AEs recording to be performed at protocol-specified visits, as specified in the Schedule of Assessments, Table 6.

### 12.1 Vital Signs

Vital signs (body temperature, heart rate, systolic and diastolic blood pressure measurements) will be evaluated at the visits indicated in the Schedule of Assessments, Table 6. All vital signs will be measured after the subject 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 6.

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 (HEENT), heart, lungs, abdomen, skin, lymph nodes, extremities and nervous system) will be performed at Screening (Visit 1). Physical examinations will be performed by a physician. In addition, medical history will be recorded at screening.

A limited physical examination to verify continued subject eligibility and to follow up on any change in medical history will be performed at the visits indicated in the Schedule of Assessments, Table 6. All changes not present at baseline or described in the past 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 6.

At the Screening Visit, the Investigator will examine the 3 ECG traces for signs of cardiac disease that could exclude the subject 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 CRF. If an ECG demonstrates a prolonged QTcF interval (i.e.>450 msec) at the defined timepoints (see Schedule of Assessments, Table 6), 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, the subject has to interrupt the study drug, the Medical Monitor must be informed and the mean QTcF value must be confirmed by central reading.

During the study, ECG should be performed at 2,5 hours ± 30 minutes after drug intake.

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 ECHOs will be conducted at the visits indicated in the Schedule of Assessments, **Table 6**, and more often if clinically indicated.

### 12.5 Respiratory 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 6 and more often if clinically indicated.

At each assessment, three studies with maximal effort will be attempted by each subject, and the experienced technician performing the testing will make the determination if the effort will be acceptable. All the study results will be recorded in the eCRF.

### 12.6 Cognitive Function Test

A general cognitive function test will be performed at the visits indicated in the Schedule of Assessments, Table 6 using the Raven coloured progressive matrices test.

## 12.7 Acceptability/Palatability Evaluation

At Visit 7 (i.e. at the end of the first month of treatment) and 15 (i.e. at the end of the study), the palatability of the oral suspension will be evaluated using the Five-Point Hedonic Scale palatability.

The investigator will explain to the child that he would be asked "how much did you like the taste of this medicine?" and encourage to indicate his preference by pointing to the appropriate face that depicts five degrees of pleasure:

5= like very much

4= like a little

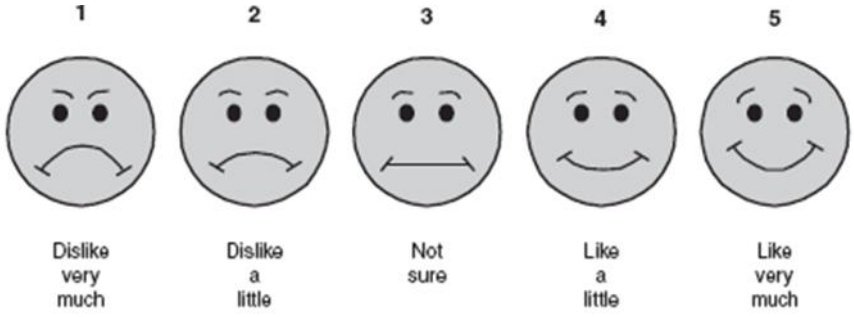
3= not sure

2= dislike a little

1= dislike very much.

The explanation will be repeated if the child did not understand.

**Figure 12-2: Five Point Hedonic Scale - Face Descriptions**




In addition, the acceptability/ palatability will be also indirectly assessed in parents with two questions:

1. "On the basis of reaction / facial expression of your child, do you think that the medication is: pleasant= 3; not sure= 2; or unpleasant= 1?"

2. "Do you sometimes have problems in giving the medication to your child because he refuses to take it or throws it up? (Yes / No)"

## 12.8 Laboratory Assessments

Laboratory assessment samples, Table 7, will be obtained at designated visits as detailed in the Schedule of Assessments, Table 6.

**Table 7: Laboratory Assessment Samples**

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

Blood and urine samples will be analysed at a central laboratory facility. Urine samples will be analyzed by dipstick, and a microscopic analysis will be performed if the results of dipstick indicate abnormalities to be further investigated. 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.

## 12.9 Adverse Events

### *12.9.1 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 drug includes both the investigational drug under evaluation and the comparator drug or placebo. Medical conditions that were present before starting study drug are only considered AEs if they worsen after the subject has started the study drug. 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 CRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE CRF 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 hospitalisation or prolongation of existing hospitalisation
- 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 hospitalisation, 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.

A pre-planned hospitalisation should not be considered an SAE.

