25 April 2025
EMA/CHMP/176222/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Duvyzat

International non-proprietary name: givinostat

Procedure No. EMEA/H/C/006079/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
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List of abbreviations

10MWR	10-metre walk/run
4SC	4-stair climb
6MWT	6-minute walking test
ADR	Adverse drug reaction
AE	Adverse event
AESI	Adverse event of special interest
ALK	Alkaline phosphatase
ALT	Alanine aminotransferase
AML	Acute myeloid leukaemia
ANCOVA	Analysis of covariance
API	Active Pharmaceutical Ingredient
APTT	Activated partial thromboplastin time
ASM	Active Substance Manufacturer
AST	Aspartate aminotransferase
b.i.d	Bis in die (twice/two times daily)
BAE	Bronchopulmonary adverse event
BCS	Biopharmaceutics Classification System
BMD	Becker muscular dystrophy
BMI	Body mass index
C_{ave}	Average concentration
CLL	Chronic lymphocytic leukaemia
cMPN	Chronic myeloproliferative neoplasms
CoA	Certificate of Analysis
CQA	Critical Quality Attribute
CRP	C-reactive protein
CRS	Chemical Reference Substance (official standard)
CSA	Cross-sectional area
CSR	Clinical study report
CVD	Cardiovascular disease
CYP	Cytochrome P450
DGC	Dystrophin–glycoprotein complex
DL	Dose level
DLBCL	Diffuse large B-cell lymphoma
DLT	Dose-limiting toxicities
DMD	Duchenne muscular dystrophy
DMF	Drug Master File = Active Substance Master File
DP	Decentralised (Application) Procedure
DSC	Differential Scanning Calorimetry
EDQM	European Directorate for the Quality of Medicines
E_{max}	Maximum effect
E-R	Exposure-response
ESR	Erythrocyte sedimentation rate
FAP	Fibroadipogenic progenitors
FEV1	Forced expiratory volume at 1 second
fT3	Free triiodothyronine
fT4	Free thyroxine
FU	Follow-up
FVC	Forced vital capacity
GLSmean	Geometric least squares mean
HDAC	Histone deacetylase
HDPE	High Density Polyethylene
HHD	Hand-held-dynamometry
HPLC	High Pressure Liquid Chromatography
IPC	In-process control test
IQ	Interquartile range
IR	Infrared
ITT	Intent-to-treat
JIA	Juvenile idiopathic arthritis
LDH	Lactate dehydrogenase
List of Quality related abbreviations	
LLN	Lower limit of normal
LOD	Limit of Detection
LOQ	(1) Limit of Quantification, (2) List of Questions
LPS	lipopolysaccharide
LS	Least squares

Ltbp4	Latent transforming growth factor-β (TGF-β)-binding protein 4
MCID	Minimal clinically important difference
MEB	Medicines Evaluation Board
MedDRA	Medical Dictionary for Regulatory Activities
MFA%	Muscle fibre area fraction
MRS	Magnetic resonance spectroscopy
MS	Mass Spectrometry
MuSC	Muscle satellite cells
ND	Not detected
NMR	Nuclear Magnetic Resonance
NMT	Not more than
NSAA	North Star Ambulatory Assessment
o.d	Once daily
OOS	Out of Specifications
PDE	Permitted Daily Exposure
PE	Polyethylene
PEF	Peak expiratory flow
PFT	Pulmonary function test
P-gp	P-glycoprotein
Ph.Eur.	European Pharmacopoeia
PIL	Patient Information Leaflet
popPK	Population pharmacokinetics
PP	Polypropylene
PSD	Particle size distribution
PT	Preferred term
PTT	Prothrombin time
PUL	Performance of upper limb
PV	Polycythaemia Vera
PVC	Poly vinyl chloride
QC	Quality Control
QoL	Quality of life
QOS	Quality Overall Summary
QTcB	QT interval corrected using Bazett's formula
QTcF	QT interval corrected using Fridericia's formula
RH	Relative Humidity
RP	Restricted Part (or Closed Part) of a DMF
RRT	Relative retention time
RSD	Relative standard deviation
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SE	Standard error
SMQ	Standardised MedDRA query
SOC	System organ class
SOJIA	Systemic onset juvenile idiopathic arthritis
TEAE	Treatment-emergent adverse event
TGA	Thermo-Gravimetric Analysis
TNFα	Tumour necrosis factor alpha
TQT	Thorough QT
TSH	Thyroid stimulating hormone
TTR	Time to rise from floor
UDP	Uridine diphosphate
UPLC	Ultra Pressure Liquid Chromatography
UV	Ultraviolet
VL MFF	Vastus lateralis muscle fat fraction
WBC	White blood cell
XRPD	X-ray powder diffraction (= PXRD)

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Italfarmaco S.p.A. submitted on 29 June 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Duvyzat, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 24 February 2022.

Duvyzat was designated as an orphan medicinal product EU/3/12/1009 on 04 July 2012 in the following condition: Duchenne Muscular Dystrophy.

The applicant applied for the following indication: Treatment of Duchenne Muscular Dystrophy.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Duvyzat as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/duvyzat

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is

composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0240/2023 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0240/2023 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. New active substance status

The applicant requested the active substance givinostat contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
23 July 2015	EMEA/H/SA/3097/1/2015/PA/PED/III	*Fernando de Andrés Trelles, Kerstin Wickström*
30 January 2020	EMEA/H/SA/3097/2/2019/PA/PED/III	*Fernando de Andrés Trelles, André Elferink*

The protocol assistance pertained to the following aspects:

EMEA/H/SA/3097/1/2015/PA/PED/III - Non-clinical and clinical development

- The proposed toxicological package including the planned carcinogenicity study to support a marketing authorisation application (MAA); the proposal not to perform a thorough QT/QTc study.
- Adequacy of the clinical development plan to support a MAA; the proposed dose selection strategy, primary efficacy endpoint, secondary and exploratory endpoints, statistical analysis plan, and population in the pivotal study; the proposed plan to ask for a conditional marketing authorisation; adequacy of the safety and tolerability assessments to be implemented in the clinical development programme; adequacy of the safety database for MAA.

EMEA/H/SA/3097/2/2019/PA/PED/III - Non-clinical and clinical development

- Suitability of the proposed non-clinical package to support a MAA.
- The revisions to the design of Study DSC/14/2357/48 (major changes concern the introduction of permanent and/or temporarily stopping rules for safety, and revision of in- and exclusion criteria in the context of a safety issue), including potential revision of the sample size at the first interim analysis; a revised dose regimen implemented in Study DSC/14/2357/48; the approach to the assessment of drug-drug interaction (DDI) potential liability with givinostat.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Janet Koenig Co-Rapporteur: Ewa Balkowiec Iskra

The appointed CHMP co-rapporteur had no such prominent role in Protocol assistance relevant for the

indication subject to the present application.

The application was received by the EMA on	29 June 2023
The procedure started on	17 August 2023
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	6 November 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	20 November 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	14 December 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	17 July 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	26 August 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	5 September 2024
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	19 September 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	08 November 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	27 November 2024
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	11 December 2024
The CHMP agreed on a second list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	12 December 2024
The applicant submitted the responses to the CHMP second List of Outstanding Issues on	21 February 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the second List of Outstanding Issues to all CHMP and PRAC members on	12 March 2025
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	26 March 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Duvyzat on	25 April 2025
The CHMP adopted a report on similarity of Duvyzat with Translarna and Agamree on (see Appendix on similarity)	25 April 2025

Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	25 April 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Givinostat is intended for the treatment of Duchenne muscular dystrophy (DMD) in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment.

2.1.2. Epidemiology

DMD is a severe, progressive paediatric neuromuscular disorder that is ultimately lethal. It occurs almost exclusively in males (X-linked recessive disorder) with an estimated male birth prevalence of 1/3,500-1/9,300. The estimated prevalence in the EU is ~ 15,000 cases.

2.1.3. Biologic features

DMD is caused by several types of mutations in the gene for dystrophin located on chromosome Xp21. Most mutations are deletions, duplications and point mutations, which produce a shift in the open reading frame of the dystrophin mRNA leading to the absence of functional dystrophin protein.

Dystrophin is a cytoplasmic protein, which associates with other proteins to form the dystrophin-associated protein complex that connects the actin cytoskeleton with the extracellular matrix. Functional dystrophin is critical for the structural stability of myofibers in skeletal, diaphragm and cardiac muscle and is also of importance for smooth muscles. In DMD, both dystrophin and the dystrophin-associated protein complex proteins are missing, leading to excessive membrane fragility and permeability, dysregulation of calcium homeostasis, oxidative damage. These factors play a crucial role in muscle cell necrosis. During the progression of the disease, the regenerative capacity of the muscles appears to be exhausted, and connective and adipose tissue gradually replaces muscle fibers.

2.1.4. Clinical presentation, diagnosis

The onset of DMD occurs in early childhood and initial findings may include delays in reaching developmental milestones such as sitting or standing without assistance, toe walking, unusual gait, difficulty climbing stairs or rising from a sitting position (Gower's sign), and repeated falling leading to an increased incidence of fractures in ambulatory subjects. Weakness is more pronounced in proximal than distal muscles and the lower limb more than the upper limb. Affected boys may show a delay in walking (after 18 months of age) accompanied with speech and/or global developmental delay. Autism and behavioural problems, such as ADHD (attention deficit hyperactivity disorder), anxiety, obsessive compulsive disorder, are relatively common. Untreated children with DMD rarely achieve the ability to run or jump. Loss of independent ambulation occurs between the ages of 6 and 13 years, the average being 9.5 years in non-steroid treated patients. Once ambulation is lost, joint contractures and scoliosis develop rapidly, which leads to an impaired pulmonary function. There is gradual loss of upper

limb, trunk, and neck function, severely affecting patient quality of life, as well as that of caregivers and families (Bendixen 2012; Magliano 2014; Uzark 2012). Complications from this loss of ambulation have a major cascading effect, including scoliosis.

Children with DMD have reduced bone density and an increased risk of developing fractures of certain bones, such as hips and spine. Many affected individuals will display mild to moderate degrees of non-progressive intellectual impairment and learning disabilities.

Symptoms of cardiomyopathy (persistent tachycardia and heart failure) can develop in early teens and are present in almost all patients in their twenties. In affected patients, dilated cardiomyopathy is characterised by extensive fibrosis of the posterobasal left the ventricular wall. As the disease progresses, fibrosis can spread to the lateral free wall of the left ventricle. With the involvement of the posterior papillary muscle, significant mitral regurgitation can occur. Inter- and intraatrial conduction abnormalities, possibly involving the AV node, can be seen. Arrhythmias, particularly supraventricular arrhythmias, are also associated with the developing cardiomyopathy (Venugopal, 2022).

Another serious complication associated with DMD is weakness and deterioration of muscles in the rib cage. This can result in an increased susceptibility to respiratory infections (e.g., pneumonia), difficulty coughing, and, ultimately, respiratory failure.

Without physical therapy treatment, leg braces may be needed by the age 8-9 to assist walking in affected individuals. Most affected individuals require a wheelchair between 10 and 12 years of age. Untreated patients die during late teens to early twenties from respiratory failure and or cardiomyopathy.

Achieving a timely and accurate diagnosis of DMD is a crucial aspect of care. The method for diagnosing DMD has not changed during the last decade. The diagnostic process typically begins in early childhood after suggestive signs and symptoms. Diagnosis is suspected on the basis of the clinical picture, family history and laboratory findings (serum creatine kinase (CK) is 100-200 times the normal level). Genetic testing is the gold standard and involves multiplex-ligation dependent probe amplification (MLPA) for detection of deletions and duplications of exon (s) and full gene sequencing for detecting small deletions and duplications and non-sense or point mutations. Given the value of information provided by genetic analysis muscle biopsy is now recommended when genetic analysis in inconclusive. Genetic testing is therefore a critical tool in the accurate diagnosis of DMD (https://www.orpha.net/).

2.1.5. Management

No medical cure exists for DMD, and the disease has a poor prognosis. Treatment is still centered on corticosteroid therapy, e.g. glucocorticoids, prevention of contractures, and medical care of cardiomyopathy and respiratory compromise (Shimizu-Motohashi 2019; McMillan 2019). Glucocorticoids (GC) should continue after loss of ambulation according to current guideline recommendations (Birkrant et al. 2018) and have been shown to improve strength and motor function, delay loss of ambulation by 2 to 3 years, preserve upper limb and respiratory function, avoid scoliosis surgery, and delay the onset of cardiomyopathy.

Recent studies confirm the benefits of starting glucocorticoids in younger children, before significant physical decline (Merlini 2012; Lamb 2016). Complications of corticosteroid therapy must be managed and include weight management, gastric protection, monitoring and treatment of osteoporosis, ophthalmic assessment for cataracts and glaucoma. The two common corticosteroid drugs used to treat individuals with DMD are prednisone and deflazacort. Prednisone is recommended at a dosage of 0.75 mg/kg per day, and deflazacort at 0.9 mg/kg/day.

Deflazacort was approved by the FDA in 2017 for the treatment of DMD in patients aged 5 years and older. In 2019, the indication was extended to include patients 2 years of age and older. Prednisone is available and widely used in the US and in the European Union but is not specifically indicated for DMD. Deflazacort was recently approved for "*treatment of Duchenne muscular dystrophy (DMD) in patients 2 years and older*" across five EU/EEA countries via the mutual recognition procedure.

On 14 December 2023, a marketing authorisation was granted for the medicinal product Agamree (active substance: vamorolone), indicated for the treatment of Duchenne muscular dystrophy (DMD) in patients aged 4 years and older. Vamorolone is a synthetic corticosteroid analogue that structurally diverges from other members of the class of glucocorticoid agents. Vamorolone shares the effective transcriptional repression at the glucocorticoid receptor (i.e. inhibition of NFκB-mediated inflammations) with established corticosteroids. Due to its structural difference, however, vamorolone shows limited transactivation of genes, whose expression is driven by the glucocorticoid response element. This may lower its potential for typical GC side effects. The structural peculiarity of vamorolone further entails that it acts as mineralocorticoid receptor antagonist, which contrasts the agonistic MR activity of other GCs. In addition, vamorolone is not a substrate for 11-β HSD enzymes, thereby potentially reducing the risk for typical GC side effects (muscle atrophy, bone loss, insulin resistance, hypertension and weight gain).

There is still an unmet medical need for (mutation-independent) treatments for DMD with an acceptable benefit-risk profile.

2.2. About the product

Givinostat is an orally active hydroxamic acid derivative that belongs to the pharmacological class of histone deacetylase (HDAC) inhibitors. Multiple HDAC enzymes have been identified in eukaryotes that differ in terms of cellular localisation (nucleus and/or cytoplasm) and co-factor dependency for their catalytic activity (zinc vs. nicotinamide adenine dinucleotide (NAD^+)). HDACs deacetylate lysine residues from both histone and non-histone proteins and, hence, epigenetically control gene expression by euchromatin to heterochromatin remodelling and modification of transcription factor activity (Seto and Yoshida, 2014). The interplay of histone acetylase and HDACs with myogenic transcription factors generally directs muscle development and differentiation (McKinsey *et al*., 2001). In dystrophic muscle, the intramuscular generation of nitric oxide by the dystrophin-associated protein complex is decreased, which interferes with HDAC inactivation by Snitrosylation. As a consequence, the increased HDAC activity in satellite cells constitutively represses myogenic transcriptional activators and follistatin that normally counteracts the inhibition of myogenesis by myostatin (Consalvi *et al*., 2011). Conversely, HDAC inhibitors were shown to induce follistatin and promote myoblast fusion into myotubes *in vitro* and of myogenic regeneration markers after experimental muscle injury or in dystrophin-deficient *mdx* mice *in vivo* (Iezzi *et al*., 2004; Minetti *et al*., 2006). Givinostat demonstrated dose-dependent inhibition of HDACs resulting in anti-fibrotic and anti-inflammatory activities *in vitro*. Accordingly, givinostat reduced the fibrosis and fatty infiltration in two murine models of human DMD *in vivo* (Licandro SA *et al*., 2021). Givinostat also promoted the formation of multinucleated myotubes from primary human skeletal myoblasts *in vitro*. Thus, HDAC inhibitors like givinostat are thought to delay DMD progression by stimulating the activation of muscular satellite cells and regeneration factor expression as well as by inhibiting fibrosis and inflammation.

2.3. Type of application and aspects on development

The givinostat clinical development programme for DMD includes five Phase 1 studies in healthy volunteers (i.e., a single and multiple ascending dose study, a food effect study, a thorough QT [TQT]

study, and a drug-drug interaction study [DDI]), as well as pharmacokinetic (PK) data in patients with DMD, preclinical and clinical studies characterising metabolism and drug interactions, population PK (popPK) analyses, and pharmacometric assessments, including exposure-response [E-R] analyses of key efficacy and safety endpoints. Evaluation of givinostat in DMD patients is based on three clinical trials contributing to efficacy and safety, i.e. a double-blind, placebo-controlled pivotal phase 3 study DSC/14/2357/48 (hereafter referred to as Study 48), a single-arm, open-label, two-part, phase 2 study DSC/11/2357/43 (hereafter referred to as Study 43), and an ongoing open-label long-term study DSC/14/2357/51 (hereafter referred to as Study 51) including patients previously enrolled in one of the aforementioned DMD givinostat studies (i.e., Studies 43 and 48) and givinostat-naïve patients, who could not been enrolled in study 48.

Protocol assistance regarding non-clinical and clinical development was granted by the CHMP on 23rd July 2015 and on 30th January 2020, respectively (EMEA/H/SA/3097/1/2015/PA/PED/III and EMEA/H/SA/3097/2/2019/PA/PED/III). National scientific advice on quality, non-clinical and clinical aspects was given each by the German Federal Institute for Drugs and Medical Devices (BfArM) on 17th February 2022 and by the Swedish Medical Products Agency (MPA) on 14th March 2022. A pre-submission meeting was held with the Rapporteurs and EMA concerning the clinical and regulatory requirements for the marketing authorisation application (MAA) on 6th October 2022. Further interaction with EMA took place on regulatory and procedural issues ahead of the MAA submission.

Overall, the CHMP and the national agencies had acknowledged during protocol assistance that a standard non-clinical development had been performed for givinostat. In view of the severity of DMD, the proposal to defer carcinogenicity testing post approval was in principle agreed along ICH S1A recommendations (CPMP/ICH/140/95) provided that genotoxicity and chronic toxicity studies would support the absence of a relevant concern.

Central topics regarding the clinical development pertained to the adequacy of the phase 3 study design regarding the choice of primary and secondary endpoints, the patient population, the dose selection strategy and the finally revised dose regimen implemented in the pivotal study, the introduction of permanent and/or temporarily stopping rules for safety management and potential revision of the sample size at the first interim analysis. It was further advised to focus on functional clinical outcome parameters and to expand the planned 12 months study duration. Moreover, the approach to the assessment of DDI potential liability with givinostat was discussed. Another relevant issue pertained to the intended target population for givinostat that was considered much broader than the study population requiring justification and substantiation of extrapolation of the results, obtained in a selected DMD population, to the broad target population as part of the Marketing Authorisation Application.

Moreover, advice has also been obtained by FDA on several occasions between 2015 and 2019.

There were two PIPs presented since 2014 before the current one was agreed in 2023. The agreed PIP (decision no. P/0240/2023) covers the paediatric population from 2 years of age, and includes the following studies:

- Study 1: a quality study to assess the suitability of the oral suspension formulation, of intragastric administration via feeding tubes.

- Study 2: a non-clinical study in juvenile animals.

The PIP includes 5 clinical studies, of which Studies No. 3 and 4 are non-deferred. These two studies have been completed and are part of the MAA:

- Study 3: 2-part open-label study to assess the safety, tolerability, pharmacokinetics (PK), effects on histology and clinical parameters of givinostat in ambulant paediatric patients from 7 years to less than

*11 years of age with DMD (**DSC/11/2357/43**). The study was already performed when the PIP was submitted, conclusion November 2017. Compliant with the PIP.*

*- Study 4: Randomised, double-blind safety and efficacy study of givinostat versus placebo in ambulant paediatric patients from 6 years to less than 18 years of age with DMD (**DSC/14/2357/48**). The study was ongoing when the PIP was presented, conclusion May 2022. Compliant with the PIP.*

- Study 5: Randomised, double-blind safety and efficacy study of givinostat versus placebo in non-ambulant paediatric patients from 9 years to less than 18 years of age with DMD (**DSC/14/2357/50**), first patient in on 19 February 2024; conclusion expected March 2026.

- Study 6: Open-label, long term safety study of givinostat in paediatric patients from 6 years to less than 18 years of age with DMD (**DSC/14/2357/51**), conclusion March 2026. The study was ongoing at the time of the MAA with interim data provided as of the data cut-off 31 December 2021 for the initial submission. New interim data as of 31 December 2023 have been provided as part of the responses. Initiation compliant with the PIP.

- Study 7: Open-label, safety and pharmacokinetic study of givinostat in paediatric patients from 2 years to less than 6 years of age with DMD (**DSC/14/2357/52**); date of completion by December 2026. A PIP modification request (000551-PIP04-21-M03) with regard to the study design has been approved on 8 March 2024. At present, the study protocol for Study 7 is under review.

In addition, there are a number of PK/PD models to support dose selection, to estimate the risk of experiencing a reduction in platelets and extrapolation studies to correlate clinical parameters with PK data to support extrapolation of efficacy data in patients with DMD younger than 6 years of age.

Study 10: Population pharmacokinetic (PK)-pharmacodynamic (PD) model to correlate clinical parameters with PK data in DMD patients treated with givinostat – No study identifier. Date of completion 2015. Compliant with the PIP.

Cardiovascular effect and effect on platelets were to be kept under monitoring.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as oral suspension containing 8.86 mg/mL givinostat as active substance. The product contains the salt and hydrate form, givinostat hydrochloride monohydrate in a concentration of 10 mg/mL.

Other ingredients are: polysorbate 20, glycerol, tragacanth gum, sodium benzoate, peach flavour, cream flavour, saccharin sodium, liquid sorbitol, tartaric acid, sodium hydroxide and purified water. The flavours contain propylene glycol (E1520).

The product is available in an amber polyethylene terephthalate (PET) bottle closed with a high-density polyethylene (HDPE) child-resistant closure with low-density polyethylene (LDPE) syringe adapter as described in section 6.5 of the SmPC. Each bottle contains 140 mL of oral suspension intended for multidose use. Each pack also contains one 5 mL oral syringe, graduated from 1 to 5 mL by increments of 0.5 mL.

2.4.2. Active substance

2.4.2.1. General information

The chemical name of givinostat is 6-(diethylaminomethyl)naphthalen-2-yl]methyl [4(hydroxycarbamoyl) phenyl]carbamate hydrochloride monohydrate corresponding to the molecular formula $C_{24}H_{27}N_3O_4.HCl.H_2O$. It has a relative molecular mass of 475.98 and the following structure:

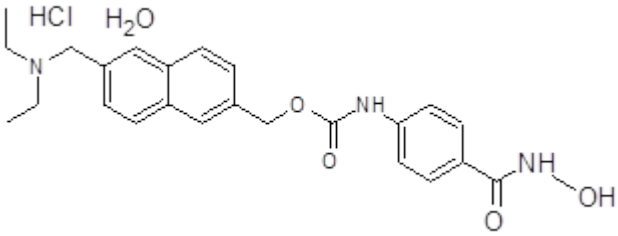


Figure 1 Active substance structure

The chemical structure of givinostat was elucidated by a combination of elemental analysis, mass spectroscopy, NMR spectroscopy (^1H, ^{13}C), IR spectroscopy, UV spectroscopy. The solid-state properties of the active substance were measured by PXRD, TGA, DSC, IR, TGA/FTIR, KF and DSC/TGA analysis.

The active substance is a non-hygroscopic white to off-white powder soluble in dimethyl sulphoxide and 1,3-Propanediol. It is slightly soluble in water and its solubility depends on the counter-ion and the pH of the solution. It has low solubility at pH 1 and 6.8 (< 1 mg/mL), whereas its solubility increases at pH 4.5 (~5 mg/mL). Polymorphism has been observed for the active substance. There is no monograph for givinostat in the European Pharmacopoeia.

2.4.2.2. Manufacture, characterisation and process controls

The active substance master file (ASMF) procedure has been used. Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory. Givinostat is synthesised in five main steps using well defined starting materials with acceptable specifications.

A sufficient description of the manufacturing process has been presented.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.
The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The active substance is packaged in double bags placed inside HDPE drums. The primary packaging complies with Commission Regulation (EU) 10/2011, as amended.

2.4.2.3. Specification

The active substance specifications applied by the finished product manufacturer include tests for description (visual), identification (IR, HPLC), chloride identification (Ph. Eur.), appearance of solution (Ph. Eur.), water content (Ph. Eur.), sulphated ash (Ph. Eur.), particle size distribution (Ph. Eur.), assay (HPLC), impurities (HPLC), residual solvents (HSGC) and microbial quality (Ph. Eur.).

The finished product manufacturer's specification for the active substance is set in line with the specification of the active substance manufacturer. It contains relevant parameters for the control of the active substance quality, which were adequately justified.

During the procedure it was requested to the applicant and ASMF holder to tighten the limits for the identified impurities, total impurities and assay. In response, the limits were tightened as requested.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data from 3 batches at commercial scale of the active substance analysed by the finished product manufacturer are provided. All results are within the specifications and consistent from batch to batch.

2.4.2.4. Stability

Stability data from 3 commercial scale batches of active substance from the proposed manufacturer stored in a container closure system representative of the intended commercial package for up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. In addition, stability data from 9 pilot scale batches stored at the same conditions have been provided. Also, stability data from 3 commercial scale batches and 1 pilot scale batch at 5 ± 3 ºC storage condition for up to 36 months have been provided.

The following parameters were tested: description, identification, appearance of solution, water content, purity, assay, crystalline form and microbiological contamination.

The analytical methods used were considered stability indicating.

All tested parameters complied with the specifications.

Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions under thermal (60° and 105°C), photolytic (1.31 million lux hours + UV light 201.22 watts hrs square meter), oxidative (5% hydrogen peroxide), acid (1N hydrochloric acid), base (1N sodium hydroxide) stress have been provided. Significant degradation was observed under 1N sodium hydroxide. Results demonstrated that the current HPLC test method for assay and purity is stability indicating.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 36 months when stored at room temperature in the proposed container.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

The finished product Duvyzat consists of a white to off-white or faintly pink 10 mg/mL givinostat hydrochloride monohydrate oral suspension (equal to 8.86 mg/mL givinostat) packaged in a 150 mL amber PET bottle closed with a red-white HDPE child-resistant closure with LDPE syringe adapter. Each

bottle contains 140 mL of oral suspension intended for multidose use. Each pack also contains one 5 mL oral syringe, graduated from 1 to 5 mL by increments of 0.5 mL.

All excipients, with exception of the flavoring agents, are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC. No materials of animal or human origin are used.

It was demonstrated that the used excipients are compatible with the active substance.

The pharmaceutical development of the product has been described. The choice of excipients is sufficiently justified and their functions explained. The excipients are considered justified according to the "Guideline on pharmaceutical development of medicines for paediatric use" EMA/CHMP/QWP/805880/2012 Rev. 2.

The pharmaceutical development studies were aimed at the selection of a suitable pharmaceutical dosage form for the paediatric population, at the selection of adequate excipients and of an effective preservative concentration. A suspension dosage form has been chosen to allow for an appropriate volume of administration, wide dosage flexibility and an acceptable chemical stability. Dosage was expected to be based upon patient body weight for the whole paediatric population. Hence a wide dose range was considered.

Excipients selection involved the selection of pH adjusting agents, of suspending agents and of a preservative agent. Experimental activities were performed to investigate the optimal preservative concentration. Additional studies have been performed during product development. Detailed information has been provided, confirming the robustness of the formulation.

The palatability of the oral suspension was evaluated to justify the choice of flavours. Good acceptability of the formulation was shown in terms of taste and easiness of administration. Adequate information related to the development of the dissolution method has been provided. The dissolution method was shown to have discriminatory power.

The commercial formulation and the formulation used in phase 3 are identical.

During the procedure, the CHMP requested information regarding the investigation of the administration of the product via enteral feeding tubes. The applicant committed to investigate it post-approval (REC1).

The dose accuracy for a 5 mL syringe with fine scaling has been demonstrated for the proposed dosing range. However, as multiple dosing is required for older patients at the highest dose (more than 5 mL), the 5 mL syringe is not considered optimal for these patients. The applicant committed to introduce a 10 mL dosing device post approval (REC2).

The manufacturing process of the finished product has been developed through three stages: laboratory scale design, pilot scale design, industrial scale design. Critical process step and relevant process parameters have been identified and thoroughly evaluated allowing the establishment of a suitable manufacturing process. The development of the manufacturing process has been described in sufficient level of detail.

The primary packaging is amber polyethylene terephthalate bottle containing 140 mL oral suspension closed with a high-density polyethylene child-resistant closure with low-density polyethylene syringe adapter. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured by one manufacturing site: Italfarmaco S.A., Calle San Rafael, 3, Polígono Industrial Alcobendas, 28108 Alcobendas, Madrid, Spain.

The process is considered to be a standard manufacturing process.

The manufacturing process has been validated for the commercial scale batch size. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

2.4.3.3. Product specification

The finished product release and shelf-life specifications include appropriate tests for this kind of dosage form: appearance and re-dispersibility (visual), pH (Ph. Eur.), density/specific gravity (Ph. Eur.), identifications of givinostat hydrochloride monohydrate (HPLC), identification of sodium benzoate (HPLC), assay of givinostat hydrochloride monohydrate (HPLC), assay of sodium benzoate (HPLC), impurities (HPLC), dissolution (in-house), particle size (Ph. Eur.), deliverable volume (USP), container closure integrity (in-house), viscosity (Ph. Eur.), uniformity and accuracy of delivered doses from multidose containers (Ph. Eur.), microbiological quality (Ph. Eur./USP) and packaging control (visual).

A comprehensive set of relevant parameters has been established for the control of the finished product. The acceptance limits are in compliance with ICH guidelines and are supported by relevant batch data.

The initially proposed limits for the dissolution test were not considered acceptable since they were not representative of the dissolution profile of the clinical batches. Therefore, a Major Objection (MO) was raised in order to tighten these limits. The revised limits were considered justified and acceptable.

Particle size has been identified as critical parameter. A one-sided acceptance limit has been defined, which was initially not considered acceptable. During the procedure, the inclusion of additional limits was requested. In response, the applicant has thoroughly discussed the performed studies investigating the effect of particle size distribution on dissolution and justified the proposal. The justification was deemed acceptable.

Upon CHMP request, the acceptance criteria for sodium benzoate content have been tightened. Also, the acceptance limits for single specified impurities and total impurities have been tightened reflecting batch release and stability testing results. The limit for unknown impurities remains at the qualification threshold. This is considered acceptable.

The limit for pH at shelf-life specification has been tightened. In addition, the applicant proposed more restrictive limit for IPC pH control (bulk suspension) and for pH at release specification. Proposed limits are in line with batch analyses and stability studies data and are considered acceptable.

The limit for viscosity at release specification has been tightened, as requested. No change in shelf-life specification limit for viscosity test has been proposed by the applicant, however a justification based on results of stability studies and pharmaceutical development data has been provided.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. The risk evaluation allowed to conclude that level of elemental impurities in the finished product is below 30% of the established PDE. The outcome of the assessment was supported by batch analysis data on three batches using a validated ICP-MS method, demonstrating that each relevant elemental impurity was

not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Initially, the risk evaluation concluded that no confirmatory testing was deemed necessary. During the procedure a MO has been raised since the active substance and its degradation products contain tertiary amines. In response to the MO, the applicant provided results of confirmatory testing obtained with a sufficiently sensitive method, demonstrating the absence (below LOQ) of nitrosamines. Based on the information provided, it is acceptable to conclude that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures in the active substance specification are deemed necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards has been presented.

Batch analysis results are provided for three commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

2.4.3.4. Stability of the product

Stability data from three commercial scale batches of finished product stored for up to 24 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. In addition, the applicant conducted studies under intermediate conditions (30°C/ 65% RH & 30°C/ 75%) and refrigerator conditions (5°C) at commercial scale up to 36 months at 30°C/65% RH, at 30°C/75% RH, at 25°C/60% RH and up to 60 months at 5°C.

The batches of Duvyzat are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for the same specifications parameters as for release. The analytical procedures used are stability indicating.

The stability results were within the specification for all conditions and batches. Sight fluctuations in pH and density/specific gravity were observed but remained within specification and were attributed to normal sample-to-sample variation. Dissolution test results showed a single point specification. This limit was tightened during the procedure, as discussed above.

No trend of assay decrease or increase has been observed, and small changes were observed for impurities (slight increase), whereas no changes were observed for the identified impurity and for the unspecified impurity.

With respect to the preservative agent, sodium benzoate, assay values were within the pre-established shelf-life specification for all batches at all condition and orientation tested. The performed AET studies assure a suitable preservative efficacy throughout the whole product shelf-life.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Based on the photostability results, it was concluded that the finished product is not sensitive to light, and no restrictions are needed with respect to light exposure.

Water loss stability studies were performed to demonstrate that the finished product can withstand low relative humidity environments as it is packaged in a semi permeable container. All timepoint results showed the good stability of the finished product.

In-use stability was performed to establish the period of time during which the product can be used whilst retaining quality within the accepted specifications after first opening. It has been demonstrated that the product can be used up to 60 days after first opening.

Stability studies will continue according to the proposed stability protocol. In accordance with EU GMP guidelines, any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

Based on available stability data, the proposed shelf-life of 3 years and 60 days after first opening with no special storage condition as stated in the SmPC (section 6.3) are acceptable.

2.4.3.5. Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During the procedure, two MOs have been raised by the CHMP with respect to finished product in relation to the nitrosamine risk evaluation and to the dissolution test limits. The responses from the applicant to the MOs were considered satisfactory and all the issues were considered resolved.

At the time of the CHMP opinion, there were two unresolved quality issues having no impact on the Benefit/Risk ratio of the product, which pertain to the lack of investigation on the administration of the product via enteral feeding tubes and to the lack of a 10 mL dosing device to ensure dose accuracy when the dose is higher than 5 mL. These points are put forward and agreed as recommendations for future quality development.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation as post authorisation measures:

1) The applicant should investigate the administration of the product via enteral feeding tubes according to PIP timeline (March 2026).

2) The applicant should introduce a 10 mL dosing device post approval within 12 months from the date of approval of the MAA.

2.5. Non-clinical aspects

2.5.1. Introduction

Givinostat is an orally administered HDAC inhibitor with anti-inflammatory and anti-fibrotic activities, which is claimed to potentially improve regeneration of dystrophic muscles.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Givinostat potently inhibited the ubiquitously expressed human class I as well as class IIa and class IIb HDAC enzymes *in vitro*. The IC_{50} values ranged from 8.6 to 120 nM for class I and class IIb members to lower inhibition between 330-560 nM for class IIa enzymes. Givinostat did not significantly interact with the sole class IV member HDAC11 that shows different expression pattern and target specificity. In contrast, the givinostat metabolites ITF2374, ITF2375, ITF2955, ITF2440 and ITF2563 did not show any significant pharmacological activity.

Givinostat more effectively interfered with the release of various pro-inflammatory cytokines than dexamethasone upon bacterial lipopolysaccharide (LPS) stimulation of cellular preparations *in vitro*, which was confirmed by the reduction of TNFα-related inflammations in LPS-stimulated mice and rats *in vivo*. The efficacy of givinostat depended on the time interval before inflammatory induction and the oral ED_{50} was approximately 1 mg/kg in mice and 0.3 mg/kg in rats when given 60 min before LPS stimulation. Similarly, oral givinostat doses ≥1 mg/kg in rats and ≥5 mg/kg in mice dose- and time-dependently reduced colonic and systemic inflammations in animal models of human colitis and arthritis. Of note, givinostat did not significantly suppress T-cell activation and antibody formation in a mouse model of delayed-type hypersensitivity.

In fibroblasts derived from the muscle biopsy of a DMD patient, 250 nM givinostat decreased pro-fibrotic and increased anti-fibrotic gene expression. In addition, 150 nM givinostat or 50 nM of the established HDAC inhibitor trichostatin A (TSA) promoted the formation of multinucleated myotubes in primary human skeletal myoblasts *in vitro*. The higher 250 nM givinostat concentration also prevented degeneration and increased myosin heavy chain gene expression in human skeletal myotubes. Concomitantly, the transcription of HDAC4 and HDAC6 (class IIa and IIb) and the activation of NF-kB inflammatory responses were reduced.

Givinostat was tested in two established mouse models of human DMD: 1) the most widely tested *mdx* mice and 2) the more severely affected D2.B10 mice with the same dystrophin nonsense mutation in another genetic background that additionally leads to increased TGFβ-mediated pro-fibrotic processes.

In *mdx* mice, oral administration of 0.1 to 37.5 mg/kg/day givinostat dose-dependently improved muscle mass and histology (larger cross-sectional area, lower fat infiltration and fibrosis) independent of the treatment duration (45 to 90 days), which was accompanied by better motor performance in functional tests (exhaustion treadmill exercise, maximal forelimb grip strength) at ≥1 mg/kg/day

doses for up to 7 weeks. Thereafter, motor performance successively deteriorated, although givinostat administrations remained superior to vehicle-treated *mdx* controls. In general, givinostat doses between 5 to 10 mg/kg/day were required to ameliorate the muscular phenotype of *mdx* mice and the 50 % improvement of histological and functional muscle deficiencies correlated with a blood AUC of approximately 600 nmol·h/l givinostat (300 ng·h/ml).

The combination of 25 mg/kg/day p.o. givinostat with 1 mg/kg i.p. prednisone for 15 weeks comparably ameliorated the functional outcome of *mdx* mice as observed with the same givinostat dose alone, but was clearly superior to individual prednisone treatment. After 15 weeks of treatment, only the 37.5 mg/kg/day givinostat high dose group showed a 2-fold higher cross-sectional area of the *gastrocnemius* muscle compared to controls, whereas no major improvements were detected in other muscles or at lower givinostat doses including the combination with prednisone. Moreover, no clear histological findings of muscle regeneration were evident. Still, givinostat alone or in combination with prednisone effectively reduced fibrosis at ≥1 mg/kg/day in the diaphragm (-40 %), ≥10 mg/kg/day in the *gastrocnemius* (-30 %) and at 37.5 mg/kg/day in the *tibialis anterior* muscles (-30 %) of *mdx* mice, while cardiac fibrosis was not significantly alleviated.

In D2.B10 mice, ≥5 mg/kg givinostat administered via drinking water for 15 weeks dose-dependently augmented muscular function (maximal forelimb grip strength) to that of healthy wildtype controls from day 21 till day 62, whereas lower functional improvements were determined for the weekly i.p. injected 1 mg/kg prednisone or deflazacort comparators. The transiently improved motor performance was accompanied by temporary decreases of muscular fibrosis in givinostat-, prednisone- and deflazacort-treated mice after 8 weeks, which was lost after 15 weeks of treatment. Only the 37.5 mg/kg/day givinostat high dose non-significantly stabilized motor performance in the exhaustion treadmill apparatus from week 9 to 15, whereas lower doses of givinostat, prednisone or deflazacort were ineffective.

2.5.2.2. Secondary pharmacodynamic studies

Secondary pharmacodynamic investigations only suggested significant inhibition of adrenergic α2A- and α2B-receptors as well as muscarinic M1-, M2- and M4-receptors receptors at elevated 10 µM givinostat concentrations.

2.5.2.3. Safety pharmacology programme

The safety pharmacological effects of givinostat were investigated in core battery studies in accordance with ICH S7A and ICH S7B guidelines (CPMP/ICH/539/00 and CPMP/ICH/423/02). Single oral doses up to 100 mg/kg givinostat did not induce any behavioural or physiological changes in an Irwin test in mice and did not affect gastrointestinal motility.

Givinostat inhibited hERG currents with an IC_{50} of 1.4 µM *in vitro*, whereas lower or no hERG inhibition was determined for its metabolites ITF2374, ITF2375, ITF2563 and ITF2440. Givinostat also slightly prolonged action potential duration (APD_{50} and APD_{90}) in rabbit Purkinje fibre preparations at higher concentrations of 3 and 10 µM during simulated bradycardia conditions (0.2 Hz: APD_{50} prolongations of 26 and 51 %, APD_{90} prolongations of 22 and 46 %, respectively) suggesting the potential to interfere with potassium channels during repolarisation. No relevant changes on ECG parameters, heart rate and respiratory parameters were evident in the safety assessment of anaesthetised Beagle dogs and in repeat-dose toxicity studies in dogs and monkeys.

2.5.2.4. Pharmacodynamic drug interactions

In pharmacodynamic *in vitro* drug interaction studies, givinostat did not significantly compete with the binding of the glucocorticoid receptor agonist triamcinolone or the anti-inflammatory activity of dexamethasone. The known immunosuppressant cyclosporine A did not interfere with the anti-inflammatory effect of givinostat. Thus, givinostat is not expected to interact with the anti-inflammatory activity of concomitant glucocorticoid or cyclosporine A medications.

2.5.3. Pharmacokinetics

Single oral dose pharmacokinetic parameters of givinostat were determined in rats, monkeys and dogs. Peak plasma levels were reached after around 2 h. Subsequently, givinostat showed rapid and biphasic elimination with half-lives of approximately 1 h in rats, 1.5 h in monkeys and 1.8 h in dogs. The bioavailability of the givinostat oral solution was 21.4 % in rats, but lower in dogs (12 %) and monkeys (13.2 %).

Givinostat revealed extensive plasma protein binding of 93.3 % in mice, 93.7 % in rats, 96 % in dogs, 97.9 % in monkeys and 95.9 % in humans. Givinostat slightly partitioned into blood cells as indicated by a slightly increased blood to plasma ratio of 1.4 in rats, 1.1 in dogs and 1.3 in monkeys and human, respectively, which was confirmed in *mdx* mice.

In rats, givinostat primarily distributed into gastrointestinal tract tissue (intestine > duodenum > stomach) and in organs involved in elimination (liver > kidney > bladder), but not significantly into the brain. In *mdx* mice, single oral administration of 5 to 37.5 mg/kg givinostat resulted in dose-proportional AUC-related muscle exposure, which was 4.5-fold higher than in plasma and contravenes the low muscular amounts of givinostat determined in rats.

The placental transfer of givinostat and its potential excretion into milk has not been elucidated.

Givinostat was widely metabolised in primary rat and human hepatocytes, cytosolic, mitochondrial or microsomal fractions with and without co-factor supplementation *in vitro*, although the respective metabolic proportions were clearly different between mice, rats, dogs, monkeys and humans. The givinostat metabolism presumably involves various enzyme classes including flavin-containing monooxygenases, CYP enzymes, mitochondrial amidoxime and cytochrome reductases, UDP-glucuronosyltransferases and possibly carboxylesterases.

The *in vivo* metabolic profile of givinostat in rats and *mdx* mice was partially congruent with previous *in vitro* evaluations. The ITF2955 glucuronide was the major metabolite in rats (~63 % of the total AUC) but was only produced in minor amounts in other animals and humans. The naphthalene metabolite ITF2440 formed by oxidative cleavage of the carbamate bond was also prominent in rats and *mdx* mice (each ~31 % and ~45 % of total AUC), whereas from the benzene counterparts mainly ITF2563 was confirmed in both species (each 13.4 % and 9.7 % of total AUC). However, the amide ITF2374 formed by the reduction of the hydroxamic acid and the carboxylic acid ITF2375 were more prevalent metabolites in *mdx* mice than in rats (6.7 % vs. 0.5 % and 29.5 % vs. 3.5 % of total AUC). In addition, low levels of the dealkylated metabolite ITF2447 and the Ndeethylated metabolite ITF3594 were detected in *mdx* mice, while minor amounts of the tertiary Noxide ITF2431 were confirmed in both rats and *mdx* mice. In contrast, the metabolic profiling in dogs and monkeys solely focussed on the identification of ITF2374 and ITF2375, but did not include other metabolites.

The excretion of givinostat was followed with radiolabelled and unlabelled compound to enable the tracing of naphthalene- and benzene-containing metabolites. Radiolabelled givinostat was found to be primarily eliminated within 24 h as metabolites by the faecal (~68 %) compared to the renal route (~30 %) after oral administration in rats. Unchanged givinostat was only excreted in minor amounts

(1 % in urine and ~4 % in faeces). Unlabelled oral givinostat was eliminated in different proportions with urinary recovery of ~20.5 % in rats, ~5 % in dogs, ~14.5 % in monkeys and ~38.6 % in humans. The naphthalene ITF2440 was the major urinary metabolite (4.3 % in dogs, ~14 % in monkeys, 17.6 % in rats and ~32 % in humans), whereas only minor amounts of unchanged givinostat were detected (0.13 – 1.27 %). No analysis for corresponding benzene-containing urinary metabolites was presented after administration of unlabelled givinostat (e.g. ITF2363, ITF2563 or ITF2537). However, the mass-balance study with radiolabelled givinostat in rats indicated that the benzene fragments ITF2563 and ITF2537 were the predominant urinary metabolites within 24 h post dose in rats (18 % and 3.1 %).

The potential of givinostat for drug interactions was investigated in accordance with current European guidance (CPMP/EWP/560/95/Rev.1 Corr.2) considering also the recommendations of the pertinent FDA and draft ICH M12 guidelines. Givinostat and its main human metabolites ITF2374, ITF2375, ITF2440 and ITF2563 did not indicate a clinically relevant potential for inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6. However, the potential inhibition of CYP3A4 and the induction of CYP1A2, CYP2B6 and CYP3A4 by givinostat could not be excluded. Givinostat served also as substrate for both P-gp and BCRP efflux transporters and may inhibit P-gp (IC_{50} of 10 µM). Hence, the possible interaction of givinostat with CYP3A4 and P-gp was clinically evaluated.

Givinostat was no substrate for OATP1B1 and OATP1B3 and did not inhibit these hepatic uptake transporters. Despite the inhibition of OCT1 with IC_{50} of 14.1 µM, a clinical risk has been reliably excluded based on the maximum unbound hepatic inlet concentration of givinostat. Givinostat did not interfere with renal uptake of OAT1, OAT3, but inhibited OCT2, MATE1 and MATE2-K with IC_{50} of each 0.091 µM, 5.1 µM and 9.3 µM. The givinostat metabolites ITF2375, ITF2440 and ITF2563 did not reveal clinically relevant inhibition of the same set of intestinal, hepatic and renal transporters, except the inhibition of OCT2, MATE1 and MATE2-K by ITF2375 (IC_{50} of 1.13 µM, 14.4 µM and 9.37 µm) and the inhibition of MATE2-K by ITF2440 (IC_{50} of 61.6 µM).

In two investigations in male mice, givinostat and prednisolone did not significantly impact on the exposure of each other.