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

### *12.9.2 Adverse Event Reporting*

The Investigators or their designees are requested to collect and assess any spontaneous AE reported by the subject or their parent/legal guardian and to question the subject or their

parent/legal guardian 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 subject or their parent/legal guardian 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 CRF as baseline condition, while any AE occurring after a subject has intake the first study treatment up to the follow-up study visit, whether volunteered by the subject/parent/legal guardian, 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 CRF.

Each AE will be described by:

- seriousness;
- duration (start and end dates);
- severity;
- relationship with the study drug;
- action taken.

The severity of 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$^{th}$ 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 CRF.

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, 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:

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

Abnormal Laboratory Findings and Other Objective Measurements:

Abnormal laboratory findings and other objective measurements should not be routinely captured and reported as AEs as they will be collected and analysed 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 "anaemia" in addition to "haemoglobin = 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.

### *12.9.3 Serious Adverse Event Reporting*

Any SAE, including death from any cause that occurs after subject/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 in the SAE form that will be automatically opened if the answer to the question "Was adverse event serious?" in the AE section is "yes." A paper SAE Form will be also available as backup.

Initial completion and follow-up reporting updates of the SAE eCRF page within the Electronic Data Capture (EDC) system will automatically send the SAE report directly to the Syneos Health Global Safety and Pharmacovigilance (GSPV) via secure email.

This automated e-mail alert process supports the Investigator in the notification of SAEs to the Syneos Health GSPV. 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 Italfarmaco S.p.A. Corporate Drug Safety, or its designee or the Medical Monitor of the study or Clinical Research and Development Director, or his designee.

The Investigator must notify the Sponsor and his designee of the SAE by completing the SAE reporting form available within the eCRF or, as a backup ONLY in case of technical issues, by faxing the SAE reporting form at the number specified below, 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:

Syneos Health Pharmacovigilance Email: SafetyPV@syneoshealth.com

Syneos Health Pharmacovigilance Fax Numbers:

North America: 866-880-9343
All other countries: +44-1628-461184

If notification cannot be made via these means due to technical delivery problems, initial notification may be made by telephone, using the SAE Hotline number:

**888-750-8020**

The same procedure must be applied to the SAE follow-up information. Preliminary reports of SAEs must be followed by detailed descriptions later on, 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 in order 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 subject'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 Drug Safety Manager 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.

### *12.9.4 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, it 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 subject 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.

### *12.9.5 Pregnancy reporting*

Since it is possible to randomize adolescent fertile trial male subjects in this trial, the subject must be informed by the investigator that if it is suspected that his partner becomes pregnant during the study treatment or within 3 months days 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 subject who is participating to the study become pregnant, he/she is required to report within 24 hours the pregnancy, using the Pregnancy Notification Form available in the Investigator Study File, to Syneos Health Pharmacovigilance by mailing to the mail address or faxing numbers specified below:

- Syneos Health Pharmacovigilance Email:

**SafetyPV@syneoshealth.com**

- Syneos Health Pharmacovigilance Fax Numbers:

North America: **866-880-9343**

All other countries: **+44-1628-461184**

If notification cannot be made via these means due to technical delivery problems, initial notification may be made by telephone, using the SAE Hotline number:

- **888-750-8020**

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 first 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.

The statistical evaluation will be performed using the latest available version of the Statistical Analysis Software (SAS®) (SAS Institute, Cary, NC).

All data will be listed, and summary tables will be provided. Summary statistics will be presented by dose group. For continuous variables, data will be summarized with the number of subjects (N), mean, SD, median, minimum, and maximum by treatment group. For categorical variables, data will be tabulated with the number and proportion of subjects for each category by treatment group.

## 13.1 Overall Statistical Strategy

This trial design a single planned interim analysis. The interim will be governed by an IDMC. The interim analysis will be performed when the first N=50 subjects randomised in the target population have reached the 12 month time point. The purpose of this interim is to solely assess futility on VL MFF and execute a blinded sample size re-estimation procedure, **there will be no formal analyses for efficacy and no early stopping for efficacy and hence no alpha spend**.

## 13.2 Determination of Sample Size

The sample size was originally calculated to provide 90% power and a 1-sided alpha of 2.5% to detect a true difference between givinostat and placebo in the target population in 4SC 18 month change from baseline of 3 seconds, assuming a common SD of 6 seconds. The

estimated SD is based on publicly available Phase 3 study data on ataluren and drisapersen in subjects with DMD in addition to internal Italfarmaco data.

The pre-planned interim analysis was performed in January 2020; it was concluded by the IDMC that futility on VLFF was not met and the trial should continue; hence the pre-planned blinded sample size re-assessment was performed. The blinded within treatment SD for the change in 4CS from baseline to 18 months was estimated to be 3.094 seconds, approximately half of that assumed in the original power calculation. Based on this SD estimate, the revised sample size (utilizing a 2:1 randomisation scheme) N= 102 was calculated to provide 90% power and a 1-sided alpha of 2.5% to detect a true difference between givinostat and placebo in the target population, in 4SC 18 month change from baseline, of 2 seconds. With an estimated drop-out rate of 8%, a total of 110 subjects in the target population (Group A) will be randomized.

For the MR cohort, the blinded SD estimate was 5.941%, being in line with the 6% SD assumed in the original power calculation, hence all the subjects in the target population will be included in the MR cohort.

A total of 110 male ambulant subjects will be randomised in the target population (Group A). Up to 50 subjects (about 35% of the overall population) outside of the target may also be recruited into the study (Group B). The overall subjects population (Group A + Group B) will provide supportive data.

## 13.3 Analysis Population

### *13.3.1 Intent-To-Treat Population*

The Intent-to-Treat (ITT) target population will include all subjects in the target population who are randomised, irrespective of any deviation from the protocol or premature discontinuation. The treatment group assignment will be designated according to initial randomisation. The ITT target population will serve as the basis for the formal analysis of efficacy. The overall ITT population will include all subjects in both the target and off-traget populations (Group A + Group B) who are randomised, irrespective of any deviation from the protocol or premature discontinuation. The overall ITT population will be used for supportive efficacy analyses.

### *13.3.2 Safety Population*

The target Safety Population will include all randomised subjects in the target population who receive at least one dose of study drug. The treatment group assignment in this population will be defined by the treatment actually received. This population will be used for the evaluation of safety in the target population. The overall Safety Population will include all randomised subjects in both the target and off-target population who receive at least one dose of study drug. The overall Safety Population will be used for supportive evaluations of safety.

## 13.4 Demographic and Baseline Characteristics

Demographic data and other baseline characteristics including medical history will be analysed based on the target safety population. Summary statistics will be provided for all collected variables. Continuous variables will be summarized by descriptive statistics, including mean, SD, median, minimum, and maximum. Details of endpoint analyses will be described in the SAP.

## 13.5 Investigational Medicinal Products

The duration (days) of exposure of givinostat/placebo will be calculated for each subject in the target and overall populations, and will be summarized descriptively including the mean, SD, median, minimum, and maximum by treatment group and overall.

In addition, the daily dosage (mg) of givinostat will be summarized for each subject in the target and overall populations, and will be summarized descriptively including the mean, SD, median, minimum and maximum.

Reason for treatment discontinuation and number of subjects treated beyond protocol-specified discontinuation criteria will also be summarized in the target and overall populations. Summaries will be based on the respective safety population.

## 13.6 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 summarised in the target and overall populations. Summaries will be based on the respective safety population.

## 13.7 Efficacy Analysis

All formal efficacy analyses will be performed on the ITT target population; efficacy analyses in the overall population will be supportive and informal. Descriptive statistics for qualitative data will include frequency tabulation and presentation of percentages. Descriptive statistics for quantitative data will include mean and SD or geometric mean, coefficient of variation, median and interquartile range.

### *13.7.1 Analysis of the Primary Efficacy Endpoint*

The change in 4SC from baseline to 18 months will be evaluated in the target population. The data will be analyzed using an Analysis of Covariance (ANCOVA) model with the change in 4SC from baseline to 18 months as the dependent variable and with terms for baseline 4SC value as a covariate and randomised treatment, concomitant steroid use and age at baseline as independent class variables. Least squares means will be estimated for givinostat and placebo. The treatment effect, being the difference in Lsmeans, will be presented along with the associated 95% confidence interval and the p-value. Significance will be achieved if the 1-sided p-value is $\leq 0.025$.

### *13.7.2 Analysis of Key Secondary Efficacy Endpoints*

Analysis of Key Secondary Endpoints

Formal analyses of functional key secondary endpoints (i.e., cumulative loss of function in the NSAA, change in 6MWT, NSAA, time to rise from floor and muscle strength by knee extension and elbow flexion) and Vastus lateralis MFF will take place in the target population at 18 months and will be achieved using the same approach as described for the primary /endpoint. Possible need for log transformation of these variables will be assessed prior to unblinding by assessment of ANCOVA model residuals without a treatment effect term.