2.5.4. Toxicology

The toxicity of givinostat was investigated after single and repeated i.v. and oral administration for up to 13 weeks in mice, 26 weeks in rats 4 weeks in dogs and 39 weeks in monkeys. The acute i.v. toxicity of the metabolites ITF2374 and ITF2375 was also tested in rats. Mice and rats were chosen as pharmacologically responsive rodent species, whereas monkeys were selected due to the limited gastrointestinal tolerability of givinostat in the 4 weeks repeat-dose toxicity study in dogs. These investigations are complemented by genotoxicity evaluations, reproductive and developmental toxicity studies in rats and rabbits including also juvenile animals. All pivotal studies adhered to GLP.

2.5.4.1. Single dose toxicity

The acute i.v. and p.o. toxicity in mice and rats was characterised by dyspnoea, salivation, tremors/ clonic convulsions, hypoactivity, piloerection and hunched posture between 30 to 60 min post dosing and death by respiratory arrest. Oral givinostat administration caused additionally diarrhoea, abdominal dilatation and perineum soiled with urine in rats. These clinical signs reversed in surviving animals within 1 to 24 h. The median LD_{50} of givinostat was 152 mg/kg i.v. and >1000 mg/kg p.o. in mice compared to 132 mg/kg i.v. and 2400 to 5000 mg/kg p.o. in rats. The ITF2374 and ITF2375 metabolites induced similar clinical signs at lower i.v. doses in rats with LD_{50} values of 32 mg/kg and ≥100 mg/kg in rats, respectively.

2.5.4.2. Repeat dose toxicity

Multiple dose toxicity investigations showed a largely consistent profile across species, which was dose- and time-dependently induced with species-specific severity. The target organ toxicities of givinostat predominantly comprised white and red blood cell lineages with related findings in spleen, lymphatic and bone marrow tissues as well as the liver.

The clinical signs following repeated administrations in mice and rats generally coincided with observations after single exaggerated doses (see above). In rats, the increased salivation after givinostat doses ≥40 mg/kg/day correlated with atrophy of salivary gland acini and granular ducts.

Among haematological parameters, givinostat markedly reduced while blood cell counts over time at oral doses ≥100 mg/kg/day for 13 weeks in mice (up to -87 %), ≥125 mg/kg/day for 4 weeks in rats (up to -68 %) and dose-dependently at ≥10 mg/kg/day for 13 or 26 weeks in rats (up to -84 %). This decline in white blood cells was primarily due to lower lymphocytes and neutrophils resulting in lower cellularity in thymus and mesenteric lymph nodes, but also involved decreases of basophils, eosinophils, monocytes and large unstained cells upon long-term administration. Overall, the lymphoid depletion manifested during prolonged administrations of ≥30 mg/kg/day givinostat for 13 and 26 weeks in rats or 13 and 39 weeks in monkeys by slight to moderate atrophy of thymus, bone marrow (lower cellularity with fat infiltration) and the white pulp of the spleen. Extramedullary haematopoiesis was observed in the spleen at ≥100 mg/kg/day in mice and ≥30 mg/kg/day in rats. Moderately reduced thymus weight and thymus involution was additionally identified in dogs administered 50 mg/kg/day p.o. givinostat for 4 weeks. In rats, the splenic B-cells were more severely reduced than T-cells of which CD4+ T-cells were more decreased than CD8+ numbers.

Givinostat increased the turnover of red blood cells across species as evident by anisocytosis, microcytosis, macrocytosis, polychromasia and mild to moderately increased bilirubin levels at ≥50 mg/kg/day givinostat in mice, ≥40 mg/kg/day in rats, ≥25 mg/kg/day in dogs and 30 mg/kg/day in monkeys, which was associated with hemocatheresis, hemosiderosis (mesenteric lymph nodes, spleen and hepatic Kupffer cells) and sinus histiocytosis at givinostat doses ≥50 mg/kg/day in mice and at ≥10 mg/kg/day in rats, respectively. As a consequence, capsular inflammation and subcapsular lymphoid accumulation developed in the spleen at ≥30 mg/kg/day givinostat in rats. Lower red blood cell counts of abnormal morphology (acanthocytes, schistocytes and echinocytes), decreased haematocrit, haemoglobin, mean corpuscular haemoglobin and/or volume and increased Heinz bodies and reticulocyte counts were determined at 200 mg/kg/day givinostat in mice, at 250 mg/kg/day in rats and at 30 mg/kg/day in monkeys. The reductions in red blood cell parameters largely recovered upon treatment cessation.

In rodents, givinostat also reversibly lowered platelet numbers at ≥50 mg/kg/day in mice and ≥40 mg/kg/day givinostat in rats with concomitant increases of megakaryocytes in spleen and bone marrow of mice. Consequently, prothrombin time was prolonged at ≥40 mg/kg/day givinostat in rats.

In the liver, chronic givinostat doses of 90 mg/kg/day for 26 weeks induced minimal centrilobular hypertrophy of hepatocytes in rats, whereas high 250 mg/kg/day doses for 4 weeks led to minor fatty vacuolation and monocyte infiltration. Short-term dosing of 50 mg/kg/day in dogs promoted minimal to marked focal hepatic necrosis, moderate hepatic pericholangitis, oedema and bile duct hyperplasia. Slight hepatic bile duct hyperplasia was also seen above givinostat doses ≥30 mg/kg/day in monkeys with concomitant pancreatic acinar cell degranulation at ≥60 mg/kg/day for 4 weeks. These liver impairments were reflected in increased triglycerides, moderately diminished cholesterol and increased aminotransferases or alkaline phosphatase. The liver abnormalities reversed in rats, but the hepatic bile duct hyperplasia was partially reversible in the 4 weeks recovery period in monkeys.

Blood urea was elevated in male mice and rats administered each 200 or 250 mg/kg/day givinostat or

by lower doses of 50 mg/kg/day givinostat in dogs and ≥10 mg/kg/day in monkeys. Moreover, creatinine was mildly increased at these dosages in dogs and monkeys. These biochemical changes correlated in monkeys with partially reversible slight kidney tubular basophilia, tubular dilatation, oedema and dystrophic mineralization following oral administration of ≥60 mg/kg/day givinostat for 4 weeks or 100 mg/kg/day for 14 days in a MTD study.

Other toxicities were only noticed in rats and comprised lung alveolitis, dose-dependent minimal to moderate adrenal cortex atrophy observed after givinostat doses ≥30 mg/kg/day over 13 or 26 weeks, diminished prostate and seminal vesicle at ≥40 mg/kg/day givinostat in male rats as well as increased haemorrhagic luteal cysts and higher numbers of *corpora lutea* in female ovaries at ≥90 mg/kg/day givinostat.

2.5.4.3. Genotoxicity

The genotoxic potential of givinostat was thoroughly investigated *in vitro* and *in vivo*. Tests comprised gene mutation *in vitro* in bacteria (Ames test) and mammalian cells (TK+/- in mouse lymphoma cells). In addition, a bone marrow micronucleus *in vivo* study in male rats, male transgenic F344 BigBlue rats on the cII and the Pig-a locus as well as chromosome aberration *in vitro* in peripheral human blood lymphocytes were studied. Moreover, the ability of givinostat to induce DNA repair *in vivo* in hepatocytes was investigated in male rats. The corresponding results do not provide evidence for any relevant genotoxic potential of givinostat.

2.5.4.4. Carcinogenicity

The carcinogenic potential of givinostat is currently under evaluation. In long-term toxicity studies, single cases of malignant lymphoma in rats and nephroblastoma in monkeys were interpreted as incidental without any relationship to givinostat.

2.5.4.5. Reproductive and developmental toxicity

The reproductive and developmental toxicity of givinostat including also juvenile animals was investigated according to ICH S5 guidance and fulfilled GLP regulations.

In the fertility and early embryonic developmental study, there were dose-dependent decreases of absolute organ weights and size of male accessory sex organs (epididymis, prostate, seminal vesicles). Animals dosed with ≥80 mg/kg/day of givinostat showed an increase of the pre-coital interval (>4 days of pairing), although no abnormal sperm quality, quantity and motility and no difference in the number of receptive or pregnant animals was recorded. Treatment-related changes in uterine parameters were statistically significant higher numbers of *corpora lutea*, implantations and pre-/ post-implantation losses at doses ≥80 mg/kg/day. Additionally, there was a significant number of early and late resorptions at doses ≥40 mg/kg/day compared to controls. Based on these findings, the NOAEL for fertility was considered to be 40 mg/kg/day for both sexes.

In the embryo-foetal development study in pregnant female Sprague Dawley rats, maternal toxicity at the 160 mg/kg/day givinostat high dose was characterized by reduced body weight gain and food consumption, but the number of viable foetuses was not affected. Nevertheless, a statistically significant decrease of group mean foetal weight, total weight of litters and placenta was observed in the high dose group, probably resulting from maternal toxicity rather than direct embryo-foetal toxicity. Foetal examinations revealed an increase of thoracic vertebral abnormalities at this high dose level and dose-dependently incomplete ossifications of sternebrae and thoracic vertebrae, albeit within the historical control data range. Except the undescended thymus, the observed visceral abnormalities

were also within the range of background controls. Therefore, the dose of 80 mg/kg/day is considered the NOAEL for maternal and 40 mg/kg/day for embryo-foetal development.

In the embryo-foetal development study in rabbits, the 160 mg/kg/day givinostat high dose exceeded the maximum tolerated level as evident by a high number of premature sacrifices due to marked weight loss, reduced food and water consumption and/or evidence of abortion. Since the number of litters was limited, foetal findings of this high dose group are not meaningful. Foetal anomalies (haemorrhages on the gall bladder and unossified epiphyses) were observed in a dose-dependent manner within the historical data range. Moreover, a dose-dependent incidence in offset alignment of the pelvic girdle was considered treatment-related. The NOAEL was 80 mg/kg/day for maternal toxicity and 40 mg/kg/day for embryo-foetal toxicity.

In the pre- and postnatal development study, a very high number of female rats had to be sacrificed due to total litter death/resorptions at the high dose of 160 mg/kg/day givinostat. With regard to the maternal toxicity in high dose dams, which was characterized by reductions in body weight gain, food consumption and effects on pregnancy parameters, the maternal NOAEL in the pre- and postnatal development study was set to 80 mg/kg/day. However, due to the clear dose-dependence in dams with regard to stillbirths, no foetal NOAEL could be established.

In the 4 weeks oral toxicity study in juvenile rats, a NOAEL of 60 mg/kg/day givinostat was determined, because of haematological effects at 180 mg/kg/day and transient non-adverse post-weaning developmental effects in all dose groups. In the 13 weeks juvenile toxicity study, treatment-related reductions in bone mineral density of the whole femur and lumbar vertebra were noted at the high dose level of 40/60/90 mg/kg/day, and at the mid dose of 20/30/45 mg/kg/day givinostat in the whole femur only. For the whole femur, a slight effect remained upon treatment cessation. Since most of the changes observed in the dosing period were reversible after an 8 week recovery period, the NOAEL was 40/60/90 mg/kg/day in this study.

2.5.4.6. Toxicokinetic data

Toxicokinetic plasma parameters of givinostat and its metabolites ITF2374 and ITF2375 were determined by validated HPLC-UV (4 and 13 weeks repeat-dose toxicity in rats and monkeys, respectively) as well as partially validated and apparently more sensitive LC-MS/MS (repeat-dose toxicity studies for 13 weeks in mice, 26 weeks in rats and 39 weeks in monkeys). The peak plasma concentrations of givinostat and ITF2375 were reached within 0.5-2 h in mice, rats and monkeys, whereas t_{max} of ITF2374 was variably delayed between 0.5-8 h in the three species.

The givinostat AUC increased nearly dose-proportionally in mice, rats and monkeys. The plasma levels of ITF2375 were highest in mice and rats, followed by ITF2374 and then givinostat lacking clear differences between sexes. Likewise, ITF2375 concentrations were at multiples higher than the similar levels of givinostat and ITF2374 in both sexes of monkeys.

Givinostat accumulated in rats (including pregnant females of embryo-foetal development study), but not in other animal species. Accordingly, quantifiable levels of ITF2375 and ITF2374 were found in pre-dose samples of the 26 weeks toxicity study at ≥30 mg/kg/day givinostat before administration of the next dose. In addition, tiny amounts of givinostat, ITF2374 and ITF2375 were determined in pre-dose samples of the subchronic and chronic repeat-dose toxicity studies in monkeys before the next administration.

2.5.5. Ecotoxicity/environmental risk assessment

The applicant provided an environmental risk assessment (ERA) in accordance with the *"Guideline on*

the environmental risk assessment of medicinal products for human use" (EMEA/CHMP/SWP/4447/00 corr.2). Givinostat is not a PBT substance as log Kow does not exceed 4.5. The PECsurfacewater has been refined with prevalence data from the Orphan Designation EU/3/12/1009. The PECsurfacewater value is below the action limit of 0.01 µg/L. A Phase II environmental fate and effects analysis is not required. Considering the above data, givinostat is not expected to pose a risk to the environment.

Summary of main study results

Substance (INN/Invented Name): Givinostat hydrochloride monohydrate			
CAS-number (if available): 732302-99-7			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	-0.1 at pH 5 1.5 at pH 7 2.2 at pH 9	Potential PBT N
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	2.2	not B
PBT-statement:	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$ refined with prevalence	0.0035	µg/L	>0.01 threshold N
Other concerns (e.g. chemical class)			N

2.5.6. Discussion on non-clinical aspects

Pharmacology

Givinostat is a potent inhibitor of the ubiquitous human class I, class IIa and class IIb HDAC enzymes, but not of the differently expressed sole class IV member HDAC11. The interaction of givinostat with class III HDAC enzymes (also termed sirtuins) was not tested, likely due to their structural divergence and co-factor dependency for their catalytic activity (Seto and Yoshida, 2014). Thus, givinostat appears to be specific for class I, class IIa and class IIb HDAC enzymes, whereas its metabolites show no significant HDAC inhibition.

Givinostat effectively inhibited the release of pro-inflammatory cytokines following LPS stimulation in various cell cultures *in vitro* and in mice and rats *in vivo*, whereas T-cell activation and antibody formation were not suppressed in a mouse hypersensitivity model. Comparable results had been obtained with other HDAC inhibitors (vorinostat, trichostatin A (TSA) or other hydroxamic acid analogues). Thus, the HDAC inhibition of givinostat mediates anti-inflammatory but no immunosuppressant effects.

In addition, givinostat decreased pro-fibrotic while increasing anti-fibrotic gene expression in fibroblasts derived from the muscle biopsy of a DMD patient suggesting inhibition of canonical and non-canonical TGFβ pathways, which have been implicated to trigger inflammation and fibrosis in dystrophic muscle (reviewed by Ceco E and McNally EM, 2013). The stimulation of anti-fibrotic gene expression by givinostat was apparently facilitated by increased acetylation of histone 3 in their promoter regions in line with earlier observations with the pan-HDAC inhibitor pracinostat (Jones DL *et al. J Cell Sci*. 2019; 132(20): jcs233486). Givinostat also stimulated myotube formation of human skeletal myoblasts, prevented their degeneration and increased myosin heavy chain gene expression.

Published scientific literature provided by the applicant indicates that targeting the balance between histone acetylation and deacetylation by HDAC inhibitors (HDACi) can enhance skeletal myogenesis and counteract the progression of muscular dystrophy. The mechanistic consequences of HDAC inhibition in muscular dystrophy have not been completely unravelled yet, but may involve the interference with muscular inflammations, fibrosis by altered TGFβ signalling and the induction of

mitochondrial biogenesis.

In order to support the proposed clinical therapy, givinostat was evaluated in the established *mdx* and the more severely affected D2.B10 mouse models of human DMD. Givinostat dose-dependently improved muscle mass and histology (larger cross-sectional area, lower fat infiltration and fibrosis) independent of the treatment duration (45 to 90 days) and the age of the study animals (1.5 to 5.5 months; Consalvi *et al*., 2013; Licandro *et al*., 2021). This is consistent with earlier findings of the non-selective class I and II HDAC inhibitor trichostatin A in *mdx* mice (Minetti *et al*., 2006). The improved muscular histology in givinostat-treated *mdx* mice was generally reflected in transiently better motor performance in functional tests at doses of 5-10 mg/kg/day for up to 7 weeks, but then successively deteriorated, although givinostat dose groups remained superior to vehicle-treated *mdx* controls. Comparable temporary improvements of functional outcome was noticed in *mdx* mice administered the combination of 25 mg/kg/day p.o. givinostat with 1 mg/kg i.p. prednisone for 15 weeks. Apart from effective reductions of fibrosis, no clear muscle regeneration could be histologically determined. Nonetheless, immunohistochemistry of muscles from *mdx* mice revealed a higher amount of M2 compared to M1 macrophages after 9 weeks of treatment with givinostat, prednisone or the givinostat/prednisone combination. M2 macrophages have been implicated in muscle regenerative and fibrotic stages of dystrophic muscular tissue of the *mdx* mice, while pro-inflammatory M1 macrophages are activated by infections (Villalta *et al*., 2009).

Similar to *mdx* mice, givinostat at doses ≥5 mg/kg transiently alleviated the motor function of D2.B10 mice for about 8 weeks compared to lower functional improvements by the 1 mg/kg i.p. prednisone or deflazacort comparators (Licandro *et al*., 2021). Concomitantly, the muscular fibrosis was transiently decreased by the three agents, but this benefit was lost upon study termination after 15 weeks. Neither givinostat, nor prednisone counteracted muscular adipocyte infiltration in D2.B10 mice and the proportion of M2 macrophages and necrosis was solely increased significantly in *triceps* muscle of those D2.B10 mice administered the 37.5 mg/kg givinostat high dose, but not in mice treated with prednisone. This increase of M2 macrophages with concomitant necrosis in *triceps* muscle of givinostat high dose mice compared to vehicle-treated D2.B10 controls was hypothetically related to the greater motor performance of these animals in the two functional tests, which might have concurrently increased their myofibre damage given the absence of relevant muscular regeneration in this mouse strain.

Hence, givinostat treatment only temporarily improved the muscle deficiencies of *mdx* mice, except the alleviation of muscular fibrosis. Nevertheless, the *mdx* phenotype is rather mild and characterised by robust skeletal muscle regeneration after transitory muscle necrosis between 3 to 6 weeks of age, because the dystrophin loss in the mice is compensated by the homologue utrophin (McGreevy *et al*., 2015). In D2.B10 mice with more severely compromised muscles, a stabilisation of motor deficiencies was only observed after oral administration of the 37 mg/kg/day givinostat high dose, whereas lower givinostat doses were ineffective and fibrosis was no longer attenuated following 15 weeks of treatment. It has been reported that the more severe muscle phenotype of D2.B10 mice differently affects muscle types and also seems to ameliorate with age (van Putten M *et al*., 2019). These inherent phenotypic improvements in *mdx* and D2.B10 mice might have countervailed a more meaningful givinostat outcome including the detection of the claimed promotion of myogenic regeneration *in vivo*. However, further available animal models including various double mutant mice and dystrophic dogs harbour other limitations and do not completely recapitulate the human DMD pathology (McGreevy *et al*., 2015). The efficacy of givinostat therefore needs to be demonstrated in human DMD patients.

The significant inhibition of adrenergic α2A- and α2B-receptors as well as muscarinic M1-, M2- and M4-receptors receptors by 10 µM givinostat is of unlikely clinically relevance, because this concentration is 30- to 100-fold higher than those required for effective HDAC inhibition (<100-300 nM).

Safety pharmacological investigations did not reveal any neurobehavioural or gastrointestinal impairments after single oral doses up to 100 mg/kg givinostat. Givinostat inhibited hERG currents with an IC_{50} of 1.4 µM and slightly prolonged action potential duration in rabbit Purkinje fibres at ≥3 µM during simulated bradycardia conditions *in vitro*, but did not significantly alter heart rate, ECG and respiratory parameters in dogs and monkeys. Considering the maximum recommended human starting dose of 70 mg bid givinostat for DMD patients ≥70 kg, a human plasma range of 150-180 ng/ml givinostat (about 315-380 nM) can be estimated from the peak plasma levels in paediatric DMD patients administered 50 mg bid givinostat and healthy adult subjects, who received 100 mg bid, which corresponds to a free C_{max} of 7.5 to 9 ng/ml (0.015 – 0.019 µM; 95 % plasma protein binding). Hence, the IC_{50} of givinostat for hERG channel inhibition translates into an approximately 74-fold safety margin and an impact of givinostat on cardiovascular function at therapeutic human doses seems unlikely. This is generally supported by the outcome of the thorough QT evaluation in healthy volunteers (see clinical assessment below).

Pharmacokinetics

The pharmacokinetic characteristics of givinostat were analysed *in vitro* and after oral or i.v. administration in mice, rats, dogs and monkeys as well as in *mdx* mice *in vivo*. These studies solely evaluated male animals, which is accepted with respect to the clinical predominance of DMD that almost exclusively affects boys. Nonetheless, female animals were evaluated for toxicokinetic exposure in repeat-dose toxicity studies.

The peak plasma levels in all animal species at around 2 h were followed by rapid plasma elimination with half-lives of approximately 1 h in rats, 1.5 h in monkeys and 1.8 h in dogs. Consequently, the oral bioavailability of givinostat was low in animals (~12-20 %). Apart from the high plasma protein binding, givinostat tended to partition into blood cells and principally distributed into the gastrointestinal tract and into organs involved in elimination in rats. However, single oral doses of givinostat were determined at 4.5-fold higher levels in the muscle compared to plasma of *mdx* mice, which contrasts the low muscle distribution in rats. The favourably high transfer of givinostat into the muscle of *mdx* mice might be related to the possibly compromised muscle structure of *mdx* mice or to species-specific differences in the metabolic rate of givinostat (see below).

Givinostat was extensively and differently metabolised in primary hepatocytes, cytosolic or mitochondrial components as well as microsomal fractions of mice, rats, dogs, monkeys and humans *in vitro*, which involved various enzyme classes including flavin-containing monooxygenases, CYP enzymes, mitochondrial amidoxime and cytochrome reductases, UDP-glucuronosyltransferases and possibly carboxylesterases.

The qualitative and quantitative metabolic differences were confirmed in rats and *mdx* mice *in vivo*. The major metabolite in rats was the ITF2955 glucuronide (~63 % of the total AUC), which is generated in minor amounts in other animals and humans. The carbamate bond cleavage product ITF2440 was also prominent in rats and *mdx* mice (each ~31 % and ~45 % of total AUC), whereas from the benzene counterparts mainly ITF2563 was identified in both species (each 13.4 % and 9.7 % of total AUC). However, the transformation of the hydroxamic acid into either the amide ITF2374, or the carboxylic acid ITF2375 was more prevalent in *mdx* mice than in rats (6.7 % vs. 0.5 % and 29.5 % vs. 3.5 % of total AUC). In addition, low levels of the dealkylated metabolite ITF2447 and the N-deethylated metabolite ITF3594 were detected in *mdx* mice, whereas minor amounts of the tertiary N-oxide ITF2431 were confirmed in both rats and *mdx* mice. The metabolic profile of givinostat was incompletely elucidated in dogs and monkeys and solely focussed on the identification of ITF2374 and ITF2375.

The human *in vivo* metabolism of givinostat has not been unravelled in detail. Nevertheless, analyses following single and repeated doses in healthy volunteers identified ITF2374, ITF2375, ITF2440 and

ITF2563 at ~33-35 %, ~34 %, 15-30 % and 24.6-28 % in human plasma as well as at 0.9-2.5 %, 0.2-1 %, 30-87.2 % and 18.9-57.8 % in human urine, respectively (clinical study nos. 01, 03, 54 and 55). Moreover, the minor levels of the ITF2955 glucuronide excluded a significant contribution of UGTs to the human metabolism of givinostat. Although a more thorough characterisation of the human metabolism would have been desirable, at least all major human metabolites were presumably determined and characterised in toxicity studies (see clinical section below). However, the totality of the available *in vitro* and *in vivo* metabolism data of givinostat points towards substantial differences in the metabolite quantities among animal species as well as between animals and humans.

These metabolic differences were further reflected in the excretion profile of givinostat, which involved predominantly faecal compared renal elimination in a mass-balance study in rats. The naphthalene ITF2440 was detected as major urinary metabolite (4.3 % in dogs, ~14 % in monkeys, 17.6 % in rats and ~32 % in humans) compared to minor amounts of unchanged givinostat (0.13 – 1.27 %). The possible urinary excretion of benzene-containing metabolites was not presented (e.g. ITF2363, ITF2563 or ITF2537). The absence of a mass-balance evaluation in humans precluded further metabolic comparisons.

Givinostat and its main human metabolites ITF2374, ITF2375, ITF2440 and ITF2563 did not inhibit the main CYP enzymes. However, givinostat potentially interferes with CYP3A4 and induces CYP1A2, CYP2B6 and CYP3A4. Therefore, a clinical drug interaction study with the CYP3A4 substrate midazolam was conducted (clinical study no. 55; see clinical section for details). Corresponding results have been included in section 4.5 of the proposed SmPC. In contrast, the induction of CYP1A2 and CYP2B6 required higher givinostat concentrations than for CYP3A4 and was therefore regarded clinically irrelevant.

Givinostat is also a substrate for the P-gp and BCRP efflux transporter and may inhibit P-gp. Consequently, the clinical drug interaction of givinostat was evaluated with the P-gp substrate dabigatran etexilate and the P-gp inhibitor clarithromycin in healthy human subjects (clinical study no. 55; see clinical section). Clarithromycin increased the peak plasma concentration of givinostat by about 40 %, but minimally increased givinostat AUC. In view of these clinical results, the higher IC_{50} of 150 µM for BCRP inhibition of givinostat as well as the similarity and co-expression of the P-gp and BCRP transporter, a comparable weak clinical effect of BCRP inhibition can be reasonably expected, which obviates the need for evaluation of BCRP inhibition by givinostat *in vivo*.

Among hepatic and renal uptake transporters, givinostat inhibited OCT2, MATE1 and MATE2-K with IC_{50} of each 0.091 µM, 5.1 µM and 9.3 µM, respectively. The metabolite ITF2375 inhibited these renal transporters with slightly higher IC_{50} values of 1.13 µM, 14.4 µM and 9.37 µm, whereas ITF2440 demonstrated lower inhibition of MATE2-K. In the light of the maximum unbound kidney concentration of ITF2375 and ITF2440 in humans (clinical study no. 55), these inhibitory activities do not translate into a clinically relevant hazard. The applicant did not discuss, why possible transporter interactions were not investigated for the ITF2374 metabolite. Nevertheless, the C_{max} of ITF2374 was about 10-fold lower than that of ITF2375, so a significant clinical risk seems unlikely.

Considering the maximum unbound kidney concentration of givinostat, the inhibition of MATE1 and MATE2K raises no concern, whereas a clinical risk cannot be excluded for OCT2. This is already mentioned in section 4.5 of the proposed SmPC.

Toxicology

The toxicity of orally administered givinostat was investigated in single and repeat-dose studies for up to 13 weeks in mice, 26 weeks in rats 4 weeks in dogs and 39 weeks in monkeys. Although only the givinostat batch used in the 13 weeks repeat-dose toxicity study in mice (17P0448) has been tested in European clinical trials, the lots used in all other repeat-dose toxicity studies in rats, dogs and

monkeys, which were derived from an earlier production process, were also comparable in terms of their impurity profiles.

Consistent clinical signs were observed after single and multiple oral administrations of givinostat including salivation, hypoactivity, piloerection and hunched posture, which were generally in line with observations in rats and dogs administered other HDAC inhibitors (see EPARs of romidepsin, EMEA/H/C/2122, and panobinostat, EMEA/H/C/3725). Similarly, the dose-dependent emesis and loose to liquid faeces induced by ≥12.5 mg/kg/day givinostat in dogs was earlier observed for the HDAC inhibitor vorinostat (see EPAR, EMEA/H/C/947). This gastrointestinal intolerability corresponds to the vomiting, salivation and resistance to dosing noted at ≥60 mg/kg/day in the 4 weeks toxicity study in monkeys as well as the very common emesis and diarrhoea in human DMD patients (see sections 4.4 and 4.8 of the proposed SmPC).

The most prominent toxicity target of givinostat were marked reductions of white blood cell counts, particularly lymphocytes and neutrophils, which developed dose- and time-dependently after oral doses ≥100 mg/kg/day for 13 weeks in mice, at ≥10 mg/kg/day for 13 or 26 weeks in rats and at 30 mg/kg/day for 13 or 39 weeks in monkeys. This lymphoid depletion led to atrophies of thymus, lymph nodes, bone marrow and spleen. This interference of givinostat with the white blood cell lineage likely reflects its anti-inflammatory activity and was partially reversible in rats and monkeys.

Givinostat additionally increased the turnover of red blood cells across species at ≥50 mg/kg/day givinostat in mice, ≥40 mg/kg/day in rats, ≥25 mg/kg/day in dogs and 30 mg/kg/day in monkeys, whereas platelets were only reversibly lowered at ≥50 mg/kg/day in mice and ≥40 mg/kg/day givinostat in rats. These haematological changes coincide with earlier toxicity reports of other HDAC inhibitors (see EPARs of vorinostat, romidepsin and panobinostat). Decreased blood cell counts were also observed in human DMD patients, but were mainly related to very common thrombocytopenia necessitating clinical dose adjustments (see clinical assessment and sections 4.4 and 4.8 of the proposed SmPC).

Histological changes in the liver included minimal centrilobular hypertrophy of hepatocytes after chronic givinostat doses of 90 mg/kg/day for 26 weeks in rats, whereas 4 weeks treatment induced fatty vacuolation and monocyte infiltration in rats, focal hepatic necrosis, hepatic pericholangitis and oedema in dogs. Moreover, bile duct hyperplasia was identified in these dogs and at ≥30 mg/kg/day in monkeys. The hepatic abnormalities tended to reverse and were accompanied by increased liver enzymes, most notably triglycerides. The applicant attributed these liver findings to the hepatic metabolism of givinostat. It should be noted that hypertriglyceridemia was very common in DMD patients as reflected in the proposed product information (see sections 4.4 and 4.8 of the SmPC).

Furthermore, blood urea was increased in all species, while creatinine was mildly increased in dogs and monkeys. Slight transient increases of creatinine were also noticed in DMD patients without further impairments of renal function and can be ascribed to the inhibition of the renal uptake of creatinine due to the inhibition of the OCT2 transporter by givinostat (Ciarimboli G *et al*., 2012; see clinical assessment).

Other toxicities comprised alveolitis in the lung of rats, which corresponded to the observed acute respiratory deficiencies, but are regarded clinically irrelevant given the lack of respiratory impairments in DMD patients. Similarly, the dose-dependent minimal to moderate adrenal cortex atrophy observed after givinostat doses ≥30 mg/kg/day over 13 or 26 weeks in rats may be a rat-specific finding in view of the low severity and the absence of adrenal effects in other species including human DMD patients. Furthermore, the diminished prostate and seminal vesicle at ≥40 mg/kg/day givinostat in male rats and the increased haemorrhagic luteal cysts and higher numbers of *corpora lutea* in female ovaries at ≥90 mg/kg/day givinostat are of unlikely clinical relevance, because the paediatric target population almost exclusively concerns boys and givinostat did not impact on the fertility of male rats (see below).

Toxicokinetic determinations confirmed the exposure of animals in the toxicology program. However, no relevant C_{max}- and AUC-based safety margins could be established for givinostat at the respective NOAELs in subchronic and chronic toxicity studies in mice, rats and monkeys compared to the envisaged therapeutic human doses of 25 to 70 mg bid in DMD patients. For this reason, non-clinical repeat-dose toxicity studies cannot significantly support the clinical safety of givinostat, which is adequately reflected in section 5.3 of the SmPC.

Comprehensive *in vitro* and *in vivo* investigations did not indicate a relevant genotoxic potential of givinostat. The carcinogenic potential of givinostat is currently under evaluation. It seems noteworthy that single cases of malignant lymphoma in rats and nephroblastoma in monkeys in long-term toxicity studies were interpreted as incidental without any relationship to givinostat. This may be followed considering their occurrence at the respective intermediate dose levels, the abovementioned lack of a relevant genotoxic potential of givinostat, cellular differences between both types of tumours and the previous evaluation of other HDAC inhibitors as anti-cancer treatments in the EU. The carcinogenicity data will become available in December 2025.

The reproductive and developmental toxicity program, which also tested juvenile animals was conducted in compliance with ICH S5 guidance and GLP. Toxicokinetic data are available for nearly all reproductive and juvenile toxicity studies or can be extrapolated from repeat-dose toxicity studies. However, embryo-fetal toxicity was observed at lower givinostat exposures in embryo-fetal and pre-/ postnatal development studies in rats than achieved at the MRHD. Thus, no exposure-based safety margins could be established, except for the EFD study in rabbits with an exposure margin of about 10 at the embryonic NOAEL towards human therapeutic exposures at the MRHD.

The toxicological findings observed in juvenile rats generally coincide with those observed in repeat-dose studies in adult rats and Cynomolgus monkeys (see above). Discrepancies in the NOAELs between adult and juvenile rats were attributed to the different sensitivity of the bone marrow as the main target organ. The intense activity of the red bone marrow in the first three weeks of postnatal ontogenesis seems to compensate for the pharmacological effects induced by treatment of givinostat in the juvenile animals.

Givinostat $PEC_{surfacewater}$ value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5. Therefore, givinostat is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

All concerns have been sufficiently resolved, the marketing authorisation can be recommended from a non-clinical point of view.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Table 1 Overview of Givinostat clinical studies for the treatment of DMD

Study code/ Phase/ Type of study	Study Design	Number of Subjects and Population to be studied	Study Drug Regimen, Schedule, and Route	Study Objectives/ Primary Endpoint	Safety Assessments	No. of Centers Countries
Controlled clinical studies in DMD						
DSC/14/2357/48 Pivotal Phase 3 study - **completed**	Randomised, double-blind, placebo-controlled, multi-centre study to evaluate the efficacy, safety, PK and PD of givinostat vs. placebo in ambulant patients with DMD. The study comprised 2 periods: 1. Screening period: starting *4 weeks* before randomisation 2. Treatment period: *18 months of treatment* Follow-up period: *1 month* after last dose of study drug	Ambulant boys with DMD aged ≥ 6 years at baseline, who were on stable systemic corticosteroid treatment for a minimum of 6 months and at screening: - able to complete two 4SC assessments with results within ±1 second of each other and a mean of the 2 4SC assessments of ≤ 8 sec. - able to complete the time to rise from floor test between ≥ 3 and < 10 sec. - manual muscle testing of quadriceps of grade ≥ 3 - Target Population: VL MFF (MRS) > 5% to ≤ 30% Screened: 359 Randomised: Overall: Givinostat: 118 (compl 111) Placebo: 61 (compl 59) Target Population* Givinostat: 81 (compl 77) Placebo: 39 (compl 37) Off-target Population Givinostat: 37 (compl 34) Placebo: 22 (compl 22)	Subjects were randomised (2:1 ratio) to receive (in addition to standard-of-care corticosteroid regimen): Givinostat oral suspension 10 mg/mL or placebo oral suspension b.i.d (fed) Dose administered based on individual subject's weight Duration: 18 months of treatment	Primary objective - To establish the effects of givinostat vs. placebo administered chronically over 18 months to slow disease progression in ambulant DMD subjects Secondary objectives - To assess the safety and tolerability of givinostat versus placebo administered chronically in DMD subjects - To evaluate the pharmacokinetic (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. Primary endpoint: mean change in time to 4SC (seconds) before and after 18 months of treatment of givinostat versus placebo	Evaluation of TEAEs, SAEs, physical examination, vital signs and body weight, BMI, 12-lead ECGs, ECHO, pulmonary function tests (FEV1, FVC, FEV1/FVC, and PEF), laboratory parameters (haematology, blood chemistry, coagulation and urinalysis), and cognitive function evaluated by the Raven coloured progressive matrices.	A total of 44 investigators received approval to participate, of which 43 sites screened subjects and 41 sites enrolled subjects into the study. Belgium Canada France Germany Italy The Netherlands Spain UK USA Israel Serbia
Uncontrolled clinical studies in DMD						
DSC/11/2357/43 Phase 2 study - **completed**	A multicenter, open-label, non-randomised, uncontrolled, dose-ranging, two-part	Ambulant boys with DMD between 7 and <11 years of age with immunohistochemical and molecular diagnosis of DMD who were able to complete 2 screening	**Part 1**: Givinostat Capsules (for 50 mg dose) Givinostat	**Part 1 and Part 2**: Primary objective: - To establish the histologic effects of givinostat	Evaluation of TEAEs, SAEs, physical exam., vital signs and body weight, 12-lead	4 sites in Italy

Study code/ Phase/ Type of study	Study Design	Number of Subjects and Population to be studied	Study Drug Regimen, Schedule, and Route	Study Objectives/ Primary Endpoint	Safety Assessments	No. of Centers Countries
Controlled clinical studies in DMD						
	study to assess the safety, tolerability, PK and effects on muscle histology and clinical parameters of givinostat in ambulant children with DMD. Based on efficacy and tolerability results, extension phases of up to additional 40 months were added to allow patients to continue treatment with givinostat.	6MWTs with a minimal distance of 250 m each (results must have been within 30 m of the other), who had at least 6 months of available 6MWT data, and who had been on a stable dose of systemic corticosteroids for at least 6 months. Planned: 20; Actual: 20; Completed: 19; Analysed: 18 *Extension 1: 19 patients* *Extension 2: 18 patients* *Extension 3: 18 patients*	Oral suspension (other doses) 25 mg b.i.d (2 weeks) 37.5 mg b.i.d (2 weeks) 50 mg b.i.d (2 weeks) **Part 2**: Givinostat Oral Suspension 25 mg b.i.d or 37.5 mg b.i.d for 12 months **Extension phase**: Givinostat Oral Suspension Weight based dosing between 25 mg - 60 mg b.i.d up to further 40 months	administered chronically at the selected daily dose Secondary objectives (excerpt): - To establish the effects of givinostat administered chronically at the selected daily dose on functional parameters, such as the 6MWT, North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL) - To establish the safety and tolerability of givinostat administered chronically at the selected daily dose in children with DMD. **Extension Phase** Secondary Objectives: To establish the effects of givinostat administered chronically on 6MWT, NSAA and PUL (Extensions 1, 2, and 3)	ECGs, ECHO, pulmonary function tests (FEV1, FVC, FEV1/FVC, and PEF), laboratory parameters (haematology, blood chemistry, urinalysis)	
DSC/14/2357/51 Long-term extension study - **ongoing**	Multi centre, open label, long-term safety, tolerability, and efficacy study of givinostat in DMD patients previously treated with givinostat in	Ambulant male subjects aged ≥ 6 years at baseline affected by DMD. 194 patients enrolled at the cut-off date 31 Dec 2021: Givinostat = 110 Delayed givinostat = 54 Naïve givinostat = 30	Givinostat oral suspension 10 mg/mL or placebo oral suspension b.i.d (fed) Dose was adjusted based on patient weight[c] Givinostat	Primary Objective: To assess long-term safety and tolerability of givinostat in DMD subjects following core protocols program and with naïve givinostat DMD subjects, i.e.,	Evaluation of TEAEs, SAEs, physical examination, vital signs and body weight, BMI, 12-lead ECGs, ECHO, laboratory parameters	39 study centers with subjects located in 11 countries: USA (10), Italy (7), Spain (4), Canada (3), Germany (3), Belgium

Study code/ Phase/ Type of study	Study Design	Number of Subjects and Population to be studied	Study Drug Regimen, Schedule, and Route	Study Objectives/ Primary Endpoint	Safety Assessments	No. of Centers Countries
Controlled clinical studies in DMD						
	Study 43 or Study 48 and givinostat-naïve DMD patients eligible for the Off-target Population in Study 48.		oral suspension	subjects screened in study 48 who met: - all the inclusion criteria and none of the exclusion criteria, and - have never been randomised because the enrollment in the off-target group was completed. Secondary Objectives: To evaluate the effects of long-term administration of givinostat on muscular function and strength, on respiratory function and the impact on daily activities and QoL. Exploratory Objective: To compare the functional effects of long-term administration of givinostat between the "delayed givinostat" (patients who received placebo in study 48) and the "givinostat" (patients who received givinostat in their previous study) treatment group.	(haematology, blood chemistry, coagulation, and urinalysis).	(2), France (2), The Netherlands (2), UK (4), Israel (1) and Serbia (1)

Abbreviations: compl = completed; 4SC = Time to Climb 4 standard-sized stairs test; 6MWT = 6-minute walking test; NSAA = North Star Ambulatory Assessment; PK = pharmacokinetic; PUL = performance of upper limb; QoL = quality of life; AE = adverse event; b.i.d = twice a day; BMI = body mass index; CRP = C-reactive protein; ECG = electrocardiogram; ECHO = echocardiogram; FEV1 = forced expiratory volume at 1 second; FVC = forced vital capacity; FU = follow-up; i.v. = intravenous; MRS = magnetic resonance spectrometry; MTD = maximum tolerated dose; o.d = once a day; PD = pharmacodynamic; PEF = peak expiratory flow; PK = pharmacokinetic; SAE = serious adverse event; sec = seconds; TEAE = treatment-emergent adverse event.

* Target Population: patients with a baseline vastus lateralis muscle fat fraction [VL MFF] assessed by MRS in the range > 5% and ≤ 30%

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Overall, the pharmacokinetics of givinostat, as well as its major metabolites, have been well characterized in healthy volunteers and patients with DMD. Additional studies in patients with different diseases (plaque psoriasis, Crohn's disease, children affected by systemic onset juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, Janus kinase 2 (JAK2) V617F positive Polycythemia Vera [PV], and BMD) have also been carried out. Givinostat has been administered at single and repeated oral doses of up to 600 mg (single doses) and up to 200 mg (repeat doses) in different regimens.

Absorption

Givinostat is well absorbed after oral administration; maximum plasma concentrations are achieved approximately 2-3 hours after both single and repeat administration. Givinostat displays a bi-phasic elimination profile with a mean apparent terminal elimination phase (half-life) of approximately 6 hours. Bioavailability has not been investigated, and no human mass balance study was conducted. Differences in absorption and bioavailability between animal species and humans can best be explained with a significantly higher metabolic rate in animals.

A high-fat standard meal slightly affected the pharmacokinetics of givinostat, resulting in increased plasma concentrations (~ 30%), rate of absorption (~ 20%), and delayed time taken for a drug to reach the maximum concentration (T_{max}) (from 2 to 3 hours) when compared with fasting conditions (Study 04). The applicant considers the effect of food on absorption to be moderate and believes that no formal recommendations concerning dietary restrictions are necessary.

Distribution

Givinostat is characterized by high values of the apparent elimination clearance (117 L/h) and a large volume of distribution (apparent volume of distribution at steady-state, Vss/F, was approximately 768 L), indicating that it is widely distributed in tissues. Givinostat was shown to be extensively bound to human plasma protein (95.9 ±1.3%).

Metabolism

Givinostat is extensively metabolized, forming several metabolites. The main reactions involve the hydroxamic acid group and the carbamic bond, while those involving the tertiary amine have a minor impact on givinostat clearance. The major metabolites identified are ITF2440 (the naphtalenic fragment formed by the oxidative cleavage of the carbamic bond; plasma ratio to givinostat 15-fold; recovered in urine 30.2%), ITF2375 (formed by the hydrolysis of the hydroxamic acid into a carboxylic acid, plasma ratio to givinostat 5.0-fold; recovered in urine <1%), ITF2563 (the remaining part of the molecule following the oxidative cleavage of the carbamic bond - the counterpart of ITF2440; plasma ratio to givinostat 5.0-fold; recovered in urine 21.5%), and ITF2374 (formed by the reduction of the hydroxamic acid into an amide; plasma ratio to givinostat 0.7-fold; recovered in urine 1.6%).

While not all enzymes that are involved in the formation of the primary metabolites of givinostat have been identified, in-vitro studies allow to exclude the most common metabolizing enzymes. The involvement of polymorphically expressed enzymes can be excluded.

No studies were conducted to investigate the potential impact of impaired renal or hepatic function on givinostat's pharmacokinetics.

Dose proportionality

The pharmacokinetics of givinostat appear to be linear (dose-proportional and time independent). There was minimal drug accumulation (accumulation ratio 1.0 - 1.2) seen in the multiple ascending dose study (Study 03) following repeated once-daily dosing and in the Phase 2 DMD study (Study 43), although the area under the curve (AUC) after 12 months was smaller than that after one week. There was some accumulation (accumulation ratio 1.7) seen for twice daily (b.i.d) dosing in the drug-drug interaction study (Study 55). Steady-state concentrations were achieved within 5 to 7 days for both the parent compound and its major metabolites (ITF2374, ITF2375, ITF2440, and ITF2563).

Drug-drug interaction

Following in vitro results, a drug-drug interaction study (Study 55) indicated a weak inhibition of CYP3A4 activity by givinostat. When co-administered with IV midazolam, the effects were minimal; however, co-administration of givinostat with oral midazolam increased exposure of the latter by 63% (based on AUC from time 0 extrapolated to infinite time, $AUC_{0-\infty}$), suggesting that the effect of givinostat is mainly due to inhibition of the intestinal CYP3A4, confirming the in vitro data. No inductive effect of CYP3A4 was seen after 14 days of givinostat administration. Coadministration of givinostat with dabigatran etexilate, a probe for intestinal P-gp activity, caused a decrease in rate (26%) and extent (30%) of exposure of total dabigatran already at Day 6 of the study, after 3 days of givinostat administration. On Day 17 of the study a slight further decrease was reported for the rate (35%) but not for the extent of exposure, in comparison to dabigatran etexilate administration alone. In vitro data suggest that givinostat may be an inhibitor of the intestinal P-gp transporter, but the reduction of dabigatran exposure might suggest an increase in Pgp activity rather than an inhibition. However, this reduction occurring as early as three days after givinostat administration might not be compatible with the biological time course of the induction expected for the transporter protein. Based on these data, a definitive conclusion on givinostat inhibition and induction potential on intestinal P-gp could not be derived. The study also evaluated givinostat as a substrate for intestinal P-gp transporter; co-administration with the strong P-pg inhibitor clarithromycin caused an increase of about 40% in givinostat maximum plasma concentration (C_{max}) but had a minimal effect on givinostat AUC. Based on in vitro studies, givinostat has the potential to inhibit the renal transporter OCT2, a clinical investigation in DMD patients (Studies 48 and 51) concluded that givinostat is a weak inhibitor of OCT2. Caution is advised if givinostat is co-administered with CYP3A4, OCT2, and P-gp substrates with a narrow therapeutic index.

In both the clinical efficacy trials and drug-drug interaction evaluations, the concomitant administration of givinostat and ataluren was not considered.

Population PK modelling

The PK analysis dataset included data from 242 subjects (105 healthy adult subjects from phase 1 trials and 137 subjects with DMD from phase 2 and pivotal trials). Overall, the dataset was composed by 3569 observations, 3325 of which were above LLOQ (2509 observations were from healthy adult subjects and 816 from subjects with DMD). A two-compartment population PK model with first order input with lag and first order elimination from the central compartment was used to describe the PK of givinostat. The plasma concentration-time profile followed a bi-exponential decline with estimated t1/2 of first and terminal phases of approximately 1h and 12h. Functional half-life was approximately 6 h.