### *13.7.3 Analysis of Exploratory Efficacy Endpoints*

Exploratory efficacy endpoints will be supportive only and not subject to alpha control; therefore nominal p-values will be presented.

The mean change from baseline to 18 months will be compared between givinostat and placebo in the target population for:

- Time to walk/run 10 meters;
- Quality of life assessed by Paediatric Outcomes Data Collection Instrument (PODCI);
- %-predicted 6MWT;

These endpoints will be analyzed using methods in line with those described for the primary and key secondary endpoints.

The fraction of subjects achieving the following endpoints at 18 months will be compared between givinostat and placebo in the target population:

- Proportion of subjects with ≥10% worsening in 6MWT at end of study;
- Proportion of subjects who loose ambulation during the study.

These endpoints will be analysed using a logistic regression model with terms for randomised treatment, concomitant steroid use and age at baseline. The odds for each treatment group will be extracted along with the odds ratio, associated 95% confidence interval and p-value.

The following endpoints will be compared between givinostat and placebo in the target population using Cox proportional hazards modelling:

- Time to 10% persistent worsening in 6MWT (Baseline through end of study);
- Time to loss of standing (Baseline through end of study);

The model will include terms for randomised treatment, concomitant steroid use and age at baseline.The hazard ratio will be extracted, along with the associated 95% confidence interval and p-value.

Moreover, exploratory analyses will be done in the target population to explore whether the effects of givinostat versus placebo administered chronically may be related to the type of DMD mutation, LTBP4 and Osteopontin genotype or to the biomarkers.

## 13.8 Pharmacokinetic Analysis

Nonlinear mixed effect models will be used for the evaluation of the population PK profile. The population PK analysis will be performed using NONMEM software (Version VI or later).

The evaluation will be performed for describing the PK of givinostat and its major metabolites (ITF2374 and ITF2375) in the subject population and for identifying the relevant demographic and pathophysiological covariates. Separate PK models for givinostat and its metabolite will be developed.

Additionally, the relationship of givinostat PK exposure to efficacy and safety endpoints in the target population will be explored as exploratory analyses.

The Population PK approach will be based on the development of the following 3 models:

1. Identification of the structural model that best describes the PK data in absence of covariates;
2. Random Effect Models: defining the inter-individual variability and the residual error. Different forms of residual error (additive, multiplicative or both) will be explored;
3. Covariate Model: identifying the relevant demographic and pathophysiological covariates affecting the PK profile of the compound. The selection of the covariates will be performed using an appropriate statistical methodology.

Population PK procedures and analyses will be detailed in a separate "Population PK data analysis plan."

## 13.9 Safety Analysis

Safety data will be evaluated descriptively in both the target and overall patient populations. The following parameters will be summarized: rates of discontinuation, AEs, and laboratory abnormalities. Adverse event data will be coded using the most updated version of Medical Dictionary for Competent Activities (MedDRA) dictionary. Adverse events will be summarized by treatment group for the number of subjects reporting the AE and the number of AEs reported. Summaries will be performed for treatment-related AEs (TEAEs), SAEs, AEs that lead to withdrawal, severity of TEAEs, and relationship of TEAEs to study drug.

They will be presented by MedDRA System Organ Class (SOC) MedDRA Preferred Term and treatment group.

Serious adverse events and AEs leading to discontinuation will be listed separately.

A by-subject AE data listing including verbatim term, coded term, treatment group, severity, and relationship to treatment will be provided.

Summary statistics will be presented for changes in vital signs, weight, height and BMI, in ECG parameters, in ECHO parameters, in respiratory function parameters, and cognitive function parameters. Laboratory test values will be summarised similarly but will also include tabulation of the number of subjects shifting from within the reference range at baseline to outside of the reference range on randomised treatment.

## 13.10 Interim Analysis

A single interim analysis is planned and will be performed by the IDMC in the target population. An IDMC charter will be prepared to describe the role, function and operations of the IDMC and the rules to be used when reviewing interim data.

### *13.10.1 Interim Analysis*

The interim analysis will be performed when the first N=50 subjects randomised in the target population have reached the 12 month time point. In this interim analysis, the IDMC will evaluate the effects of givinostat versus placebo on the vastus lateralis MFF (VL MFF) in terms of futility. Futility will be considered if the mean change from baseline to 12 months in VLMFF in the givinostat group is equal to or worse than that seen in the placebo group since the biologic plausibility of a subsequent treatment effect on 4SC time would be greatly diminished. The IDMC will only communicate to the Sponsor whether the study should proceed or it should be stopped having met the futility criteria.

If the IDMC indicates that the study should proceed, a blinded sample size re-estimation will be conducted for MFF and 4SC in the target population as follows:

- To maintain the blind on the primary endpoint, the within treatment SD for the change from baseline will be estimated as $\hat{\sigma}_{within} = \sqrt{\hat{\sigma}^2_{overall} - \left(\frac{4}{2}\right)^2}$ where $\hat{\sigma}_{overall}$ is the overall SD for the change from baseline in 4SC based on the n=50 subjects in

the interim and $\Delta$ is the originally hypothesized treatment effect, i.e., $\Delta = 3$ seconds. Dependent upon the blinded SD estimate, the sample size may be decreased or increased to maintain 90% power. Any increase in sample size for 4SC will be limited to 1.5 times maximum of the initial target population sample size, i.e., to a maximum of $1.5 \times 192 = 288$ subjects. For a 20% increase in the SD, this degree of increase in sample size will maintain 90% power, and for a 40% increase in SD power will be maintained at 80%.

- With respect to VL MFF, a similar approach will be applied; the within treatment SD for the change from baseline will be estimated as $\hat{\boldsymbol{\sigma}}_{\boldsymbol{within}} = \sqrt{\hat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{overall}} - \left(\frac{\Delta}{2}\right)^{2}}$ where $\hat{\boldsymbol{\sigma}}_{\boldsymbol{overall}}$ is the overall SD for the change from baseline in VL MFF and $\Delta$ is the originally hypothesized treatment effect, i.e. $\Delta = 3.63\%$ (i.e., 55% of 6.6%). Dependent upon the blinded SD estimate, the sample size may be decreased or increased to maintain 90% power. Any increase in sample size required to assess MFF will also be limited to 1.5 times the required target population sample size stated in the preceding power calculation, to a maximum of $1.5 \times 99 = 150$ subjects.

At the interim futility analysis, there will be no efficacy assessment, no unblinded analysis of the primary endpoint and, hence, no alpha spend nor early stopping of the study for efficacy.

As noted above in Section 13.2, the interim analysis took place in January 2020; futility was passed and the blinded sample size reassessment followed. The estimated SD for 4SC was approximately half of that assumed in the original power calculation. Consequently, it was determined that a total of N=102 patients would be sufficient to test the hypothesis that 4SC was improved by at least 2 seconds with givinostat compared placebo. With allowance for a small dropout rate, a total of 110 male ambulant subjects will be randomised in the target population (Group A). Up to 50 subjects (about 35% of the overall population) outside of the target may also be recruited into the study (Group B). The overall subjects population (Group A + Group B) will provide supportive data.

For the MR cohort, the blinded SD estimate was 5.941%, being in line with SD assumed in the original power calculation for VL MFF, hence all the subjects in the target population will be included in the analysis of the MR cohort.

### *13.10.2 Final Analysis*

The final analysis will be performed when N=110 patients in the target population subjects have achieved 18 months follow-up. The p-value applicable to the final analysis of 4SC will be p≤0.025 1-sided.

## 13.11 Independent Data Monitoring Committee

The IDMC will be established by the Sponsor to review accumulating safety data at regular intervals throughout the study and monitor overall study conduct. The IDMC will review, evaluate, and categorise safety findings every 3 months during the study and will be responsible for oversight of the planned interim analysis as described in Section 13.10.

Members will include at least 3 person, but are not limited to, a chairman, a clinician experts in DMD and a biostatistician, who are not participating in this study and do not have affiliation with the Investigators or the Sponsor. The IDMC can recommend in writing to the Sponsor whether to continue, modify, or stop the clinical study on the basis of safety considerations.

An IDMC charter will be prepared to describe the role, function and operations of the IDMC and the rules to be used when reviewing interim data.

# 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 the 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.

For EU/EEA countries this study will be performed in accordance with EU and country regulation in force.

Moreover, this study will be performed in accordance to all relevant federal regulations, as set forth in Parts 50 “Protection of Human Subjects”, 56 “Institutional Review Boards”, 312 “Responsibilities of Sponsors and Investigators”, Subpart D “GCP”, of Title 21 of the Code of Federal Regulations (CFR), and in compliance with Good Clinical Practice (GCP) guidelines.