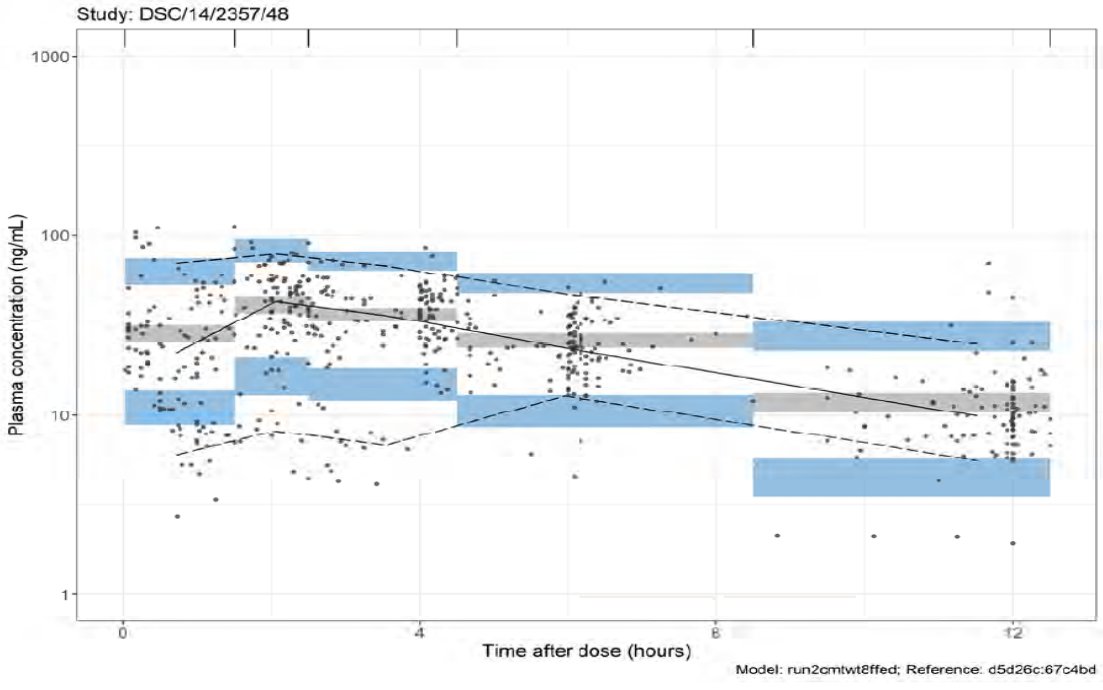
The effect of a meal was described categorically with a 27% increase in bioavailability.

Table 2 Parameters of the final Givinostat population PK model (2cmtwt8ffed)

Parameter	Description	Estimate (Shrinkage %)	RSE(%)	Estimate (Natural Scale)
CL/F (L/h)	Estimated on log scale	4.76	1.0	117
V_2/F (L)	Estimated on log scale	5.33	1.4	206
Q/F (L/h)	Estimated on log scale	3.80	1.4	44.7
V_3/F (L)	Estimated on log scale	6.33	0.7	561
F1 (unitless)	Natural scale	1 (FIX)	NA	1 (FIX)
k_a (1/h)	Estimated on log scale	-1.19	2.4	0.304
Lag time (h)	Estimated on log scale	-1.49	1.8	0.225
Allometric coefficient on CL	*(WT/medianWT)$^{\theta}$	0.326	16.4	0.326
Meal on F1	*(1+ θ)	0.276	29.2	0.276
IIV on CL/F	Variance	0.037 (30.4)	29.7	
IIV on V_1/F	Variance	0.466 (17.6)	21.4	
IIV on F1	Variance	0.0687 (19.8)	15.7	
RUV	Additive variance	0.0001 FIX		
RUV	Proportional variance	0.104 (5.5)	8.0	

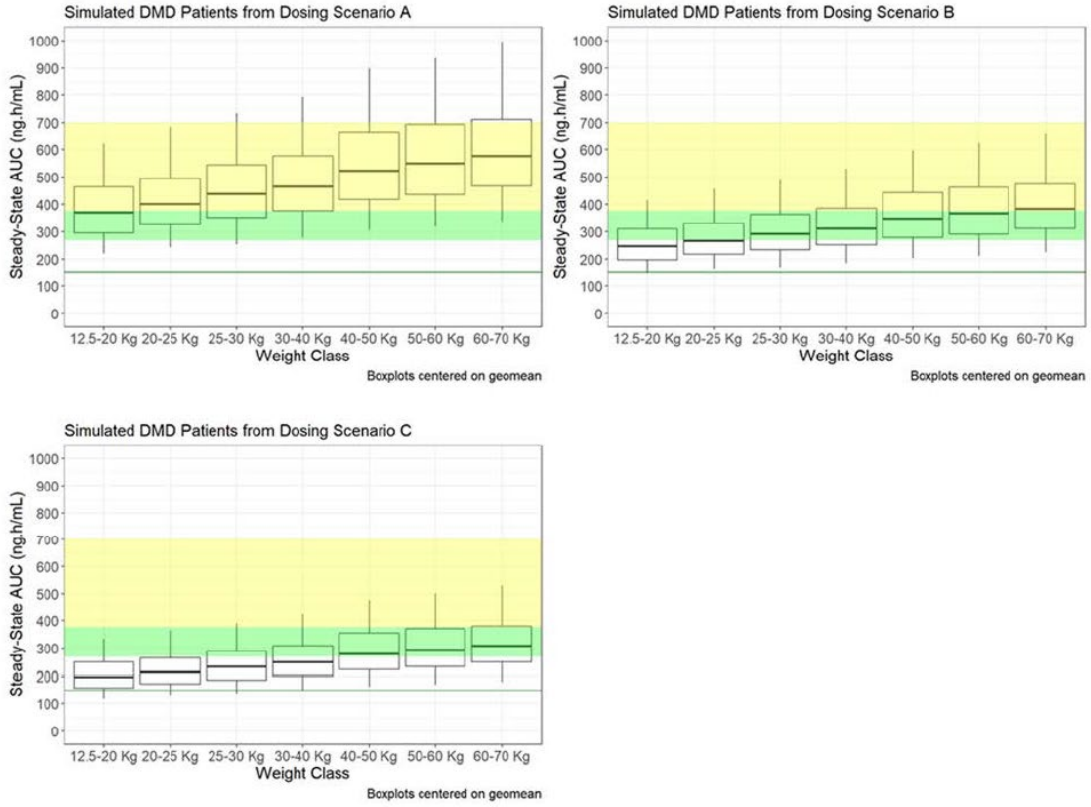
Source: pirana_sum_run2cmtwt8ffed.html
Note: median WT was 40.5 kg
Abbreviations: CL/F = apparent elimination plasma clearance, F1=relative bioavailability; FIX=fixed in the model; IIV=inter-individual variability; k_a=first order absorption rate constant; NA=not available; PK=pharmacokinetic; Q/F=apparent inter-compartmental clearance; RSE=relative standard error; RUV= residual unexplained variability; V_2/F= apparent volume of the central compartment, V_3/F=apparent volume of the compartment; WT=body weight; θ=fixed effect parameter

Figure 2 pcVPC of the Givinostat final model (2cmtwt8ffed) for study 48



Source: ital-pmx-givinostat-3392-ppk-run2cmtwt8ffedsim-2022-09-26.html
Notes: symbols represent observations, solid thick line represents the median of the observations; dashed thick lines represent the 5th and 95th percentiles of the observations; colored bands represent 50th (grey), 5th and 95th (blue) percentiles of the model predictions with 95% CI. On the top left of each plot, reference is made to the study number.

Figure 3 Givinostat week 26 (steady state) AUC within the dosing interval obtained by simulations in subjects receiving 3 different dosing scenarios (A, B, C)



Notes: Boxes are the 25th and 75th PIs, the line is the geometric mean, and whiskers are the 5th and 95th PIs of the steady state AUC within the dosing interval. The dark green line is the preclinical target AUC_{ss} within the dosing interval (150 ng.h/mL, corresponding to a daily AUC of 300 ng.h/mL); green and yellow areas correspond to the 90%CI of the AUC_{ss} within the dosing interval at 25 mg (268-376 ng.h/mL) and 37.5 mg (377-702 ng.h/mL) b.i.d, respectively, obtained in the phase 2 study.

Abbreviations: AUC=area under the plasma concentration-time curve; PI=prediction interval; ss=steady-state

Source: Figure 12 Modeling and Simulation Report ITAL-PMX-GIVINOSTAT-3392

Pharmacodynamics

Givinostat (ITF2357) is an orally active hydroxamic acid derivative possessing a potent HDAC inhibitory activity and the anti-tumoral properties typical of this class of inhibitor. It is hypothesised that HDAC inhibitors delay DMD disease progression by stimulating activation of muscle satellite cells, increasing expression of muscle regeneration factors, and inhibiting fibrosis and inflammation through reduction of cytokine production and signalling.

Primary pharmacodynamics

Effects on Muscle Morphology Measured Histologically

The primary objective of Part 2 of Study 43 was to confirm proof-of-concept in humans, i.e., to demonstrate that givinostat can counteract the histological signs of the disease. Muscle fibre area fraction (MFA%) increased in all children, with a mean relative increase of 29.1% ($p < 0.0001$). Endomysial and perimysial fibrosis decreased in all children with a mean relative decrease in total fibrosis of 27.4% ($p < 0.0001$). Significant improvements were observed also for other DMD histological features, with reductions in necrotic and hypercontracted fibre number as well as

reductions in fat tissue replacement ($p < 0.0005$). MFA% increased secondary to a homogeneous increment of the cross-sectional area (CSA) value of all fibres. These results demonstrate that givinostat counteracts histological disease progression in children aged ≥ 7 to < 11 years, increasing muscle fibre area, reducing necrosis, fatty replacement and fibrosis, and decreasing the number of hypercontracted fibres.

Effects on Muscle Morphology Measured by Magnetic Resonance

Muscle fat infiltration was assessed in Study 48 at Baseline, 12 and 18 months. VL MFF measured by magnetic resonance spectroscopy (MRS) was a key secondary endpoint. In addition, fat fraction was assessed in 5 specific thigh muscle districts by Dixon MRI, and these served as exploratory endpoints. Regardless of the technique used, givinostat consistently (nominal p-value 0.0354 to 0.0009) reduced the increase in fat infiltration in the selected muscles. These results confirm the histological results and demonstrate that givinostat can prevent the muscle degeneration typical of DMD.

Effects on Biomarkers

The concentrations of the following molecular markers were assessed as exploratory endpoints in subjects enrolled in Study 48: LAP-TGFβ1, IL-1b, TNF-α, IL-18bp, IP-10, osteopontin, leptin, and FABP3. Givinostat produced statistically significant reductions in the concentrations of LAP-TGFβ1 at Months 12 and 18 and of IP-10 at Month 12 with a positive trend at Month 18. These results confirm nonclinical results and results in Study 43 showing that givinostat reduces the inflammation and pro-fibrotic signals in patients with DMD.

Secondary pharmacodynamics

Effect on QTc

The effects of therapeutic and supratherapeutic doses of givinostat on the QT/QTc interval were studied in a single-centre, randomised, partially double-blinded, single-dose, placebo-corrected, 12-sequence, 4-period, crossover study. The highest mean effect on ΔΔQTcF with a therapeutic dose of 100 mg givinostat was 5.5 ms (90% CI: 1.99 to 9.01). With this dose, the effect on heart rate was small and of no clinical concern. The supratherapeutic dose (300 mg) of givinostat had a clinically relevant effect on heart rate. The highest mean effect on ΔΔQTcF with this dose was 13.6 ms.

Overall, the study showed that QTc prolongation may occur at doses ≥ 6-fold and concentrations ≥ 10-fold higher than those expected in DMD children treated with the proposed givinostat dose. Therefore, givinostat should be used with caution where there is an increased risk for ventricular arrhythmias (including torsades de pointes) such as coronary artery disease, electrolyte disturbance, concomitant use of other medicinal products known to cause QT prolongation disturbance.

Pharmacodynamic interactions and genetic differences

The applicant has not provided any data for the potential of pharmacodynamic interactions of givinostat with other substances or genetic differences in PD response.

Relationship between plasma concentration and effect

A population PK-PD model was developed to describe the time course of platelet counts (Table 3). The original PD dataset data was composed of platelet count observations from all studies that were included in the PK dataset for the popPK analysis. Single dose data (Studies 01, 04 and 54) informed the model only with regards to the baseline platelet count. Considering this, PK/PD modelling was

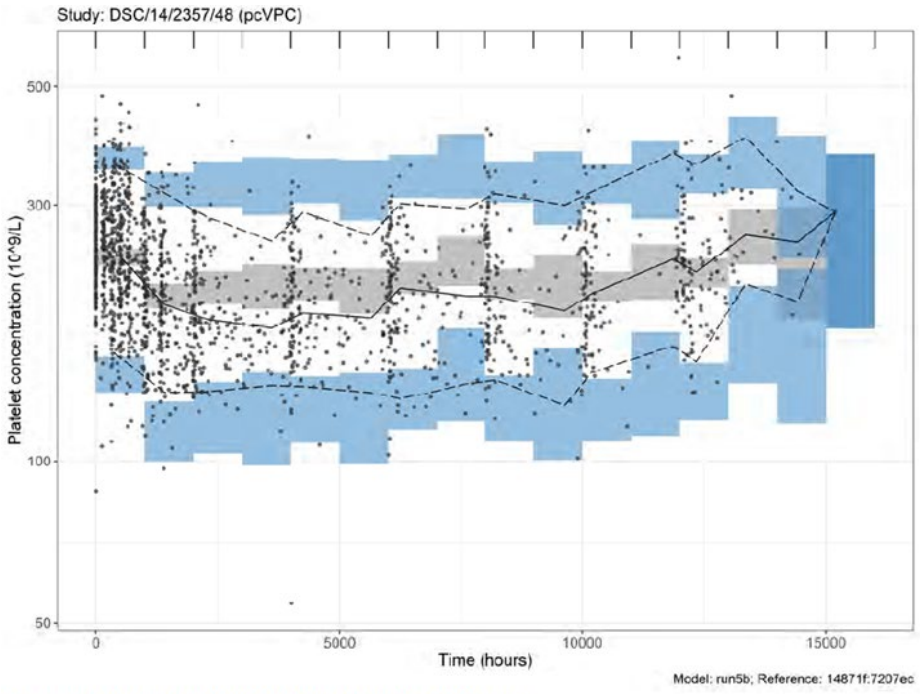
performed only using the data obtained in the multiple dose studies (based on 3241 observations in 169 subjects). PK-PD simulations of platelet counts were performed at the end of a 26-week dosing period and indicated that the incidence of thrombocytopenia grade >1 was predicted to be 17%, 6%, and 2% in the worst case scenario for patients enrolled in Study 48 weighting ≥ 60 - < 70 kg, who received the highest dose levels of Dose A, B, and C, respectively. The incidence of thrombocytopenia was predicted to be lower in subjects with lower body weight (receiving lower dose levels).

Table 3 Model parameters of the final PK-PD model for platelet counts following Givinostat treatment

Parameter	Description	Estimate (Shrinkage %)	RSE(%)	Estimate (Natural Scale)
Baseline platelets (10^9/L)	Estimated on log scale	5.76	0.3	316
MTT	Estimated on log scale	4.36	1.0	78.1
Slope mL/ng	Estimated on log scale	-7.0	1.09	0.00091
Proportional RUV	Variance	-1.89	2.1	0.150
Gamma	Estimated on logit	-1.86	4.9	0.0640
Weight on baseline	Log-linear	-0.251	12.8	-0.251
IIV on Baseline	Variance	0.0314 (7.7)	13.5	
IIV on MTT	Variance	0.0297 (50.1)	31.3	
IIV on Slope	Variance	0.183 (13.8)	22.9	
Additive error	Variance	0.00001 FIX	NA	

Source: pirana_sum_run5b.html
Abbreviations: FIX= fixed in the model; IIV=inter-individual variability; MTT= mean transit time; NA=not available; PD=pharmacodynamic; PK=pharmacokinetic; RSE=relative standard error; RUV= residual unexplained variability

Figure 4 pcVPC of the final PK-PD model for platelet counts following Givinostat treatment for study 48



Source: ital-pmx-givinostat-3392-pkpd-run5bsim-2022-09-02.html
Notes: In study 48, givinostat treatment was continuous. Symbols represent the observations; solid thick line represents the median of the observations; dashed thick lines represent the 5th and 95th percentiles of the observations; dashed line represents the median of the model predictions; dotted lines represent the 5th and 95th percentiles of the model predictions and colored bands represent 50th (grey), 5th and 95th (blue) percentiles of the model predictions with 95% CI. On the top left of each plot, reference is made to the study number.
Abbreviations: pc-VPC=prediction-corrected visual predictive checks; PD pharmacodynamic; PK=pharmacokinetic

No meaningful results were obtained by exposure response modelling for efficacy endpoints and model parameters were characterised by a high uncertainty. Exposure-response modelling for safety showed that higher systemic exposure was significantly correlated with higher probability of diarrhoea.

2.6.3. Discussion on clinical pharmacology

The clinical program for givinostat PK characterization involves PK after single and multiple dose administration, the effect of food on givinostat PK, drug-drug interaction and metabolism of the drug. The pharmacology package provided by the applicant can be considered comprehensive. The analytical methods, primarily based on LC/MS/MS detection, were validated according to the recommendations. Several issues, such as long-term stability, carry-over effect, the impact of haemolysis, and the effect of lipemic plasma on analysis, have been addressed by the applicant.

During bioanalyses AA02477, AA02324, and A124710A, supporting clinical studies DM-00-2357-01, DM-00-2357-03, and DM-00-2357-04 respectively, incurred sample reproducibility was not conducted. However, the impact on the missing ISR early phase 1 studies is considered negligible.

The longest storage conditions did not exceed time period covered by long-term stability data, except for study 48 (DSC/14/2357/48). However, the percentage of samples in this study tested beyond the proven stability is low (<5%) and this was no confirmative pharmacokinetic study.

Givinostat is well absorbed after oral administration; maximum plasma concentrations are achieved approximately 2-3 hours after both single and repeat administration. Bioavailability in humans was not investigated in a dedicated study or through a human mass balance study and can only be estimated through PK modelling. Significant differences between animal species and humans can best be explained by a higher metabolic rate in animals. While the formulation was changed during clinical development and bioequivalence was not formally investigated within a dedicated study, popPK modelling and comparison of PK data across studies indicate a sufficient degree of bioequivalence between the formulations. In addition, the influence of food on the pharmacokinetic parameters following a single dose of givinostat was investigated. The applicant noted a moderate effect of food on absorption; as such, it is believed that no formal recommendation concerning dietary restrictions is necessary.

An overview of givinostat metabolites and the involved enzymes, as far as they have been identified, was provided and compared across species. Metabolic profiles appear comparable except for an additional metabolite in rats. Several potential interactions with givinostat as both perpetrator and victim have already been identified. Although information about the metabolic pathways remains incomplete the overall risk for additional drug-drug-interaction potential is low.

No specific clinical studies directed to characterisation of givinostat's PK in special populations were performed. However, according to the popPK modelling, no dose adjustment is required according to age, race and gender. Body weight was found to be the strongest covariate of givinostat PK, and the recommended dose of givinostat is based on body weight.

During the drug development program, no studies were conducted to investigate the potential impact of impaired renal and hepatic function on givinostat's pharmacokinetics. However, long-term exposure to metabolites from damaged muscles might increase the risk of kidney impairment in both adult and paediatric DMD patients. Furthermore, given that givinostat is extensively metabolized, the effects of impaired hepatic function cannot be ruled out. However, although givinostat is extensively metabolised by the liver, a contribution of extrahepatic metabolism has been demonstrated. In cases of hepatic impairment, compensation by extrahepatic metabolism may be expected. Nevertheless, due to the lack of data on the pharmacokinetics of givinostat in patients with hepatic impairment, appropriate

statements have been included in the SmPC. Furthermore, the applicant informed that a study is currently underway to evaluate the effect of hepatic impairment on givinostat exposure. With regard to renal impairment, the SmPC includes a statement about the lack of data in this patient population. Additionally, since the renal route has very limited significance in the drug's excretion, the impact on drug exposure is not expected to be significant.

The mechanism of action of givinostat is well characterized but not disease specific. The primary cause for efficacy in DMD would probably be anti-inflammatory effects. Consequently, pharmacodynamic biomarkers for efficacy are also unspecific and it was not possible to describe a meaningful relationship between plasma concentration and effect.

Non-compartmental PK analyses were conducted using conventional software and methods. The standard methodology is acceptable for PK data analysis. General methods have been applied and are considered acceptable for statistical analyses. In the popPK modelling givinostat's PK was correctly described by a 2-compartment model with first-order input (with lag time) and first-order elimination from the central compartment. The PK of givinostat was characterised as dose-proportional and time-independent. The PK-PD model accurately depicted the time-course of platelets post-givinostat administration. The wording in the SmPC regarding linearity is adequate.

The results regarding the interaction between glucocorticoids and givinostat are ambiguous. Specifically, Study 0428-2012-R did not indicate any influence of steroids on the PK of givinostat, whereas Study 0159-2012 demonstrated the contrary. Furthermore, no dedicated drug-drug interaction studies assessing the co-administration of glucocorticoids and givinostat in a cohort of healthy subjects have been conducted. The applicant provided justification for the lack of potential interaction between the administered steroids and givinostat, and explained that the observed impact of steroids on the pharmacokinetics of givinostat was based on data from a population of patients with Crohn's disease. The justification regarding the possible interaction through CYP3A4 activity is convincing, particularly given that almost all patients participating in the pivotal studies were using steroids.

With regard to the population PK modelling, the following limitations have been noted:

Compared to the previously developed model, some differences were found regarding the final model. No formulation effect was found as significant covariate in the final model. Paediatric patients predominantly used the givinostat suspension, whereas adults predominantly used the capsule formulation, which further complicates the analysis. Furthermore, the estimated allometric exponent on CL/F was even smaller than in the previously developed model (0.625 vs. 0.326) and no effect of bodyweight on the volume of distribution was included. No modelling results for standard allometric exponents were shown. The applicant argued that changes to the previously developed model may have occurred due to the exclusion of other indications than DMD. The applicant compared results for the final model a) with the previous model using the updated dataset and b) with results using standard allometric exponents on CL/F and volume (0.75 and 1, respectively) to ensure that the popPK model is suited to appropriately predict paediatric patient PK of givinostat. Results were compared to the final model using GOF plots and pcVPC stratified by age- and weight groups showing a better fit for the model using estimated exponents.

To show that simulations across all weight bands are appropriate, the applicant submitted boxplots corresponding to Figure 3 adding individual estimates for AUC depicted as symbols in the plot. The lowest weight band was changed to 15-20 kg body weight. The youngest patient was 6.3 years of age and the lowest body weight included was 17.5 kg. The model should not be used to extrapolate outside this age- and weight range. Additional diagnostic plots were submitted to ensure that the model is appropriate to describe paediatric data adequately.

The PD modelling for platelet count over time indicated that individuals with a higher body weight might be at greater risk of experiencing a significant decrease in platelet count. The SmPC recommends the use of a dose reduction based on the platelet level, specifically applying scenario B and then scenario C sequentially. Given that, for scenario A, a significant number of patients with a body weight greater than 30 kg meet the dose reduction criteria due to low platelet levels, the applicant proposed a revised dosing regimen.

In the revised dosing regimen, the weight bands were reduced from 9 weight bands to 4 weight bands. For patients with higher body weights, this is supported. For patients with lower body weights, the width of the weight bands is considered to be rather broad as it would be changed from 5 kg in the clinical study to 20 kg in the proposed dosing regimen. The aim of a simplified dosing regimen, i.e., to be able to use only one syringe instead of two syringes is acknowledged (see also quality part). The applicant discussed whether additional weight bands (width = 10 kg body weight) would be beneficial. The applicant provided simulations for the resulting exposure (AUC0-12, Cmax) after using dosing scheme based on 4, 6 or 9 weight bands. As modelling predicts similar exposures for all dosing schemes, the applicant prefers to use only 4 weight bands. The dosing scheme was revised regarding the lower weight cut-off, which was changed from 10 kg body weight to 15 kg bodyweight. This is agreed as the indication includes paediatric patients 6 years of age and older.

In addition, for the exposure data of the new proposed posology all data (A, B and C dosing) were combined. The applicant presented the data for subjects having their final dose with A, B and C separately.

Effects on biomarkers have been assessed. The widely recognised and currently used biomarker for DMD is the serum activity level (units/L) of creatine kinase. The applicant explained that although CK levels are a standard marker in the diagnosis of DMD, in later stages, due to muscle mass loss causing decreases in CK levels, they do not appear to be an optimal biomarker for muscle condition. This position is shared in accordance with the EMA Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy (EMA/CHMP/236981/2011, Corr. 1).

In Study DM/00/2357/01, oral administration of givinostat caused rapid decrease of LPS-induced TNFα in whole blood that was sustained over 2 to 8 hours after drug administration. Givinostat decreased TNFα in a dose-depended manner with a maximum effect starting from 200 mg dose. TNFα levels were measured as a PD marker in the initial phase of givinostat studies because the inhibitory effect of HDAC inhibitors on the synthesis of inflammatory cytokines had been identified.

The effects of therapeutic and supratherapeutic doses of givinostat on cardiac repolarisation were assessed in the QTc study ITF/2357/54 and results do not indicate a clinically relevant concern at therapeutic givinostat doses. However, in DMD patients, the primary cause of mortality is cardiopulmonary failure. A known risk factor for ventricular arrhythmias and arrhythmic sudden death in DMD patients is a high degree of QT dispersion (Yotsukura et al., 1999). The applicant presented a discussion about the role of QT dispersion as a method to assess the benefits and risks of antiarrhythmic treatments. According to some publications, it has been demonstrated that the measure of QT dispersion may be less reliable than the QT interval. Pharmacometric modelling (Emax model) confirmed that a QT placebo-corrected change from the pre-dose baseline in QTcF (ΔΔQTcF) exceeding 10 msec could be excluded across the range of observed givinostat plasma concentrations. These results indicate that QT prolongation is not expected at therapeutic doses used in DMD.

The applicant has provided a discussion of the potential of pharmacodynamic interactions of givinostat with other substances commonly co-administered with givinostat. Overall, the potential for interaction appears to be low.

Regarding the relationship between plasma concentration and effect, no meaningful results were obtained by exposure response modelling for the primary efficacy endpoint 4SC and model parameters were characterised by a high uncertainty. The applicant provided placebo data for 4SC depicted as symbols for comparison in a plot corresponding to the figure showing the relationship between the 4SC time change from baseline and steady state average concentration at the dose at Month 18 showing no significant exposure-response relationship. The applicant discussed that no exposure response relationship could be shown due to the large overlap in exposures of different weight groups. It is agreed that the results should be interpreted with caution because of the small number of patients in each subgroup. However, for the secondary endpoint NSAA total score change from baseline the relationship was statistically significant. This could be regarded as supportive.

2.6.4. Conclusions on clinical pharmacology

There are no objections to MAA approval based on the clinical pharmacology dossier.

2.6.5. Clinical efficacy

In total 3 studies have been conducted with 1 pivotal phase 3 study, Study DSC/14/2357/48 to serve for the primary evidence of efficacy. Additional supportive data is provided by a proof-of-concept, dose-ranging study, Study DSC/11/2357/43 as well as the ongoing long-term extension study DSC/14/2357/51. Further, efficacy results from study DSC/14/2357/51 were compared with results of two Natural History Studies, ImagingDMD study and The Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study.

2.6.5.1. Dose response study(ies)

The doses to be used were established based on pre-clinical data and on the results of the phase I studies (see also Clinical Pharmacology (PK/PD) section 3.3.1 as well as the Non-clinical aspects, section 3.2).

Based on the provided exposure response modelling, no clear exposure response relationship for the examined clinical endpoints was shown.

One open-label, Phase 2, proof-of-concept and dose-ranging study has been performed.

A Two-Part Study to Assess the Safety and Tolerability, Pharmacokinetics, and Effects on Histology and Different Clinical Parameters of Givinostat in Ambulant Children with Duchenne Muscular Dystrophy (Study DSC/11/2357/43)

Study DSC/11/2357/43 was conducted from 2 April 2013 to 17 November 2017. It was a first-in-patient, proof of concept, multi-center, single-arm, open-label, two-part study of givinostat doses of 25, 37.5 or 50 mg b.i.d. for 2 weeks (part 1), followed by givinostat doses of 37.5 mg b.i.d. and/ or 25 mg b.i.d. for 12 months (Part 2) in boys aged 7 to < 11 years with DMD.

Twenty children were to be enrolled in the study as follows: the first 4 children were to be treated at a low dose level of givinostat (25 mg b.i.d. for children who weighed 20 kg to 49 kg, and 37.5 mg b.i.d. for children who weighed ≥ 50 kg). Dose escalation was determined based on PK data and the observed safety and tolerability profile. If none of the pre-defined stopping criteria were met after 2 weeks of treatment, the review team determined the escalated dose level to be used for the treatment of additional 8 children who were to be treated at the second dose level. The 4 children previously treated at the low dose level were also planned to be switched to that second dose level. If none of the

stopping criteria were met after 2 weeks of treatment at the intermediate dose, the review team determined the subsequent dose level to be used for the treatment of additional 8 children who were to be treated at the high dose level. All children treated at the intermediate dose level were also planned to be switched to the high dose level. Once all 20 children who were enrolled during Part 1 had been treated for at least 2 weeks, the review team determined the RD to be used in Part 2 based on the observed safety and tolerability profile and PK analyses. All enrolled children were to be switched to the RD level, which was planned to be administered for the subsequent 12 months of the study (Part 2).

Permanently and temporarily stopping criteria were implemented for safety reasons, e.g., diarrhoea, reduction in platelet counts or any drug-related SAE.

Subjects who completed Part 2 of the study were treated for up to additional 40 months in the extension phase (Part 3) (Extensions 1, 2, and 3 up to month 52). Each extension generally lasted 12 months; however, extension 3 was expanded by amendment from 12 to 16 months to allow subjects to continue treatment with givinostat without any interruptions until the further planed long-term study, study 51 started. During Extensions 2 and 3, the givinostat dose was adjusted by patient's weight.

Male subjects 7 to < 11 years of age at study entry, diagnosed with DMD on the basis of immune-histochemical analyses and genetic confirmation, who were able to perform 2 screening 6MWTs with a minimal distance of at least 250 meters each with results of the 2 tests within ± 30 m of each other were included into the study.

Establishment of histologic effects of givinostat administered chronically at the selected daily dose was defined as primary objective (Parts 1 and 2). Secondary objectives (Parts 1 and 2) encompassed to establish the effects of givinostat administered chronically at the selected daily dose on functional parameters, i.e., the 6MWT, North Star Ambulatory Assessment (NSAA), and performance of upper limb (PUL); to establish the safety and tolerability of givinostat, to explore the effects of givinostat on MRI parameters and biomarkers; to explore the acceptability/palatability of the oral suspension and to explore whether the effects of givinostat on disease progression may be related to the type of DMD mutation. Primary objective of the Extensions was to evaluate the safety and tolerability of long-term administration at the selected daily dose of givinostat; secondary objectives of the Extensions were to establish the effects on the 6MWT, NSAA, and PUL (Extensions 1, 2, and 3); to explore the effects of givinostat on MRI parameters (Extension 1); to collect information related to 2 biomarkers, latent TGF β binding protein 4 (LTBP4) and osteopontin genotype (at the beginning of Extension 2 only); to collect information related to time to wheelchair and the time the children spend in a wheelchair (Extension 3, only for children who were not able to complete the 6MWT).

The primary endpoint was change in the value of muscle fibre area % (MFA%) comparing the histology biopsies before and after 12 months of treatment with givinostat. Secondary efficacy endpoints were change in additional histological endpoints based on evaluation of the biopsies (i.e., CSA, inflammation, necrosis, fibrosis, and muscle regeneration) and change in muscular function based on the 6MWT, the NSAA and the PUL, all after 12 months of treatment with givinostat at the selected daily dose. The secondary efficacy endpoints of the Extensions were the change of muscular function after 24, 36, and 52 months of treatment (Extension 1, Extension 2, and Extension 3, respectively) with givinostat as assessed by 6MWT, NSAA, and PUL. For children who were not able to complete the 6MWT, the time to wheelchair and how much time the children spent in the wheelchair was assessed. Exploratory endpoints included amongst others the change in muscle biomarker (e.g., miRNA), PK-PD correlations, evaluation of any correlation between the effect of givinostat on disease progression and the type of DMD mutation. For the assessment of Quality of Life, the PedsQL test was performed before treatment start, at Visit 10 (12 months), and at months 24, 36, and 52 for the Extensions 1, 2,

and 3, respectively.

A first brachial biceps biopsy (baseline) was taken prior to the first dose of study drug. A second brachial biceps biopsy was taken at Visit 10 (12 months) from the opposite arm. The mean cross-sectional area (CSA) was obtained by the CSA average of all intact fibres, whereas the muscle fibre area fraction (MFAF) was obtained from the contribution of all fibres, both intact and partial (localised at the edges of the field) fibres, in each microscopic field. Myofibres having CSA values below to 600 μm^2 (i.e., lower than CSA values and corresponding mean diameters of healthy fibres from 7-year-old controls) have been included in the "hypotrophic" fibre group. All sectioning and staining steps were performed by a single technician, and the confocal imaging was carried out by another operator. Morphometric analysis was performed and double-checked by two operators.

Disposition of subjects:

Twenty-one patients were screened, 1 patient failed screening. Twenty patients were enrolled; 19 patients received study drug in Part 1. One patient discontinued the study due to an adverse event of decreased platelet count and 18 patients continued to Part 2. Another patient was enrolled in Part 1 but started treatment in Part 2. Hence, 19 patients completed Part 2 and no patients discontinued. One subject was excluded from the evaluable population due to major protocol deviations. All 19 patients who completed Part 2 continued to Extension 1. One patient withdrew consent and discontinued from the study at the beginning of Extension 2, the remaining 18 patients completed Extension 2 and Extension 3.

Demography and baseline characteristics:

All patients (N = 20) were ambulant Caucasian males and were treated with corticosteroids. Patients were in the age range of 7 to 10 years with a mean age of 8.2 years, a median age of 8.0 years and mean (SD) BMI of 18.09 (2.648) kg/m^2. The type of DMD mutation was deletion for 10 (50.0%), duplication for 5 (25.0%) and point mutation for 5 (25.0%) patients. No patients with point mutations received the 25 mg b.i.d. dose. In the baseline biopsies, variable degrees of endomysial and perimysial fibrosis as well as fat tissue proliferation, and necrotic and hypercontracted fibres were observed. Patients had a mean MFA% of ~51%, mean total fibrosis of ~46%, mean necrosis of ~2.0%, mean fatty replacement of approximately 0.9% and a mean CSA of the fibres of approximately 1190 μm^2.

The RD of givinostat was determined as 37.5 mg b.i.d. While 7 subjects completed Part 2 of the study with the RD dose, 12 subjects who started on the RD reduced their dose to 25 mg b.i.d. due to decreased platelets during Part 2. However, efficacy results have only been provided for the entire population.

Efficacy results:

Table 4 Summary statistics for change in muscle fibre area (MFA) % (evaluable population) statistics for change in muscle fibre area (MFA) % (evaluable population)

Visit/Change	Statistic	Overall (N = 19)
Baseline	n	18
	Mean (SD)	51.003 (9.6138)
	Median	50.903
	Minimum, Maximum	34.35, 66.15
Part 2 – End of Study	n	18
	Mean (SD)	64.909 (8.3469)
	Median	66.930
	Minimum, Maximum	42.00, 76.11
Change from Baseline to Part 2 – End of Study	n	18
	Mean (SD)	13.906 (4.7065)
	Median	12.768
	Minimum, Maximum	6.17, 21.23
	95% CI	11.5657, 16.2466
	p-value	p < 0.0001

Abbreviations: CI = confidence interval; MFA% = muscle fiber area %; N = number of patients; n = number of patients in the sample; SD = standard deviation.
Reference: Table 14.2.1.1.1, Table 14.2.1.1.2, Listing 16.2.6.1

Table 5 Histological endpoints, changes from baseline at end of part 2 (evaluable population)

Parameter	N	Baseline Mean (SD)	End of Study Mean (SD)	Absolute Change Mean (SD)	95% CI; p-value
CSA (µm²)	18	1191.087 (400.9813)	2056.356 (781.3925)	865.269 (555.3543)	- < 0.0001[a]
Total fibrosis %	18	46.128 (9.6129)	33.488 (8.2430)	-12.640 (4.6493)	-14.953, -10.328; < 0.0001[a]
Perimysial fibrosis %	18	23.469 (8.6600)	15.884 (5.5629)	-7.585 (6.4779)	-10.8062, -4.3634; 0.0001[a]
Endomysial fibrosis %	18	22.660 (6.5066)	17.604 (3.7859)	-5.056 (6.2531)	-8.1653, -1.9461; 0.0032[a]
Fatty replacement %	18	0.886 (0.6970)	0.584 (0.6000)	-0.302 (0.2756)	-0.4391, -0.1650; 0.0002[a]
Necrosis %	18	1.983 (0.7311)	1.019 (0.3172)	-0.964 (0.6260)	-1.2750, -0.6524; < 0.0001[a]
N° of hypercontracted fibers[b]	18	1.977 (0.7139)	0.773 (0.5429)	-1.204 (0.6621)	-1.5334, -0.8749; < 0.0001[a]

The primary endpoint was the change from baseline in Muscle Fibre Area (MFA) % obtained from histology biopsies until 12 months of treatment with givinostat. The mean MFA% at baseline was 51.003% and increased to 64.909% at EoS (End of Part 2) with a mean change from baseline of 13.906%.

Cross-sectional area (CSA) of the muscle fibres increased from mean (SD) 1191.087 µm² (400.9813) at baseline to 2056.356 µm² (781.3925) at EOS with a mean change from baseline of 865.269 µm² (555.3543). There were observed decreases between baseline and EOS visit in mean (SD) percentages of total fibrosis (-12.640 ± 4.6493), perimysial fibrosis (-7.585 ± 6.4779), endomysial fibrosis (-5.056

± 6.2531), fatty replacement (-0.302 ± 0.27569, and necrosis (-0.964 ± 0.6260). The mean (SD) number of hypercontracted fibres decreased from 1.977 (0.7139) at baseline to 0.773 (0.5429) at EOS with a mean change from baseline of -1.204 (0.6621). An increase could also be observed in regenerative fibres with no depletion in the pool of satellite cells. The histology analysis of macrophages showed an increase in the M1 macrophages component of the total macrophages and a decrease in the M2 component, while the double stained M1M2 component did not change. Histology data also showed an increase, about 3% of the total number of fibres, in regenerative fibres assessed by primary antibodies anti-myosin developmental.

Efficacy results for the secondary endpoints 6MWT distance, the NSAA total score and the PUL total score decreased over time until months 52. While the 6MWT distance and the NSAA score followed a gradual decline, the PUL decreased after months 36. Moreover, 6/20 subjects lost ambulation until week 52.

Quality of Life: No important changes were reported until the end of Part 2. At Month 52, scores indicated a decline by patients in PedsQL generic score and by parents/caregivers in all 3 PedsQL modules, i.e., fatigue, neuromuscular scale and generic score.

Exploratory endpoints: Histology Changes by Type of DMD Mutation: Summary statistics for MFA% by DMD mutation type showed that for all of the 3 DMD mutation groups, the mean (SD) change from baseline to EoS (Part 2) was comparable, ranging from 11.422 (3.8458) to 17.076 (5.5781). Summary statistics for MFA% by Arm Sequence, i.e., left-right: n = 11, right-left: n = 7 showed, that the arm sequence did not affect MFA% in a clinically meaningful way. No relevant changes from baseline to EoS have been observed in any Micro Ribonucleic Acid (miRNA) parameter. The oral formulation of givinostat appears to be adequate in terms of acceptability and palatability for children. Parents reported no difficulties in administering givinostat to children.

In a Post-hoc Analysis the distances covered in the 6MWT at baseline and EoS were directly correlated with the MFAF% and indirectly correlated with the % of total fibrosis at the corresponding time-point ($p < 0.05$). No significant correlations were seen with the other parameters and, in particular, no correlations were seen between age, corticosteroids duration, and histology parameters.

2.6.5.2. Main study

Study DSC/14/2357/48

A Phase 3, Randomised, Double Blind, Placebo Controlled, Multicentre Study to Evaluate the Efficacy and Safety of Givinostat in Ambulant Patients with Duchenne Muscular Dystrophy

This study was conducted at 44 centres in 11 countries.

Date first subject enrolled: 06 June 2017 and Date last subject completed: 22 February 2022

Methods

The study was 19 months in duration and consisted of 2 phases: 1) screening period: starting 4 weeks (± 2 weeks) before randomisation; and 2) treatment period: 18 months of treatment.

Subjects underwent pre-study screening assessments up to 4 weeks before the first scheduled dose of study treatment. The standard-of-care corticosteroid treatment regimen was continued. At the randomisation visit subjects were randomised (2:1 ratio) to receive givinostat oral suspension 10 mg/mL or placebo oral suspension twice daily (in a fed state) with randomisation stratified for their

concomitant use of steroids in 4 strata: 1) deflazacort daily regimen; 2) deflazacort intermittent regimen; 3) other steroids daily regimen; and 4) other steroids intermittent regimen.

An independent data monitoring committee (IDMC) reviewed, evaluated, and categorised safety findings every 3 months during the study and was responsible for the oversight of the interim analysis at which futility was assessed, and a blinded sample size re-estimation was made. The IDMC had access to un-blinded data, if necessary, and operated according to the rules defined in the IDMC charter.

The following efficacy assessments were performed at screening and randomisation and afterwards as follows: Time to Climb 4 standard stairs (4SC) test , NSAA, 6MWT, other timed function tests (i.e. time to walk/run 10 meters, time to rise from floor) and Muscle strength assessments were performed at week 12, 24, 36, 48, 60 and 72. Quality-of-life tests (assessed by PODCI) were conducted at week 48 and 72.

During the screening period, time to 4SC assessments were to be performed at screening visit 1 (V1) and screening visit 2 (V2). The second 4SC test could be performed 1 day after V1 up to a maximum of 2 weeks (+3 days). Therefore, subjects had to go to the centre 2 times (i.e., Visit 1 and Visit 2). The test was video recorded during Visit 1, Visit 3 (randomisation), Visit 13 (week 48) and Visit 15 (EOS/Early Withdrawal). Muscle strength tests at screening were performed by HHM (i.e., knee extension and elbow flexion) and by MMT (i.e., knee extension) while muscle strength tests during the study and at the end of the study were solely performed by HHM (i.e., knee extension and elbow flexion).

The initial dose suggested for the pivotal study was chosen to achieve similar exposure as the 37.5 mg b.i.d. dose in study 43 targeting a daily exposure of 300 ng*h/mL.

Study participants

Main inclusion criteria relating to efficacy were DMD diagnosed subjects confirmed by genetic testing. Subjects were $\geqslant$ 6 years of age at randomisation with DMD-characteristic clinical signs or symptoms (e.g., proximal muscle weakness, Gowers' manoeuvre, elevated serum CK level), were able to complete two 4-stairs climb (4SC) screening assessments with results within ±1 second of each other and a mean of the 2 4SC assessments of $\leqslant$ 8 seconds, were able to complete the time to rise from floor test between $\geqslant$ 3 and < 10 seconds at screening, had manual muscle testing of quadriceps of grade $\geqslant$ 3 at screening and had used systemic corticosteroids for a minimum of 6 months immediately prior to start of study treatment, with no significant changes in corticosteroid type or dosage or dosing regimen. Additionally, the so called "Target Population" had to have a vastus lateralis muscle fat fraction (VL MFF) on magnetic resonance spectroscopy (MRS) of > 5% to ≤ 30%.

Main exclusion criteria: Exposure to other investigational drugs within 3 months prior to start of study treatment with the exception of deflazacort in the US as part of the Expanded Access Program and in Canada as part of the Special Access Program; exposure to idebenone within 3 months or exposure to any dystrophin restoration product (e.g., ataluren, exon-skipping) within 6 months prior to the start of study treatment; use of any pharmacologic treatment, other than corticosteroids, that might have had an effect on muscle strength or function within 3 months prior to start of study treatment (e.g., growth hormone); vitamin D, calcium, and any other supplements were allowed as long as their intake had been stable for 3 months prior to start of study treatment; testosterone was also allowed if it was used with stable dose and regimen as a replacement therapy for the treatment of delayed puberty, and its circulating testosterone levels were within the normal ranges for the subject' s age; had surgery that might have had an effect on muscle strength or function within 3 months before study entry or planned surgery at any time during the study; loss of $\geq$ 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); change in contracture treatment such as serial casting, contracture control devices, night splints, stretching exercises (passive, active, self) within 3 months prior to enrolment, or expected need for such intervention during the study; had platelet, white blood cell, and haemoglobin counts at screening < lower limit of normal (LLN); had symptomatic cardiomyopathy or heart failure (New York Heart Association functional class III or IV) or left ventricular ejection fraction <50% at screening; had a current or history of liver disease or impairment, including but not limited to an elevated total bilirubin (i.e., > 1.5 × upper limit of normal [ULN]), unless secondary to Gilbert disease or pattern consistent with Gilbert's disease; had inadequate renal function, as defined by serum cystatin C >2 × the ULN. If the value was >2 × ULN, the serum cystatin C was repeated once; if the repeated test result was still >2 × ULN, the subject was excluded); had triglycerides > 300 mg/dL (3.42 mmol/L) in fasting condition at screening visit; had a baseline-corrected QT interval, Fridericia's correction (QTcF) of >450 msec, or history of additional risk factors for torsade de pointes (e.g, heart failure, hypokalaemia, or family history of long QT syndrome).

Treatments

Givinostat (10 mg/mL) or placebo was administered as an oral suspension twice daily (b.i.d.) under fed conditions. Before its use, the suspension was to be shaken for at least 30 seconds by rotating the bottle by 180 degrees and the homogeneity of the obtained suspension verified. The suspension was administered by means of a graduated dosing syringe. The dosage to be administered was based on the subject' s weight.

Study drug had to be permanently interrupted in case of severe drug-related diarrhoea (i.e., increase of ≥7 stools per day); any drug-related SAE; QTcF >500 msec; platelets count ≤50 x 10^9/L. White blood cells ≤2.0 x 10^9/L; haemoglobin ≤8.0 g/dL.

Study drug had to be temporarily stopped if any of the following occurred: 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, white blood cell <3.0 x 10^9/L but >2.0 x 10^9/L, haemoglobin <10.0 g/dL but > 8.0 g/dL with observed laboratory parameters to be re-measured after 1 week and re-tested until they were normalised; Triglycerides >300 mg/dL (3.42 mmol/L) in fasting condition (triglycerides had to be measured every 2 weeks until triglycerides return to levels below 300 mg/dL (3.42 mmol/L).

Treatment regimens:

Patients who were included into the study under Study Protocol Versions ≤4 were dosed under Treatment Regimen 1: All patients started on Dose A and during the trial, the study drug could be reduced to Dose B 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 haemoglobin were normalised and/or triglycerides return to levels below 300 mg/dL (3.42 mmol/L), or diarrhoea was mild. The subject may have had more than the usual number of scheduled visits if needed, until the AE resolves, and then continued the study as per scheduled visits.

Also in case a subject had a consistent (e. g., at least 2 consecutive evaluations) platelet count of ≤ 150 × 10^9/L and did not meet the stopping criteria due to platelet reduction, the Investigator had to reduce the dose by one-third of the starting dose. If a subject was randomised with baseline triglycerides above 300 mg/dL (3.42 mmol/L), the Investigator could discuss how to manage the case with the medical monitor; however, the final decision remained with the Investigator or its authorised designee only. Moreover, for subjects who already had a dose reduction by 1/3, an additional reduction by 20% was allowed for safety reasons as implemented subsequently with study amendment 5

(protocol version 6.0). If a subject has a medical event not necessarily drug related that required interruption of study drug dosing for >4 weeks, the Investigator could discuss the case with the medical monitor if the subject could resume study drug treatment, however the final decision remained with the Investigator only or its authorised designee.

Switch from Treatment Regimen 1 to Treatment Regimen 2: A blinded safety review of haematological data conducted during the study showed that approximately 50% to 60% of those subjects who had at least 8 weeks of treatment at the above starting doses had to reduce dose due to a decrease in platelet counts. Considering these emerging data, the applicant determined that it was necessary to implement changes to the starting dose of the study. Therefore, revised starting doses (known as Treatment Regimen 2), equivalent to Dose B in protocol ≤4, with a permitted 20% dose reduction (Dose C) based on protocol-specified safety/tolerability criteria, were implemented in Protocol Versions > 4.0.

For subjects who were randomised under protocol version 6.0, 7.0 or 8.0 the study drug could 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 haemoglobin were normalised, and/or triglycerides returned to levels below 300 mg/dL (3.42 mmol/L), or diarrhoea was mild. The subject may have had more than the usual number of scheduled visits if needed, until the AE resolved, and then continued the study as per scheduled visits.

Also, in case a subject had a consistent (e. g., at least 2 consecutive evaluations) platelet count of ≤ 150 × 10^9/L but did not meet the stopping criteria for platelets, the Investigator had to reduce the dose by 20% of the current dose. For subjects randomised under protocol version 6.0, 7.0, or 8.0, only 1 dose reduction was allowed during the treatment period.

Dose Modification (all subjects): If subjects (i.e., those already treated or those who were randomised under protocol version 6.0, 7.0 or 8.0) gained weight during the study, the drug dose must be kept unchanged. If subjects (i.e., those already in treatment or those who were randomised under protocol version 6.0, or 7.0 or 8.0) lost weight during the study, the drug dose was to be adjusted accordingly. All subjects were asked to have platelet count assessments weekly during the first month of treatment and performed every 2 weeks during the second month of treatment, in order to strictly monitor this parameter for safety reasons. If the dose was reduced due to a platelet count of ≤ 150 × 10^9/L and/or white blood cell count of < 3.0 × 10^9/L and/or haemoglobin of < 10.0 mg/dL, a complete blood cell count test was to be performed weekly for 8 consecutive weeks.

Table 6 Summary of dosing in study 48

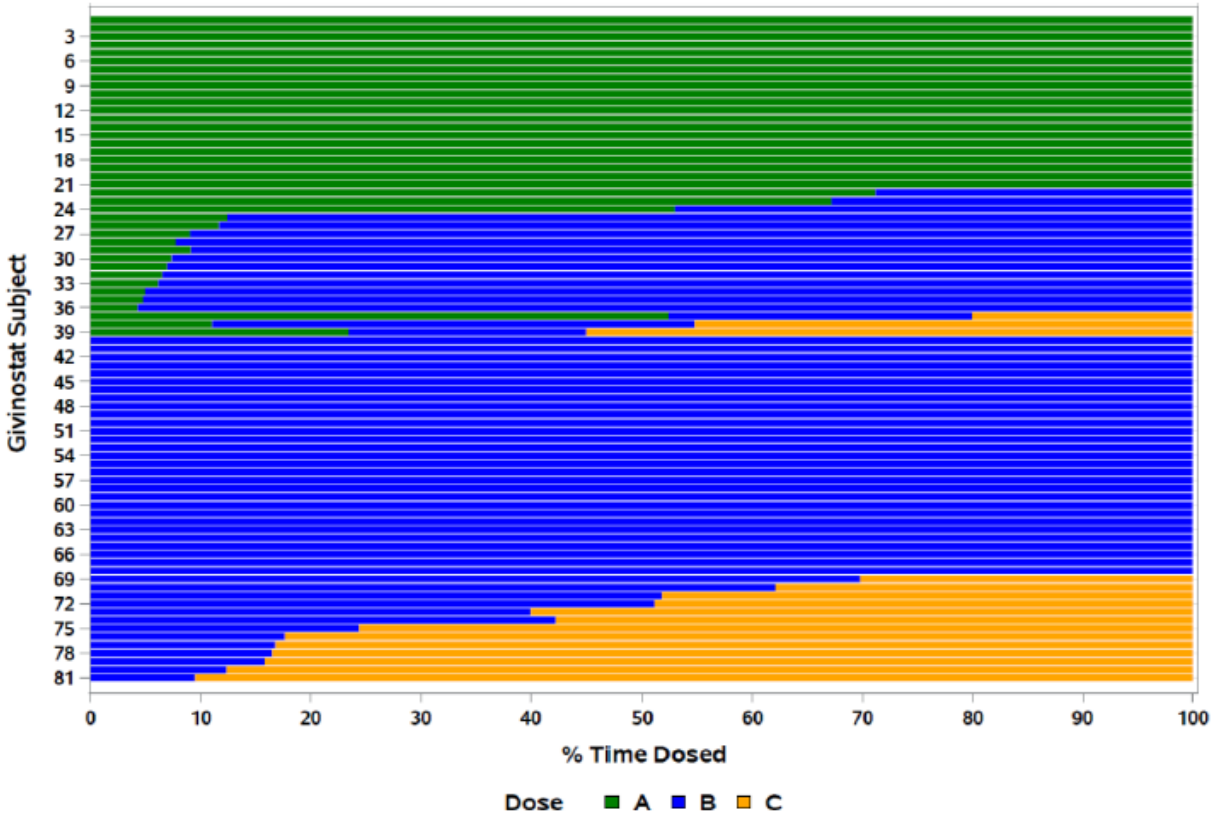
Treatment Regimen	ITT Population			Target Population		
	Givinostat (N=118)	N	%	Givinostat (N=81)	N	%
Treatment Regimen 1	Started at Dose A	55	100	Started at Dose A	39	100
	Remained at Dose A	23	41.8	Remained at Dose A	21	53.8
	Reduced from Dose A to Dose B	32	58.2	Reduced from Dose A to Dose B	18	46.2
	Reduced from Dose B to C	5	9.1	Reduced from Dose B to C	3	7.7
Treatment Regimen 2	Started at Dose B	63	100	Started at Dose B	42	100
	Remained at Dose B	46	73.0	Remained at Dose B	29	69.0
	Reduced from Dose B to Dose C	17	27.0	Reduced from Dose B to Dose C	13	31.0

ITT = intent-to-treat; N = number.
Source: Study 48 Post Hoc Statistical Analysis, Tables 14.1.3.1 and 14.1.3.2.

In the primary efficacy population (Target Population), approximately half of the patients in Treatment

Regimen 1 (53.8%) tolerated Dose A and stayed on it until the end of the study. For Treatment Regimen 2, 69.0% of patients in the Target Population tolerated Dose B and stayed on it until the end of the study.

Figure 5 Waterfall plot for Givinostat dosing (study 48 target population)



A waterfall plot has been provided showing changes in starting dose as function of time for each individual patient in the Study 48 Target Population (the primary efficacy analysis set). The plot shows that for Treatment Regimen 1, patients who reduced from Dose A to Dose B did so early in the study (approximately 80% of reductions from Dose A to Dose B occurred within the first 3 month in the study, i.e., 15% of time dosed). Similar results were observed in the ITT Population. As a result, Dose B accounted for 57% of the total patient exposure in the Study 48 Target Population.

With respect to inclusion criteria for this study, subjects were to be on systemic corticosteroid therapy for at least 6 months prior to initiation of study drug (i.e., there were 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).

Objectives

Primary objective

- To establish the effects of givinostat versus placebo administered chronically over 18 months to slow disease progression in ambulant Duchenne Muscular Dystrophy (DMD) subjects

Secondary objectives

- To assess the safety and tolerability of givinostat versus placebo administered chronically in DMD subjects

- To evaluate the pharmacokinetic (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

Exploratory objectives

- To evaluate the correlation between PK profile of givinostat and pharmacodynamic (PD) data
- To explore whether the effects of givinostat versus placebo administered chronically may be related to the type of DMD mutation or to the biomarkers

Outcomes/endpoints

Primary efficacy endpoint

- The mean change in time to 4SC before and after 18 months of treatment of givinostat versus placebo

Key secondary efficacy endpoints

Function:

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

Imaging:

- Mean change in vastus lateralis muscles fat fraction (VL MFF)

Exploratory endpoints:

Mean changes in: Time to walk/run 10 metres; PODCI scores; Percent-predicted 6MWT (the predicted value is against healthy subjects and is calculated using the equation of Geiger); only in the MR cohort: MRI parameters (e.g., fat fraction (FF) of thigh muscles, CSA of vastus lateralis (VL) and other thigh muscles). Time to 10% persistent worsening in 6MWT (baseline through EOS); Proportion of subjects with $\geq$ 10% worsening in 6MWT at EOS; Time to loss of standing (baseline through EOS); Proportion of subjects who lost 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.

Specific Safety Endpoints: Changes from baseline to EOS in: 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 ECHO, Cognitive function evaluated by the Raven-coloured progressive matrices, Weight, Evaluation of acceptability/palatability of the oral suspension

Based on previous studies, the following minimally clinically important differences are accepted: TTSTAND velocity - 0.023 rise/sec, TTRW velocity - 0.212 m/sec and TTCLIMB velocity (corresponds to 4SC) - 0.035 tasks/sec (Duong et al, 2021), 6MWTD of around 30 meters (McDonald et al, 2013) and

NSAA total score 2.32 points (Haberkamp et al, 2019). With respect to the calculation of 4SC velocity Duong states, that "speed was calculated for the entire activity; therefore, 4SC was calculated as 1 /x instead of 4/seconds, implying that boys with DMD do not complete each step at the same rate." It is therefore considered, that the generally accepted MCID of -0.035 which is in the unit of "tasks/second" is not applicable to the 4SC velocity in the unit of "stairs/second" used by the applicant. All functional and strength assessments were evaluated by qualified functional evaluators (i.e., physiotherapists) who were different from the site personnel who reviewed subjects' safety results; safety results were not to be shared with site personnel. To limit variability associated with functional evaluators "drift" in performing trial assessments across the course of the study and to ensure compliance with functional evaluator manual procedures and valid conduct of assessments, some screening, baseline, 12-month, and final study visits were videoed and reviewed for quality assurance across all subjects. Videos were uploaded to a central secure repository and reviewed only by an expert and independent team engaged to train and qualify the site functional evaluators.

MRI Cohort: Fat infiltration in the vastus lateralis muscle of the thigh was measured using a non-invasive objective imaging method called MRS to assess the efficacy of givinostat versus placebo in the MR cohort. The MRI using the Dixon technique and the MRS were centralised in a referred qualified site. Therefore, some subjects were asked to undergo the examination at a site that was different from the study site. The MRI/MRS personnel involved were different from the site personnel. They did not review subjects' safety results; safety results were not to be shared with them. Moreover, the MRI/MRS images were centrally read by an Independent Central Review team. A qualification process was implemented in order to improve data quality and decrease inter-site variability and the test was performed in a standardised manner described in a specific site manual. The baseline MRI/MRS test had to be performed when all inclusion/exclusion criteria had been already evaluated and the subject was eligible. All subjects were to undergo the baseline MRI scan of thigh and MRS of vastus lateralis. All planned subjects (110 randomised subjects) in the target population (i.e., Group A: subjects with a baseline VL MFF assessed by MRS in the range of > 5% to ≤ 30%) were included in the MR cohort and received the MRI scan of thigh and the MRS of vastus lateralis also at week 48 and week 72. The MRI/MRS images were read to evaluate the FF of each muscle. A cross-sectional area (CSA) of muscles was evaluated as well.

During the COVID-19 pandemic, monitoring activities were re-assessed. Therefore, on-site monitoring visits by CRAs could have been temporarily interrupted or visit frequencies could have changed from the planned frequency or been replaced by remote review of data. Monitoring visits were performed routinely whenever possible. Remote source data verifications were performed during database lock run due to ongoing on-site visit restrictions. The applicant arranged independent audits as part of the implementation of quality assurance to ensure that the study was conducted in compliance with the protocol, standard operating procedures, Good Clinical Practice, and all applicable regulatory requirements. Quality assurance audits were performed in the most of investigational study sites to ensure that safety and efficacy assessments were well conducted and documented. Considering the patients population in study, the trial continued during the COVID-19 pandemic emergency, and various operative challenges (i.e., restrictions of visits to health care facilities, change in investigational medicinal product (IMP) supply, changes to trial staff availability, etc) were identified (COVID-19 FDA/EMA guidelines) and Upper Management has requested to increase the percentage of site audits.

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 of 3 seconds between givinostat and placebo in the Target Population 4SC change from baseline to 18 months, assuming a common standard deviation (SD) of 6 seconds. The estimated

SD was 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. This gave rise to an estimated sample size of N=192 subjects in the Target Population. Based on internal Italfarmaco S.p.A. data, a total of N=99 subjects were originally calculated as sufficient to provide 80% power to detect a 55% reduction in the annual mean increase in VL 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 VL MFF of 6%.

A pre-specified interim analysis was conducted in January 2020 based upon the first 50 randomised subjects reaching 12 months of treatment to evaluate the effects of givinostat versus placebo on the VL MFF in terms of futility. Futility was not met and the IDMC concluded that the study should continue. A pre-planned blinded sample size re-assessment was then performed which provided a revised, blinded within-treatment SD estimate for the change from Baseline in time to 4SC at 18 months of 3.094 seconds, approximately half of that assumed in the original power calculation. Based on this revised SD estimate, the revised sample size (utilising a 2:1 randomisation scheme) is N=102, which provides 90% power and a 1-sided alpha of 2.5% to detect a true difference of 2 seconds between givinostat and placebo in the Target Population, for the change from baseline in 4SC at 18 months. With an estimated drop-out rate of 8%, a total of 110 male ambulant subjects were to be randomised in the Target Population (Group A). Up to 50 subjects (about 35% of the overall population) outside of the Target Population may also be recruited into the study (Group B).

A total of 120 male ambulant subjects were actually randomised in the Target Population (Group A). Fifty-nine subjects (about 35% of the overall population) outside of the Target Population were also recruited into the Off-Target Population (Group B). The overall population (Group A + Group B) provided supportive data.

Randomisation and blinding (masking)

At Visit 3, subjects were randomised via interactive response technology (IRT). During the randomisation procedure, the Investigator was asked to record the type of steroid used by the subject (ie, deflazacort or other type of steroids) and to record the regimen of intake (ie, daily regimen or intermittent regimen) following the instructions provided in the IRT manual. The IRT assigned the subject to one stratum according to the collected information (ie, deflazacort daily regimen, deflazacort intermittent regimen, other steroids daily regimen, other steroids intermittent regimen) and then to a treatment arm in a 2:1 ratio to receive givinostat or placebo.

Subjects receiving givinostat or placebo received medication indistinguishable in appearance. Personnel involved in the study remained blinded at all times, unless under exceptional circumstances when knowledge of the study drug was essential for treating the subject, such as in case of an AE. If the Investigator or authorised designee decided to break the code of a subject, it was suggested that the monitoring team (medical monitor or CRA) would be consulted if possible before breaking the code; however, this was not mandatory because the decision of the Investigator could not be influenced, deleted, or approved by the monitoring team.

Code breaking could be performed by the Investigator or authorised designee at any time by using the proper module of the IRT system. If the code was broken, treatment with the study drug was to be stopped. In any case, after breaking the code, the Investigator was to record the date, time, and reason for breaking the code in the eCRF, and was to notify the monitoring team as soon as possible.

To prevent subject unblinding with interruption of study treatment or increased laboratory monitoring due to for instance platelets count reduction or increase of triglycerides, the study site personnel who collected the primary and secondary efficacy data such as data from functional tests/strength and MRI/MRS was different from the personnel who reviewed subjects' safety results. Moreover, safety

results were not to be shared with the personnel responsible for the functional tests and MRI/MRS. For documentation, clinical sites were requested to sign the blinded plan for each role. Specific rules were also set up for Veristat data management and statistical teams. To mitigate the risk of subject unblinding, a blinding plan was also followed by the applicant´s team based on different role and access to the clinical data.

The IDMC reviewed, evaluated, and categorised safety findings every 3 months during the study and was responsible for the oversight of the interim analysis at which futility was assessed, and a blinded sample size re-estimation was made. The IDMC had access to un-blinded data, if necessary, and operated according to the rules defined in the IDMC charter.

Statistical methods

Sample Size Determination

A pre-specified interim analysis was conducted to evaluate the effects of givinostat versus placebo on the VL MFF in terms of futility. Futility was not met and the IDMC concluded the study should continue. A pre-planned blinded sample size reassessment was then performed which provided a revised, blinded within treatment standard deviation (SD) estimate for the change from Baseline in time to 4SC at 18 months of 3.094 seconds, and a revised sample size (utilizing a 2:1 randomization scheme) of N = 102, which provides 90% power and a 1-sided alpha of 2.5% to detect a true difference of 2 seconds between givinostat and placebo in the Target Population, for the change from Baseline in 4SC at 18 months. Therefore, with an estimated drop-out rate of 8%, a total of 110 ambulant male patients were to be randomised in the Target Population (Group A). Up to 50 patients (approximately 35% of the Overall Population) could also be recruited in the Off-target Population (Group B).

Analysis Sets

The intent-to-treat (ITT) Analysis Set - Target Population served as the basis for the formal analysis of efficacy. The MR Cohort formed the basis for analysis of all MRI/MRS parameters. Demography and Baseline characteristics were summarised based on the Safety Analysis Set for the Overall and Target Populations.

- ITT Analysis Set: includes all randomized patients who received at least one dose of study drug and who had at least one post-Baseline 4SC measure or missing post-Baseline 4SC measure due to being either non-ambulatory or otherwise physically unable to perform the assessment, irrespective of any deviation from the protocol or premature discontinuation. Each patient treatment group was assigned at randomisation. The treatment group assignment in this analysis set was defined as the treatment group assigned at randomisation.
- MR Cohort: includes all patients in the Target Population who were randomized to study treatment and completed at least one post-Baseline MRI/MRS assessment.
- Safety Analysis Set: includes all randomized patients who received at least one dose of the study drug. The treatment group assignment in this analysis set was defined as the treatment actually received.

Analysis Methods

For the continuous efficacy variables, change from Baseline to 18 months after treatment with givinostat or placebo was analysed using an analysis of covariance (ANCOVA). Least squares (LS) means and associated 2-sided confidence intervals (CIs) were estimated for givinostat and placebo. Treatment effect, i.e., the difference in LS means (givinostat-placebo), is presented with the associated 2-sided CI and p-value. Where necessary, log transformation of variables for analysis was discussed prior to unblinding and performed with back transformation applied for the presentation of results.

Analysis of cumulative loss of function on the NSAA over 18 months of treatment with givinostat or placebo was performed using negative binomial regression. The estimated cumulative number of failures at 18 months and associated 2-sided CIs are presented for each treatment group. The treatment effect, i.e., the ratio of cumulative failures (givinostat/placebo) is also presented with the associated 2-sided CI and p-value.

Binary efficacy variables, such as the proportion of patients losing ambulation during the study, were analysed using logistic regression. The odds ratio comparing the treatment groups is presented, together with its associated 2-sided CI and p-value.

Time to event variables (e.g., time to persistent loss of standing) were analysed using Cox proportional hazards modelling. The hazard ratio is presented, together with its associated 2-sided CI and p-value.

Missing data for continuous values for a subject were classified and imputed as shown below:

Table 7 Handling of missing data

Classification	Definition	Imputation Method
1	Patient had missing data for reasons other than being non-ambulatory or physically unable to perform the test/assessment.	Mean of all non-missing values for the respective measurement and time point across all patients randomized to the respective treatment and in the respective concomitant steroid stratum and Group (i.e., Group A or Group B) was used.
2	Patient had missing data due to being non-ambulatory or otherwise physically unable to perform the test/assessment.	Setting the value to zero or to twice the maximum non-missing value recorded across all patients depending on the directionality of the test.

Source: Study 48 CSR, Appendix 16.1.9, Section 4.4.4.

In addition to the methods described for handling missing data, to assess the impact of missingness a 'tipping point' sensitivity analysis of the primary outcome was performed.

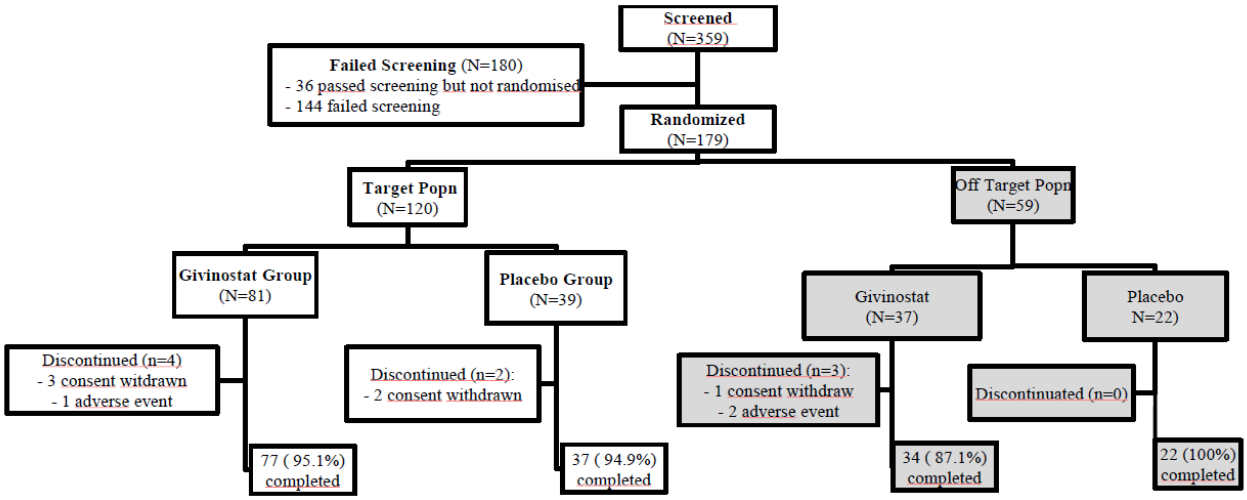
The p-value applicable to the analysis of 4SC at 18 months (ITT Analysis Set - Target Population) is ≤ 0.05 (2-sided). The formal analysis of the key secondary efficacy endpoints at 18 months was performed using an adjustment for multiple comparisons (Hochberg) procedure in the Target Population of the ITT Analysis Set, see Study 48 CSR, Appendix 16.1.9, Section 4.4.5. All other analyses are considered supportive only and therefore, nominal p-values are presented with a significance level of ≤ 0.05 (2-sided).

Summaries, plots or analyses of efficacy or safety data presented by subgroup (total compliance, total weight-adjusted exposure, starting dose and Baseline age) were produced where there were ≥ 5 patients per treatment group in at least 2 of the respective subgroup categories.

Results

Participant flow

Figure 6 Disposition of subjects (overall population)



Abbreviations: N, number of subjects randomized; n, number of subjects meeting the criterion; Popn, population.
Note: Target population included subjects with a baseline vastus lateralis muscle fat fraction in the range of > 5% to ≤ 30%, as assessed by magnetic resonance spectroscopy.
Source: Study data listings

The most common reasons for screen failure were not meeting inclusion criterion #8* of protocols version ≤4.0 (subjects who were eligible according to the protocol-specified functional algorithm predictive of VL MFF that should be in the range > 10% but < 30%) (79 [22.0%] subjects) and inclusion criterion #6 (subjects who had time to rise from the floor of <10 seconds at screening) (25 [7.0%] subjects), and meeting exclusion criterion #6 (subjects who had a 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]) (20 [5.6%] subjects). There were 36 subjects who past screening but were not randomised, e.g. those who were unable to travel for MRI/MRS; failed screening as off-target group or the OFF target population was already closed.

Recruitment

Target population: A total of 81 subjects were enrolled in the givinostat group, of whom 77 (95.1%) subjects completed the study (see Table 12, clinical AR). Of the 4 (4.9%) subjects who prematurely discontinued the study, 3 (3.7%) subjects withdrew consent, and 1 (1.2%) subject withdrew because of AEs. A total of 39 subjects enrolled in the placebo group, of whom 37 (94.9%) subjects completed the study. Two (5.1%) subjects prematurely discontinued the study because of withdrawal of consent.

Overall population: A total of 118 subjects were enrolled in the givinostat group, of whom 111 (94.1%) subjects completed the study). Of the 7 (5.9%) subjects who prematurely discontinued, 4 (3.4%) subjects withdrew consent, and 3 (2.5%) subjects withdrew due to AEs. A total of 61 subjects were enrolled in the placebo group, of whom 59 (96.7%) subjects completed the study. Two (3.3%) subjects prematurely discontinued due to withdrawal of consent.

Conduct of the study

The original study protocol was dated 07 May 2016. The study protocol was revised multiple times throughout the study, i.e. 7 global amendments, with the most notable changes being the revision of stopping rules for safety issues, revision of in- and exclusion criteria and the treatment regimen, e.g., dose modification rules. There were additional some amendments requested by the French Agency that were implemented only for the French patients.

In addition, before protocol 6.0, analyses were based on the included patient population i.e. patients with a VL MFF range > 10% and ≤ 30%. With amendment 4.0 resulting in protocol 6.0 (dated 19 October 2018) the "predictive enrichment" approach has been implemented, i.e. the primary, target population for formal statistical analysis has been defined to be those children with a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range > 5% and ≤ 30%, defined as Group A, while the off-target population (Group B) was defined to include subjects with a baseline VL MFF in the range ≤ 5% or >30%. This has changed the study population. A sensitivity analysis was requested in EMA protocol assistance to evaluate whether there is no effect modification before and after change in study population (EMEA/H/SA/3097/2/2019/PA/PED/III). This has been provided and there was no impact.

For both, Target Population and the Overall Population, the proportion of subjects who had at least 1 major protocol deviation was overall balanced across the treatment groups. In the Target Population, major protocol deviations were reported in 14 (17.3%) subjects and 6 (15.4%) subjects in the givinostat and placebo groups, respectively. The most common major protocol deviations in both groups were related to assessments performed outside the time window because of COVID-19 or categorised as "Other." For the Overall Population, a total of 38 major protocol deviations were reported in 20 (16.9%) subjects and 12 (19.7%) subjects in the givinostat and placebo groups, respectively. Two (1.7%) subjects in the givinostat group had major protocol deviations which were related to "eligibility criteria not met", of whom both reported major protocol deviations because of change of steroid treatment before the start of the study drug. Of these 2 subjects, 1 subject was in the Target Population and the other subject was not in the Target Population, both randomised to givinostat.

Baseline data

Target Population: The majority of subjects in the Safety Analysis Set were White and the most common ethnicity was not Hispanic or Latino (91.7% and 92.5%, respectively). 79 (65.8%) subjects were enrolled in Europe, 1 (0.8%) in Israel, 12 (10%) in Canada and 28 (23.3%) in the United States. Across the treatment groups, the mean (SD) age was 9.67 (1.944) years (min 6.1, max 14.2). The mean (SD) height was 124.4 (7.37) cm, the mean (SD) body weight was 30.70 (7.960) kg and the mean (SD) body mass index (BMI) was 19.68 (4.062) kg/m^2.

Overall Population: The majority of subjects in the Safety Analysis Set were White and the most common ethnicity was not Hispanic or Latino (91.1% and 93.3%, respectively). 120 (67%) subjects were enrolled in Europe, 1 (0.6%) in Israel, 15 (8.4%) in Canada and 43 (24.0%) in the United States. Across the treatment groups, the mean (SD) age was 9.84 (2.039) years (min 6.1, max 15.9). The mean (SD) height was 125.6 (8.62) cm, the mean (SD) body weight was 31.68 (9.554) kg and the mean (SD) body mass index (BMI) was 19.77 (4.192) kg/m^2.

Baseline clinical characteristics

Table 8 Target and overall populations (safety analysis set)

	Target Population		**Overall Population**	
	Givinostat (N = 81)	Placebo (N = 39)	Givinostat (N = 118)	Placebo (N = 61)
Time since diagnosis (years) Mean (SD) Median Min ; Max	 5.70 (2.523) 5.50 0.4 ; 10.6	 5.60 (2.890) 5.30 0.5 ; 12.3	 5.45 (2.601) 5.40 0.1 ; 11.4	 5.62 (2.720) 5.30 0.5 ; 12.3
DMD mutation, n (%) Deletion Duplication Point mutation	 58 (71.6) 13 (16.0) 10 (12.3)	 28 (71.8) 9 (23.1) 2 (5.1)	 83 (70.3) 17 (14.4) 18 (15.3)	 40 (65.6) 14 (23.0) 7 (11.5)
Time since steroid initiation (years) Mean (SD) Median Min ; Max	 3.71 (2.055) 3.40 0.5 ; 8.9	 3.76 (2.136) 3.60 0.5 ; 8.2	 3.62 (2.095) 3.40 0.4 ; 8.9	 3.97 (2.131) 4.00 0.5 ; 9.2
Type of steroid, n (%) Prednisone Deflazacort Other	 10 (12.3) 66 (81.5) 5 (6.2)	 10 (25.6) 29 (74.4) 0	 20 (16.9) 91 (77.1) 7 (5.9)	 15 (24.6) 45 (73.8) 1 (1.6)
Steroid schedule n (%) Daily Intermittent Every other day 10 days ON- 5 days OFF 10 days ON- 10 days OFF Only during the weekend 20 days ON- 10 days OFF Other	 69 (85.2) 12 (14.8) 1 (1.2) 1 (1.2) 8 (9.9) 2 (2.5) 0 0	 31 (79.5) 8 (20.9) 2 (5.1) 1 (2.6) 2 (5.1) 3 (7.7) 0 0	 99 (83.9) 19 (16.1) 1 (0.8) 1 (0.8) 14 (11.9) 3 (2.5) 0 0	 48 (78.7) 13 (21.3) 4 (6.6) 1 (1.6) 4 (6.6) 4 (6.6) 0 0
4 SC (seconds) Mean (SD) Min, Max	 3.39 (1.16) 1.8 ; 6.8	 3.48 (1.16) 1.8 ; 6.7	 3.58 (1.25)	 3.60 (1.27)
6MWT (meters) Mean (SD) Min; Max	 403.31 (72.11) 196.0 ; 551.0	 399.64 (63.83) 304.0 ; 550.0	 398.27 (70.93)	 393.72 (61.43)
Time to rise from floor (seconds) Mean (SD) Min; Max	 5.57 (1.86) 2.8 ; 12.3	 5.59 (1.96) 2.9 ; 11.0	 6.89 (7.43)	 6.76 (7.31)
VL MFF (%) Mean (SD) Min ; Max	MR Cohort (n = 77) 15.45 (6.37) 5.0 ; 29.0	MR Cohort (n = 37) 15.95 (6.66) 6.0 ; 29.0	15.45 (6.37)	15.95 (6.65)
NSAA score Mean (SD) Min; Max	 24.44 (5.09) 14.0 ; 34.0	 24.67 (4.79) 13.0 ; 33.0	 23.92 (5.37)	 24.16 (4.95)
R/L knee extension strength (knee strength) (N/kg) Mean (SD)	 R: 1.83 (0.81) L: 1.78 (0.80)	 R: 1.76 (0.80) L: 1.70 (0.81)	 R: 1.80 (0.82) L: 1.73 (0.80)	 R: 1.77 (0.92) L: 1.70 (0.91)
R/L elbow flexion strength (elbow strength) (N/kg) Mean (SD)	 R: 1.24 (0.50) L: 1.23 (0.50)	 R: 1.23 (0.51) L: 1.22 (0.48)	 R: 1.25 (0.49) L: 1.23 (0.48)	 R: 1.27 (0.51) L: 1.28 (0.53)

Numbers analysed

Table 9 Number of subjects by analysis populations (target population)

	Givinostat (N=81) n (%)	Placebo (N=39) n (%)	Overall (N=120) n (%)
All enrolled subjects	81 (100.0)	39 (100.0)	120 (100.0)
Intent-to-treat analysis set	81 (100.0)	39 (100.0)	120 (100.0)
Safety analysis set	81 (100.0)	39 (100.0)	120 (100.0)
PK analysis set	80 (98.8)	0	80 (66.7)
MR cohort	77 (95.1)	37 (94.9)	114 (95.0)

Abbreviations: MR, magnetic resonance; N, number of subjects randomized; n, number of subjects meeting the criterion; PK, pharmacokinetic.
Note: (%) = n/N × 100.
Source: Table 14.1.1.1.1.3

Table 10 Number of subjects by analysis populations (overall population)

	Givinostat (N=118) n (%)	Placebo (N=61) n (%)	Overall (N=179) n (%)
All enrolled subjects	118 (100.0)	61 (100.0)	179 (100.0)
Intent-to-treat analysis set	118 (100.0)	61 (100.0)	179 (100.0)
Safety analysis set	118 (100.0)	61 (100.0)	179 (100.0)
PK analysis set	117 (99.2)	0	117 (65.4)
MR cohort[5]	77 (65.3)	37 (60.7)	114 (63.7)

Abbreviations: MR, magnetic resonance; N, number of subjects randomized; n, number of subjects meeting the criterion; PK, pharmacokinetic.
Note: (%) = n/N × 100.
Source: Table 14.1.1.1.1.1

Outcomes and estimation

Primary efficacy endpoint: mean change in time to Climb 4 Stairs (4SC) before and after 18 months of treatment – Givinostat versus Placebo

Primary analysis:

Table 11 Time (seconds) to 4SC and change from baseline at 18 months of treatment: Givinostat versus placebo (ITT analysis set - target population)

	Givinostat (N = 81)		Placebo (N = 39)	
	Observed	CFB	Observed	CFB
Baseline (seconds), mean (SD)	3.39 (1.162)	-	3.48 (1.158)	-
18 months (seconds), mean (SD)	4.70 (3.154)	1.31 (2.626)	6.37 (7.698)	2.89 (7.069)

4SC, climb 4 standard stairs; CFB, change from Baseline; ITT, intent-to-treat; N, number of patients in the analysis set; SD, standard deviation.
Note: Baseline 4SC was defined as the measurement taken at the randomisation assessment unless missing, in which case Baseline was taken as the last non-missing value recorded prior to or on the date of first study treatment. At each time point, only patients with a value at both Baseline and that time point were included in the CFB column.
Source: Study 48 CSR, Table 14.2.1.1.1.

Table 12 Analysis of time (seconds) to 4SC, change from baseline at 18 months (ITT analysis set – target population)

Time to 4SC	Givinostat (N = 81)	Placebo (N = 39)
Log Scale Analysis		
GLS mean (log scale SE) (95% CI)	1.27 (0.040) (1.172, 1.372)	1.48 (0.058) (1.317, 1.657)
GLS mean ratio (givinostat/placebo) (log scale SE) (95% CI) p-value	0.86 (0.071) (0.745, 0.989) 0.0345	
No Log Scale Analysis		
LS mean (95% CI)	1.25 (0.311, 2.181)	3.03 (1.666, 4.394)
Difference in LS means (givinostat-placebo) (95% CI) p-value	-1.78 (-3.462, -0.106) 0.0374	

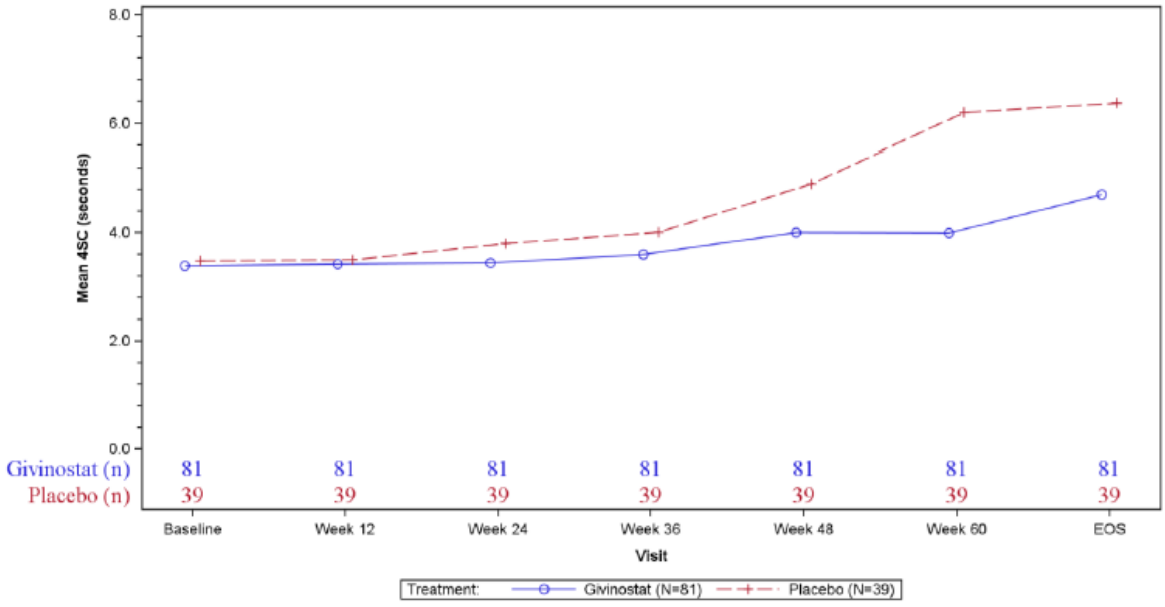
4SC, climb 4 standard stairs; 10MWR, walk or run 10 metres; CI, confidence interval; EOS, end of study; GLS mean, generalised least square mean; ITT, intent-to-treat; LS, least square; N, number of patients in the analysis set; SE, standard error.
Notes: Baseline 4SC was defined as the measurement taken at the randomisation assessment unless this was missing in which case Baseline was taken as the last non-missing value recorded prior to or on the date of first study treatment.
LS means, CIs, and p-values were obtained from an analysis of covariance model on change from Baseline in 4SC at 18 months with Baseline values for: 4SC, time to rise from floor, time to 10MWR, distance walked in 6 minutes and re-derived age at first dose fitted as covariates, with concomitant steroid use and treatment group as independent classification factors. For the analysis where log transformation was applied, data were log transformed prior to calculating change from Baseline and the results from the analysis were then back transformed for presentation. GLS mean change from Baseline should therefore be interpreted as a rate change (EOS/Baseline).
Source: Study 48 CSR, Table 14.2.1.2.1

In the primary analysis (analysis of log-transformed values) of change from baseline in time to climb 4 stairs (4SC) in seconds at month 18, a statistically significant difference was demonstrated when givinostat was compared against placebo (p = 0.0374). While the time to 4SC increased in the givinostat group from 3.39 (SD 1.162) to 4.70 (SD 3.154) seconds it increased even more in the placebo group from 3.48 (SD 11.158) to 6.37 (7.698) seconds. The GLS mean (log scale SE) was 1.27

(0.040) and 1.48 (0.058) for givinostat and placebo, respectively, with a GLS mean ratio (95% CI) of 0.86 (0.071). This represents a 14% less increase in the time to climb 4 stairs at month 18 in favour of givinostat when compared to placebo.

In the analysis performed on non-log-transformed data, the LS mean was 1.25 and 3.03 seconds for givinostat and placebo, respectively, with a difference in LS means of - 1.78 seconds in favour of givinostat (p=0.0374).

Figure 7 Plot of mean time (seconds) to climb 4 standard stairs by treatment over time (ITT analysis set – target population)



Abbreviations: 4SC, 4-stair climb; EOS, end of study; ITT, Intent-to-Treat population; N, number of subjects in the analysis set; n, number of subjects meeting the criterion.
Source: Figure 14.2.1.1.1

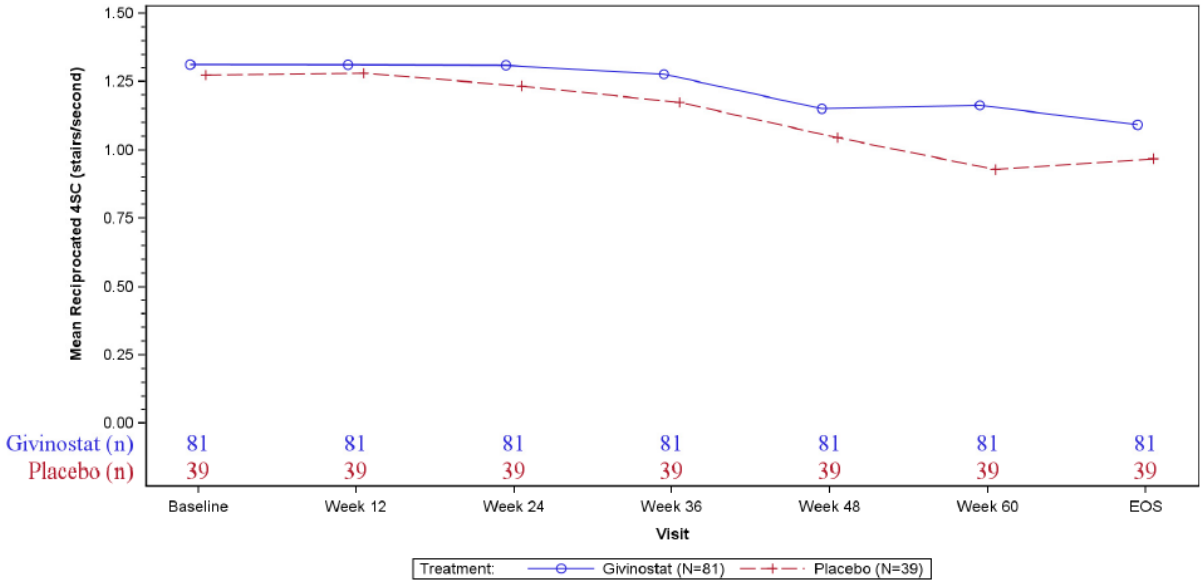
Mean time (seconds) to 4SC by treatment over time is plotted in the figure above. A clear separation is observed from week 48 onwards.

Supportive analysis:

Functional Adaptation Ascent Grading Assessments for the Four Stairs Climb: No subject had a deterioration to Grade 1 (unable to climb 4 standard stairs) for functional adaptation of 4SC from baseline to 18 months after treatment with givinostat, whereas 1 (2.6%) subject in the placebo had a deterioration to Grade 1 from Grade 5 (climbs 4 standard stairs alternating feet, needs handrail for support) at baseline. Overall, a lower backward shift (indicator of treatment effect) in grades for functional adaptation of 4SC was observed in the givinostat group compared with the placebo group.

Velocity (stairs/second) to Climb 4SC

Figure 8 Plot of mean velocity (stairs/second) to climb four standard stairs (reciprocated 4SC) by treatment over time (ITT analysis set - target population)



Imputation of missing data has been applied as described in sections 4.4.4.1 and 4.4.4.2 of the SAP, prior to reciprocal transformation to convert the timed results to units of velocity (where Velocity (stairs/second) = 4 / Completion time (seconds)).
EOS=End of Study.
N = the number of subjects in the analysis set.
n = the number of subjects with a measurement.
Source: Listing 16.2.6.1.1
PROGRAM NAME:F_4SC_03.SAS DATE OF RUN:06JUL2022 TIME OF RUN:11:03:05 DATE OF EXTRACTION:09MAY2022

Mean changes in 4SC velocity from baseline until week 48 were - 0.161 and - 0.229 stairs/second and - 0.220 and - 0.306 stairs/second until EOS for the givinostat and the placebo group, respectively.

Baseline 4SC velocity was included as a covariate in the ANCOVA model to compare the change from baseline in 4SC velocity between givinostat and placebo. 4SC velocity decreased in the givinostat group from 1.311 stairs/second at baseline to 1.091 stairs /second at EOS (LS mean change from baseline -0.22 stairs/second) and decreased in the placebo arm from 1.273 stairs/second at baseline to 0.967 stairs/second at EOS (LS mean change from baseline -0.31 stairs/second), demonstrating a non-statistically significant difference in LS means for 4SC velocity of 0.09 stairs /second; p-value = 0.1696. Hence, results on 4SC velocity are not consistent with those on the primary analysis of the primary endpoint.

A transformation of the primary endpoint result on the log scale to the reciprocal scale of velocity using the delta theorem was performed post-hoc. This analysis provided an estimate of the difference in 4SC velocity between givinostat and placebo of 0.03 tasks/second [95% CI: 0.00, 0.07] which is nominally statistically significant (p-value = 0.0285).

The random coefficient model estimated annual change (slope) of -0.12 and -0.19 stairs/second (p value: 0.0717) for givinostat and placebo, respectively for 4SC velocity (calculated as reciprocal 4/completion time [seconds]).