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 Institutional Review Board/Independent Ethics Committees*

The protocol, informed consent form, assent form, recruitment materials, and all participant materials will be submitted to the Independent Ethics Committee (IEC) or Institutional review Board (IRB) for review and approval. This study will be undertaken only after approval of the protocol, informed consent form, assent form and all other materials described above, have been obtained from the appropriate IEC or IRB 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 IRB/IEC must be supplied to Italfarmaco S.p.A. or its designee. The IRB/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 IRB/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 IRB/IEC and to Italfarmaco S.p.A.

### *14.1.3 Independent Data Monitoring Committee*

An IDMC will be utilized in this study to ensure external objective medical and/or statistical review of safety issues in order to protect the ethical and safety interests of subjects and to protect the scientific validity of the study. The IDMC also has the responsibility to monitor

risk versus benefit considering factors external to the study when relevant information becomes available.

The members of the IDMC act as advisors to the Sponsor to monitor subject safety and study progress. The IDMC will have the responsibility to provide the Sponsor with recommendations related to the protection of the subject's safety, including stopping or amending the study protocol, the consent/assent forms, and the IB.

During the study, the IDMC will review, evaluate and categorise safety findings every three months and will be responsible for the interim analyses (see section 13.10.10), which could result in recommendations to the Sponsor. The IDMC will meet at predefined times and more frequently as needed.

The IDMC will be also responsible to review any protocol amendments.

The IDMC will operate under a written charter that includes a well-defined standard operating procedure. The charter will be prepared to describe the role, function, and operations of the IDMC and the rules to be used when reviewing interim data.

All members should have no financial, scientific, or other conflicts of interest with the study.

IDMC meeting/phone call will include both an open and closed session, while the meeting/phone call with the Sponsor and Investigators will always be blinded session.

### *14.1.4 Subject Informed Consent*

Prior to the beginning of the study, the Principal Investigator (PI) must have the IRB/IEC written approval/favourable opinion of the written Informed Consent/ Assent Form and any other written information to be provided to subject and legally accepted representative. The approved subject information letter/Informed Consent/Assent Form must be filed in the study files (clinical Trial Master File [TMF] and Investigator File [IF]). For each study subject, a written Informed Consent Form the legally accepted representative will be obtained prior to any protocol related activities. Informed assent or consent, if applicable, may be obtained from subjects who are capable of providing assent/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 subject, the parent and, if applicable, appointed guardian are aware of the potential risks, inconveniences, or

adverse effects that may occur. Subjects and their legally accepted representatives should be given ample time and opportunity to inquire about the details of the study prior to deciding whether to participate in the study. It is the responsibility of the Investigator to ensure that all questions about the study are answered to the satisfaction of the subjects and their legally accepted representatives.

Subjects and their legally accepted representatives should be informed that subjects 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 IRB/IEC approved ICF prior to the start of the study.

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

For details of the information provided, refer to the ICF.

### *14.1.5 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 subject 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 subjects;
- poor adherence to protocol and regulatory requirements;
- plans to modify or discontinue the development of the study drug.

### *14.1.6 Data Handling*

Data on subjects collected on eCRFs during the trial will be documented in an anonymous fashion and the subject will only be identified by the subject number. All the information

required by the protocol should be provided and any omissions require explanation. All eCRFs must be completed expeditiously after the subject's visit.

Any data to be recorded directly on the eCRFs (to be considered as source data) will be identified at the start of the study. 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, unless the study site makes direct data entry to the databases for which no other original or source documentation is maintained. In such cases, the study site should document which eCRFs are subject to direct data entry and should have in place procedures to obtain and retain copies of the information submitted by direct data entry. 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.

Any changes made to data after collection will be made through the use of Data Clarification Forms. 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 the relevant regulatory authority.

In addition, because this is an international study, the retention period must meet the requirements of the most stringent authority.

The 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 subjects 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 subject 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 in order to check the completeness, clarity, consistency of the data recorded in the eCRFs/CRFs for each subject 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 complete eCRFs/CRFs. No information in these records about the identity of the subjects will leave the study centre. 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/assent, 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 CRFs 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.

## 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 impact one of the following criteria:

- The safety or physical or mental integrity of the subject;
- 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 IRB/IEC before implementation.

In case of urgent safety measures to protect the subject against any immediate hazard, these measures may be taken without prior authorization from the Competent Authority or favourable opinion of the IRB/IEC. In this case, the Competent Authority and IRB/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 IRB/IECs 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 IRB/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 IRB/IEC will review and approve this protocol, the informed consent form, the assent 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 subjects' anonymity will be maintained and that their identities will be protected from unauthorized parties. On eCRFs or other documents submitted to the Sponsor, subjects 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 IRB/IECs. 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 subjects who wish to participate in the study. For USA sites, the Investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of subject health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR, Parts 160 and 164 (the Health Insurance Portability Accountability Act of 1996 [HIPAA] Privacy Regulation). The Investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with HIPAA Privacy Regulation and in a form satisfactory to the Sponsor.

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: 12 September 2018. Accessed 9 October 2018.

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 have a Conditional Risk of TdP (CR). The full description of these categories can be found on the CredibleMeds.org website.

| Generic Name | Brand Name |
|---|---|
| Abarelix (PR) | Plenaxis |
| Aclarubicin (KR) | Aclacin and others |
| 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 |
| Amsacrine (acridinyl anisidide) (CR) | Amsidine |
| Anagrelide (KR) | Agrylin and others |
| Apalutamide (PR) | Erleada |
| 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 |

| Generic Name | Brand Name |
|---|---|
| Bendroflumethiazide or bendrofluazide (CR) | Aprinox and others |
| Benperidol (PR) | Anquil and others |
| Bepridil (KR) | Vascor |
| Betrixaban (PR) | Bevyxxa |
| Bortezomib (PR) | Velcade and others |
| Bosutinib (PR) | Bosulif |
| Buprenorphine (PR) | Butrans and others |
| Cabozantinib (PR) | Cometriq |
| Capecitabine (PR) | Xeloda |
| Ceritinib (PR) | Zykadia |
| Chloral hydrate (CR) | Aquachloral and others |
| Chloroquine (KR) | Aralen |
| Chlorpromazine (KR) | Thorazine and others |
| Cilostazol (KR) | Pletal |
| Cimetidine (CR) | Tagamet and others |
| Ciprofloxacin (KR) | Cipro and others |
| Cisapride (KR) | Propulsid |
| Citalopram (KR) | Celexa and others |
| Clarithromycin (KR) | Biaxin and others |
| Clofazimine (PR) | Lamprene |
| Clomipramine (PR) | Anafranil |
| Clotiapine (PR) | Entumine |

| Generic Name | Brand Name |
|---|---|
| Clozapine (PR) | Clozaril and others |
| Cocaine (KR) | Cocaine |
| Crizotinib (PR) | Xalkori |
| Cyamemazine (cyamepromazine) (PR) | Tercian |
| Dabrafenib (PR) | Tafinlar |
| Dasatinib (PR) | Sprycel |
| Degarelix (PR) | Firmagon and others |
| Delamanid (PR) | Deltyba |
| Desipramine (PR) | Pertofrane and others |
| Deutetrabenazine (PR) | Austedo |
| 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 |
| Eliglustat (PR) | Cerdelga |

| Generic Name | Brand Name |
|---|---|
| Eperisone (CR) | Myonal and others |
| Epirubicin (PR) | Ellence and others |
| Eribulin mesylate (PR) | Halaven |
| Erythromycin (KR) | E.E.S. and others |
| Escitalopram (KR) | Cipralex and others |
| Esomeprazole (CR) | Nexium and others |
| Ezogabine (Retigabine) (PR) | Potiga and others |
| Famotidine (CR) | Pepcid and others |
| Felbamate (PR) | Felbatol |
| Fingolimod (PR) | Gilenya |
| Flecainide (KR) | Tambocor and others |
| Fluconazole (KR) | Diflucan and others |
| Fluorouracil (5-FU) (PR) | Adrucil and others |
| Fluoxetine (CR) | Prozac and others |
| Flupentixol (PR) | Depixol and others |
| Fluvoxamine (CR) | Faverin and others |
| 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) | Hysinglaå„¢ ER and others |
| Hydroxychloroquine (CR) | Plaquenil and others |

| Generic Name | Brand Name |
|---|---|
| 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 |
| Inotuzumab ozogamicin (PR) | Besponsa |
| Isradipine (PR) | Dynacirc |
| Itraconazole (CR) | Sporanox and others |
| Ivabradine (CR) | Procoralan and others |
| Ketanserin (PR) | Sufrexal |
| Ketoconazole (CR) | Nizoral and others |
| Lacidipine (PR) | Lacipil and others |
| Lansoprazole (CR) | Prevacid |
| Lapatinib (PR) | Tykerb and others |
| Lenvatinib (PR) | Lenvima |
| Leuprolide (PR) | Lupron and others |
| Levofloxacin (KR) | Levaquin and others |
| Levomepromazine (methotrimeprazine) (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 |
| Lopinavir and ritonavir (PR) | Kaletra and others |
| Maprotiline (PR) | Ludiomil and others |
| Melperone (PR) | Bunil and others |
| Memantine (PR) | Namenda XR and many others |
| Mesoridazine (KR) | Serentil |