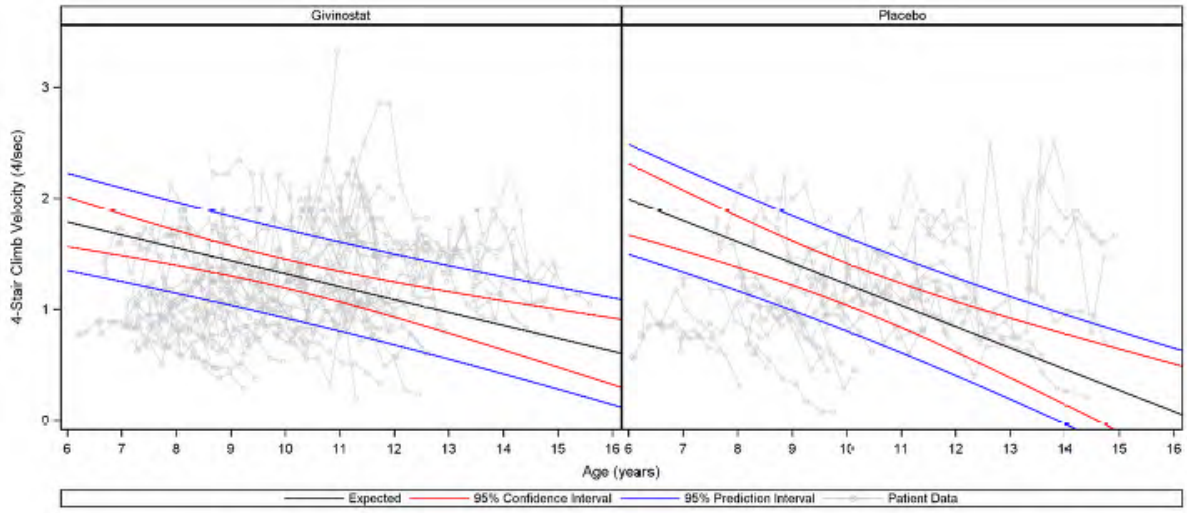
Table 13 Random coefficient analysis 4-stair climb velocity / group 1 ITT set – target population

Parameter	Number of subjects in the analysis	Term	Estimate (95% CI)	p-value
4-Stair Climb Velocity (4/sec)	120	Intercept	3.11 (2.445, 3.782)	<0.0001
		Age	-0.19 (-0.251, -0.127)	<0.0001
		Treatment group	-0.61 (-1.430, 0.201)	0.1376
		Age x Treatment group	0.07 (-0.006, 0.146)	0.0717

Note 1: Imputation of missing data has been applied as described in sections 4.4.4.1 and 4.4.4.2 of DSC/14/2357/48 SAP.
Note 2: Baseline 4SC is defined as the measurement taken at the randomisation assessment unless this is missing in which case baseline will be taken as the last non-missing value recorded prior to or on the date of first study treatment.
Note 3: Estimates, CIs and p-values are obtained from on a mixed random coefficient analysis with 4SC velocity (4/sec) as dependent variable, age as random effect, treatment group as fixed effect, an interaction between treatment and age and a random intercept. Pre-Baseline assessments have been discarded from the analysis.
Cross-reference: Listing 16.2.6.1.1 from study DSC/11/2357/48

Program (Date Time): t14 2 1 1 3.sas (19Jan2023 14:54) SAS Version: 9.4
Page 1 of 1

Figure 9 Study 48: random coefficient analysis, 4SC velocity (ITT analysis set – target population)



4SC, climb 4 standard stairs; ITT, intent-to-treat.
Note: Annual change (slope) for the givinostat group = Age coefficient estimate + Age × Treatment Group coefficient estimate, in Study 48 Post-hoc Analysis, Table 14.2.1.1.3
Source: Study 48 Post-hoc Analysis, Figure 14.2.1.10.

The provided random coefficient analysis indicated that age was the nominally statistically significant covariate for decrease in 4SC velocity (p-value: < 0.0001). The model demonstrated that 4SC velocity worsens with advancing age. Givinostat did not significantly prevent the decline in 4SC velocity as compared to placebo (difference in annual change in 4SC velocity between givinostat and placebo: -0.07 stairs/second; p-value: 0.0717).

Sensitivity Analyses:

Analyses Assessing the Impact of the COVID-19 Pandemic on the Study

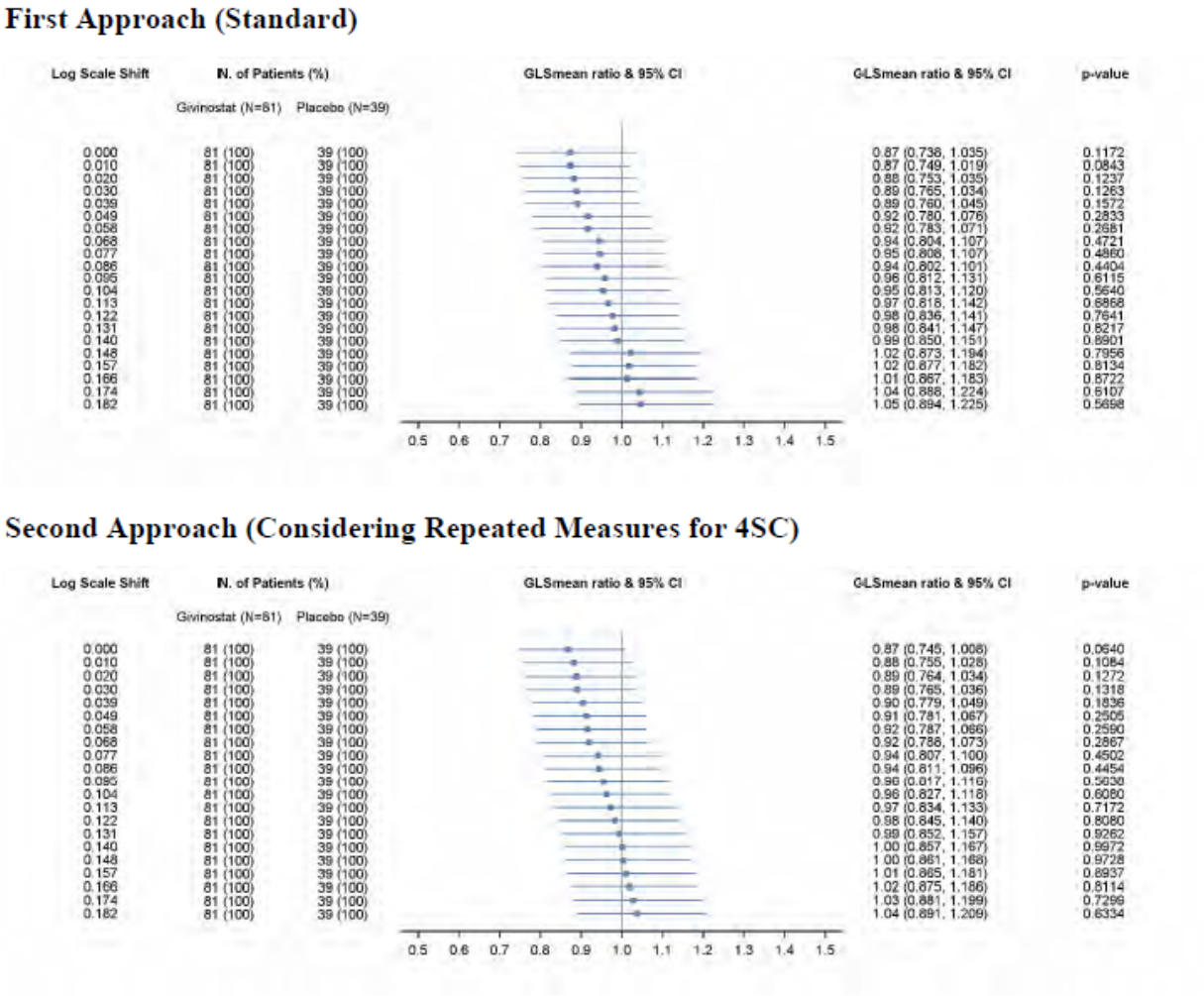
An analysis of time (seconds) to 4SC on change from baseline at 18 months excluding the patients who completed their Month 18 time to 4SC assessment outside of the protocol defined window was performed. Similar to the primary analysis, the treatment effect was statistically significantly higher for givinostat (n = 61) versus placebo (n = 28) (GLS mean ratio: 0.83 [95% CI: 0.701, 0.990]; p = 0.0385).

Additionally, an analysis of time (seconds) to 4SC on change from baseline at 18 months excluding subjects completing the Month 18 assessment outside of the extended visit window has been performed (n = 75, n = 37 for the givinostat and the placebo group, respectively). The treatment effect was comparable to the one observed in the main analysis, although it missed statistical significance (GLS mean ratio: 0.87 [95% CI: 0.746, 1.004]; (p = 0.0570).

Results of analyses of time to 4SC with and without adjustment for MRS VL MFF were in line with those of the primary analysis. The treatment effect from baseline until EOS in both analyses was comparable to the one observed in the main analysis, although it missed statistical significance in the analysis adjusted for MRS VL MFF [unadjusted analysis: (GLS mean ratio: 0.84 [95% CI: 0.729, 0.972]; (p = 0.0196); adjusted analysis: (GLS mean ratio: 0.88 [95% CI: 0.758, 1.022]; (p = 0.0923)].

Tipping Point Analysis

Figure 10 Study 48: forest plot for tipping point analysis results considering log scale shift (ITT analysis set – target population)



4SC, climb 4 standard stairs; CI, confidence interval; N, number of patients in the analysis set; SE, standard error.
Notes: Plotted mean difference and CIs were standardised to be presented on the same scale. In addition, for 4SC, a natural log transformation has been applied before standardising for SE. First approach is standard approach. Second approach considered repeated measures for 4SC.
Source: Study 48 Post-hoc Analysis, Figure 14.2.1.7 and Figure 14.2.1.8.

The results of the tipping point analysis considering no shift showed a treatment effect of 0.87 (GLSmean ratio) for both Tipping Point analysis approaches similar to that obtained in the primary 4SC analysis (0.86; GLSmean ratio). Standard error on the other hand increased to 0.085 and 0.077, respectively, for both Tipping Point analysis approaches compared to 0.071 from the primary analysis. Difference between first and second approach stands in the inclusion of 4SC repeated measures for the Class 1 imputation in the latter.

As the primary analysis utilised single imputation methods for handling all missing data, the standard error may have been underestimated due to ignoring the uncertainty of the imputed values. This is confirmed by the tipping point sensitivity analysis using a multiple imputation, which resulted in greater standard errors. As the results from the sensitivity analysis are no longer statistically significant, the robustness of the results from the primary analysis is questionable (see section **2.6.6 Discussion on clinical efficacy** for further evaluation).

Key secondary endpoints

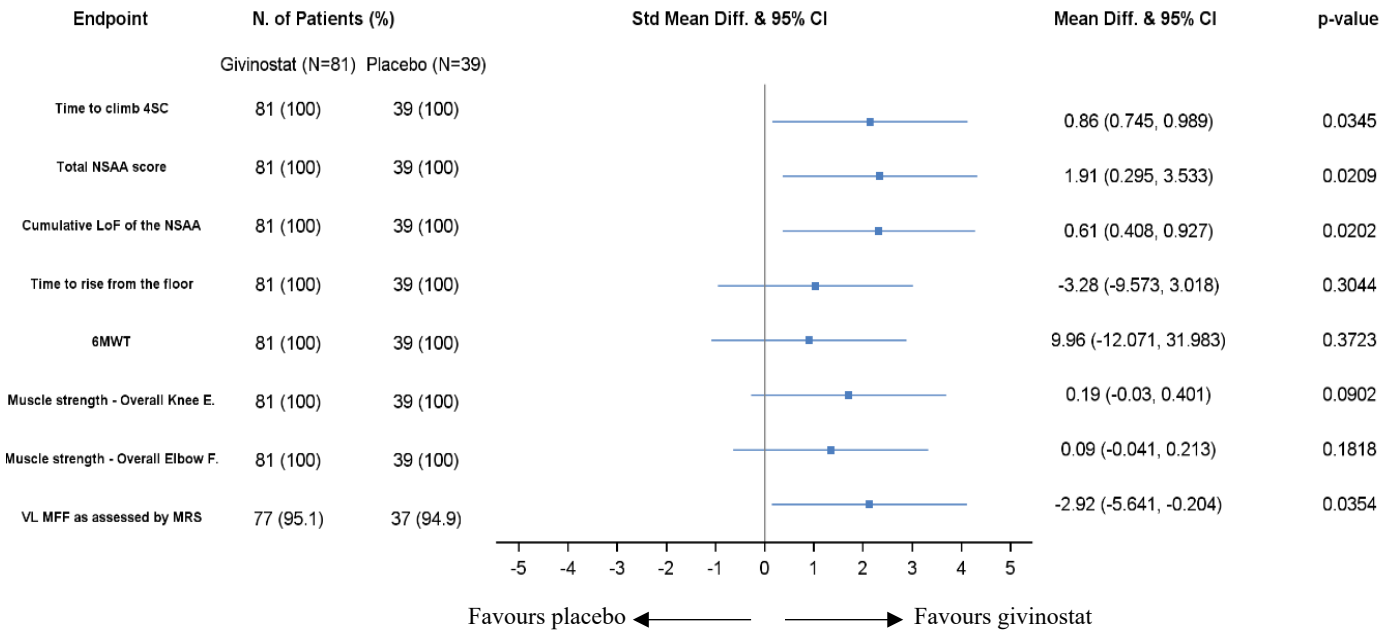
Final analysis:

Table 14 Summary of P-values and adjusted confidence intervals for the treatment effect on each key secondary efficacy endpoint at 18 months following the Hochberg procedure (ITT analysis set - target population)

Endpoint	p-value	Adjusted CI	Adjusted Alpha	Significant
Distance walked in 6 minutes	0.3723	-12.071, 31.983	0.0500	No
Time to rise from floor	0.3044	-10.497, 3.942	0.0250	No
Muscle strength: overall elbow flexion	0.1818	-0.069, 0.241	0.0167	No
Muscle strength: overall knee extension	0.0902	-0.090, 0.462	0.0125	No
Fat fraction of vastus lateralis muscles	0.0354	-6.519, 0.674	0.0100	No
NSAA total score	0.0209	-0.282, 4.110	0.0083	No
Cumulative loss of function	0.0202	0.350, 1.081	0.0071	No

CI, confidence interval; ITT, intent-to-treat; MR, magnetic resonance; NSAA, North Star Ambulatory Assessment.
Notes: Two-sided p-values were presented for the relevant treatment effect obtained from the appropriate analysis of each endpoint. Following the Hochberg procedure, significance was determined by comparing each 2-sided p-value with a significance level of 0.05/i, where i represents the position of the endpoint in descending order of p-values from least to most significant. As soon as the ith endpoint had a corresponding p-value <0.05/i, the treatment effects for that endpoint and all the endpoints following it were considered significant.
Adjusted 2-sided 95%/i CIs for the treatment effects were presented, where i represents the position of the endpoint in descending order of p-values from least to most significant.
For the endpoint of "Fat fraction of vastus lateralis muscles," analysis was performed on the MR cohort.
Source: Study 48 CSR, Table 14.2.6.3

Figure 11 Forest plot of primary and key secondary efficacy endpoints - Givinostat versus placebo (ITT analysis set - target population)



4SC, climb 4 standard stairs; 6MWT, 6-minute walk test; CI, confidence interval; E., extension; F., flexion; GLS, generalised least square; ITT, intent-to-treat; LoF, loss of function; MR, magnetic resonance; MRS, magnetic resonance spectroscopy; N, number of patients in the analysis set; NSAA, North Star Ambulatory Assessment; SE, standard error.
Notes: Plotted mean difference and CIs were standardised to be presented on the same scale. In particular each GLS/LS mean difference, upper and lower limit has been divided by its related SE. In addition, for 4SC and cumulative loss of function, a natural log transformation has been applied before standardising for SE.
Cumulative LoF of the NSAA estimates are obtained from a negative binomial regression. Direction of interpretation has been fixed accordingly.
VL MFF analysis is performed under the MR cohort population.
Source: Study 48 Post-hoc Analysis, Figure 14.2.1.1.

Based on the pre-specified Hochberg analysis, none of these endpoints reached formal statistical significance. However, results for NSAA total score, NSAA cumulative loss of function and VL MFF reached nominally statistical significance.

North Star Ambulatory Assessment

Mean (SD) total NSAA scores decreased (worsened) until EOS (Week 72) and were 21.89 (6.481) and 19.87 (6.974) in the givinostat and placebo groups, respectively. Mean (SD) changes from baseline for givinostat were -1.32 (4.248) at Week 48, -1.46 (4.402) at Week 60, and -2.56 (4.450) at EOS, whereas mean (SD) changes from baseline for placebo were -3.41 (3.837) at Week 48, -5.49 (5.675) at Week 60, and -4.79 (4.652) at EOS.

Overall, a higher number of subjects had NSAA assessments completed outside of visit windows at EOS (31 [25.8%] subjects) followed by Week 60 (13 [10.8%] subjects) and Week 48 (10 [8.3%] subjects).

The LS mean (95% CI) change from baseline in the NSAA total score for givinostat and placebo was - 2.66 (-3.563, -1.759) and -4.58 (-5.891, -3.260), respectively. The LS means difference in the NSAA total score of 1.91 in favour of givinostat was nominally statistically significant (p = 0.0209). However, the difference is lower than a published minimally clinically important difference of 2.32 points (Haberkamp 2019).

In the sensitivity analysis (n =61 and n = 28 for givinostat and placebo, respectively), excluding those patients who completed their 18 Month assessment outside of the protocol defined window the LS mean difference was 1.57 (p = 0.1053). In the sensitivity analysis (n= 75 and n = 37 for givinostat and placebo, respectively) excluding those subjects who completed the Month 18 assessment outside

of the extended visit window, results were comparable to those of the main analysis (LS mean difference: 1.82; p = 0.0372).

Cumulative loss of function on the NSAA

Mean (SD) NSAA items failed scores at baseline were 1.0 (1.49) and 0.9 (1.52) in the givinostat and placebo groups, respectively. Mean (SD) cumulative post-baseline NSAA items failed were 4.1 (4.93) and 7.4 (8.03) in the givinostat and placebo groups, respectively. The percentage of patients with NSAA failures varied for different items over time, always favouring the givinostat group.

The ratio of cumulative failures of NSAA scores (givinostat/placebo) was 0.61 (p = 0.0202).

Sensitivity analyses to address the impact of the COVID-19 pandemic, i.e. excluding the patients who completed their Month assessment outside of the protocol-defined window (ratio of cumulative failures: 0.61; p = 0.0435) and excluding subjects who completed the Month 18 assessment outside of the extended visit window (ratio of cumulative failures: 0.62; p = 0.0194) were in line with the primary analysis.

Time to rise from floor

The time to rise from floor in seconds from baseline through EOS increased in the givinostat group from 5.57 to 14.43 seconds and in the placebo group from 5.59 to 19.17 seconds with mean changes of 8.86 seconds and 13.59 seconds, respectively.

In the analysis of change from baseline at 18 months as presented above, the LS mean was 9.33 and 12.61 seconds for givinostat and placebo, respectively, with a LS mean difference of -3.28 seconds in favour of givinostat which was not nominally statistically significant (p-value = 0.3044).

Sensitivity analyses to address the impact of the COVID-19 pandemic, i.e. excluding the patients who completed their Month assessment outside of the protocol-defined window (LS mean difference: -4.45 seconds [95% CI: -12.522, 3.629]; p = 0.2764) and excluding subjects who completed the Month 18 assessment outside of the extended visit window (LS mean difference: -2.64 seconds [95% CI: -9.254; 3.972]; p = 0.4301) were in line with the primary analysis.

Time to rise from floor velocity decreased from baseline to EOS in the givinostat group from 0.198 (0.0600) to 0.151 (0.0854) rises/sec and in the placebo arm from 0.198 (0.0612) to 0.118 (0.0744) rises/sec with mean changes of -0.047 rises/sec and -0.080 rises/sec for givinostat and placebo, respectively.

LS mean change in time to rise from floor velocity from baseline to 18 months was – 0.05 rises/sec in the givinostat group and – 0.08 rises/sec in the placebo group. The LSM difference for givinostat from placebo was 0.03 rises/sec and reached nominal statistical significance (p = 0.0124). The slower decrease in time to rise from the floor velocity under givinostat can be considered clinically relevant as it is greater than the published minimally clinically important difference for time to rise from floor of 0.023 rises/sec (Duong 2021).

6-Minute Walking Test

From baseline to 18 months, the mean (SD) 6MWT distance decreased from 403.31 (72.110) to 365.11 (81.083) metres in the givinostat group and from 399.64 (63.832) to 350.78 (91.248) metres in the placebo group.

In the analysis of change from baseline in the 6MWT distance at 18 months, the LS mean change for givinostat and placebo was –38.43 and -48.38 meters, respectively. The LSM difference for change from baseline for givinostat against placebo was 9.96 meters (p = 0.3723) and therefore not clinically relevantly different.

Sensitivity analyses to address the impact of the COVID-19 pandemic, i.e. excluding the patients who completed their Month assessment outside of the protocol-defined window (LSM difference: 18.18; p = 0.1805) and excluding subjects who completed the Month 18 assessment outside of the extended visit window (LSM difference: 11.35; p = 0.3344) were in line with the primary analysis.

Muscle Strength Evaluated by Knee Extension and Elbow Flexion

In the analysis of change from baseline at 18 months no statistical difference was observed between treatment groups when muscle strength was evaluated by knee extension or elbow flexion and normalised by subject weight (N/kg).

Knee extensor and elbow flexor muscle strength overall slightly decreased but lower in the givinostat group compared to placebo. With respect to the overall knee extension, the LS mean change for givinostat and placebo was – 0.32 and -0.50, respectively. The LSM difference for givinostat against placebo was 0.19 (p = 0.0902). With respect to the overall elbow flexion, the LS mean change for givinostat and placebo was – 0.10 and -0.19, respectively. The LSM difference for givinostat against placebo was 0.09 (p = 0.1818).

Sensitivity analyses to address the impact of the COVID-19 pandemic, i.e. excluding the patients who completed their Month assessment outside of the protocol-defined window and excluding subjects who completed the Month 18 assessment outside of the extended visit window were in line with the primary analysis, except for the analyses, when muscle strength was evaluated by knee extension after excluding subjects completing the Month 18 assessment outside of the protocol-defined window (LS mean difference: 0.25 N/kg [95% CI: 0.051, 0.456]; p = 0.0146). However, the mean difference of 0.10 N/kg (95%CI: 0.036,0.244) was not nominally statistically significant (p=0.1429) when muscle strength was evaluated by elbow flexion.

Fat Fraction of Vastus Lateralis Muscle (Only in the Magnetic Resonance Cohort)

The LS mean (95% CI) in change from baseline in VL FF for the Target Population in the MR cohort at 18 months for givinostat and placebo was 7.63 (6.098, 9.172) and 10.56 (8.331, 12.783), respectively. The LSM difference for givinostat against placebo was -2.92 and nominally statistically significant (p = 0.0354). Results indicated that treatment with givinostat delayed fat infiltration by approximately 30% after 18 months.

Exploratory endpoints

Time to Walk/Run 10 Metres

Mean time to 10MWR increased (worsened) over time in both the givinostat group (from 5.17 seconds at Baseline to 6.04 seconds at EOS) and the placebo group (from 5.18 seconds at Baseline to 7.99 seconds at EOS). However, mean (SD) change from Baseline in time to 10MWR at EOS was lower in the givinostat group (0.63 [1.489] seconds) compared with placebo (2.81 [8.214] seconds). Analysis of change from Baseline in mean time to 10MWR at 18 months showed a nominally statistically significant treatment effect (LS mean difference: -2.35 seconds, p-value: 0.0140).

Time to 10MWR velocity decreased (worsened) over time in both the givinostat group (from 2.01 metres/second at Baseline to 1.80 metres/second at EOS) and the placebo group (from 1.99 metres/second at Baseline to 1.60 metres/second at EOS). However, mean (SD) change from Baseline in 10MWR velocity at EOS was lower in the givinostat group (-0.134 [0.3419] metres/second) compared with the placebo group (-0.390 [0.3263] metres/second). Analysis of change from Baseline in 10MWR velocity at 18 months showed a nominal statistically significant treatment effect (LS mean difference: 0.21 metres/second; p-value: 0.0011), indicating clinically relevance.

PODCI scores: In an analysis of change from Baseline in PODCI standardised global function scale

scores at 18 months, the difference in LS means (givinostat-placebo) was not nominally statistically significant (LS mean difference: 2.96 points; p-value: 0.1824).

Percent-Predicted 6-minute Walking Test: Mean (SD) percent-predicted 6MWD at baseline was 65.80% (11.462%) and 65.10% (10.509%) for the givinostat and placebo groups, respectively. The mean (SD) percent-predicted 6MWD at EOS was 59.56% (13.094%) and 57.07% (14.808%) for the givinostat and placebo groups, respectively. At EOS, mean (SD) changes from baseline percent-predicted 6MWD were -6.24% (9.149%) and -8.03% (11.675%) for the givinostat and placebo groups, respectively. In an analysis of percent-predicted 6MWT by change from baseline at 18 months the difference in LS means (givinostat-placebo) percent-predicted 6MWT was not statistically significant (LS mean difference: 1.65; p-value: 0.3715) for change from baseline at 18 months.

Fat Fraction of Thigh Muscles by Magnetic Resonance Imaging: An analysis of change from Baseline in fat fraction (%) in 5 thigh muscles (VL, biceps femoris long head, semitendinosus, quadriceps and hamstrings) as measured by MRI at 18 months was performed. Difference in LS means (givinostat-placebo) was nominally statistically significant in all 5 thigh muscles (VL: -3.95% [p-value: 0.0033]; biceps femoris long head: -4.44% [p-value: 0.0235]; semitendinosus: -4.43% [p-value: 0.0009]; quadriceps: -4.19% [p-value: 0.0009]; hamstrings: -4.38% [p-value: 0.0026]), demonstrating treatment effect.

Time to 10% Persistent Worsening in the 6-minute Walking Test: The median time to ≥ 10% persistent worsening of 6MWT (months) was 14.4 months and not calculable in the givinostat and placebo groups, respectively. The difference among treatment groups (givinostat vs placebo) was not statistically significant (hazard ratio 0.86; 95% CI: 0.485, 1.514; p-value: 0.5942).

Proportion of Subjects With ≥ 10% Worsening in the 6-minute Walking Test: The number of subjects who achieved ≥ 10% persistent worsening in the 6MWT at EOS were 41 (50.6%) in the givinostat group and 19 (48.7%) in the placebo group. The difference among treatment groups (givinostat vs placebo) was not statistically significant (odds ratio: 1.09; 95% CI: 0.466, 2.563; p value: 0.8378).

Time to Loss of Standing: A subject was considered to have persistent loss of standing if they were unable to stand for at least 2 consecutive assessments. Because there was only 1 subject who lost the ability to stand up (met the criterion of persistent loss of standing) during the study, the time to loss of standing could not be evaluated.

Proportion of Subjects Who Lost Ambulation During the Study: There was only 1 subject in the placebo group who lost 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: There were no strong signals that givinostat might be more efficacious in a given DMD mutation, LTBP4 genotype or SPP1 genotype.

Ancillary analyses

A composite endpoint, defined by ranking each endpoint separately at Baseline and Month 18 (using Wilcoxon rank sum test ranking approach), such that low rank is equal to poor and high rank is equal to good, and then performing the sum was evaluated post-hoc. The following endpoints formed the basis of the composite endpoint: Time to 4SC (seconds), Physical function as measured by NSAA total score, Time to rise from floor (seconds), 6MWT (metres), 10MWR (seconds). The composite endpoint analysis was conducted to check for trending clinically meaningful givinostat effect. A comparison between treatment groups (givinostat vs placebo) was performed using an ANCOVA model with the sum of the change from baseline (at Month 18) as dependent variable, treatment group and

concomitant steroid use (according to the 4 strata: Deflazacort daily regimen, Deflazacort intermittent regimen, Other steroids daily regimen, Other steroids intermittent regimen) included as fixed effects and age at first dose with study treatment and baseline composite score as covariates. The difference between the adjusted means for givinostat and placebo was calculated with the relative 95% CI and p-value, p = 0.0039). The proposed composite endpoint is not clinically validated and could therefore not be considered meaningful in the context of supportive evidence of efficacy. Furthermore, the rank-based treatment effect is not based on a clinically interpretable scale.

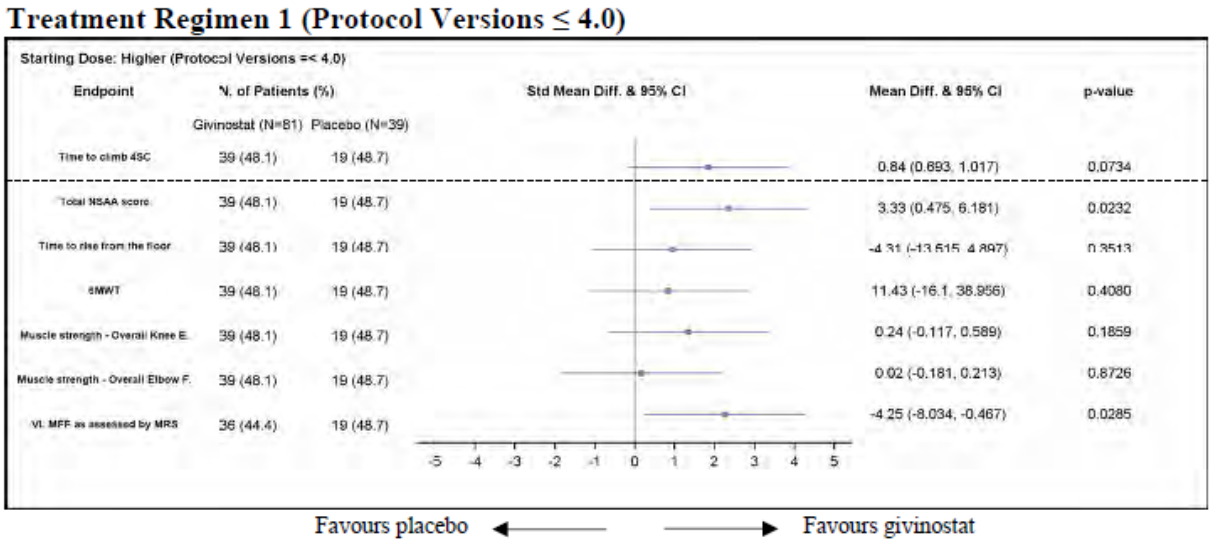
Pre-specified subgroup analyses in the Target Population for the primary (time to 4SC) and the secondary functional endpoints (NSAA, time to rise from floor and 6MWT), muscle strength and muscle morphology (VL MFF) were performed to further explore the treatment effect of givinostat on treatment compliance (> 80% / ≤ 80%), total weight-adjusted exposure (≥ Median/< Median), starting dose (Higher / Lower) and age (6 to 7 years; > 7 years).

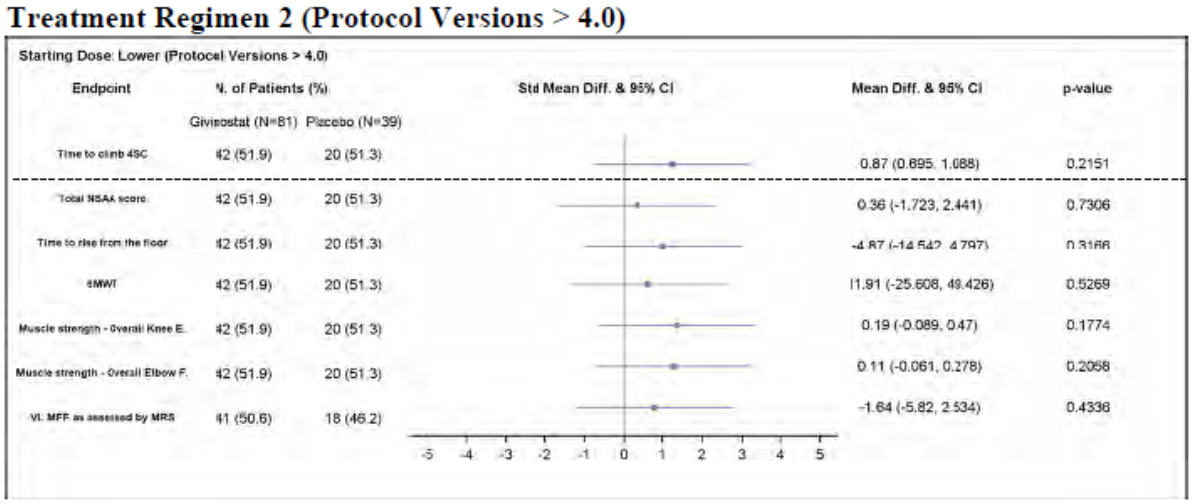
Since only 3 patients had a treatment compliance ≤ 80%, i.e., 2 patients in the givinostat group and 1 patient in the placebo group, subgroup analyses of **total compliance** were not applicable.

Overall, only subgroups based by total **weight-adjusted exposure** ≥ median were in support of the main analyses. However, according to the applicant, these results should be interpreted with caution. PopPK analyses of givinostat showed a non-linear relationship between givinostat exposure and weight. As a result, givinostat dose was adjusted to patient's weight with larger weight-adjusted doses administered to patients with lower body weight. Therefore, according to the applicant, the two subgroups by total weight-adjusted exposure (mg/kg) were not well balanced with respect to baseline characteristics, making it difficult to draw conclusions.

Starting dose

Figure 12 Forest plot for primary and key secondary results by starting dose (ITT analysis set – target population)





4SC, climb 4 standard stairs; 6MWT, 6-minute walk test; CI, confidence interval; E, extension; F, flexion; GLS, generalised least square; ITT, intent-to-treat; LS, least square; MR, magnetic resonance; MRS, magnetic response spectroscopy; N, number of patients in the analysis; NSAA, North Star Ambulatory Assessment; SE, standard error; VL MFF, vastus lateralis muscle fat fraction.

Note: Plotted mean difference and CIs were standardised to be presented on the same scale. In particular each GLS/LS mean difference, upper and lower limit has been divided by its related standard error. In addition, for 4SC a natural log transformation has been applied before standardising for SE.

VL MFF analysis is performed in the MR Cohort.

Source: Study 48 Post-hoc Analysis, Figure: 14.2.1.2.

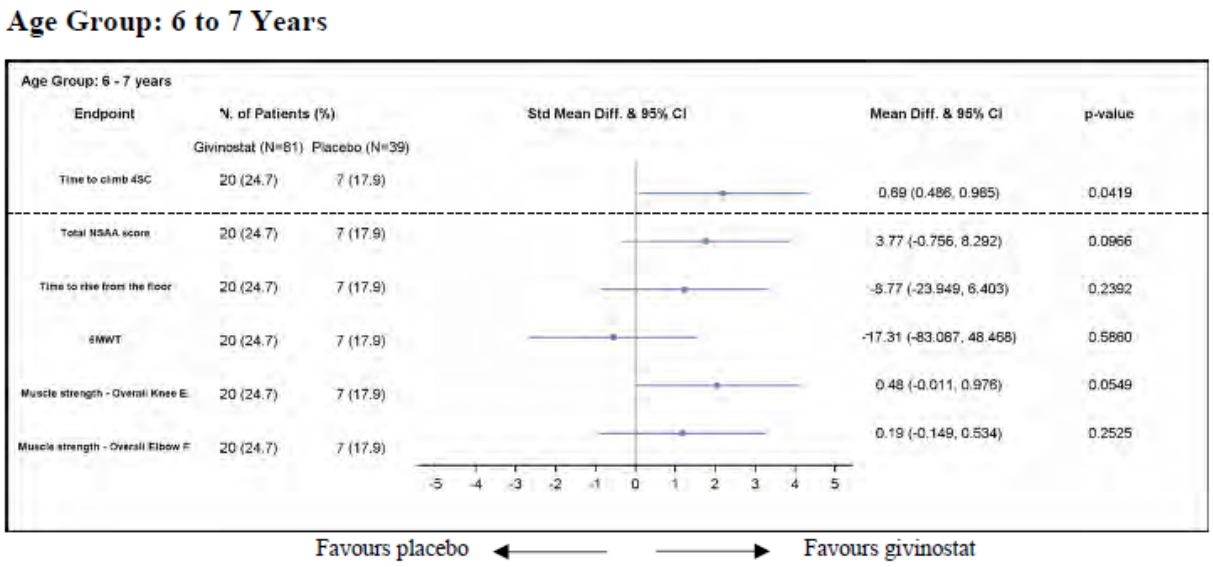
To determine the impact of the starting dose on the efficacy of givinostat, subgroup analyses for change from Baseline at 18 months were performed, comparing the subgroup of patients who started on the higher dose as specified in protocol versions ≤ 4.0 (Treatment Regimen 1) with the subgroup of patients who started on the lower dose as specified in protocol versions > 4.0 (Treatment Regimen 2).

There were 39 and 19 subjects in the givinostat and the placebo group, respectively who started treatment under protocol versions ≤ 4 compared to 42 and 20 subjects in the givinostat and the placebo group, respectively, who started treatment under protocol version >4.

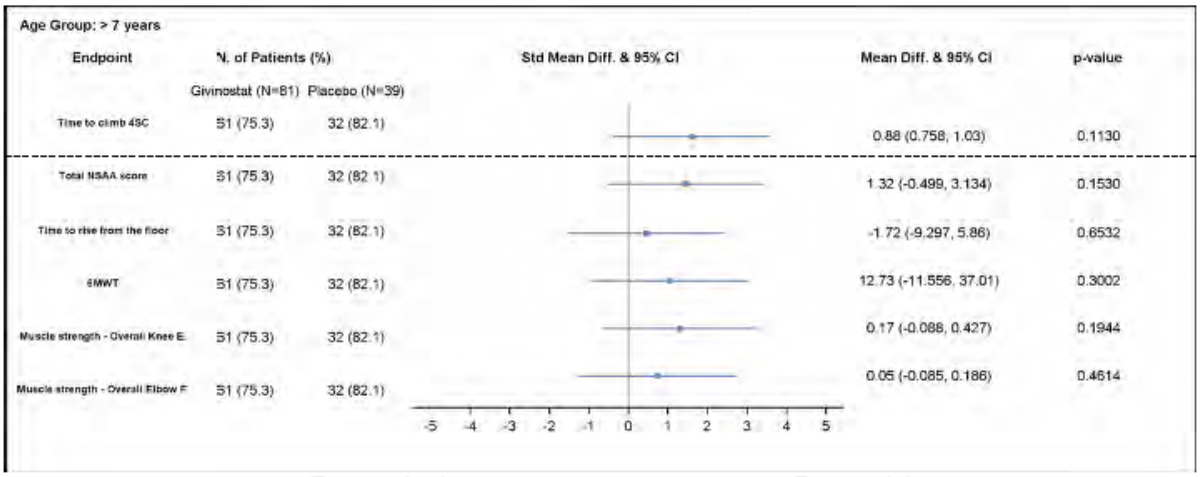
For the NSAA Total Score, the treatment effect was quantitatively smaller under Treatment Regimen 2; this result was at least in part due to a better performance of the placebo group under this Treatment Regimen. For Treatment Regimen 1, LS mean change from Baseline in NSAA total score was -2.24 points in the givinostat group and -5.57 points in the placebo group (LS mean difference: 3.33 points; p-value: 0.0232), whereas, for Treatment Regimen 2, LS mean change from Baseline in NSAA total score was -3.13 points in the givinostat group and -3.49 points in the placebo group (LS mean difference: 0.36 points; p-value: 0.7306). Overall, results in these two subgroups were in line with those in all patients of the target population, showing a positive trend in the givinostat group when compared to the placebo group for the primary and the key secondary endpoints. Nevertheless, no statistical significance could be shown as the study was not powered for any subgroup analyses.

Baseline age

Figure 13 Forest plot for primary and key secondary results by age group (ITT analysis set – target population)



Age Group: > 7 Years



4SC, climb 4 standard stairs; 6MWT, 6-minute walk test; CI, confidence interval; E, extension; F, flexion; GLS, generalised least square; ITT, intent-to-treat; LS, least square; MRS, magnetic response spectroscopy; N, number of patients in the analysis; NSAA, North Star Ambulatory Assessment; SE, standard error.
Note: Plotted mean difference and CIs were standardised to be presented on the same scale. In particular each GLS/LS mean difference, upper and lower limit has been divided by its related SE. In addition, for 4SC a natural log transformation has been applied before standardising for SE.
Source: Study 48 Post-hoc Analysis, Figure 14.2.1.3.

To determine the impact of Baseline age on the efficacy of givinostat, subgroup analyses for change from Baseline at 18 months were performed by baseline age 6 to 7 years of age and above 7 years of age.

Overall, results in these two subgroups were in line with those in all patients of the target population, except that a negative trend was observed in the givinostat group when compared to the placebo group for 6MWT in the subgroup of patients aged 6 to 7 years at baseline. Nevertheless, no statistical significance could be shown for any positive or negative treatment effect in the subgroup analyses.

Analysis on Change in Dosing Instructions

The dosing regimen was changed from Treatment Regimen 1 (starting Dose A with the option to reduce to Dose B) to Treatment Regimen 2 (starting Dose B with the option to reduce to Dose C) during the conduct of Study 48. The change in treatment regimen was introduced in Protocol Amendment 4, therefore, the Sponsor has conducted analyses to determine whether the change in dosing instructions affected the efficacy results in Study 48. An analysis of covariance (ANCOVA) was performed by adding class factor for 'pre' and 'post' protocol amendment along with an interaction term between dosing schema and randomised treatment. The interaction between randomised treatment and treatment regimen was non-significant.

Table 15 ANCOVA test of study 48 primary endpoint assessing the potential impact of changes to dosing instructions introduced in protocol amendment 4

		With Interaction		Without Interaction					
Endpoint	**Treatment Regimen**	**N**	**Random Treatment x Treatment Regimen p-value**	**N**	**LS Means Givinostat**	**LS Means Placebo**	**Treatment Effect Givinostat vs Placebo**	**95% CI**	**p-value**
4SC Log (ratio)	1	58	0.628	120	1.27	1.48	0.86	0.75, 0.99	0.035
	2	62							

CI, confidence interval; 4SC, 4-stair climb; LS, least square; N, number of patients.
Source: Study 48 Post Hoc Statistical Analysis, Tables 14.2.1.1.4.

In order to evaluate whether the givinostat treatment effect of the primary endpoint was disproportionately driven by the results of Dose A, patients on givinostat were grouped into those who had stayed on Dose A for the full study (Group A) and those patients in Treatment Regimen 1 who reduced the dose, together with patients in Treatment Regimen 2 (Group Not A). The results show that givinostat treatment effect versus placebo was comparable in Groups A and Not A.

Table 16 Treatment comparison of group A vs group not A

Endpoint	Treatment Comparison	N	Treatment Effect	95% CI
4SC Log (Ratio)	Group A vs Placebo	21 vs 39	0.82	0.67, 1.00
	Group Not A vs Placebo	60 vs 39	0.87	0.75, 1.01

CI, confidence interval; 4SC, 4-stair climb; N, number of patients.
Source: Study 48 Post Hoc Statistical Analysis, Tables 14.2.1.1.11.

2.6.5.3. Summary of main efficacy results

The following table summarises the efficacy results from the main study, study DSC/14/2357/48. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 17 Summary of efficacy for trial DSC/14/2357/48 (i.e. study 48)

Title: Randomised, Double Blind, Placebo Controlled, Multicentre Study to Evaluate the Efficacy and Safety of Givinostat in Ambulant Patients with Duchenne Muscular Dystrophy.		
Study identifier	DSC/14/2357/48 EudraCT number 2016-000401-36 IND number 126598	
Design	Randomised, double-blind, placebo-controlled, multicentre study in ambulant patients with Duchenne muscular dystrophy	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	18 months not applicable not applicable
Hypothesis	Superiority	

Treatments groups	givinostat (G)		Givinostat, 18 months of treatment, 118 randomised patients
	placebo (P)		Placebo, 18 months of treatment, 61 randomised patients
	In- target givinostat (G)		Givinostat, 18 months of treatment, 81 randomised patients
	In- target placebo (P)		Placebo, 18 months of treatment, 39 randomised patients
Endpoints and definitions	Primary endpoint	4SC (sec)	Mean change in 4 Stairs Climb (4SC) before and after 18 months of treatment of givinostat versus placebo
	Key Secondary endpoint	TTR (sec)	Mean change in time to rise from floor (TTR) before and after 18 months of treatment of givinostat versus placebo
	Key Secondary endpoint	6MWT (m)	Mean change in 6 minutes walking test (6MWT) before and after 18 months of treatment of givinostat versus placebo
	Key Secondary endpoint	NSAA	Mean change in North Start Ambulatory assessment (NSAA) before and after 18 months of treatment of givinostat versus placebo
	Key Secondary endpoint	NSAA cumulative loss	Cumulative loss of function on the NSAA in 18 months of treatment
	Key Secondary endpoint	HHM (N/Kg)	Mean change of normalized muscle strength evaluated by knee extension, elbow flexion as measured by hand-held myometry before and after 18 months of treatment of givinostat versus placebo
	Key Secondary endpoint	VL MFF (%)	Mean change in vastus lateralis muscle fat fraction (VL MFF) as measured by Magnetic Resonance before and after 18 months of treatment of givinostat versus placebo
Database lock	09 May 2022		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent-to-treat / Target, time point= 18 months The **all-enrolled subjects** set for the target population included all subjects in the target population who were randomised to study treatment. The **intention-to-treat** (ITT) analysis set for the target population included all subjects in the target population who were randomised, received at least 1 dose of study drug, and had at least one non missing post-baseline 4SC measure or missing post-baseline 4SC measure due to being either non ambulatory or otherwise physically unable to perform the assessment, irrespective of any deviation from the protocol or premature discontinuation.			
Descriptive statistics and estimate variability	Treatment group	givinostat (G)	placebo (P)	
	Number of subjects	81	39	
	Change in 4 Stairs Climb (4SC) (mean) (SD)	1.31 (2.626)	2.89 (7.069)	

Effect estimate per comparison	Primary endpoint	Comparison groups	givinostat (G) / placebo (P)	
		Geometric Least Square Mean Ratio	0.86	
		Confidence Interval	(0.745, 0.989)	
		P-value	0.0345	
Notes	Estimates are obtained from an analysis of covariance (ANCOVA) model on change from baseline in 4SC at Month 18. Since log transformation was applied, data were log transformed prior to calculating change from baseline and the results from the analysis were then back transformed for presentation. GLS mean change from baseline should therefore be interpreted as a rate change (EOS/baseline). The pre-specified primary analysis used single imputation methods for handling missing data.			
Analysis description	**Key Secondary analysis**			
Analysis population and time point description	Intent-to-treat /Target, time point= 18 months The **all-enrolled subjects** set for the target population included all subjects in the target population. The **intention-to-treat** (ITT) analysis set for the target population included all subjects in the target population who were randomised, received at least 1 dose of study drug, and had at least one non missing post-baseline 4SC measure or missing post-baseline 4SC measure due to being either non ambulatory or otherwise physically unable to perform the assessment, irrespective of any deviation from the protocol or premature discontinuation. The **Magnetic Resonance** (MR) analysis set for the target population included all subjects in the target population who were randomised to study treatment and completed at least one post-baseline MRI/MRS assessment. These subjects were be expected to perform the magnetic resonance assessment at baseline, 12 and 18 months.			
Descriptive statistics and estimate variability	Treatment group	givinostat (G)	placebo (P)	
	Number of subjects (ITT/Target)	81	39	
	Change in TTR (mean)	8.86	13.59	
	Change in TTR (Standard Deviation)	17.649	20.528	
	Change in 6MWT (mean)	-38.20	-48.86	
	Change in 6MWT (Standard Deviation)	55.516	70.836	
	Change in Total NSAA (mean)	-2.56	-4.79	
	Change in Total NSAA (Standard Deviation)	4.450	4.652	
	Cumulative loss in NSAA (mean)	4.1	7.4	

	Cumulative loss in NSAA (Standard Deviation)	4.93	8.03	
	Change in HHM (mean) • Knee Extension ○ Left: ○ Right: • Elbow Flexion ○ Left ○ Right	 -5.98 -6.47 -0.47 0.12	 -8.93 -6.50 -0.59 -0.72	
	Change in HHM (Standard Deviation) • Knee Extension ○ Left ○ Right • Elbow Flexion ○ Left ○ Right	 20.295 21.542 12.526 12.309	 20.413 21.791 9.824 11.160	
	Treatment group	givinostat (G)	placebo (P)	
	Number of subjects (MR/Target)	77	37	
	Change in VL MFF (mean)	7.48	10.89	
	Change in VL MFF (Standard Deviation)	7.099	8.674	

Effect estimate per comparison	Key Secondary endpoint – TTR	Comparison groups	givinostat (G) - placebo (P)
		Least Square Mean	-3.28
		Confidence Interval	(-9.573, 3.018)
		P-value	0.3044
	Key Secondary endpoint – 6MWT	Comparison groups	givinostat (G) - placebo (P)
		Least Square Mean	9.96
		Confidence Interval	(-12.071, 31.983)
		P-value	0.3723
	Key Secondary endpoint – Total NSAA	Comparison groups	givinostat (G)- placebo (P)
		Least Square Mean	1.91
		Confidence Interval	(0.295, 3.533)
		P-value	0.0209
	Key Secondary endpoint – NSAA cumulative loss	Comparison groups	givinostat (G) / placebo (P)
		Ratio	0.61
		Confidence Interval	(0.408, 0.927)
		P-value	0.0202
	Key Secondary endpoint – HHM		
	• Overall Knee Extension	Comparison groups	givinostat (G) - placebo (P)
		Least Square Mean	0.19
		Confidence Interval	(-0.030, 0.401)
		P-value	0.0902
	• Overall Elbow	Comparison groups	givinostat (G) - placebo (P)

	Flexion	Least Square Mean	0.09
		Confidence Interval	(-0.041, 0.213)
		P-value	0.1818
	Key Secondary endpoint – VL MFF	Comparison groups	givinostat (G) - placebo (P)
		Least Square Mean	-2.92
		Confidence Interval	(-5.641, -0.204)
		P-value	0.0354
Notes	Estimates are obtained from an analysis of covariance (ANCOVA) model on change from baseline at Month 18, except for NSAA cumulative loss where they are obtained from a negative binomial regression. Formal analysis of key secondary endpoints at 18 months was performed using adjustment for multiple comparisons (Hochberg) procedure.		