| Generic Name | Brand Name |
|---|---|
| Methadone (KR) | Dolophine and others |
| Metoclopramide (CR) | Reglan and others |
| Metolazone (CR) | Zytanix and others |
| Metronidazole (CR) | Flagyl and many others |
| Midostaurin (PR) | Rydapt |
| Mifepristone (PR) | Korlym and others |
| Mirabegron (PR) | Myrbetriq |
| Mirtazapine (PR) | Remeron |
| Moexipril/HCTZ (PR) | Uniretic and others |
| Moxifloxacin (KR) | Avelox and others |
| Necitumumab (PR) | Portrazza |
| Nelfinavir (CR) | Viracept |
| Nicardipine (PR) | Cardene |
| Nilotinib (PR) | Tasigna |
| Norfloxacin (PR) | Noroxin and others |
| Nortriptyline (PR) | Pamelor and others |
| Nusinersen (PR) | Spinraza |
| Ofloxacin (PR) | Floxin |
| Olanzapine (CR) | Zyprexa and others |
| Omeprazole (CR) | Losec and others |
| Ondansetron (KR) | Zofran and others |
| Osimertinib (PR) | Tagrisso |
| Oxaliplatin (KR) | Eloxatin |
| Oxytocin (PR) | Pitocin and others |
| Paliperidone (PR) | Invega and others |
| Palonosetron (PR) | Aloxi |
| Panobinostat (PR) | Farydak |
| Pantoprazole (CR) | Protonix and others |

| Generic Name | Brand Name |
|---|---|
| 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 and others |
| Perphenazine (PR) | Trilafon and others |
| Pilsicainide (PR) | Sunrythm |
| Pimavanserin (PR) | Nuplazid |
| Pimozide (KR) | Orap |
| Pipamperone (PR) | Dipiperon (E.U) and others |
| Piperacillin/Tazobactam (CR) | Tazosyn and Zosyn |
| Posaconazole (CR) | Noxafil and others |
| Primaquine phosphate (PR) | |
| Probucol (KR) | Lorelco |
| Procainamide (KR) | Pronestyl and others |
| Promethazine (PR) | Phenergan |
| Propafenone (CR) | Rythmol SR and others |
| 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 |

| Generic Name | Brand Name |
|---|---|
| Ribociclib (PR) | Kisqali |
| Rilpivirine (PR) | Edurant and others |
| Risperidone (PR) | Risperdal |
| Romidepsin (PR) | Istodax |
| Roxithromycin (KR) | Rulide and others |
| Saquinavir (PR) | Invirase(combo) |
| Sertindole (PR) | Serdolect and others |
| Sertraline (CR) | Zoloft and others |
| Sevoflurane (KR) | Ultane and others |
| 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 |

| Generic Name | Brand Name |
|---|---|
| Terodiline (KR) | Micturin and others |
| Tetrabenazine (PR) | Nitoman and others |
| Thioridazine (KR) | Mellaril and others |
| Tiapride (PR) | Tiapridal and others |
| Tipiracil and Trifluridine (PR) | Lonsurf |
| Tizanidine (PR) | Zanaflex and others |
| Tolterodine (PR) | Detrol and others |
| Toremifene (PR) | Fareston |
| torsemide (torasemide) (CR) | Demadex and others |
| Tramadol (PR) | Crispin and others |
| Trazodone (CR) | Desyrel (discontinued 6/13) and others |
| Trimipramine (PR) | Surmontil and others |
| Tropisetron (PR) | Navoban and others |
| Valbenazine (PR) | Ingrezza |
| 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 |
| Zuclopenthixol, Zuclopentixol (PR) | Cisordinol and others |

## 16.2 Drugs knows to be a substrate of OCT2 transporter

OCT2 transporter = Organic Cation Transporter 2

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

Accessed October 9th, 2018.

| **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.

*P-glycoprotein inhibitors*

- Amiodarone (Cordarone®)
- Atorvastatin (Lipitor®)
- Azithromycin (Zithromax®)
- Boceprevir (Victrelis®) - Drug PI states possible inhibitor
- 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
- Telaprevir (Incivek®)
- Ticagrelor (Brilinta®)
- Velpatasvir (Epclusa®)
- Verapamil (Calan®,Covera-HS®, Isoptin®, Verelan®)
- Vorapaxar (Zontivity®) (weak inhibitor)
- Voxilaprevir (Vosevi™)