2.6.5.4. Clinical studies in special populations

No dedicated clinical studies in special populations have been performed in the Duchenne population. Since the target patient population in Duchenne is a paediatric population, no elderly patients were included. This is in accordance with the life expectancy for this patient population.

2.6.5.5. In vitro biomarker test for patient selection for efficacy

Not applicable.

2.6.5.6. Analysis performed across trials (pooled analyses and meta-analysis)

Random Coefficient Analyses of 4SC Velocity

According to the applicant, comparison of the results of the post-hoc mixed-effect-random coefficient analyses of 4SC velocity from Study 51 (i.e., including Study 48 patients only) with those from Study 48 shows that decline in 4SC velocity over age is: similar in the givinostat and delayed givinostat groups in Study 51; similar in the givinostat group in Study 51 and the givinostat group in Study 48 and different between the givinostat and placebo groups in Study 48 (p-value: 0.0717) thus, demonstrating the persistence of efficacy of givinostat with long-term treatment. Interpretation of the results presented is limited. With regard to study 48, the model showed that 4SC velocity worsens with advancing age. Givinostat did not significantly prevent the decline in 4SC velocity as compared to placebo (p-value: 0.0717).

Results from these analyses from study 51 suggest similarity of givinostat treatment effect in the givinostat and delayed givinostat group. However, analysis also included in the givinostat arm subjects from the former off-target population.

Comparison of efficacy results from studies DSC/14/2357/48 and DSC/14/2357/51 with results from the natural history studies ImgagingDMD and CINRG

Integrated Analysis of Long-term Efficacy (Age at Persistent Disease Milestones)

The main objective of the ISE was to provide supportive analyses for the long-term efficacy evaluation of givinostat for the treatment of DMD, based on data from clinical studies. i.e., Studies 48 and 51, and the Natural History Studies ImagingDMD and CINRG.

Data was extracted from ImagingDMD and CINRG for patients who had Baseline characteristics aligning with the key inclusion/ exclusion criteria of Study 48 (i.e., ambulant males aged ≥ 6 years

with a confirmed diagnosis of DMD, Baseline 4SC assessments ≤ 8 seconds, Baseline time to rise from floor ≥ 3 to < 10 seconds, who were on stable corticosteroid treatment with no exposure to another investigational drug or to any dystrophin restoration product [e.g., ataluren, Exon-skipping] and no diagnosis of other uncontrolled neurological disease or presence of relevant uncontrolled somatic disorders unrelated to DMD).

The ITT Analysis Set defined in each study protocol was the primary population for the ISE. For the NH studies, patients with a Baseline and at least one follow-up assessment were included in the analysis. A propensity score analysis was implemented to establish a control group composed of 'matched' NH data, however, matching, was not sufficiently effective and therefore not further pursued.

As a result, the analyses were conducted in the 'full givinostat group' (ITT Analysis Set N = 148) and the 'full control group' (Natural History Data, N = 197) in the supportive integrated analysis of long-term efficacy of givinostat in patients with DMD.

Table 18 ISE groups

Group	Analysis	Givinostat	Control
1	Post-hoc analysis (Study 48)	Givinostat (dosages not taken into consideration)	Placebo
2	Integrated analysis (Study 48 and Study 51)[a]	Givinostat (dosages not taken into consideration)	ImagingDMD and CINRG

CINRG, Cooperative International Neuromuscular Research Group; ImagingDMD, Magnetic Resonance Imaging and Biomarkers for Muscular Dystrophy.

[a] Group 2 includes Study 51 givinostat-naïve patients (i.e., patients screened in Study 48 who were eligible for the Off-target Population but were never randomized as enrollment in the off-target group was complete); for whom Day 1 is considered the first day of givinostat dosing and excludes Study 51 delayed givinostat patients (i.e., patients who received placebo in Study 48).

Source: ISE SAP, Section 4.3

This integrated analysis evaluated the long-term effects of givinostat on function, including age at persistent disease milestones (e.g., loss of ambulation) through comparison of the givinostat group (i.e., all patients who received givinostat from the first day they were recruited to givinostat in Studies 48 or 51), with the control group comprised of NH data from patients in the ImagingDMD and CINRG studies who met the key inclusion/exclusion criteria of Study 48.

Selection of the following endpoints for the integrated analysis, for further evaluation of the long-term efficacy of givinostat was driven by the progressive nature of DMD: Age at persistent rise from floor > 10 seconds; Age at persistent 10MWR > 10 seconds; Age at persistent loss of rise from floor; Age at persistent loss of 4SC; Age at persistent loss of ambulation.

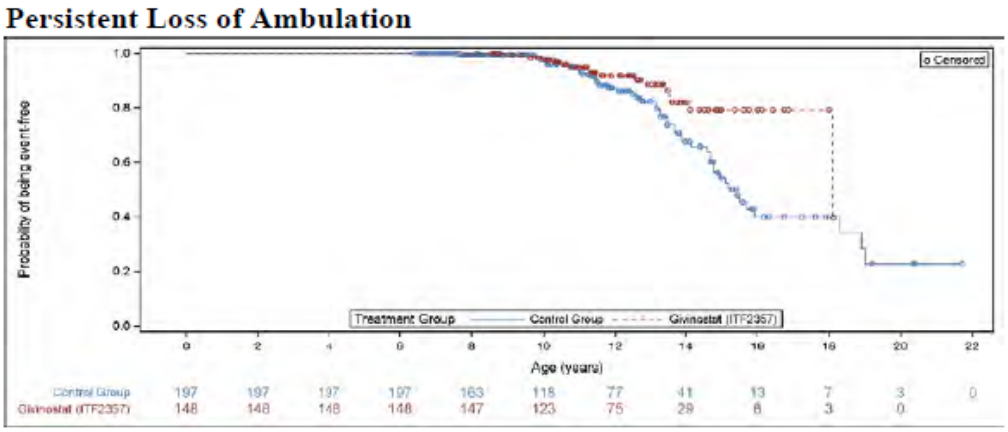
All patients in the integrated analysis were male (givinostat group [N = 148]; control group [N = 197]). Median (range) age of patients was higher in the givinostat group (9.95 [6.1 to 15.9] years) than in the control group (7.51 [5.9 to 14.1] years) however, median (range) age at last assessment was similar across both groups (12.13 [7.6 to 19.0] years and 11.34 [6.4 to 22.9] years, in the givinostat group and control group, respectively), indicating that the longer follow-up in the control group compensates for the younger age at Baseline. All the other baseline characteristics reflected the younger age of the control group at baseline. Deflazacort was the steroid used by the majority (79.7%) of patients in the givinostat group, whereas most patients (51.3%) in the control group used other steroids. Baseline values for functional assessments also reflect the higher median age of patients in the givinostat group. Although mean Baseline time to 4SC was the same (3.58 seconds) in both the givinostat group and the control group; mean Baseline 6MWT (NH data based on ImagingDMD only), mean Baseline time to rise from floor and mean Baseline 10MWR were higher in the givinostat group (396 metres, 6.62 seconds and 8.28 seconds, respectively) than in the control group (389

metres, 4.97 seconds and 5.38 seconds, respectively).

With regard to all disease milestones results indicate a delay by givinostat. In particular: Median age at persistent rise from floor > 10 seconds was delayed by 1.6 years (Hazard Ratio [95% CI]: 0.62 [0.45, 0.84]; p-value: 0.003); Median age at persistent 10MWR > 10 seconds was delayed by 3.5 years (Hazard Ratio [95% CI]: 0.43 [0.29, 0.64]; p-value: < 0.001); Median age at persistent loss of rise from floor was delayed by 2.2 years (Hazard Ratio [95% CI]: 0.68 [0.47, 0.97]; p-value: 0.034); Median age at persistent loss of 4 standard stairs climb (4SC) was delayed by 3.3 years (Hazard Ratio [95% CI] 0.42 [0.26, 0.67]; p-value: < 0.001); Median age at persistent loss of ambulation was delayed by 2.7 years (Hazard Ratio [95% CI] 0.48 [0.27, 0.86]; p-value: 0.014)

However, based on this approach, the results obtained are difficult to interpret and not representative, given the open-label study approach.

Figure 14 ISE*: Kaplan-Meier estimate of age at disease milestones (matching patients from ITT set and natural history data)*



4SC, climb 4 standard stairs; ITT, intent-to treat.
Notes: Patients not reaching a disease function event or lost to follow-up were censored on the last date they are known to be on study.
Circled values represent censored results.
Source: ISE, Figure 14.2.3.2.3, Figure 14.2.3.5.3 and Figure 14.2.3.6.3.

2.6.5.7. Supportive studies

An Open Label, Long-term Safety, Tolerability, and Efficacy Study of Givinostat in All DMD Patients Who Have Been Previously Treated in One of the Givinostat Studies Study (DSC/14/2357/51)

This study is an ongoing multicentre, open-label, long-term extension study to assess the safety, tolerability and efficacy of givinostat administered to previously givinostat treated and treatment naïve DMD subjects, all on concomitant corticosteroid treatment. The study initiation Date was 25 October 2017. Pre-specified interim cuts of the ongoing study 51 data were conducted with cut-off dates 30 November 2018, 15 November 2019, 31 December 2020 and 31 December 2021. The latter formed the basis for this report. New data were available with cut-off date 31 December 2023.

Baseline was defined as the last non-missing value (including scheduled and unscheduled assessments) prior to the subject receiving treatment on this study. Where applicable and possible, the End of Study visit of the subject's previous givinostat study where the subject was enrolled was the screening/baseline visit of this study.

There were three groups: 1. the givinostat group comprised patients randomised to givinostat in their previous givinostat study; 2. the delayed givinostat group comprised patients randomised to placebo in their previous givinostat study; and 3. the naïve givinostat group comprised DMD patients screened in study 48 who met all the inclusion criteria and none of the exclusion criteria but have never been randomised because enrolment in the off-target group was completed.

The primary objective was to assess the long-term safety and tolerability of givinostat in subjects with DMD following core protocols programme and with naïve givinostat DMD subjects.

Secondary objectives were to evaluate the effects of long-term administration of givinostat on muscular function and strength, on respiratory function and the impact on daily activities and quality of life (QoL) following long-term administration of givinostat.

Exploratory objectives were to compare the functional effects of long-term administration of givinostat between the givinostat and delayed givinostat treatment groups.

With regard to the requested inclusion criteria, subjects had to be ≥6 years and - had participated in study 43 or study 48 and attended the End of Study Visit or - had been screened in study 48 and met: all the inclusion criteria and none of the exclusion criteria, had a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range ≤5% or >30%, i.e., included in "off-target" group, but never been randomised because the enrolment in the off-target group was completed.

Givinostat oral suspension (10 mg/mL) was administered twice daily in a fed state. The starting dose of givinostat was the same level that the patient had received at the end of their previous givinostat study (i.e., Study 43 or Study 48). For givinostat-naïve patients, treatment was started at dose level B. The dose was adjusted per the individual patient's weight change throughout the study. Givinostat dose adjustments for safety reasons followed the criteria described in the study protocol.

Efficacy analyses were performed on the intention-to-treat (ITT) population, defined as all enrolled patients who had taken at least one dose of therapy and who had at least one post-Baseline efficacy assessment. Efficacy assessments for all subjects were: Performance of Upper Limb (PUL); Motor Function Measure (MFM); Pulmonary Function Test (PFT); Quality of life (QoL): assessed by PedsQL for paediatric patients (both child and parent versions), and SF-36 for adult patients; Time to first occurrence of major disease milestones (e.g., time to respiratory support needed during the day, time to scoliosis surgery, time to death).

All the other efficacy assessments differed between the ambulant and the non-ambulant subjects.

For ambulant subjects the following efficacy assessments were performed: Physical Function assessed by 6MWT and NSAA total score; Timed Function Tests: 4SC, Time to Walk 10 Meters (10MWR) and Time to Rise from Floor (TTR); time to loss of ambulation. Muscle Strength Assessed by Knee Extension and Elbow Flexion.

For non-ambulant subjects: (i.e., subjects who were unable to complete the 10m walk/run test in 30 seconds or less without any support or devices): Physical Function Assessed by Egen Klassification (EK) Total Score; Reports of activities of daily living (measured by Barthel Index Total Score); Upper Limbs Muscle Strength Assessed by Elbow Flexion (evaluated by handheld myometry).

Exploratory Assessments:

Comparison of the following endpoints between the delayed givinostat group (i.e. all subjects enrolled in placebo group during the Study 48 who were switched to givinostat in this study) and the results of the givinostat group (i.e., subjects already treated with givinostat in Study 48 or Study 43) of the following endpoints: Mean change from Baseline and week 48 and then yearly till the end of the study, in physical function as measured by the Performance of Upper Limb (PUL); Mean change of MFM from

Baseline and week 48 and then yearly till the end of the study; Mean change from Baseline and week 48 and then yearly till the end of the study, in respiratory function (e.g. FVC, FEV1, PEF); Time to major disease milestones (e.g. time to respiratory support needed during the day, time to scoliosis surgery, time to death) (Baseline through end of study).

For ambulant subjects: Mean change from Baseline and week 48 and then yearly till the end of the study, in physical function as measured by 6MWT, NSAA, Time function tests (e.g. time to rise from floor, time to 4SC, time to 10MWR); Mean change from Baseline and week 48 and then yearly till the end of the study in muscle strength (e.g. knee extension and elbow flexion) as measured by HHM; Time to major disease milestones (e.g. time to loss of ambulation).

For non-ambulant subjects: Mean change from Baseline and week 48 and then yearly till the end of the study in the Egen Klassifikation (EK) score; Mean change from Baseline and week 48 and then yearly till the end of the study in subject and/or parent/caregiver reports of activities of daily living as measured by Barthel Index, Mean change from Baseline and week 48 and then yearly till the end of the study in muscle strength (e.g. elbow flexion) as measured by HHM.

Statistical methods:

No hypothesis testing or statistical modelling was carried out for the secondary endpoints in Study 51. However, exploratory analyses were conducted to compare the givinostat group with the delayed givinostat group in the ITT Analysis Set.

'Change from Baseline' endpoints were analysed using a repeated measures model of change from Baseline at each timepoint. Least square means were estimated for the givinostat and delayed givinostat groups. Treatment effect, i.e., the difference between treatment group LS means, is presented, with the associated 95% CI and p-value. 'Time to event' endpoints were analysed with a Cox Proportional Hazards model. The hazard ratio (givinostat/delayed givinostat) is presented along with associated 95% CI and p-value. Proportional hazards tests were performed, examining log-log Kaplan Meier estimates over time and evaluating whether the curves were reasonably parallel. A mixed effect random coefficient analysis was used to analyse the impact of age on pulmonary function tests and physical function tests.

For the above endpoints, which were also analysed in Study 48, data was log transformed or not in alignment with the conclusions of the pivotal study. For the remaining endpoints, the need to apply log transformation was assessed after final database lock, by examining the ANCOVA model residuals from a model without a treatment effect term.

The handling of missing data mirrored the approach in Study 48; patients who lost ambulation after Baseline had results subsequent to the loss of ambulation imputed to worst case (Class 2).

All p-values presented for Study 51 are considered nominal.

For the pre-specified analyses, estimates may have been inaccurate, as patients from both Studies 43 and 48 were included in the givinostat group, whereas only Study 48 patients were included in the delayed givinostat group. Therefore, analysis of change from baseline and mixed effect random coefficient analyses were performed post-hoc for the ITT Analysis Set, excluding patients from Study 43.

At the cut-off date 31 December 2021 for the fourth interim analysis, a total of 194 subjects were enrolled in the study: 110 subjects in the givinostat group (subjects already treated with givinostat in Study 48 or Study 43), 54 subjects in the delayed givinostat group (subjects in placebo group during the Study 48 who were switched to givinostat in this study), and 30 subjects in the givinostat-naïve group. Seven subjects prematurely discontinued from the study, the majority of whom (5 [2.6%] patients) had withdrawn consent. The mean study duration was 564.7 days (SD=±374.26). At the cut-

off date 31 Dec 2023, a total of 207 subjects were screened and enrolled in the study.

Patient Demographics:

In the Safety Analysis Set (N = 194), patient demographics were almost similar across the 3 treatment groups.

Overall, patients had a median age of 11.45 years (6.1 to 17.5) with median age of 11.7 years, 11.4 years and 10.3 years in the givinostat, delayed givinostat and givinostat-naïve groups, respectively. The majority (183, 94.3%) of patients were ambulant at screening: 102 (92.7%) patients in the givinostat group, 51 (94.4%) patients in the delayed givinostat group and all patients in the givinostat-naïve group. There were 11 non-ambulant patients, i.e. 8 (7.3%) in the givinostat group and 3 (5.6%) in the delayed givinostat group. Up to the latest cut-off date, there were 12 (5.8%) non-ambulant subjects and 195 (94.2%) ambulant subjects included.

Overall, mean (SD) time since DMD diagnosis was 7.30 [2.651] years, (7.47 [2.574] years, 7.16 [2.775] years and 6.92 [2.737] years in the givinostat, delayed givinostat and givinostat-naïve groups, respectively) and mean (SD) time since steroid initiation was 5.40 (2.400) years (5.69 [2.403] years, 5.35 [2.138] years and 4.46 [2.651] years in the givinostat, delayed givinostat and givinostat-naïve groups, respectively). Overall, the majority of patients (80.9%) were taking the steroid deflazacort (72.2% to 90.0% across the treatment groups), and most patients (79.4%) were on a daily steroid schedule (76.4% to 93.3% across the treatment groups). Overall, most patients (63.9%) had a deletion DMD mutation, (56.7% to 66.7% across the treatment groups), with duplications in 13.9% of patients (11.8% to 20.0% across the treatment groups), point mutations in 11.9% of patients (3.7% to 15.5% across the treatment groups) and 'other' in 9.8% of patients (7.3% to 14.8% across the treatment groups).

Table 19 Baseline values for efficacy endpoints (ITT analysis set – ambulant patients)

Endpoint	**Mean (SD)**	
	Givinostat (N = 102)	**Delayed Givinostat (N = 51)**
Time to 4SC (seconds)	7.97 (14.58)	6.76 (10.02)
4SC velocity (stairs/second)	1.04 (0.54)	0.97 (0.48)
NSAA total score (points)	20.30 (7.08)	19.90 (6.52)
Time to rise from floor (seconds)	39.63 (58.74)	34.84 (54.96)
Rise from floor velocity (1/second)	0.14 (0.10)	0.12 (0.08)
6MWT (metres)	350.33 (104.75)	347.67 (83.82)
Time to 10MWR (seconds)	18.43 (69.24)	6.54 (2.06)
10MWR velocity (metres/second)	1.68 (0.60)	1.66 (0.45)
R/L knee extension strength (knee strength) (N)	R: 47.99 (20.73) L: 45.43 (19.94)	R: 47.37 (25.49) L 44.27 (23.68)
R/L elbow flexion strength (elbow strength) (N)	R: 35.10 (12.76) L: 34.74 (12.19)	R: 39.07 (18.62) L: 38.03 (17.52)

4SC, 4 standard stairs climb; 6MWT, 6-minute walk test; ITT, intent-to-treat; L, left; MR, magnetic resonance; NSAA, North Star Ambulatory Assessment; R, right; SD, standard deviation.
Source: Study 51 CSR, Table 14.2.1.1, Table 14.2.2.1, Table 14.2.3.1, Table 14.2.3.3, Table 14.2.4.1, Table 14.2.4.3, Table 14.2.5.1, Table 14.2.5.3 and Table 14.2.6.1.1.

Baseline disease characteristics in the givinostat-naïve group reflected the younger baseline age of

these patients compared to the givinostat and the delayed givinostat group, i.e. representing a mean 6MWT distance of 386.814 (SD 71.88) meters at baseline compared to 350.329 (104.75) and 347.673 (83.82) meters in the givinostat group and the delayed givinostat group, respectively (Table 19) or a NSAA total score of 23.6 (6.16) compared to 20.3 (7.08) and 19.9 (6.52) in the givinostat group and the givinostat-naïve group, respectively.

Table 20 Study 51: baseline values for functional efficacy endpoints (ITT analysis set – ambulant patients - excluding study 43 patients)

Endpoint	Mean (SD)	
	Givinostat (N = 89)	Delayed Givinostat (N = 51)
Time to 4SC (seconds)	6.25 (10.26)	6.76 (10.02)
4SC velocity (stairs/second)	1.06 (0.53)	0.97 (0.48)
NSAA total score (points)	20.57 (6.83)	19.92 (6.52)
Time to rise from floor (seconds)	33.58 (54.48)	34.84 (54.96)
Rise from floor velocity (1/second)	0.14 (0.09)	0.12 (0.08)
6MWT (metres)	359.37 (92.52)	347.67 (83.82)
Time to 10MWR (seconds)	15.46 (60.80)	6.54 (2.06)
10MWR velocity (metres/second)	1.72 (0.59)	1.66 (0.45)

4SC, 4 standard stairs climb; 6MWT, 6-minute walk test; ITT, intent-to-treat; L, left; MR, magnetic resonance; NSAA, North Star Ambulatory Assessment; R, right; SD, standard deviation.
Source: Study 51 Post-hoc Analysis, Table 14.1.3.3.

For ambulant patients in the ITT Analysis Set, baseline values in the givinostat (N = 102) and the delayed givinostat groups (N = 51) were almost comparable except for time to 10MWR (seconds), while 10MWR velocity was comparable. Given the observed treatment effect of givinostat in study 48 after 18 months of treatment, this finding is against expectation. To exclude the possibility, that baseline values for functional outcome measurements in the givinostat arm were affected by a more heterogeneous population in study 51, i.e., patients came from the former 43 and 48 studies with different prerequisites in functional capacities for inclusion between these two studies, the applicant provided an additional analysis set, excluding the patients from study 43. In this context it is also worth mentioning, that the 18 patients from study 43 were already treated for a duration of 52 months when rolled over to study 51 compared to those patients from study 48, who had been treated only for a period of 18 months. However, baseline values based on this additional analysis set still were comparable for those subjects who already were treated in study 48 with givinostat (givinostat treatment arm) and those randomised in study 48 to placebo (the delayed givinostat group). The applicant therefore concluded that the observed values were probably related to the fact that the analysis was done only in ambulant subjects at baseline as well as due to treatment interruptions between study 48 and study 51. Of note, a relevant portion of patients in the givinostat group experienced temporary treatment interruptions, due to delays in Study 51 approval for certain sites and study closure due to the COVID-19 pandemic. A total of 24 out of 89 ambulant patients had an interruption of more than 30 days. It is acknowledged that givinostat-naïve subjects, i.e. those who were not enrolled into study 48 as the off-target population was still completed, at baseline were generally younger than those subjects, already included in the previous studies. Of note, referencing to the table above, there were 89 patients included in the givinostat treatment arm, who rolled over from study 48, including also several subjects from the former off-target population. However, information for these subjects, i.e., baseline disease characteristics and how these patients responded on

givinostat treatment in the context of study 48 has not been provided. Given the fact, that these patients differed from the other patients, e.g. due to baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range ≤5% or >30%, the mean baseline values in study 51 for the givinostat arm could also have been affected by those patients from the former off-target population of study 48.

Efficacy Results with data cut-off 31 December 2021:

Overall, descriptive analyses indicated that, ambulant patients in the ITT Analysis Set (N = 183) deteriorated over the course of the study as shown by mean change from Baseline in functional tests at 4 years. Increases of approximately 20 seconds in time to 4SC, 204 seconds in time to 10MWR, and 58 seconds in time to rise from floor and decreases in 6MWT and NSAA Total Score (approximately -140 metres and -12.5 points, respectively) were observed. Velocity of 4SC, 10MWR velocity and rise from floor velocity decreased, by approximately -0.6 stairs/second, -0.9 meters/second and -0.08 1/sec, respectively. Mean knee extension and elbow flexion muscle strength also worsened over time.

Overall, non-ambulant patients in the ITT Analysis Set (N = 11) showed a slight deterioration in both EK score and Barthel Index Total Score at 2 years and at 4 years of treatment; upper limb muscle strength was similar throughout the study.

In the ITT Analysis Set (N = 194), PUL (version 2) results showed a slight decline in total score. Motor Function as measured with MFM showed slight deterioration during the study until Month 24 for the total score. At 4 years, mean motor function total score was approximately 10.5 points lower than at Baseline (n = 16). Pulmonary function tests (PFTs) showed a minimal increase from Baseline for forced vital capacity (FVC) while % predicted FVC showed a steady decrease. Peak expiratory force (PEF) showed a steady increase throughout the study period with a minimal decrease in % predicted PEF observed.

Pre-specified exploratory analyses comparing the givinostat group and the delayed givinostat group indicated neither significant nor relevant differences between treatment groups for the functional endpoints of time to 4SC, 4SC velocity, NSAA total score, time to rise from floor, rise from floor velocity, time to 10MWR, 10MWR velocity, and 6MWT, and in muscle strength in all muscles analysed, at Month 12 and Month 24. Similarly, neither significant nor relevant differences between treatment groups were noted for all analysed PUL version 2 dimensions, all MFM Scores, and Pulmonary Function Tests (PFTs).

As study 51 included patients of different ages and with different extents of givinostat treatment, a mixed-effect random coefficient analysis was performed to fit the efficacy dependent variables against age, in which age was treated as a random effect, treatment group (givinostat/delayed givinostat) as fixed effect, treatment by age as interaction term and a random intercept was fitted. Results from these analyses with baseline defined as the last non-missing value prior to the subject receiving treatment on this study suggests the similarity of givinostat treatment effect in the givinostat and delayed givinostat groups for physical function tests related to lower limb function. The models showed that the physical and pulmonary functions worsened with advancing age, similarly between the two treatment groups. No significant differences between the two treatment groups were noted also in the annual change for each pulmonary function and physical function parameters. However, as analysis have also included subjects in the givinostat arm from the former off-target population, baseline values are not representative for the two treatment arms and results therefore considered of limited value to demonstrate persistence of efficacy.

Post-hoc analyses were also conducted to repeat the comparison between the givinostat group and the delayed givinostat group excluding givinostat patients from Study 43; similar results were observed. No significant differences between the two treatment groups were noted also in the annual change for

each pulmonary function and physical function parameters evaluated with the Random Coefficient Analyses. The same results were noted for all physical parameters when the analysis was done excluding the missing values.

For the disease milestones of first rise from floor over 10 seconds, first 10MWR over 10 seconds, loss of ability to rise from floor, loss of ability to 4SC and loss of ambulation, median age at first occurrence was similar in the givinostat and delayed givinostat groups. Median age in the naïve givinostat group was in many cases not calculable due to the small number of subjects enrolled in this group, the younger age at Baseline and the limited number of events.

A consistent trend was observed for the analyses of age at first rise from floor > 10 seconds, and first time to 10MWR > 10 seconds for ambulant patients in the ITT Analysis Set. None of the patients reached the other major disease milestones (age at first occurrence of respiratory support, age at first scoliosis surgery and age at death).

Overall, 85/183 ambulant patients lost the ability to rise from floor (55/102 [53.9%] patients, 24/51 [47.1%] patients and 6/30 [20%] patients in the givinostat, delayed givinostat, and givinostat-naïve groups, respectively) with Kaplan-Meier (KM) median estimates of age at loss of rise from floor of 14.0 years, 14.3 years and 15.2 years in the givinostat, delayed givinostat, and givinostat-naïve group, respectively.

Overall, 40/183 (21.9%) ambulant patients lost the ability to perform the 4SC assessment (28/102 [27.5%] patients, 10/51 [19.6%] patients and 2/30 [6.7%] patients in the givinostat, delayed givinostat and givinostat-naïve groups, respectively). Median age at loss of 4SC was calculable only in the givinostat group (16.6 years [95% CI: 16.09, 17.97]).

In Study 51 with regard to the cut-off date 31 December 2021, overall, 37/194 patients lost ambulation (26/110 [23.6%] patients, 10/54 [18.5%] patients and 1/30 [3.3%] patient in the givinostat, delayed givinostat, and givinostat-naïve groups, respectively. Median age at loss of ambulation was 18.0 years in the givinostat group, however, median age at loss of ambulation was not calculable in the other 2 groups. With regard to the latest cut-off date, 78 subjects lost ambulation (19.1%), the median age at loss of ambulation was 16.7 years in the givinostat group, 17.3 years in the delayed givinostat group, while in the naïve givinostat group was not calculable.
Since study 51 has an open-label design data from this study do not have the convincing value of controlled data. However, the provided data indicate a median age at loss of ambulation of about 18.0 years in the givinostat group.

With regard to the latest data cut-off 31 December 2023 ambulant subjects (n=195) deteriorated by an overall mean (SD) decrease of the 6MWdistance of 196.3 (140.1) meters at fourth year, a mean decrease from baseline to fourth year in the NSAA total score of about 10.7, and more time taken for the time function tests. Indeed, the mean velocity of 4SC decreased by about 0.579 steps/sec at fourth year, the mean velocity of walk 10 meters decreased by 0.98 m/s at fourth year, and the mean velocity for rise from floor decreased by 0.06 1/sec at fourth year. Mean knee extension and elbow flexion muscle strength worsened over time as well.

For the total 12 non-ambulant subjects, the mean score of EK was increased from baseline to each year till sixth year of treatment showing decrease in physical function. The mean total score of Barthel Index showed that, from baseline to year 4 the total score was decreased of about 8 and continue to decrease in the givinostat group until 6 years of treatment. The upper limb muscle strength including left and right elbow flexion remained almost the same.

The Imaging DMD study (Barnard, 2020) is an ongoing, observational (case-control), prospective, multicentre study performed in the US. With respect to this ongoing study, the submitted information only covers the publication by Barnard, 2020.

From 2010 to 2020, 160 individuals with DMD, aged 4 to 30 years were recruited. Patients were followed-up annually for up to 7 years. At the annual visits assessments include amongst others medical history, treatment history, functional tests and MR measurements of the thigh and lower leg.

Objective of the study is to investigate the potential of the lower extremity MR biomarkers to serve as endpoints in clinical trials of therapeutics for DMD by characterising the longitudinal progression of MR biomarkers over 48 months and assessing their relationship to changes in ambulatory clinical function.

Ambulatory and non-ambulatory males with a confirmed diagnosis of DMD aged 5 to 30 years were included. Patients were initially required to walk at least 100 m and to be able to 4SC at the time of enrolment, but inclusion criteria were later expanded to include non-ambulatory patients. Ambulatory males with a confirmed diagnosis of BMD/DMD aged 18 to 62 years without disease or injury to the lower extremities are also enrolled. At Baseline, patients underwent MRI and MRS examination of the lower leg and thigh, followed by clinical assessments of ambulatory function (10MWR, 4SC, rise from floor and 6MWT). Patients are followed for up to 7 years, returning annually (every 12 ± 2 months) for MR, functional, and medical history data collection. A subset of patients had additional follow-up visits 3 and 6 months after Baseline.

Study population: At the time of the analysis, 160 patients had been enrolled. At Baseline, patients mean age was 8.6 (range 4.8 to 18.8) years; 97% of patients were ambulatory, 95% were able to 4SC and 88% were able to rise from floor. Mean (SD) Baseline VL MFF as measured by MRS was 20% (19).

According to Barnard et al, 2020, overall, participants reported taking corticosteroids at 87% of the visits. Of those taking steroids, 74% took deflazacort, and 26% took prednisone/prednisolone. A number of participants also took conditionally approved drugs (11.9% ataluren, 9.3% eteplirsen) or were enrolled in ongoing clinical trials at the same point during the course of their study participation (7.5%).

The Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (McDonald 2018) is an ongoing observational (case-control), prospective, longitudinal, international multicentre Natural History study. Data reported in this narrative are based on a data cut-off of 2016. With respect to this ongoing study, the submitted information only covers the publication by McDonald, 2018.

The primary objective of the study is to establish the largest long-term assessment of people with DMD. Additionally, the study aims to determine if small, normal differences in the genetic makeup of people with DMD (single nucleotide polymorphisms [SNPs]) affected how their disease progressed and related to muscle strength/size and steroid response, to determine genetic variations associated with DMD and to determine whether certain biomarkers are present in people with DMD and not in healthy controls.

Patients with documented DMD aged 2 to 28 years are enrolled. Patients aged 2 to 4 years are required to have a diagnosis of DMD confirmed by immunofluorescence/immunoblot or genetic laboratory findings. Patient's physical abilities, the medical problems they experience, and how they use health care services are observed for ≥ 8 years. Physical abilities are compared to a group of healthy controls. Patients are assessed using timed function tests (time to stand from supine, time to 4SC, time to 10MWR) and the Brooke upper extremity functional rating scale, PFTs and the PODCI health-related QoL assessments, at Baseline and Months 3, 6, 9, and 12 (ambulatory), or Months 6 and 12 (non-ambulatory) with long-term follow-up visits at Months 18, 24, and annually thereafter. Functional milestones were selected to represent sequentially lost abilities associated with disease progression.

Study populations: During the period 2006 to 2016, 440 patients were enrolled. Of these, 340 patients aged 2 to 28 years with documented DMD were enrolled into the parent study (2006 to 2009) with an

additional 100 patients aged from 4 to 8 years enrolled from 2012 to 2016. At Baseline, patients median age (interquartile range) was 8.9 (6.2 to 14.0) years and 292/440 (66%) patients were ambulatory. A total of 369 patients had a known duration of glucocorticoid treatment; of these, 58 (16%) patients had no history of glucocorticoid use and 311 (84%) patients had ≥ 12 months of treatment. Of 397 patients with a known glucocorticoid regimen, 53 (13%) had never been treated, 40 (10%) received daily prednisone or prednisolone, 63 (16%) received intermittent prednisone or prednisolone and 107 (27%) received daily deflazacort.

Both studies were used as an external control for the long-term comparison versus givinostat.

2.6.6. Discussion on clinical efficacy

Givinostat is proposed to be indicated in the treatment of Duchenne muscular dystrophy (DMD). The applicant submitted 1 pivotal study, study (DSC/14/2357/48), a Phase 2, single-arm, open-label, two-part study (DSC/11/2357/43) and an ongoing open-label long-term extension study (DSC/14/2357/51) that included patients previously enrolled in studies 43 or 48 and patients, who could not be enrolled in study 48 due to the completion of the off-target group. Further, efficacy results from studies DSC/11/2357/48 and DSC/11/2357/51 were compared with results from two natural history studies, ImagingDMD and CINRG.

One open-label phase 2 study has been conducted:

Study DSC/14/2357/43 was a first-in-patient, single-arm, 2-part, phase 2 study to provide proof-of-concept and to explore dose ranging for givinostat in patients with DMD.

The single **pivotal study**, Study DSC/14/2357/48 was a randomised, double-blind, parallel-group study of 19 months duration to evaluate the efficacy and safety of givinostat in ambulatory, corticosteroid treated, ≥ 6 years of age boys with DMD. The study consisted of 2 phases: 1) screening period: starting 4 weeks (± 2 weeks) before randomisation; and 2) treatment period: 18 months of treatment. The study duration of 18 months treatment is welcomed. It is considered adequate to provide clinically relevant (and not merely statistically significant) effect sizes on function, i.e. on the primary and secondary endpoints.

Key inclusion criteria encompassed Duchenne patients diagnosed based on genetic testing. Included patients were defined to be ≥ 6 years of age at randomisation, able to complete two 4-stairs climb (4SC) screening assessments with results within ±1 second of each other and a mean of the 2 4SC assessments of ≤ 8 seconds, able to complete the time to rise from floor test between ≥ 3 and < 10 seconds at screening, had manual muscle testing of quadriceps of grade ≥ 3 at screening and were treated on stable systemic corticosteroid dosages for at least 6 month. Considering the study duration and the included patient population, i.e., patients ≥ 6 years of age, the add-on setting to administer givinostat on top of corticosteroids is comprehensible. Since corticosteroids are routinely used in boys affected by DMD and recent treatment guidelines, recommend starting steroid therapy "before substantial physical decline" (Birnkrant et al., 2018), which could be translated into a recommended start at 4-6 years of age, it can be assumed that most of the ambulant DMD patients intended for study enrolment will most probably be treated with corticosteroids. Moreover, a potentially required discontinuation of the previous corticosteroid treatment would have entailed the risk of iatrogenic adrenal insufficiency and ethical implications. Enrolment of glucocorticoid treated patients therefore addresses this issue. Overall, mean (SD) cumulative concomitant corticosteroid dosing was similar between the givinostat and placebo groups with a small imbalance in the cumulative prednisone/prednisolone dose in favour of the placebo group. Although during protocol assistance the CHMP suggested to include a subset of corticosteroid naive subjects to be evaluated outside the primary analysis population (EMEA/H/SA/3097/1/2015/PA/PED/III), this has not been followed by the

applicant. Patients in the target population, defined to be the primary analysis population additionally had to have a vastus lateralis muscles fat fraction (VL MFF) in the Magnetic resonance spectroscopy (MRS) > 5% to ≤ 30%.

In principle, the choice of the inclusion and exclusion criteria are considered appropriate. The study population represents an enriched, rather heterogeneous ambulant patient population. In light of the chosen broad age range despite some restrictions with respect to functional capacities, this in sum is indicative for a rather later ambulatory stage in the disease process.

Despite progressive muscular impairment, DMD boys are not significantly declining in motor function tests from 4 - <7 years of age (McDonald et al, 2013). While prior to 7 years of age, young DMD boys improve their motor function, after the age of 7 motor function starts to decline (Ricotti v et al 2016). With regard to the chosen inclusion criteria, patients are considered to be in a decline phase and might already deteriorate in the study with a minimum amount of severity at baseline. It is further acknowledged that with respect to the included age of ≥ 6 years and above, there is a risk of losing the ability to walk during the course of the study. Most patients become wheelchair dependent around 10 -12 years of age (Duan, 2021).

However, in view of the initially intended indication, it was questioned, whether the studied population is representative for the full ambulatory DMD population, e.g. also patients that are younger than 6 years and in an early stage in the disease process. This issue is not further pursued as during the procedure the applicant proposed to restrict the indication to patients aged 6 years and older. Patients, who were non-ambulant at treatment initiation as well as patients with symptomatic cardiomyopathy and patients not taking steroids were excluded from the study. It is understood that the applicant was expecting the effect of givinostat at all stages of the disease, which was reflected in the initially proposed broad indication text. However, the initially intended population is much broader than the study population. A Major Objection has been raised with regard to extrapolating study results obtained in a selected DMD population to the intended broad DMD patient population. With the responses, the applicant agreed to restrict the indication to ambulatory patients with the additional wording that "*patients who become non ambulant during treatment with Duvyzat should continue treatment*". However, a decision regarding continuation of treatment in subjects who become non-ambulant during treatment with Duvyzat should be at the discretion of the treating physician. The applicant's proposal to add the statement above in section 4.1 of the SmPC was not fully agreed as it is not in line with the current SmPC guideline. It was revised and moved to section 4.2 as follows: "*The decision to continue treatment in patients who become non-ambulatory should be taken at the discretion of the physician based on the overall benefit and risk assessment".*

Clinical data from the phase 2 study 43 informed on the doses to be administered in the phase 3 study 48. The initial dose suggested for the pivotal study was chosen to achieve similar exposure as the 37.5 mg b.i.d. dose in study 43 targeting a daily exposure of 300 ng*h/mL. Essential study protocol amendments refer to changes in the dosing regimen due to tolerability and safety aspects. Doses applied were adjusted on the patient's weight. During the study the treatment regimen 1 (protocol ≤ version 4) was replaced by treatment regimen 2 (protocol > version 4). While under treatment regimen 1 the starting dose was Dose A, that depending on safety and tolerability aspects could be reduced by 1/3 to Dose B and thereafter could be further reduced by 20% to Dose C, the starting dose under treatment regimen 2 was Dose B that could be reduced by 20% to Dose C.

Overall, with respect to dose A doses ranged from 20 mg bid to 70 mg bid. Dose B ranged from 13.3 mg bid to 46.7 mg bid and Dose level C ranged from 10.6 mg bid to 37.4 mg bid. The following five treatment options in study 48 need therefore to be considered when interpreting the study results: Start of treatment with Dose A that was kept unchanged during the study; Start of treatment with Dose A that was reduced to Dose B; Start of treatment with Dose A that was reduced to Dose B and

thereafter to Dose C; Start of treatment with Dose B that was kept unchanged during the study; Start of treatment with Dose B that was reduced to Dose C. Subjects who gained weight during the study did not have their drug dose increased. However, it is noted that a total of 36% of subjects had moved up one weight band in the givinostat group, one additional patient had moved up 2 weight bands and 4 subjects had moved down one band, compared to baseline. Nevertheless, considering that similar exposures were reported between patients whose weight increased and subjects whose weight remained unchanged, no additional analyses are considered necessary in this context.

Major changes in study drug dose adjustment during the study also relate to the inclusion of safety rules for permanent or temporary discontinuation of treatment with givinostat. Amongst others, temporary stopping criteria due to triglyceride limits had first been added under protocol version 6.

With regard to the changes of the flexible dosing regimen from treatment regimen 1 to treatment regimen 2, the applicant could demonstrate that - based on former provided statistical analyses for the primary endpoint 4SC – there are no differences in effects between the different treatment regimens, e.g. for patients who remained on Dose A compared to those who reduced their dose (Not A).

To demonstrate that dosing of givinostat during the study had no impact on the efficacy results, the applicant further performed subgroup analyses as requested for the primary and the key secondary endpoints based on the final doses patients received during the study, i.e. Dose A, B and C. Generally, results across the subgroups numerically favoured givinostat when compared against placebo. However, results are difficult to interpret, as the number of subjects for the different doses was small and differed notably, i.e., n = 21 for Dose A; n = 44 for Dose B and n = 16 for Dose C compared to Placebo n = 39. Although the results across the final dose groups indicate no dose-response relationship the effect sizes under Dose B were generally smaller than for Dose A and/or Dose C. With the answers, the applicant could sufficiently demonstrate, that in all cases exposures at Dose B are within the range of exposures at Doses A and C. However, as the study was not powered to evaluate differences in givinostat dose regimens while patients were not randomized to Doses A, B or C this might have limited the interpretation in the results received. However, in this context it needs to be considered that similar exposures were shown in patients receiving Dose A, B and C.

The applicant could also show that patients in study 48 with treatment 1 achieved comparable exposures compared to patients with treatment 2. With both treatment regimens, the overall exposures were higher than the predicted threshold level for efficacy that had been established based on preclinical results and pharmacometrics analyses, i.e., a target minimum daily exposure of 300 ng*h/mL.

Moreover, the applicant could demonstrate by simulation that exposures across the 9 different weight bands used in study 48 were also comparable.

Likewise, exposures were found to be comparable when patients had to reduce their dose due to side effects. The applicant conducted analyses to assess the likelihood of a patient for experiencing dose reductions depending on the starting dose and also on the patient's weight. Heavier patients, who started at Dose A, reduced the dose more frequently than patients with lower body weights due to higher-than-expected exposures in patients with higher body weight. Based on the data from Study 48, the estimated allometric coefficient was adjusted. The smaller allometric coefficient resulted in lower predicted givinostat exposures in patients with lower body weights and higher predicted givinostat exposures in patients with higher body weights. This has been addressed in the revised dosing regimen, i.e., patients with higher body weight will receive lower doses of givinostat per kg body weight right from the beginning of treatment resulting in comparable exposures across weight bands and fewer dose reductions. Population PK based simulations suggested that the revised dosing regimen leads to comparable exposures in the weight bands 10 mg to 20 kg and 20 mg to 40 kg and

slightly lower exposures in the weight bands 40 mg to 60 kg and > 60 kg as compared to the previously proposed dose regimen.

However, in the revised dosing regimen, the weight bands were reduced from 9 weight bands to 4 weight bands. For patients with higher body weights, this is supported. For patients with lower body weights, the width of the weight bands is considered to be rather broad as it would be changed from 5 kg in the clinical study to 20 kg in the proposed dosing regimen. The aim of a simplified dosing regimen, i.e., to be able to use only one syringe instead of two syringes is acknowledged (see also quality part). However, the applicant was asked to discuss whether additional weight bands (width = 10 kg body weight) would be beneficial. Furthermore, the lowest body weight of subjects included in the study was 17.5 kg. Therefore, the model should not be used to extrapolate outside this weight range, i.e., the lower limit of the lowest weight band starting at 10 kg body weight was regarded as not acceptable. The applicant provided simulations for the resulting exposure (AUC_{0-12}, C_{max}) after using dosing scheme based on 4, 6 or 9 weight bands. As modelling predicts similar exposures for all dosing schemes, the applicant prefers using only 4 weight bands. The dosing scheme was revised regarding the lower weight cut-off, which was changed from 10 kg body weight to 15 kg body weight. This is agreed as the indication includes paediatric patients 6 years of age and older.

With respect to exposure-response modelling, no significant relationship between givinostat exposure and the primary efficacy endpoint 4SC has been confirmed within the tested exposures. However, for the secondary endpoint NSAA total score change from baseline the relationship was statistically significant that could be considered supportive for the effect observed in the clinical study. According to the applicant no exposure-response relationship could overall be shown due to the large overlap in exposures of different weight groups. It is agreed that the results should be interpreted with caution because of the small number of patients in each subgroup.

Overall, the applicant could sufficiently justify, that based on the different treatment regimens that have been implemented in study 48 there was no substantial variability in exposures that might have affected the outcome on efficacy parameters. Therefore, it is agreed with the applicant, that switching from Treatment Regimen 1 to Treatment Regimen 2 is not considered to affect the interpretability of the final study results with respect to study integrity and interpretation of efficacy results.

Moreover, the proposed dosing regimen with tightening the 9 weight bands to 4 is sufficiently justified.

Primary efficacy endpoint was the mean change in time to 4SC before and after 18 months of treatment of givinostat versus placebo with velocity to climb 4SC as supportive analysis. Key secondary efficacy endpoints included the mean change in time to rise from floor, the mean change in the 6MWT distance, the mean change in the NSAA score, the cumulative loss of function on the NSAA score, the mean change of muscle strength evaluated by knee extension and elbow flexion as measured by hand-held myometry (HHM) and the mean change in vastus lateralis muscles fat fraction (VL MFF) as assessed by MRS.

Overall, the choice of endpoints is acceptable and in line with the EMA Guideline for the Treatment of Duchenne Muscular Dystrophy (EMA/CHMP/236981/2011, Corr.1) as well as EMA advice recommendations. While the NSAA is considered nowadays the preferred functional efficacy endpoint by experts in the field, it has at least been included as a key secondary endpoint in the study. The use of the 6MWT is agreed as it allows cross comparison with other medicinal products in the field.

With respect to the minimal clinically important difference in 4SC velocity, it is considered, that the MCID of 0.035 published by Duong 2021 which is in the unit of "tasks/second" is not applicable to the 4SC velocity in the unit of "stairs/second" used by the applicant. See also below under efficacy endpoints. Thus, the applicant was asked to discuss the MCID for the results obtained in 4SC velocity in the unit of "stairs/second" in study 48. Duong 2021 derived MCIDs for timed function tests including

4SC velocity (tasks/second) using an anchor-based approach, with the Vignos scale as an anchor to determine clinical change in functional status, which was defined as a binary variable: 0=no decline (Vignos score remained the same or decreased by 1 or more points over the 12-month interval) or 1=decline (Vignos score increased by 1 or more points over the 12-month interval). For each timed function test, an optimal cutoff point for the 12-month change in timed function test that best distinguished patients with and without decline in function was estimated by the maximum point reached from the receiver operating characteristic curve (ROC) analysis, with the largest value defined by dividing sensitivity (true positive rate) by 1 minus specificity (false positive rate). These estimated cutoff points were then used as MCIDs for respective timed function tests. For example, for 4SC velocity, this means that patients with a 12-month change in 4SC velocity ≤ 0.035 tasks/second are considered to be patients with a decline in function, and patients > 0.035 without a decline in function.

The sample size of 120 subjects in the Target Population is considered sufficient, taking into consideration the prevalence of this orphan condition. Randomisation is acceptable.

Potential development of thrombocytopenia, diarrhoea and also temporary treatment interruptions and/or increases in scheduled visits due to adverse events that overall seem dose-dependent, carry the risk of unblinding. As generally the study site personnel who collected the data for the primary and the secondary endpoints was different from the personnel who reviewed subjects' safety results and safety results must not be shared with the personnel who was responsible for the functional tests, there is no signal that the study personal was not blinded in the most possible way. The applicant provided a comprehensible justification that blinding has sufficiently been maintained during the study.

The intent-to-treat (ITT) Analysis Set - Target Population served as the basis for the formal analysis of efficacy. The MR Cohort formed the basis for analysis of all MRI/MRS parameters. The definition of the ITT analysis set is not acceptable, as excluding patients without post-baseline measurements from the ITT analysis set may lead to bias. Nevertheless, as no enrolled patients were actually excluded from the ITT analysis set, there are no further concerns.

In the target population, 8 of 81 patients in the givinostat group and 5 of 39 patients in the placebo group had missing values for time to 4SC at 18 months. One patient in the placebo group was non-ambulant at 18 months, so missing data were classified as 2 (i.e. missing data due to being either non-ambulatory or otherwise physically unable to perform the test / assessment). Other missing data due to reasons such as early withdrawal from the study, COVID-19, fracture/injury, patient being not cooperative were classified as 1 (i.e. missing data due to reasons other than being non-ambulatory or physically unable to perform the test / assessment).

In the primary analysis of the primary efficacy endpoint, single imputation methods were utilised for handling all missing data. In this case, the standard error may have been underestimated due to ignoring the uncertainty of imputed values, leading to a narrower confidence interval for the treatment effect. In the tipping point sensitivity analysis, a multiple imputation was applied for handling Class 1 missing data, whereas Class 2 missing data were handled as in the primary analysis. Although the treatment effect from the tipping point sensitivity analysis considering no shift was similar to that from the primary analysis, it was no longer statistically significant due to the increased standard error.

In the tipping point sensitivity analysis, Class 1 missing data were imputed based on the distribution of non-missing values in the respective treatment group. The implied assumption that patients with missing data are similar to those who completed the assessment is questionable. At least, patients who withdrew from the study (and also the treatment) early are not expected to further benefit from the treatment and their outcomes would therefore be different from those of patients who adhered to the treatment.

Therefore, the primary analysis of the primary efficacy endpoint was not considered the most

appropriate and plausible one with respect to the handling of missing data. Sensitivity analyses using more conservative missing data handling strategies that would not likely lead to biased results in favour of givinostat were requested. The applicant was asked to provide sensitivity analyses for the primary and key secondary endpoints: time and velocity to 4SC (on both non-log-transformed and log-transformed data), time and velocity to rise from floor, 6MWT, muscle strength evaluated by knee extension and elbow flexion, with all Class 1 missing data imputed using a jump-to-reference multiple imputation.

The rationale for handling Class 1 missing NSAA item scores using different strategies (imputation with mode or zero depending on the missing patterns) was unclear. The applicant was asked to comment and to provide sensitivity analyses for the NSAA total score and the cumulative loss of function on the NSAA, with all missing item scores imputed with zero. The applicant explained why the imputation method for the missing NSAA items was different from that for the missing continuous endpoints. It was expected that the applicant could explain why Class 1 missing NSAA items were imputed using different values (mode or zero) within the NSAA analyses.

The applicant provided the requested sensitivity analyses using a zero imputation for all missing NSAA items for both the Target and Overall Population. The results of the sensitivity analyses generally assured the direction of the treatment effect estimates from the primary analyses, i.e. numerically in favour of givinostat.

For the endpoints analysed via ANCOVA, an unblinded assessment of ANCOVA model residuals without a treatment effect term was performed to determine whether data should be log-transformed. However, a log-transformation of data is not considered an optimal strategy, as it changes the endpoint from the difference to the ratio, rendering the interpretation of the results difficult.

The assessment of multiple key secondary efficacy endpoints at 18 months in the target population at the final analysis was accounted for using the Hochberg procedure (Hochberg, 1988) that is considered appropriate. Following the Hochberg procedure, significance was determined by comparing each 2-sided p-value with a significance level of 0.05/i, where i represents the position of the endpoint in descending order of p-values from least to most significant. As soon as the ith endpoint had a corresponding p-value < 0.05/i, the treatment effects for that endpoint and all the endpoints following it were considered significant.

Several sensitivity analyses and supportive analyses were performed to show robustness of the primary analysis. Amongst others, 2 analyses assessing the Impact of the COVID-19 Pandemic on the Study, i.e. excluding the patients who completed their Month assessment outside of the protocol defined window and excluding subjects who completed the Month 18 assessment outside of the extended visit window; analyses of time to 4SC with and without adjustment for MRS VL MFF and tipping Point Analysis.

Subgroup analyses were performed for the primary (time to 4SC) and the secondary (NSAA, time to rise from floor and 6MWT) functional endpoints, muscle strength and muscle morphology (VL MFF) the following subgroup analyses based on: treatment compliance (> 80% / ≤ 80%), total weight-adjusted exposure (≥ Median/< Median), subjects who started on the higher dose specified in Protocol Versions =< 4.0 versus those who started on the lower dose specified in Protocol Versions > 4.0 and age (6 to 7 years; > 7 years).

Study DSC/14/2357/51 is an ongoing multicentre, open-label, long-term extension study to assess the safety, tolerability and efficacy of givinostat administered to previously givinostat treated and treatment naïve DMD subjects, all on concomitant corticosteroid treatment.

Baseline was defined as the last non-missing value (including scheduled and unscheduled assessments) prior to the subject receiving treatment on this study.

There were three groups defined: 1. the givinostat group comprised patients randomised to givinostat in their previous givinostat study; 2. the delayed givinostat group comprised patients randomised to placebo in their previous givinostat study; and 3. the naïve givinostat group comprised DMD patients screened in study 48 who met all the inclusion criteria and none of the exclusion criteria but have never been randomised because enrolment in the off-target group was completed.

With regard to the requested inclusion criteria, subjects had to be ≥6 years and - had participated in study 43 or study 48 and attended the End of Study Visit or - had been screened in study 48 and met: all the inclusion criteria and none of the exclusion criteria, had a baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range ≤5% or >30%, i.e., included in "off-target" group, but never been randomised because the enrolment in the off-target group was completed.

The starting dose of givinostat was the same level that the patient had received at the end of their previous givinostat study (i.e., Study 43 or Study 48). For givinostat-naïve patients, treatment was started at dose level B. The dose was adjusted per the individual patient's weight change throughout the study. Givinostat dose adjustments for safety reasons followed the criteria described in the study protocol.

Efficacy assessments for all subjects were: Performance of Upper Limb (PUL); Motor Function Measure (MFM); Pulmonary Function Test (PFT); Quality of life (QoL): assessed by PedsQL for paediatric patients (both child and parent versions), and SF-36 for adult patients; Time to first occurrence of major disease milestones (e.g., time to respiratory support needed during the day, time to scoliosis surgery, time to death). For ambulant subjects the following efficacy assessments were additionally performed: Physical Function assessed by 6MWT and NSAA total score; Timed Function Tests: 4SC, Time to Walk 10 Meters (10MWR) and Time to Rise from Floor (TTR); time to loss of ambulation. Muscle Strength Assessed by Knee Extension and Elbow Flexion. For non-ambulant subjects: (i.e., subjects who were unable to complete the 10m walk/run test in 30 seconds or less without any support or devices): Physical Function Assessed by Egen Klassification (EK) Total Score; Reports of activities of daily living (measured by Barthel Index Total Score); Upper Limbs Muscle Strength Assessed by Elbow Flexion (evaluated by handheld myometry).

Comparison of efficacy results from studies DSC/14/2357/48 and DSC/11/2357/51 with results from the natural history studies ImgagingDMD and CINRG

To provide a comparison of long-term efficacy evaluation of givinostat based on data from clinical studies, i.e., studies 48 and 51, outcomes on specific disease milestones parameters, i.e., age at persistent rise from floor > 10 seconds; age at persistent 10MWR > 10 seconds; age at persistent loss of rise from floor; age at persistent loss of 4SC and age at persistent loss of ambulation were compared against outcomes in boys from the Natural History Studies Imaging DMD and the CINRG study.

The ImagingDMD study is an ongoing, observational (case-control), prospective, multicentre study performed in the US to investigate the potential of the lower extremity MR biomarkers to serve as endpoints in clinical trials of therapeutics for DMD by characterising the longitudinal progression of MR biomarkers over 48 months and assessing their relationship to changes in ambulatory clinical function.

Objective of the study is to investigate the potential of the lower extremity MR biomarkers to serve as endpoints in clinical trials of therapeutics for DMD by characterising the longitudinal progression of MR biomarkers over 48 months and assessing their relationship to changes in ambulatory clinical function.

From 2010 to 2020, 160 individuals with DMD, aged 4 to 30 years were recruited. Patients were followed-up annually for up to 7 years. Ambulatory and non-ambulatory males with a confirmed diagnosis of DMD aged 5 to 30 years were included.

With regard to patient characteristics it is noted, that subjects taking corticosteroids at 87% of the visits. Of those taking steroids, 74% took deflazacort, and 26% took prednisone/prednisolone. A number of participants also took conditionally approved drugs (11.9% ataluren, 9.3% eteplirsen) or were enrolled in ongoing clinical trials at the same point during the course of their study participation (7.5%)

The Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study is an ongoing observational (case-control), prospective, longitudinal, international multicentre Natural History study. Published data are based on a data cut-off of 2016.

The primary objective of the study is to establish the largest long-term assessment of people with DMD. Additionally, the study aims to determine if small, normal differences in the genetic makeup of people with DMD (single nucleotide polymorphisms [SNPs]) affected how their disease progressed and related to muscle strength/size and steroid response, to determine genetic variations associated with DMD and to determine whether certain biomarkers are present in people with DMD and not in healthy control.

During the period 2006 to 2016, 440 patients were enrolled. Of these, 340 patients aged 2 to 28 years with documented DMD were enrolled into the parent study (2006 to 2009) with an additional 100 patients aged from 4 to 8 years enrolled from 2012 to 2016. At Baseline, patients median age (interquartile range) was 8.9 (6.2 to 14.0) years, and 292/440 (66%) patients were ambulatory. A total of 369 patients had a known duration of glucocorticoid treatment; of these, 58 (16%) patients had no history of glucocorticoid use and 311 (84%) patients had ≥ 12 months of treatment. Of 397 patients with a known glucocorticoid regimen, 53 (13%) had never been treated, 40 (10%) received daily prednisone or prednisolone, 63 (16%) received intermittent prednisone or prednisolone and 107 (27%) received daily deflazacort.

A matched comparison of efficacy from study 48 with the historical controls was planned (ImagingDMD and CINRG), however this was not feasible as matching based on a propensity score analysis was not effective. Instead, comparisons were based on ITT Analysis sets including subjects who had baseline characteristics aligning with key inclusion and exclusion criteria from study 48. With the responses, the applicant provided an update of the integrated analysis of long-term efficacy (ISE). In the updated ISE, the applicant has considered the following covariates in an updated matching approach: baseline 4SC, baseline TTR, baseline 10MWRT and baseline steroid use (Deflazacort, Other).

Random Coefficient Analyses of 4SC Velocity were performed to demonstrate persistence of efficacy of givinostat with long-term treatment.

Efficacy data and additional analyses

Study DSC/11/2357/43

Twenty-one patients were screened, 1 patient failed screening. Twenty patients were enrolled; 19 patients received study drug in Part 1. One patient discontinued the study due to an adverse event of decreased platelet count and 18 patients continued to Part 2. Another patient was enrolled in Part 1 but started treatment in Part 2. Hence, 19 patients completed Part 2 and no patients discontinued. One subject was excluded from the evaluable population due to major protocol deviations. All 19 patients who completed Part 2 continued to Extension 1. One patient withdrew consent and discontinued from the study at the beginning of Extension 2, the remaining 18 patients completed Extension 2 and Extension 3.

In the analysis of the primary endpoint, the mean MFA% at baseline was 51.003% and increased to 64.909% at EoS (End of Part 2) with a mean change from baseline of 13.906%. While in change from

baseline until month 12 under the treatment with givinostat an obvious improvement in histological muscle parameters could be observed based on the primary and further structural secondary endpoints, the observed morphological effects could not compellingly be transferred into effects on functional outcome parameters, i.e. there were consistent declines in the 6MWT distance and the total NSAA score until week 52, while the PUL at least remained almost stable until week 36. Moreover, 6/20 subjects lost ambulation until week 52.

The RD of givinostat was determined as 37.5 mg b.i.d. While 7 subjects completed Part 2 of the study with the RD dose, 12 subjects who started on the RD reduced their dose to 25 mg b.i.d. due to decreased platelets during Part 2. However, efficacy results have only been provided for the entire population.

Generally, there are several methodological limitations of this study that renders the interpretation of the efficacy results difficult. This was a single-arm, open-label study, small numbers of subjects and an enriched study population. Results on histological muscle parameters that only had been collected in the first year indicate that the doses of 37.5 mg and/or 25 mg b.i.d after 1 year of givinostat treatment provided a pharmacodynamics effect in the studies patient population. However, contrary to improvement on morphologic measurements, there was a decrease in change from baseline until study end for all functional outcome parameters that however needs to be put into relation to treatment naïve patients for interpretation of the obtained results. However, a placebo control arm was not available and the study was not powered to demonstrate an effect on function.

Pivotal Study DSC/14/2357/48

A total of 359 subjects were screened for participation in study DSC/14/2357/48 and of these, 179 (49.9%) subjects were randomised: 120 subjects in the Target Population (81 subjects in the givinostat group and 39 subjects in the placebo group) and 59 subjects in the Off-Target Group (37 subjects in the givinostat group and 22 subjects in the placebo group).

Thirty-six (10%) subjects passed screening but were not randomised, and 144 (40.1%) subjects failed screening, predominantly due to the specific inclusion criteria. Overall, completion rates were high and comparable across the treatment arms. 170 (95.0%) of the randomised subjects completed the study.

The number of subjects with at least 1 major protocol deviation in the Target Population was 20 (16.7%) out of 120 who were dosed. There was one (1.2%) subject, randomised to the givinostat treatment arm with a major protocol deviation due to changes in the steroid treatment before the start of study treatment. One additional subject was included into the Non-Target Population who also changed steroid treatment before start of study treatment. The applicant could sufficiently justify that inclusion of these two subjects did not have any impact on the study results.

Overall, the two treatment groups were well balanced with regard to demographic and patient characteristics in the Safety Analysis Sets across the target and the overall population and were also comparable for these two populations.

The included patients were ≥ 6 years of age with mean age around 10 years. The study population consisted of male subjects with an overall mean (SD) time since diagnosis of 5.67 (2.636) years for the target population and an overall mean (SD) time since diagnosis of 5.51 (2.636) years for the overall population.

According to the study protocol patients had to have a confirmed diagnosis of DMD by genetic testing, type of DMD mutation for the majority of subjects across the treatment groups was deletion. Subjects should be on systemic corticosteroid therapy for at least 6 months prior initiation of the study treatment. The mean time since steroid treatment initiation was 3.74 years in the target population and 3.8 years in the overall population with deflazacort as the steroid used by the majority of the

subjects (79.2%) with a daily treatment regimen (83.3%).

Baseline values for the chosen primary and the key secondary endpoints were comparable across the treatment groups in the target population and were also in line with those of the overall population.

In the primary analysis (analysis of log-transformed values) of change from baseline in time to climb 4 stairs (4SC) in seconds at month 18, a statistically significant difference was demonstrated when givinostat was compared against placebo (p = 0.0374). While the time to 4SC increased in the givinostat group from 3.39 to 4.70 seconds it increased even more in the placebo group from 3.48 to 6.37 seconds. The GLS mean (log scale SE) was 1.27 and 1.48 for givinostat and placebo, respectively, with a GLS mean ratio (95% CI) of 0.86 (0.071). This represents a 14% less increase in the time to climb 4 stairs at month 18 in favour of givinostat when compared to placebo.

In the analysis performed on non-log-transformed data, the LS mean was 1.25 and 3.03 seconds for givinostat and placebo, respectively, with a difference in LS means of - 1.78 seconds in favour of givinostat (p=0.0374).

The post-hoc analysis using jump-to-reference multiple imputation failed to show statistical significance for the primary and the key secondary endpoints, however this was a post-hoc analysis that might be considered too conservative in the context of a disease-modifying treatment.

To justify the minimum clinically important difference (MCID) for the primary endpoint "time to 4SC", the applicant referred to the publication by Ayyar Gupta (2023). The article discusses several statistical approaches to determine the MCID for functional outcome measures, i.e., (1) distribution-based approaches [a) estimates of one-third standard deviation (SD) of baseline scores and b) 1 standard error of measurement (SEM) calculated as SD as baseline x], (2) an anchor based method with 6 MWD as the anchor, and (3) evaluation of patient and parent perception using participant-tailored questionnaires. Although it is noted that the publication only relates to the MCID for the North Star Ambulatory Assessment (NSAA) in ambulant boys with DMD between the ages of 7 to 10 years, the described statistical approaches are general approaches considered to be applicable also to other functional outcome measures.

While it is acknowledged, that obviously for the results obtained in the primary analysis of the primary endpoint (analysis of log-transformed values), no generally agreed MCID is available, the treatment effect of -1.78 seconds in favour of givinostat (untransformed (non-log)scale analyses) is not considered to be irrelevant based on the above described distribution based approaches, e.g. the difference of 1.78 seconds is larger than the MCID calculated as 1/3 of the SD of the 4SC baseline values of the study (1/3 baseline SD = 0.385) and larger than the MCID calculated as the 4SC baseline SEM of the study (baseline SEM = 0.373).

Supportive analyses:

4SC velocity decreased in the givinostat group from 1.311 stairs/second at baseline to 1.091 stairs /second at EOS (LS mean change from baseline -0.22 stairs/second) and decreased in the placebo arm from 1.273 stairs/second at baseline to 0.967 stairs/second at EOS (LS mean change from baseline -0.31 stairs/second), demonstrating a non-statistically significant difference in LS means for 4SC velocity of 0.09 stairs /second; p-value = 0.1696.

With regard to the clinical relevance for velocity, the applicant referred to the publication by Duong, 2021 who describe a specific anchor-based MCID of 12-month change values for 4SC velocity of 0.035 tasks/sec. in boys with DMD. The anchor used in this study was the Vigos lower extremity scale which is an ordinal scale used to describe lower extremity functional ability in boys with DMD. However, no

analysis using an anchor based approach has been provided by the applicant to put the results for 4SC velocity into context of clinical relevance.

The applicant also referred to the results of the additionally performed post-hoc analysis for velocity using the delta theorem [difference in LS means in change from baseline in 4SC velocity between givinostat and placebo of **0.03** tasks/second (p-value = 0.0285)]. However, the plausibility and appropriateness of the post-hoc transformation is questionable; i.e., the applicant's statement that "if 4SC is non-Normal then $v = 1/4SC$ is, by definition, also non-Normal" is generally invalid. A counterexample is that, if a variable is normally distributed, its reciprocal is usually not normally distributed. It is also unclear, whether the log scale of 4SC would be better than the reciprocal scale of 4SC to approximate a normal distribution.

Therefore, the initially performed and pre-specified analysis of 4SC velocity (stairs /second) [difference in LS means for 4SC velocity of 0.09 stairs/second (p = 0.1696); treatment effect of 0.09 stairs/sec corresponds to 0.0225 tasks/sec.] is more convincing even if the obtained effect is smaller.

The provided random coefficient analysis indicated that age was the nominally statistically significant covariate for decrease in 4SC velocity (p-value: < 0.0001). The model demonstrated that 4SC velocity worsens with advancing age. Givinostat did not significantly prevent the decline in 4SC velocity as compared to placebo (difference in annual change in 4SC velocity between givinostat and placebo: -0.07 stairs/second; p-value: 0.0717).

Results of sensitivity analyses to assess the impact of the COVID-19 pandemic on Study 48 were in line with the primary analysis. Results of analyses of time to 4SC with and without adjustment for MRS VL MFF were in line with those of the primary analysis.

As the primary analysis utilised single imputation methods for handling all missing data, the standard error may have been underestimated due to ignoring the uncertainty of the imputed values. This is confirmed by the tipping point sensitivity analysis using a multiple imputation, which resulted in greater standard errors. As the results from the sensitivity analysis are no longer statistically significant, the robustness of the results from the primary analysis was not confirmed.

Based on the pre-specified Hochberg analysis, none of the key secondary endpoints reached formal statistical significance. However, results for NSAA total score, NSAA cumulative loss of function and VL MFF were nominally statistically significant and these are important endpoints for DMD.

The LS mean (95% CI) change from baseline in the NSAA total score for givinostat and placebo was -2.66 (-3.563, -1.759) and -4.58 (-5.891,-3.260), respectively, with a LS mean difference in the NSAA total score of 1.91 in favour of givinostat (p = 0.0209). Regarding cumulative loss of function, for each subject at each post-baseline visit, failure to perform each of the 17 items of the NSAA was assessed, where "failure" was defined as a score transition from 2 or 1 at baseline to 0 at the respective visit. The total number of failed items for the visit was calculated (maximum of 17 failed items per visit per subject). The subject´s cumulative number of failures across all visits was the sum of the total failures at each post-baseline visit. The ratio of NSAA cumulative loss of function (givinostat/placebo), obtained from a negative binominal regression on the subject's cumulative number of failures across all post-baseline visits, was 0.61 (p = 0.0202).

To justify the clinical relevance of the outcome obtained on the NSAA total score, the applicant referred to an often-applied distribution based approach to evaluate minimal clinically important differences (MCID). As discussed by the applicant, the difference of 1.91 points in change in NSAA total score is larger than the estimate of one-third standard deviation (SD) of baseline scores in the pivotal study (1/3 baseline SD = 1.638).

It is noted that the obtained difference of 1.91 points compared to the MCID of 2.32 points that has been described by Haberkamp 2019 (also using a distribution-based approach that refers to the original NSAA raw scale) is slightly smaller and gets even smaller in relation to the MCID of 3.5 points proposed by Ayyar Gupta 2023 (using an anchor-based approach on the 6 MWD).

However, it is important to consider that the reference to the published MCIDs bears the risk of several uncertainties, e.g. Ayyar Gupta states/concludes, that "*there are limitations of using a single approach to determining MCID, e.g. the lack of patient perspective itself in the estimation of MCID is considered an important limitation. It is therefore recommended to combine statistically derived metrics, clinical data and patient insight to derive estimates which are empirically sound and clinically relevant*". Moreover, it needs to be considered that the MCID of 2.32 described by Haberkamp relates to the correlation between the NSAA total score and a new functional endpoint (Stride Velocity 95th Centile (SV95C)) and therefore needs primarily to be interpreted in this context. Moreover, as described by Mayhew et al 2013, estimated values for MCIDs more reflect/refer to ranges (mean) as they also vary in relation to subjects´ age or stage of the disease. Based on these considerations both described MCIDs for the NSAA total score can only serve as a landmark in interpreting the clinical relevance of the obtained difference of 1.91 points in change from baseline at months 18 in the pivotal study, which however, is considered to be in a clinically meaningful range as it is close to the described MCIDs.

With respect to the results obtained for the cumulative loss of function on the NSAA score after 18 months, the estimated cumulative failures (95% CI) were 3.42 (2.692, 4.334) and 5.56 (4.002, 7.715) under givinostat and placebo, respectively.

The complete loss of function in a single item or deterioration of function in one to two items has been described to be an important change perceived by patients and parents (Ayyar Gupta 2023). However, no data/analyses have been provided by the applicant, to evaluate the number of patients with loss of function in at least one or two items at EOS as compared to baseline or the mean number of items with loss of function at EOS as compared to baseline per patient. With the responses, the applicant provided the requested analyses. Overall, these results are in favour of givinostat compared with placebo and supportive of efficacy.

To further justify the clinical relevance of the cumulative loss of 2 items in NSAA score (as well as the mean change of 2 points in the NSAA total score) in predicting a meaningful disease milestone, i.e. loss of ambulation in the declining group and loss of the ability to rise from floor in the stable group, the applicant further refers to an analysis of 5 natural history studies and 3 placebo arms from controlled trials. However, although these data are supportive the published information only refers to an abstract with limited information of the described analyses (McDonald et al Development and evaluation of a time to event endpoint for clinical trials in Duchenne muscular dystrophy (DMD). Neuromuscul Disord 2022; 32:S69) that limits the interpretation.

In addition, the applicant provided results for an analysis of the NSAA cumulative loss of function performed by separate items. The largest effects were observed for the items hop, run and jump that according to a publication by Mayhew 2011, are more difficult for patients to perform.

The time to rise from floor (TTR) in seconds from baseline through EOS increased in the givinostat group from 5.57 to 14.43 seconds and in the placebo group from 5.59 to 19.17 seconds with mean changes of 8.86 seconds and 13.59 seconds, respectively. In the analysis of change from baseline at 18 months, the LS mean was 9.33 and 12.61 seconds for givinostat and placebo, respectively. Although the LS mean difference of -3.28 seconds in favour of givinostat was not nominally statistically significant (p = 0.3044), the observed effect size might not be considered irrelevant.

Using the TTR velocity (rises/sec) is supportive to address the increased variability caused by patients who were unable to perform the TTR due to disease progression. **Time to rise from floor velocity**

decreased from baseline to EOS in the givinostat group from 0.198 to 0.151 rises/sec and in the placebo arm from 0.198 to 0.118 rises/sec with mean changes of -0.047 rises/sec and -0.080 rises/sec for givinostat and placebo, respectively. LS mean change in time to rise from floor velocity from baseline to 18 months was – 0.05 rises/sec in the givinostat group and – 0.08 rises/sec in the placebo group. The LSM difference for givinostat from placebo was 0.03 rises/sec and reached nominally statistical significance (p = 0.0124). Referring to the former defined MCID for TTR velocity of 0.023 rises/sec by Duong 2021 (i.e. annual decline in TTR velocity of 0.023 rises/sec), the average decrease in TTR velocity under givinostat as compared to placebo is greater than the MCID described by Duong.

In the analysis of change from baseline in the **6MWT distance** at 18 months, a decline in the 6MWT distance over 18 months was observed in both treatment arms with a slower decline with givinostat compared to placebo, the LS mean change for givinostat and placebo was –38.43 and -48.38 meters, respectively. The LSMean difference for change from baseline for givinostat against placebo was 9.96 meters (p = 0.3723).

In the pivotal study, inclusion of subjects was not based on any cut-off point for the 6MWT distance and the mean 6MWT at baseline was 403.31 (72.11) and 399.64 (63.83) meters under givinostat and placebo.

While it is known that amongst others the variability in the rate of progression of 6MWD is influenced by baseline 6MWD, a baseline 6MWD≥350 meters has been identified as a possible cut-off point to identify different rates of progression (Mercuri 2016).

However, the studied patient population provided a mean baseline 6MWT distance of around 400 meters and therefore represents a DMD population not that sensitive to changes in the 6MWT. It is therefore agreed with the applicant, that baseline characteristics with respect to the 6MWT distance and the rather small sample size of the study may have limited the chance of a more favourable outcome on the 6MWD.

The LS mean (95% CI) in change from baseline in **VL FF for** the Target Population in the MR cohort at 18 months for givinostat and placebo was 7.63 and 10.56, respectively. The LSM difference for givinostat against placebo was -2.92 (p = 0.0354). Results indicated that treatment with givinostat delayed fat infiltration by approximately 30% after 18 months.

Although VL MFF increased in both treatment arms over 18 months, the increase was smaller under givinostat when compared to placebo at different time points, i.e., week 48 and end of study (EOS) with a mean VL MFF change from baseline until end of treatment for givinostat and placebo of 7.48% and 10.89%, respectively.

Muscles of boys with Duchenne are progressively infiltrated and replaced by fatty fibrous tissues. The vastus lateralis (VL) muscle is a key extensor muscle of the knee. According to Nair et al., 2002, the VL muscle is a sentinel muscle for loss of ambulation and functional declines. MR findings showed that proximal thigh muscles such as the VL and biceps femoris long head (BFLH) are affected early in the disease but reach a ceiling effect at later stages of the disease while other muscles such as the distal muscles of the posterior compartment such as the medial gastrocnemius (MG) and soleus (SOL) become progressively involved (Nair 2022). Findings by Barnard (2020) concluded that amongst others Vastus lateralis FF was the strongest predictor of future loss of function, including loss of ambulation.

In this context the applicant referred to a nonlinear mixed-effect model developed by Rooney (2020) to describe the progression of VL MFF. Rooney showed that based on MR modelling of disease trajectory in DMD the age when VL FF reaches half-maximum muscle involvement is strongly associated with loss-of-ambulation age. Based on the modelling approach the applicant concludes that the observed difference in fat fraction using MRS between givinostat and placebo after 18 months would be expected

to be translated into a 2-year delay in age at loss of ambulation in favour of givinostat. Based on this approach the obtained effect might be interpreted as clinically meaningful.

Although FF of the vastus lateralis is currently not accepted as a validated surrogate parameter in clinical DMD trials, it could serve as a relevant pharmacodynamic parameter. Based on the results of the pivotal study in favour of givinostat, the results for the key secondary endpoint VL MFF as obtained with MRS can be considered supportive for the results of the functional parameters.

Moreover, results for vastus lateralis fat fraction as assessed by MRS were supported by results of further exploratory endpoints; i.e., the fat fraction of 5 thigh muscles (VL, biceps femoris long head, semitendinosus, quadriceps and hamstrings) measured by Dixon MRI at 18 months. The difference in LS means (givinostat-placebo) of fat fraction was in favour of givinostat in all 5 thigh muscles and therefore in support of the key secondary endpoint for fat fraction.

Time to 10MWR velocity decreased (worsened) over time in both the givinostat group (from 2.01 metres/second at Baseline to 1.80 metres/second at EOS) and the placebo group (from 1.99 metres/second at Baseline to 1.60 metres/second at EOS). However, mean (SD) change from Baseline in 10MWR velocity at EOS was lower in the givinostat group (-0.134 metres/second) compared with the placebo group (-0.390 metres/second). Analysis of change from Baseline in 10MWR velocity at 18 months showed a LS mean difference of 0.21 metres/second (p-value: 0.0011), indicating clinically relevance.

The applicant further provided the requested analyses using a Jump-to-Reference (J2R) multiple imputation for all Class 1 missing data (due to reasons other than being non ambulatory or physically unable to perform the test / assessment) for the primary, relevant key secondary and velocity endpoints for both the Target and Overall Population.

The results of these additional analyses are generally consistent with those of the primary analyses (using the original imputation approach specified in the SAP) with regard to the direction of the treatment effect estimates, i.e. numerically in favour of givinostat but failed to show statistical significance. Overall, the point estimates became smaller and/or the confidence intervals became wider.

For the primary analysis population, the target population (characterised by a VL MFF (MRS) > 5% to ≤ 30% in addition to the generally defined inclusion criteria) it is noted, that with respect to the primary endpoint time to 4SC (both on the log and the original (non-log) scale), statistical significance could not be shown any more. The same relates to the key secondary endpoints that reached nominal statistical significance in the primary analyses, i.e., change from baseline in total NSAA score, cumulative loss of function on the NSAA score, VL MFF as assessed by MRS as well as TTR velocity.

With regard to the treatment effect estimates for the key secondary and further velocity endpoints, i.e., velocity to 4SC, time and velocity to rise from floor, 6MWT and muscle strength evaluated by knee extension and elbow flexion, most of the obtained effects were smaller when compared to the original analyses, e.g. TTR velocity decreased from initially 0.03 rises/sec to 0.02 rises/sec (still close to the accepted MCID of 0.023 rises/sec). However, the 6MWT achieved a greater walking distance compared to the initially performed analyses, i.e., 13.94 meters (95% CI: -10.152, 38.022) compared to 9.96 meters (95% CI: -12-071, 31.983).

With respect to the treatment effect estimate for 4SC velocity (stairs/sec), a slight improvement was also observed, i.e., from 0.09 (95% CI: -0.038, 0.216) stairs/sec in the original analyses to 0.12 (95% CI: -0.588, 0.823) stairs/sec. These treatment effect estimates in the unit of stairs/sec correspond to 0.0225 tasks/sec and 0.03 tasks/sec, respectively.

Overall, it is concluded that the magnitude and statistical significance of the treatment effect estimates from the primary analyses could not be confirmed for all tested endpoints in the J2R analyses.

Only efficacy results in an enriched population have initially been provided, whereas these were missing for the off-target population. With the responses the applicant provided the requested efficacy analyses for the primary endpoint, the key secondary endpoints and further velocity endpoints for the "off-target" and the "overall population". Despite limitations in interpreting these results, e.g. due to the small sample size for the "off target population" with 37 subjects in the givinostat group and 22 subjects in the placebo group), it is considered that results were in line with those of the target population showing a positive trend in the givinostat group when compared to placebo. With regard to the primary endpoint time to 4SC, results in the overall population were consistent with those of the target population [treatment effect 0.84 (95% CI: 0.733, 0.961) and 0.86 (95% CI: 0.745, 0.989), respectively].

Subgroup analyses:

Only subgroup analyses based on total weight-adjusted exposure ≥ median were in support of the main analyses. However, according to the applicant, these results should be interpreted with caution. PopPK analyses of givinostat showed a non-linear relationship between givinostat exposure and weight. As a result, givinostat dose was adjusted to patient's weight with larger weight-adjusted doses administered to patients with lower body weight. Therefore, according to the applicant, the two subgroups by total weight-adjusted exposure (mg/kg) were not well balanced with respect to baseline characteristics, making it difficult to draw conclusions.

To determine the impact of the starting dose on the efficacy of givinostat, subgroup analyses for change from Baseline at 18 months were performed, comparing the subgroup of patients who started on the higher dose as specified in protocol versions ≤ 4.0 (Treatment Regimen 1) to those patients who started on the lower dose as specified in protocol versions > 4.0 (Treatment Regimen 2). There were 39 and 19 subjects in the givinostat and the placebo group, respectively who started treatment under protocol versions ≤ 4 compared to 42 and 20 subjects in the givinostat and the placebo group, respectively, who started treatment under protocol version > 4. Overall, results in these two subgroups were in line with those in all patients of the target population, showing a positive trend in the givinostat group when compared to the placebo group for the primary and the key secondary endpoints. Nevertheless, no statistical significance could be shown as the study was not powered for any subgroup analyses.

To determine the impact of Baseline age on the efficacy of givinostat, subgroup analyses for change from Baseline at 18 months were performed by baseline age 6 to 7 years of age and above 7 years of age. Overall, results in these two subgroups were in line with those in all patients of the target population, except that a negative trend was observed in the givinostat group when compared to the placebo group for 6MWT in the subgroup of patients aged 6 to 7 years at baseline. Nevertheless, no statistical significance could be shown for any positive or negative treatment effect in the subgroup analyses.

Study DSC/14/2357/51 (Open Label Extension Study)

At the cut-off date 31 December 2021 for the fourth interim analysis, a total of 194 subjects were enrolled in the study: 110 subjects in the givinostat group (subjects already treated with givinostat in Study 48 or Study 43), 54 subjects in the delayed givinostat group (subjects in placebo group during the Study 48 who were switched to givinostat in this study), and 30 subjects in the givinostat-naïve group. New data were available with cut-off date 31 December 2023 with a total of 207 subjects who were screened and enrolled in the study. Up to the latest cut-off date, there were 12 (5.8%) non-

ambulant subjects and 195 (94.2%) ambulant subjects included.

Seven subjects prematurely discontinued from the study, the majority of whom (5 [2.6%] patients) had withdrawn consent. The mean study duration was 564.7 days (SD=±374.26).

For ambulant patients in the ITT Analysis Set, baseline values in the givinostat (N = 102) and the delayed givinostat groups (N = 51) were almost comparable except for time to 10MWR (seconds), while 10MWR velocity was comparable. Given the observed treatment effect of givinostat in study 48 after 18 months of treatment, this finding is against expectation. To exclude the possibility, that baseline values for functional outcome measurements in the givinostat arm were affected by a more heterogeneous population in study 51, i.e., patients came from the former 43 and 48 studies, with different prerequisites in functional capacities for inclusion between these two studies the applicant provided an additional analysis set, excluding the patients from study 43. In this context it is also worth mentioning, that the 18 patients from study 43 were already treated for a duration of 52 months when rolled over to study 51 compared to those patients from study 48, who had been treated only for a period of 18 months. However, baseline values based on this additional analysis set still were comparable for those subjects who already were treated in study 48 with givinostat (givinostat treatment arm) and those randomised in study 48 to placebo (the delayed givinostat group). The applicant therefore concluded that the observed values were probably related to the fact that the analysis was done only in ambulant subjects at baseline of study 51 as well as due to treatment interruptions between study 48 and study 51. Of note, a relevant portion of patients in the givinostat group experienced temporary treatment interruptions, due to delays in Study 51 approval for certain sites and study closure due to the COVID-19 pandemic. A total of 24 out of 89 ambulant patients had an interruption of more than 30 days.

It is acknowledged that givinostat-naïve subjects, i.e. those who were not enrolled into study 48 as the off-target population was already completed, at baseline were generally younger than those subjects, already included in the previous studies.

Of note, there were 89 patients included in the givinostat treatment arm, who rolled over from study 48, including also several subjects from the former off-target population. However, information for these subjects, i.e., baseline disease characteristics and how these patients responded on givinostat treatment in the context of study 48 has not been provided. Given the fact, that these patients differed from the other patients, e.g. due to baseline vastus lateralis muscle fat fraction (VL MFF) assessed by MRS in the range ≤5% or >30%, the mean baseline values in study 51 for the givinostat arm could also be affected by those patients from the former off-target population of study 48. The applicant justified that comparable baseline values in study 51 for both treatment groups, the givinostat and the delayed givinostat group, probably relate to some treatment interruptions between study 48 and study 51 for earlier treated subjects, but may also be due to those patients from the former off target population of study 48 who also were included in the givinostat group for study 51 and who showed slower performance on several function tests.

Overall, descriptive analyses indicated that, ambulant patients in the ITT Analysis Set (N = 183) deteriorated over the course of the study as shown by mean change from Baseline in functional tests at 4 years. No significant differences were observed between the two treatment groups (early givinostat group and delayed givinostat group).

Overall, non-ambulant patients in the ITT Analysis Set (N = 11) showed a slight deterioration in both EK score and Barthel Index Total Score at 2 years and at 4 years of treatment; upper limb muscle strength was similar throughout the study.

In the ITT Analysis Set (N = 194), PUL (version 2) results showed a slight decline in total score. Motor Function as measured with MFM showed slight deterioration during the study until Month 24 for the

total score. At 4 years, mean motor function total score was approximately 10.5 points lower than at Baseline (n = 16). Pulmonary function tests (PFTs) showed a minimal increase from Baseline for forced vital capacity (FVC) while % predicted FVC showed a steady decrease. Peak expiratory force (PEF) showed a steady increase throughout the study period with a minimal decrease in % predicted PEF observed.

Random Coefficient Analyses were produced to take into consideration not only the effect of age on the pulmonary (FVC, PEF, decrease in % predicted FVC) and physical (6MWD, NSAA, 4SC, 10MWR and rise from floor velocity) function tests but also the fact that subjects were of different ages in the study. The models showed that the physical and pulmonary functions worsened with advancing age, similarly between the two treatment groups. No significant differences between the two treatment groups were noted also in the annual change for each pulmonary function and physical function parameters.

As study 51 included patients of different ages and with different extents of givinostat treatment, a mixed-effect random coefficient analysis was performed to fit the efficacy dependent variables against age, in which age was treated as a random effect, treatment group (givinostat/delayed givinostat) as fixed effect, treatment by age as interaction term and a random intercept was fitted. Results from these analyses with baseline defined as the last non-missing value prior to the subject receiving treatment on this study suggests the similarity of givinostat treatment effect in the givinostat and delayed givinostat groups for physical function tests related to lower limb function. However, as analysis have also included subjects in the givinostat arm from the former off-target population, baseline values are not representative for the two treatment arms and results therefore considered of limited value to demonstrate persistence of efficacy.

To demonstrate persistence of efficacy, the applicant was asked to provide further analyses for the relevant endpoints for change from baseline with baseline defined to be the baseline of study 48 for the givinostat group (without off-target) compared to patients of the delayed givinostat treatment. The applicant provided the requested analyses of change from baseline (defined as the baseline of Study 48) over time for the primary, relevant key secondary and velocity endpoints, using a J2R multiple imputation approach for handling Class 1 missing data, with patients in Study 51 who came from the ITT Analysis Set - Target Population of Study 48 being included in the analyses.

At EOS in Study 48, the results for change from baseline in primary, relevant key secondary and velocity endpoints using a J2R approach are in line with those reported for the pre-specified analyses, demonstrating overall a slower decline in the givinostat group compared to the delayed givinostat group (patients that received placebo until EOS of Study 48).

However, after EOS in Study 48 until Month 24 in Study 51, neither continuity (no clear trend over time) of the treatment effects observed at EOS in Study 48 nor consistency with respect to the difference between the givinostat and delayed givinostat group across all analysed endpoints could be identified.

Overall, due to the different strategies in data collection applied in Study 48 and Study 51, the different objectives and endpoints as well as a limited number of included patients for both treatment arms in Study 51, the results of the provided analyses are generally difficult to interpret, and no meaningful conclusions can be drawn.

In Study 51, with regard to the cut-off date 31 December 2021, overall, 37/194 patients lost ambulation (26/110 [23.6%] patients, 10/54 [18.5%] patients and 1/30 [3.3%] patient in the givinostat, delayed givinostat, and givinostat-naïve groups, respectively. Since study 51 has an open-label design data from this study do not have the convincing value of controlled data. However, the provided data indicate a median age at loss of ambulation was 18.0 years in the givinostat group. However, median age at loss of ambulation was not calculable in the other 2 groups. With regard to

the latest cut-off date, 78 subjects lost ambulation (19.1%), the median age at loss of ambulation was 16.7 years in the givinostat group, 17.3 years in the delayed givinostat group, while in the naïve givinostat group was not calculable.

Comparison of efficacy results from studies DSC/14/2357/48 and DSC/11/2357/51 with results from the natural history studies ImgagingDMD and CINRG

Analyses of data from the integrated summary of efficacy were provided to demonstrate persistence of efficacy of givinostat with long-term treatment and to show that givinostat can delay progression to clinically meaningful DMD disease milestones. However, the comparisons of the givinostat group of study 48 to patients from the Imaging DMD and CINRG studies are of limited value. Based on this approach, the obtained results are difficult to interpret and not representative, given the low number of subjects after the age of fourteen years.

With the responses, the applicant provided an update of the integrated analysis of long-term efficacy (ISE) based on the data from clinical studies, i.e. studies 48 and 51, and the Natural History Studies ImagingDMD and CINRG.

While initially, matching of each of the givinostat treated patients with a corresponding patient in the natural history control using a propensity score matching approach was not sufficiently effective, the 'full givinostat group' (ITT Analysis Set, N = 148) and 'full control group' (Natural History Data, N = 197) were used. Compared to the approach described in the ISE SAP, the new approach (based on covariates baseline 4SC, baseline TTR, baseline 10MWRT and baseline steroid use) improved matching, i.e., standardised mean difference of the logit score was 0.23 with a variance ratio of 1.66 for the new approach as compared to 0.37 and 1.68, respectively, for the ISE SAP approach. Moreover, with the new approach only 6 patients were discarded from the matching.

Overall, as the matching procedure was selected post-hoc, and it cannot be fully ensured that the results are not biased by other confounders, uncertainties remain with respect to the plausibility of the results.

Overall, the results of the ISE analysis can only be used as supportive evidence for the efficacy of givinostat.

Random Coefficient Analyses of 4SC Velocity

Interpretation of the presented results is limited. With regard to study 48, the model showed that 4SC velocity worsens with advancing age. Givinostat did not significantly prevent the decline in 4SC velocity as compared to placebo (p-value: 0.0717).

Results from these analyses on study 51 suggest similarity of givinostat treatment effect in the givinostat and delayed givinostat group. However, analysis also included in the givinostat arm subjects from the former off-target population.

Analysis on Change in Dosing Instructions

The dosing regimen was changed from Treatment Regimen 1 (starting Dose A with the option to reduce to Dose B) to Treatment Regimen 2 (starting Dose B with the option to reduce to Dose C) during the conduct of Study 48. The change in treatment regimen was introduced in Protocol Amendment 4, therefore, the Sponsor has conducted analyses to determine whether the change in dosing instructions affected the efficacy results in Study 48. An analysis of covariance (ANCOVA) was

performed by adding class factor for 'pre' and 'post' protocol amendment along with an interaction term between dosing schema and randomised treatment. The interaction between randomised treatment and treatment regimen was non-significant. Based on the provided analyses, no differences in effects were observed between the different treatment regimens.

In order to evaluate whether the givinostat treatment effect of the primary endpoint was disproportionately driven by the results of Dose A, patients on givinostat were grouped into those who had stayed on Dose A for the full study (Group A) and those patients in Treatment Regimen 1 who reduced the dose, together with patients in Treatment Regimen 2 (Group Not A). Based on the provided results givinostat treatment effect versus placebo was similar in Groups A and Not A. Further subgroup analyses were requested for the relevant efficacy endpoints based on the final 3 doses the patients received during the study as indicated above.

Following responses, the applicant provided a further justification regarding the evidence of efficacy of Duvyzat based on statistical aspects as well as on clinical data.

With respect to statistical robustness the applicant´s argumentation covers the following aspects:

- The amount of missing data in the primary analysis of Study 48 is not substantial (13/120 patients) and there is no evidence of bias in favor of givinostat
- The assertion that analyses without MI underestimate the true variability is not entirely accurate; it is the mechanism of MI itself that inflates the true variability
- It is not appropriate to base confirmatory decisions solely on the result of the Jump to Reference, which has important limitations
- When all approaches are considered (single imputation, MI, Jump to Reference) side-by-side, the treatment effect estimates and their CIs are substantively overlapping and thus consistent

Regarding the amount of missing data, the applicant included information on the missing data at end of study (EOS) for the primary endpoint in the primary analysis population of Study 48 in the response. The applicant maintained that the amount of missing data was not substantial (13/120 patients) and was balanced between treatment groups (givinostat: 9.9% vs. placebo: 12.8%). However, as indicated in the EMA Guideline on Missing Data in Confirmatory Clinical Trials (EMA/CPMP/EWP/1776/99 Rev. 1), there is no rule regarding the maximum amount of missing data that could still be considered non-substantial/acceptable. If the unmeasured value is related to the real value of the outcome (e.g. the unobserved values have a higher proportion of poor outcomes), this may lead to a biased estimate of the treatment effect even if the missing values are not related to treatment (i.e. missing values are equally likely in all treatment arms). Furthermore, the applicant stated that there is no evidence that these limited data were informatively missing, i.e., missingness in ambulatory patients was not due to poor tolerability and/or lack of efficacy, thus, there is no evidence that ambulant patients with missing data would benefit from treatment differently from ambulant patients without missing data. However, this assumption could not be verified and may not be plausible in practice. At least for patients who withdrew early from Study 48, which also implies early withdrawal from the study treatment, it seems not plausible that similar values as those for patients who continued the study treatment until EOS would be observed , had they been further followed-up until EOS after premature treatment discontinuation (i.e. according to the treatment policy strategy). For patients with missing values due to other reasons such as "fracture" or "patient not cooperative", it could also not be excluded that missingness would not be informative of the condition and potential outcome of patients. Generally, Missing Not At Random (MNAR) data is difficult to rule out, and it is not clear whether even a small amount of MNAR data could have an impact on the study results (reference is made to the EMA Guideline on Missing Data in Confirmatory Clinical Trials, EMA/CPMP/EWP/1776/99 Rev. 1). The applicant also argued that the imputed values were either similar or larger than the last

non-missing value and did not favour givinostat. However, even based on the observed values in the open-label extension Study 51 for 3 givinostat patients (7 out of 13 patients with missing values at EOS of Study 48 transitioned to Study 51, the remaining 6 patients withdrew from Study 48 and did not transition to Study 51), it could not be fully verified whether the imputed values would be conservative enough.

Based on the above, the underlying assumption of the pre-specified primary analysis using the single imputation and the sensitivity analysis using the multiple imputation is not verifiable and may not be plausible in practice. Furthermore, it could not be excluded that the single imputation would not underestimate the standard error by ignoring the uncertainty of imputed values. As shown by the results of the single imputation and the multiple imputation, even a difference in only the third decimal place of the estimated standard error (SEs were 0.071 vs. 0.077 in the single imputation and the multiple imputation, respectively) already led to a different conclusion (i.e. statistical significance of the primary endpoint could not be demonstrated in the sensitivity analysis using the multiple imputation). The sensitivity analysis using the jump-to-reference multiple imputation is generally a more conservative approach and would not likely overestimate the treatment effect and underestimate the variability of the estimated treatment effect, considering that any assumptions about the missing response are generally not verifiable. However, in the context of a disease modifying treatment, this analysis might lead to too conservative results and artificially reduce the power to detect a potentially effective treatment. Taking into account the results of the pre-specified primary analysis which was statistically significant and various sensitivity analyses based on different assumptions and the potential caveats associated with these methods efficacy is considered to be demonstrated, even if there is some uncertainty associated with the robustness of the estimated treatment effects.

With regard to clinical relevance of the observed treatment effects the applicant´s argumentation relates to the following aspects:

- The totality of evidence demonstrates that givinostat is an effective therapy with a constellation of consistently positive treatment effect estimates that are extremely unlikely to have arisen by chance alone
- Givinostat treatment effect is clinically relevant to patients with DMD

Similarly to previous responses, the applicant provided a discussion on the clinical relevance of the observed effects for the efficacy endpoints. The effect sizes were overall small but consistently showed a smaller deterioration compared to placebo after 18 months. While the applicant could not provide confirmation that the effect of a 14% less increase in the time to 4SC test for the primary endpoint in the primary analysis (log scale analysis) represents a clinically meaningful effect per se, further analyses and approaches, i.e. difference in seconds in 4SC based on non-log transformed data and results in 4SC velocity (task/seconds) are considered in the same range as the published data.

In addition, the applicant re-discussed the evaluation of long-term effects of Duvyzat based on data from clinical studies, i.e. studies 48 and 51, compared with natural history data using a matching approach. Although, the provided post-hoc analyses for DMD disease milestones can be interpreted as a delay of 1.7 to 3.3 years in progression for loss of function milestones for givinostat compared to natural history data these data can be considered only supportive of the results obtained in the 18 months placebo-controlled pivotal study, which by default is designed to produce the most valid results.

With the responses, the applicant provided additional analyses of the Study 48 data to justify a full MAA.

The applicant displayed the probability densities for key efficacy endpoints (4SC, NSAA and VL MFF) in the On-Target ITT population (using the multiple imputation for handling missing data). The applicant

also performed a Bayesian analysis. Under this analysis, the Off-Target Population serves as independent data to the prior distribution of efficacy previously established in the On-Target Population. The prior distribution is then updated to provide the posterior distribution of efficacy. For the primary endpoint 4SC, the probability that givinostat is superior to placebo is increased in the Bayesian analysis compared with the original On-Target results (98.52% vs 96.57%, respectively). For NSAA, the probability that givinostat is superior to placebo was already very high in the original On-Target results (>97.5%). These results indicate that the level of uncertainty in the efficacy of givinostat is low.

However, available data overall are still not considered comprehensive to justify a full MAA. Therefore, a conditional marketing authorisation is recommended.

Additional efficacy data needed in the context of a conditional MA

Duchenne Muscular Dystrophy (DMD) is a rare, disabling, progressive and ultimately fatal X-linked recessive neuromuscular disorder caused by mutations in the gene for dystrophin. Despite the rarity of the disease, a randomised placebo-controlled trial over 18 months was performed. Although this study provided statistically significant results demonstrating efficacy the chosen analysis was not considered robust enough and uncertainties remain that could not be solved by the provided sensitivity analyses. Additional data to replicate the results of the pivotal study and provide reassurance on the long-term effects of Duvyzat for the treatment of DMD are needed and a CMA is warranted (see section 3.7.3).

The applicant will conduct a randomised controlled trial as well as an observational PAES study to further confirm efficacy of Duvyzat in the treatment of DMD.

2.6.7. Conclusions on the clinical efficacy

In the primary analysis (analysis of log-transformed values) of change from baseline in time to climb 4 stairs (4SC) in seconds at month 18, a statistically significant difference was demonstrated when givinostat was compared against placebo ($p = 0.0374$). While the time to 4SC increased in the givinostat group from 3.39 to 4.70 seconds it increased even more in the placebo group from 3.48 to 6.37 seconds. The GLS mean ratio of 0.86 represents a 14% less increase in the time to 4SC (log scale analysis) at month 18 in favour of givinostat.

Further analyses with respect to the handling of missing data were provided upon the CHMP request for the primary, key secondary and velocity endpoints. The applicant provided results from analyses using jump- to- reference multiple imputation of missing data to confirm the robustness of the effects as requested. However, these analyses failed to show statistical significance and therefore statistical robustness of the obtained effect sizes may be questioned.

Moreover, the effect sizes were overall small with relatively wide confidence intervals for the treatment effect estimates although consistently showing a smaller deterioration compared to placebo after 18 months.

Although the applicant could not provide confirmation that the effect of a 14% less increase in the time to 4SC test for the primary endpoint in the primary analysis (log scale analysis) represents a clinically meaningful effect per se, as a MCID has not been established and is highly population dependent, further analyses and approaches, i.e. difference in seconds in 4SC based on non-log transformed data and results in 4SC velocity (task/seconds) are in the same range as the published data.

Dosing of givinostat was modified several times during the study. Given the uncertainties with respect to modified dosing during the study, the applicant could sufficiently justify, that efficacy results are not affected. The new proposed dosing regimen is also overall agreed with.

With regard to the applied indication, the restriction of the indication for givinostat to the studied patient population, i.e. ambulant DMD patients aged 6 years and older, and with concomitant corticosteroid treatment, has been agreed and the issue was resolved. Steroid naïve patients have not been studied in the DMD clinical programme.

Non-ambulatory patients have not been prospectively studied in the clinical DMD studies 43, 48, and 51, while preliminary findings on efficacy and safety in givinostat-treated patients who lost ambulation during the clinical studies do not imply a different benefit-risk conclusion. Therefore, the possibility to continue treatment with givinostat in patients becoming non-ambulatory during treatment is supported by available data, while treatment should not be initiated in patients being in a non-ambulatory stage of the disease.

Overall, efficacy is considered to be demonstrated. However, there are uncertainties on the robustness of the observed treatment effects. The absence of replication in an additional study and the need for more robust long-term data add to the fact that the data are not considered comprehensive to support a full MA as initially requested. Therefore, a CMA is recommended in agreement with the applicant.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

In order to confirm the efficacy and safety of givinostat in the treatment of Duchenne Muscular Dystrophy in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment, the MAH should conduct and submit the results of a randomised, double blind, placebo-controlled study in ambulant patients with Duchenne Muscular Dystrophy, according to an agreed protocol.

In order to confirm the long-term efficacy and safety of givinostat in the treatment of Duchenne Muscular Dystrophy in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment, the MAH should conduct and submit the final results of a non-interventional study based on data from patient registries and site based, according to an agreed protocol.

2.6.8. Clinical safety

The body of the integrated givinostat safety database in DMD includes three clinical studies, one open-label Phase 2 proof-of-concept study in patients with DMD aged 7 to <11 years, stable on steroids for at least 6 months (doses between 25 mg and 50 mg b.i.d in two parts with 2 weeks and 12 months duration plus extension phases up to 40 months; DSC/11/2357/43), a randomised, placebo-controlled Phase 3 study in ambulant patients aged ≥ 6 years to 16 years at study entry (18 months of treatment), with doses adjusted on the patient's weight (DSC/14/2357/48), and an open-label long-term (safety) study (DSC/14/2357/51) in all DMD patients, who have previously been treated in one of the parent studies 43 or 48 or were screened in Study 48, met all the inclusion criteria and none of the exclusion criteria but were never randomised because enrolment in the off-target group was completed and started givinostat in this study (i.e., naive givinostat DMD patients). This study provides long-term safety data and it may continue up to the envisaged marketing authorisation of givinostat. Safety from the interim data cut off 31 December 2021 is included in the MAA submission.

Safety data for givinostat were presented by pooling the three clinical controlled and uncontrolled studies 43, 48, and 51 (**Group 2 – DMD studies pool**), which forms the primary set of the safety evaluation. Moreover, long-term safety data from study 51 are also presented separately for the group

of patients treated continuously with givinostat from one of the parent studies.

Supportive data from other populations and clinical development programmes with givinostat have been provided and pooled analyses have been presented (Group 1 - Healthy subjects' studies, Group 3 - Non-oncology/non-DMD indications, Group 4 – Patients' studies, and Group 5 – Haematological malignancies).

According to the SAP for the integrated summary of safety (ISS), the Safety Population defined in each study protocol (i.e., all subjects who have received at least one dose of givinostat) forms the primary population for the integrated analyses.

The dose levels used in studies 43, 48, and 51 were slightly different but were classified for the pooled analysis in dose levels A, B and C, based on the patient's weight: Patients who received 37.5 mg b.i.d or who escalated to 50 mg b.i.d in Study 43 were assigned to dose level A and patients who received 25 mg b.i.d in Study 43 were assigned to dose level B. Exposure as well as adverse events and all subsequent safety data are presented by treatment groups and daily dose categories, i.e. Dose A (patients starting and remaining on this dose), Dose B (patients starting and remaining on this dose), Dose A-B-C (patients starting on Dose A with reduction to Dose B plus patients who then further reduced to Dose C), and Dose B-C (patients starting on Dose B and who reduced to Dose C).

2.6.8.1. Patient exposure

222 patients were treated with givinostat and are included in the DMD safety set: study 43 contributes 20 patients, study 48 contributes 118 patients, and study 51 contributes 84 patients [i.e. 54 patients on placebo during study 48 and 30 patients screened in study 48 without being treated]). Overall, 61 patients were treated with placebo in study 48.

As per the data cut-off for study 51, the 222 patients in the givinostat group were in the DMD studies for a median duration of 24.90 months and 61 patients in the placebo group were in the DMD studies for a median of 18.14 months (note: Study 51 is ongoing so the number of months in this study reflects the time of the data cut-off for this analysis, i.e. 31 December 2021). The total age range across studies at the DCO for givinostat was 6 to 16 years. 77.5% of patients treated with givinostat were 6 to <12 years and 22.5% of patients were ≥12 to 18 years.

17 patients (7.7%) in the givinostat group and 2 patients (3.3%) in the placebo group had prematurely discontinued from the studies, with 187 patients (84.2%) receiving ongoing open label givinostat treatment in Study 51.

41%, 31.1%, 14.4%, and 13.5% of all givinostat-treated patients received Dose B, followed by dose level A-B-C, Dose A, and Dose B-C throughout the DMD studies. The median duration at these dose levels in the DMD studies was 18.17, 44.68, 32.30, and 17.59 months. Patients included in the Dose level A-B-C group mimicking the proposed dosing regimen in the product label had therefore the highest duration in the studies.

Median treatment duration with givinostat irrespective of dose levels and of any temporary treatment interruption in the DMD studies was 23.77 months (range 0.4 to 103.9 months) and the median treatment exposure was 23.67 months (range 0.4 to 102.7 months; taking into account temporary treatment interruption). Treatment interruptions were noted during the studies and are also advised in the product information in case of increased triglycerides. 67 of 222 patients (30.2%) in the givinostat overall group had at least one temporary dose interruption, and the percentage was highest in the dose reduction groups, ranging between 42.2% and 70.8%. Median duration of the first dose interruption was 18 days. The reason for the first dose interruption was mainly blood triglycerides increased and hypertriglyceridaemia (9% and 3.6% of patients, respectively). Most patients with

temporary treatment interruption had one or two dose pauses (18.5% and 6.3%). The cumulative median interruption duration was 29 days.

Overall, 188 of 222 patients (~85%) have been exposed to givinostat for more than 12 months and 108 of 222 patients (~49%) had an exposure of more than 24 months.

The recommended starting dose for givinostat in DMD as indicated in the product information is Dose A (20 mg – 70 mg based on a body weight ranging between 10 to 70 kg and higher) twice daily. The starting dose should then be adjusted in case of safety / tolerability issues (platelet count decreases or diarrhoea or increased triglycerides) to Dose B (13.3 mg – 46.7 mg based on a body weight ranging between 10 to 70 kg and higher; i.e. a 33% reduction in dose), and if abnormalities are persistent, further down to Dose C (10.6 mg – 37.4 mg based on a body weight ranging between 10 to 70 kg and higher; i.e. a 20% reduction from Dose B).

2.6.8.2. Adverse events

The majority of safety data derive from a double-blind, placebo controlled study (study 48) of 18 months duration. A similar proportion of subjects had at least 1 TEAE in the givinostat and the placebo group (94.9% and 93.4%), with a majority of TEAEs in both treatment groups being mild to moderate in severity. Nevertheless, the proportion of subjects with SAEs was higher in the givinostat group (6.8% vs. 3.3%). Moreover, the proportion of subjects with TEAEs leading to withdrawal and leading to permanent discontinuation of the study drug was higher in the givinostat treated patients (2.5% vs. 0.0%) and (3.4% vs. 0.0%), respectively. Of note, the proportion of TEAEs leading to dose reduction was substantially higher in givinostat-treated patients (35.6% and 1.6%).

The overall incidence of TEAEs in the **Group 2 – DMD studies pool** was 93.4% in the placebo group, and ranged between 83.5% and 100% in the four dose level groups. The higher reporting rate in the A-B-C and in the B-C dose groups as compared to the other dose groups relates to the fact that these two groups include patients for whom a dose reduction from Dose A to B (and further to dose C) and from Dose B to C became necessary due to specific TEAEs (e.g. TEAEs related to platelet decreases, triglycerides, and diarrhoea). Severe related TEAEs were not observed with placebo and ranged between 3.1% and 5.8% in the four dose level groups.

The most frequently reported SOCs were GI disorders, followed by infections and infestations and investigations. Except for the infections and infestations SOC and the nervous system disorders SOC, all reported SOCs were reported more frequently in the givinostat group as compared to the placebo group. The most frequent TEAEs by PT for givinostat overall that were also notably higher compared to placebo were diarrhoea (39.6%), vomiting (28.4%), abdominal pain (23.0%), platelet count decreased (23.0%), pyrexia (20.7%), thrombocytopenia (18%), blood triglycerides increased (16.7%), arthralgia (10.4%), hypertriglyceridaemia (9%), decreased appetite (8.1%), rash (7.7%), and hypothyroidism (5.4%). Troponin increased occurred in 7.2% patients in the givinostat group and 0% in the placebo group, but troponin was only measured in study 51 where all patients received givinostat.

TEAEs reported by ≥5% of subjects in the overall givinostat or placebo group by SOC and PT are summarised in Table 21 (including TEAEs reported in the continuous givinostat group in study 51). The percentage of patients with frequent TEAEs (with an incidence of ≥5%) was lower in the long-term study 51 as compared to the pooled givinostat data for a majority of the events except for troponin increased (8.2% vs. 7.2%) and hypothyroidism (7.3% vs. 5.4%).

Table 21 Summary of most frequent TEAEs (5% or more of patients by PT in the overall Givinostat or placebo groups) by SOC and PT in DMD studies (safety set) – amended to include Givinostat group in DMD study 51 (safety set) for comparison

	Givinostat (ITF2357)											**Placebo**	
	A N=32		**B N=91**		**A-B-C N=69**		**B-C N=30**		**Givinostat Overall N=222**		**Givinostat group – study 51 – long-term* N=110**	**Overall N=61**	
SOC Preferred Term	**Patients (%)**	**Events (per patient-year)**	**Patients (%)**	**Events (per patient-year)**	**Patients (%)**	**Events (per patient-year)**	**Patients (%)**	**Events (per patient-year)**	**Patients (%)**	**Events (per patient-year)**	**Patients (%)**	**Patients (%)**	**Events (per patient-year)**
At least 1 TEAE	31 (96.9)	387 (4.08)	76 (83.5)	591 (4.15)	69 (100.0)	1281 (4.53)	29 (96.7)	330 (7.56)	205 (92.3)	2589 (4.59)	96 (87.3)	57 (93.4)	398 (4.48)
Gastrointestinal disorders	25 (78.1)	124 (1.31)	50 (54.9)	170 (1.19)	55 (79.7)	312 (1.10)	19 (63.3)	70 (1.60)	149 (67.1)	676 (1.20)	50 (45.5)	29 (47.5)	71 (0.80)
Diarrhoea	19 (59.4)	58 (0.61)	23 (25.3)	64 (0.45)	39 (56.5)	124 (0.44)	7 (23.3)	26 (0.60)	88 (39.6)	272 (0.48)	20 (18.2)	11 (18.0)	16 (0.18)
Vomiting	8 (25.0)	17 (0.18)	19 (20.9)	44 (0.31)	30 (43.5)	53 (0.19)	6 (20.0)	8 (0.18)	63 (28.4)	122 (0.22)	16 (14.5)	8 (13.1)	12 (0.14)
Abdominal pain	10 (31.3)	22 (0.23)	10 (11.0)	13 (0.09)	27 (39.1)	58 (0.21)	4 (13.3)	9 (0.21)	51 (23.0)	102 (0.18)	12 (10.9)	9 (14.8)	16 (0.18)
Abdominal pain upper	4 (12.5)	7 (0.07)	12 (13.2)	15 (0.11)	11 (15.9)	11 (0.04)	5 (16.7)	6 (0.14)	32 (14.4)	39 (0.07)	7 (6.4)	7 (11.5)	9 (0.10)
Nausea	1 (3.1)	1 (0.01)	6 (6.6)	9 (0.06)	9 (13.0)	20 (0.07)	5 (16.7)	5 (0.11)	21 (9.5)	35 (0.06)	5 (4.5)	4 (6.6)	6 (0.07)
Constipation	0	0	4 (4.4)	4 (0.03)	7 (10.1)	9 (0.03)	2 (6.7)	2 (0.05)	13 (5.9)	15 (0.03)	6 (5.5)	1 (1.6)	1 (0.01)
Dyspepsia	0	0	1 (1.1)	2 (0.01)	3 (4.3)	4 (0.01)	0	0	4 (1.8)	6 (0.01)	1 (0.9)	4 (6.6)	4 (0.05)
Infections and infestations	11 (34.4)	38 (0.40)	38 (41.8)	80 (0.56)	47 (68.1)	159 (0.56)	21 (70.0)	48 (1.10)	117 (52.7)	325 (0.58)	40 (36.4)	40 (65.6)	117 (1.32)
Nasopharyngitis	6 (18.8)	11 (0.12)	11 (12.1)	18 (0.13)	21 (30.4)	39 (0.14)	7 (23.3)	10 (0.23)	45 (20.3)	78 (0.14)	10 (9.1)	19 (31.1)	27 (0.30)
Gastroenteritis	1 (3.1)	1 (0.01)	3 (3.3)	3 (0.02)	14 (20.3)	20 (0.07)	2 (6.7)	3 (0.07)	20 (9.0)	27 (0.05)	6 (5.5)	3 (4.9)	3 (0.03)
COVID-19	1 (3.1)	1 (0.01)	8 (8.8)	8 (0.06)	7 (10.1)	7 (0.02)	3 (10.0)	3 (0.07)	19 (8.6)	19 (0.03)	8 (7.3)	1 (1.6)	1 (0.01)
Upper respiratory tract infection	1 (3.1)	1 (0.01)	5 (5.5)	5 (0.04)	7 (10.1)	11 (0.04)	4 (13.3)	5 (0.11)	17 (7.7)	22 (0.04)	7 (6.4)	8 (13.1)	9 (0.10)
Influenza	2 (6.3)	4 (0.04)	1 (1.1)	1 (0.01)	11 (15.9)	15 (0.05)	0	0	14 (6.3)	20 (0.04)	2 (1.8)	4 (6.6)	4 (0.05)
Rhinitis	2 (6.3)	4 (0.04)	2 (2.2)	3 (0.02)	4 (5.8)	7 (0.02)	3 (10.0)	4 (0.09)	11 (5.0)	18 (0.03)	4 (3.6)	7 (11.5)	8 (0.09)
Ear Infection	1 (3.1)	2 (0.02)	4 (4.4)	6 (0.04)	4 (5.8)	5 (0.02)	0	0	9 (4.1)	13 (0.02)	3 (2.7)	4 (6.6)	4 (0.05)
Investigations	16 (50.0)	48 (0.51)	26 (28.6)	49 (0.34)	53 (76.8)	130 (0.46)	19 (63.3)	46 (1.05)	114 (51.4)	273 (0.48)	42 (38.2)	9 (14.8)	14 (0.16)
Platelet count decreased	5 (15.6)	7 (0.07)	4 (4.4)	5 (0.04)	37 (53.6)	71 (0.25)	5 (16.7)	9 (0.21)	51 (23.0)	92 (0.16)	10 (9.1)	0	0
Blood triglycerides increased	6 (18.8)	9 (0.09)	8 (8.8)	10 (0.07)	12 (17.4)	17 (0.06)	11 (36.7)	20 (0.46)	37 (16.7)	56 (0.10)	14 (12.7)	3 (4.9)	6 (0.07)
Troponin increased	6 (18.8)	8 (0.08)	8 (8.8)	8 (0.06)	2 (2.9)	3 (0.01)	0	0	16 (7.2)	19 (0.03)	9 (8.2)	0	0
Blood thyroid stimulating hormone increased	3 (9.4)	3 (0.03)	5 (5.5)	5 (0.04)	4 (5.8)	6 (0.02)	0	0	12 (5.4)	14 (0.02)	5 (4.5)	1 (1.6)	1 (0.01)
Injury, poisoning and procedural complications	14 (43.8)	35 (0.37)	29 (31.9)	66 (0.46)	43 (62.3)	131 (0.46)	12 (40.0)	43 (0.99)	98 (44.1)	275 (0.49)	49 (44.5)	19 (31.1)	49 (0.55)
Fall	7 (21.9)	9 (0.09)	13 (14.3)	18 (0.13)	22 (31.9)	33 (0.12)	7 (23.3)	16 (0.37)	49 (22.1)	76 (0.13)	22 (20.0)	13 (21.3)	18 (0.20)
Ligament sprain	3 (9.4)	3 (0.03)	6 (6.6)	6 (0.04)	9 (13.0)	14 (0.05)	2 (6.7)	2 (0.05)	20 (9.0)	25 (0.04)	6 (5.5)	3 (4.9)	4 (0.05)
Contusion	1 (3.1)	1 (0.01)	4 (4.4)	6 (0.04)	7 (10.1)	24 (0.08)	4 (13.3)	14 (0.32)	16 (7.2)	45 (0.08)	4 (3.6)	3 (4.9)	6 (0.07)
Joint injury	1 (3.1)	2 (0.02)	4 (4.4)	7 (0.05)	5 (7.2)	8 (0.03)	2 (6.7)	3 (0.07)	12 (5.4)	20 (0.04)	9 (8.2)	2 (3.3)	2 (0.02)
Limb injury	4 (12.5)	6 (0.06)	2 (2.2)	2 (0.01)	4 (5.8)	6 (0.02)	2 (6.7)	3 (0.07)	12 (5.4)	17 (0.03)	4 (3.6)	2 (3.3)	2 (0.02)
Femur fracture	1 (3.1)	1 (0.01)	2 (2.2)	3 (0.02)	7 (10.1)	8 (0.03)	0	0	10 (4.5)	12 (0.02)	6 (5.5)	0	0
Musculoskeletal and	13 (40.6)	35 (0.37)	21 (23.1)	35 (0.25)	41 (59.4)	107 (0.38)	8 (26.7)	16 (0.37)	83 (37.4)	193 (0.34)	41 (37.3)	21 (34.4)	38 (0.43)

	Givinostat (ITF2357)											Placebo	
	A N=32		B N=91		A-B-C N=69		B-C N=30		Givinostat Overall N=222		Givinostat group – study 51 – long-term* N=110	Overall N=61	
SOC Preferred Term	Patients (%)	Events (per patient-year)	Patients (%)	Events (per patient-year)	Patients (%)	Events (per patient-year)	Patients (%)	Events (per patient-year)	Patients (%)	Events (per patient-year)	Patients (%)	Patients (%)	Events (per patient-year)
connective tissue disorders													
Arthralgia	5 (15.6)	7 (0.07)	4 (4.4)	6 (0.04)	11 (15.9)	13 (0.05)	3 (10.0)	4 (0.09)	23 (10.4)	30 (0.05)	7 (6.4)	1 (1.6)	1 (0.01)
Pain in extremity	7 (21.9)	8 (0.08)	3 (3.3)	7 (0.05)	12 (17.4)	16 (0.06)	1 (3.3)	1 (0.02)	23 (10.4)	32 (0.06)	8 (7.3)	7 (11.5)	10 (0.11)
Back pain	3 (9.4)	3 (0.03)	2 (2.2)	4 (0.03)	12 (17.4)	17 (0.06)	1 (3.3)	1 (0.02)	18 (8.1)	25 (0.04)	9 (8.2)	8 (13.1)	8 (0.09)
Myalgia	2 (6.3)	2 (0.02)	4 (4.4)	6 (0.04)	5 (7.2)	10 (0.04)	3 (10.0)	4 (0.09)	14 (6.3)	22 (0.04)	1 (0.9)	2 (3.3)	2 (0.02)
General disorders and administration site conditions	10 (31.3)	31 (0.33)	19 (20.9)	26 (0.18)	40 (58.0)	81 (0.29)	8 (26.7)	13 (0.30)	77 (34.7)	151 (0.27)	34 (30.9)	12 (19.7)	14 (0.16)
Pyrexia	5 (15.6)	14 (0.15)	10 (11.0)	12 (0.08)	26 (37.7)	37 (0.13)	5 (16.7)	6 (0.14)	46 (20.7)	69 (0.12)	19 (17.3)	5 (8.2)	6 (0.07)
Fatigue	6 (18.8)	8 (0.08)	4 (4.4)	4 (0.03)	9 (13.0)	11 (0.04)	2 (6.7)	2 (0.05)	21 (9.5)	25 (0.04)	6 (5.5)	0	0
Respiratory, thoracic and mediastinal disorders	8 (25.0)	13 (0.14)	20 (22.0)	44 (0.31)	30 (43.5)	48 (0.17)	11 (36.7)	20 (0.46)	69 (31.1)	125 (0.22)	27 (24.5)	15 (24.6)	25 (0.28)
Cough	7 (21.9)	8 (0.08)	7 (7.7)	9 (0.06)	16 (23.2)	19 (0.07)	6 (20.0)	7 (0.16)	36 (16.2)	43 (0.08)	12 (10.9)	9 (14.8)	9 (0.10)
Epistaxis	2 (6.3)	3 (0.03)	4 (4.4)	14 (0.10)	5 (7.2)	6 (0.02)	4 (13.3)	8 (0.18)	15 (6.8)	31 (0.05)	5 (4.5)	5 (8.2)	9 (0.10)
Oropharyngeal pain	1 (3.1)	1 (0.01)	4 (4.4)	4 (0.03)	7 (10.1)	9 (0.03)	2 (6.7)	2 (0.05)	14 (6.3)	16 (0.03)	5 (4.5)	1 (1.6)	1 (0.01)
Nervous system disorders	5 (15.6)	7 (0.07)	18 (19.8)	38 (0.27)	28 (40.6)	69 (0.24)	10 (33.3)	12 (0.28)	61 (27.5)	126 (0.22)	19 (17.3)	17 (27.9)	33 (0.37)
Headache	2 (6.3)	3 (0.03)	18 (19.8)	37 (0.26)	25 (36.2)	49 (0.17)	9 (30.0)	11 (0.25)	54 (24.3)	100 (0.18)	16 (14.5)	14 (23.0)	30 (0.34)
Skin and subcutaneous tissue disorders	5 (15.6)	8 (0.08)	11 (12.1)	13 (0.09)	27 (39.1)	50 (0.18)	7 (23.3)	13 (0.30)	50 (22.5)	84 (0.15)	9 (8.2)	10 (16.4)	12 (0.14)
Rash	1 (3.1)	1 (0.01)	4 (4.4)	5 (0.04)	10 (14.5)	17 (0.06)	2 (6.7)	5 (0.11)	17 (7.7)	28 (0.05)	2 (1.8)	1 (1.6)	1 (0.01)
Blood and lymphatic system disorders	3 (9.4)	3 (0.03)	4 (4.4)	4 (0.03)	27 (39.1)	48 (0.17)	11 (36.7)	16 (0.37)	45 (20.3)	71 (0.13)	12 (10.9)	0	0
Thrombocytopenia	1 (3.1)	1 (0.01)	3 (3.3)	3 (0.02)	26 (37.7)	46 (0.16)	10 (33.3)	15 (0.34)	40 (18.0)	65 (0.12)	10 (9.1)	0	0
Metabolism and nutrition disorders	5 (15.6)	7 (0.07)	12 (13.2)	20 (0.14)	23 (33.3)	41 (0.14)	3 (10.0)	11 (0.25)	43 (19.4)	79 (0.14)	14 (12.7)	3 (4.9)	5 (0.06)
Hypertriglyceridaemia	1 (3.1)	1 (0.01)	5 (5.5)	7 (0.05)	11 (15.9)	24 (0.08)	3 (10.0)	9 (0.21)	20 (9.0)	41 (0.07)	9 (8.2)	1 (1.6)	3 (0.03)
Decreased appetite	2 (6.3)	3 (0.03)	3 (3.3)	3 (0.02)	12 (17.4)	14 (0.05)	1 (3.3)	2 (0.05)	18 (8.1)	22 (0.04)	2 (1.8)	0	0
Endocrine disorders	5 (15.6)	9 (0.09)	3 (3.3)	3 (0.02)	14 (20.3)	17 (0.06)	1 (3.3)	1 (0.02)	23 (10.4)	30 (0.05)	15 (13.6)	0	0
Hypothyroidism	3 (9.4)	3 (0.03)	1 (1.1)	1 (0.01)	7 (10.1)	7 (0.02)	1 (3.3)	1 (0.02)	12 (5.4)	12 (0.02)	8 (7.3)	0	0

SOC results for all patients are presented but only PTs reported in ≥5% patients are included (hence reported patients with each preferred term does not add up to the total number of patients reporting events by SOC).
Note 1: AEs with onset date ≥ date of first study medication intake are presented in this table; Note 2: AEs have been coded using the MedDRA dictionary (Version 24.1). SOC and PT are presented.
Note 3: Percentages are based on the number of patients (N); Note 4: Patients are only counted once per treatment and dosage level for each row in the column 'Patients (%)'.
Note 5: A: dose level A maintained throughout the study; B: dose level B maintained throughout the study; A-B-C: dose level A decreased to dose level B throughout the study, and dose level A decreased to dose level B and then C throughout the study; B-C: dose decreased from level B to level C.
Note 6: dose level A: ranging from 20 to 70 mg b.i.d, based on patients' weight; dose level B: ranging from 13.3 to 46.7 mg b.i.d, based on patients' weight; dose level C: ranging from 10.6 to 37.4 mg b.i.d., based on patients' weight. Data Source: Module 5.3.5.3, Table 14.3.1.2.2.1
* Summary of TEAEs (matched to the SOCs/ PTs from the Group 2 DMD studies pool) reported in the long-term givinostat group in DMD Study 51 (Safety Set), Data Source: Module 5.3.5.2, Study 51 CSR Table 14.3.1.2.1.

The median time to first dose reduction based on 97 patients with dose reductions in the A-B-C and B-C group was 105 days (15 weeks). The median time to the second dose reduction was 516.5 days (applied in 24 of 69 patients included in the A-B-C dose regimen group). The majority of TEAEs occurred before the first dose reduction (93.9%) or after the first reduction (90.9%), with only 20.2% of TEAEs occurring after the second dose reduction. Diarrhoea, thrombocytopenia, and platelet count decreased were the trigger for dose reductions, thus, most TEAEs were seen before the first reduction (e.g., for diarrhoea, 40.4% of TEAEs occurred before the first dose reduction, 21.2% occurred after the first dose reduction, and 3.0% occurred after the second dose reduction). In contrast, TEAEs from the Musculoskeletal and connective tissue disorders SOC as well as PTs of pyrexia and fall were reported more frequently after the first dose reduction.

Treatment-related TEAEs (ADRs) occurred with a higher frequency in the givinostat overall group (and most frequently for patients on dose level A-B-C [100%] and dose level B-C [86.7%]) as compared to placebo (27.9%). Related TEAEs were higher in the givinostat overall group as compared to placebo and were most frequently reported for the SOCs investigations, followed by GI disorders, and blood and lymphatic system disorders. The most frequently related TEAEs in the givinostat overall group were diarrhoea (27.5%), platelet count decreased (22.5%), thrombocytopenia (17.6%), blood triglycerides increased (15.3%), and abdominal pain (15.3%).

Most TEAEs were mild or moderate in severity. Severe TEAEs were reported in 16.7% of patients in the givinostat overall group (50 events) compared to 1 event in 1 patient in the placebo group. The only severe TEAEs reported in more than 2 patients in the givinostat group were femur fracture (5 patients, 2.3%), blood triglycerides increased (4 patients, 1.8%), back pain (3 patients, 1.4%), platelet count decreased (3 patients, 1.4%), and chest pain (3 patients, 1.4%). Severe TEAEs in study 51 were experienced by 11.8% of patients and included events of fractures, back pain, blood triglycerides increased, low density lipoprotein increased, tendinous contracture, epiphysiolysis, rhabdomyolysis, dehydration and anuria. No Grade 4 or Grade 5 TEAEs were reported up to the data cut-off.

Justification of ADRs to be included in section 4.8 of the SmPC was provided by the applicant and comprised TEAEs by PT reported > 5% of givinostat - treated patients in the Group 2 DMD pool, and reported with a frequency at least twice the frequency in the placebo group. TEAEs should then have a plausible causality with givinostat treatment.

The following TEAEs were therefore identified as potential ADRs: *Thrombocytopenia (including platelet count decreased), diarrhoea, vomiting, pyrexia, hypertriglyceridaemia (including blood triglycerides increased), and arthralgia* with a frequency "very common", and *hypothyroidism, constipation, fatigue, blood thyroid stimulating hormone increased, decreased appetite, and rash* with a frequency "common".

Troponin increased (albeit fulfilling the frequency requirement for being rated an ADR) has not been included as ADR based on recent literature review indicating that troponin increases occur to a variable extent in DMD patients and taking into consideration that troponin has only been measured in study 51 lacking a placebo comparison. COVID-19 and oropharyngeal pain met the frequency criteria outlined above but were not included as ADRs as these events are exogenous in nature and a causal relationship to givinostat was not considered plausible.

Adverse events of special interest

AESI were defined in the SAP for the ISS based on the Group 2 – DMD studies pool and include Thrombocytopenia/ platelet count decrease, Hypertriglyceridaemia/ triglyceride increase, Gastrointestinal disturbances: diarrhoea, nausea, vomiting and abdominal pain, and QT prolongation. A total of 172 patients (77.5%) reported 830 AESIs in the givinostat group compared to 26 patients (42.6%) with 68 AESIs in the placebo group.

Thrombocytopenia/ platelet count decreased

Platelet count decreased and thrombocytopenia were reported by 23% and 18% of patients in the overall givinostat group and in no subject on placebo. Based on a Standardised MedDRA query (SMQ) which includes all terms related to thrombocytopenia and platelet count decreases, a total of 157 events in 85 patients (38.3%) were reported. None of the events led to clinical signs and symptoms of thrombocytopenia. Two events of thrombocytopenia were reported as SAEs during Study 43: one occurred during the dose escalation Part 1 with a platelet nadir of 20 x 10^9/L (CTCAE Grade 4, life-threatening) after 2 weeks of treatment, rated related to givinostat, leading to discontinuation of the study drug with recovery to normal within 2 weeks. No bleeding events have been reported. The second SAE was reported ~2.5 years after initiation of givinostat during an extension phase, within 20 days of a weight-based dose increase from 25 mg b.i.d to 33 mg b.i.d. Platelet counts decreased to a nadir of 48 × 10^9/L, recovered within 3 days after treatment stop to >LLN, and no concomitant bleeding event was reported. Givinostat was restarted 12 days later at a dose of 25 mg b.i.d.

The median change from baseline to the end of treatment visit for platelet values for Group 2 studies in DMD was -93.00 x 10^9/L for the overall givinostat group (no change in the placebo group). The median decrease from baseline was greatest with the highest dose (Dose A: -116.00 x 10^9/L) and reduced with reduction in dose as indicated in Dose group A-B-C (-80.00 x 10^9/L).

The median change from baseline to each post-baseline visit was highest in the overall givinostat group at Month 6 (-123.50 x 10^9/L) and lower thereafter (with an outlier at Month 69 of -137.0 x 10^9/L). The same time course was observed for the Dose A, B, A-B-C, and B-C groups.

The median time to nadir for platelets was 88 days in the overall givinostat group (highest in Dose A group [133 days], followed by Dose B [114 days], Dose B-C [87.5 days], and Dose A-B-C [60.5 days].

Shifts from normal at baseline to low post-baseline for givinostat in study 48 were highest at Week 12 (31.4% of patients).

Changes in platelet were assessed during long-term treatment with givinostat in study 51. The mean platelet count in patients treated with givinostat in studies 43 and 48 was low at study 51 baseline and remained at this level. Five patients (4.5%) in the long-term givinostat group reduced their dose due to platelet count decreases.

Based on pharmacometric modelling of the platelet count data derived from healthy volunteer studies and studies 43 and 48 (in patients with DMD), a significant influence of body weight on baseline platelet count was found, i.e., baseline platelet count was significantly decreased with increasing body weight, which is likewise correlated with older age. Simulations indicated that the incidence of thrombocytopenia >Grade 1 was predicted to be 17%, 6%, and 2% in the worst-case scenario for subjects enrolled in Study 48 and body weight of ≥60-< 70 kg, who had the highest exposures at Dose A, B, and C, respectively. The incidence of thrombocytopenia was predicted to be lower in subjects with lower body weight (receiving lower dose levels).

Hypertriglyceridaemia/ triglyceride increase

Triglyceride increases were reversible with interruption/ discontinuation of givinostat treatment.

Blood triglycerides increased and hypertriglyceridaemia were reported in a higher number of patients on givinostat compared to placebo (16.7% vs. 4.9%, and 9.0% vs. 1.6%). Blood triglycerides increased, hypertriglyceridaemia, and blood triglycerides abnormal as individual PTs for 'triglycerides increase' were then analysed according to the SAP, which resulted in 98 events in 56 patients (25.2%) in the givinostat group and 9 events in 4 patients (6.6%) in the placebo group. The incidence of TEAEs based on this evaluation suggests a dose-dependency for Dose A and B (21.9% and 14.3% of patients) but not for Dose A-B-C and B-C (31.9% and 46.7%).

There were no SAEs related to triglycerides increased/ hypertriglyceridaemia. Three patients discontinued treatment with givinostat due to blood triglycerides increased (2 patients) and hypertriglyceridaemia (1 patient; all of them in dose reduction groups A-B-C and B-C), all of them with already high triglyceride values at baseline. First increases in triglycerides in these patients occurred between 2 and 8 months following treatment initiation with givinostat, the highest values for triglycerides recorded in these patients were between 5.08 mmol/L and 5.38 mmol/L (Grade 2), and all of them had various treatment interruptions after reoccurrence of the events with lower doses. The events were rated as related to givinostat and as recovered/ resolved for two of the three patients.

The median change from baseline to EOT visit for triglycerides for Group 2 studies in DMD was 0.43 mmol/L for the overall givinostat group and lower in the placebo-treated subjects (0.18 mmol/L). The median increase from baseline was highest for Dose A (0.61 mmol/L), followed by Dose B-C (0.55 mmol/L), and lower for Dose B (0.34 mmol/L). The maximum value achieved was 6.67 mmol/L in the givinostat group and 5.78 mmol/L in the placebo group. The median change from baseline was highest in the overall givinostat group at Month 3 and 6 and roughly plateaued thereafter, but not in the Dose A group, when triglycerides slightly increased further and were highest around Month 24.

The median time to maximum triglyceride levels was 221.0 days in the overall givinostat group (highest in the Dose A group with 385.5 days and lowest for Dose B-C with 95 days) compared to 255.0 days in the placebo group.

The mean triglyceride value in patients treated with givinostat in studies 43 and 48 during study 51 was already higher at study 51 baseline as compared to the delayed or naïve givinostat group and remained roughly stable at this increased level throughout long-term treatment except for some fluctuation. The median maximum value was 2.510 mmol/L in the givinostat group, the median time to maximum was 112.0 days, and median time to recovery was 103.0 days. Two patients in the long-term givinostat group reduced their dose due to triglyceride increases.

Shifts from normal at baseline to high post-baseline for givinostat were highest at Week 8 (42.4% of patients).

Pharmacometric modelling of the triglyceride data did not show a significant association with givinostat systemic exposure.

Gastrointestinal disturbances: diarrhoea, nausea, vomiting and abdominal pain

AESIs related to events in the GI disorders SOC were reported by 60.8% of patients in the givinostat group (573 events; 1.02 TEAEs per patient-year) and 41.0% of patients on placebo (59 events; 0.66 TEAEs per patient-year). Diarrhoea, nausea, vomiting and abdominal pain were reported in a higher number of patients in the givinostat group compared to the placebo group. Diarrhoea occurred more than twice as high with givinostat as compared to placebo (39.6% vs. 18%).

AESI of GI disorders with givinostat were mainly mild to moderate in severity (2 subjects reported a severe TEAE based on Group 2 – DMD studies pool), were reported as SAEs in two patients (abdominal pain and vomiting), and led to discontinuation of givinostat in three patients (nausea, diarrhoea, and abdominal pain). The events leading to discontinuation occurred at different time points during treatment and were rated related to givinostat.

Based on pharmacometric modelling of the diarrhoea data derived from healthy volunteer studies and studies 43 and 48 (in subjects with DMD), a significant association of increased incidence of diarrhoea with increasing givinostat systemic exposure was noted. Lower starting doses as per the protocol versions >4 in study 48 were associated with a significant reduction in the reporting of TEAEs from the GI disorders SOC, mainly for diarrhoea.

QT prolongation

No AESIs of QT prolongation were reported in patients included in the DMD studies (Group 2).

Evaluation of the effect of givinostat on cardiac repolarisation in the QTc study 54, revealed that a supratherapeutic dose of 300 mg (~6 x the recommended therapeutic dose) had clinically relevant effects on the heart rate and QTc interval, with a peak effect at 5 hours post-dose of 13.6 msec (90% CI: 10.15 to 17.05). For 100 mg givinostat, which is a therapeutic dose, the highest mean effect on ΔΔQTcF was 5.5 msec (90% CI: 1.99 to 9.01), which is lower than the clinically relevant threshold of 10 msec.

Based on an E_{max} model, a QT effect (ΔΔQTcF) exceeding 10 msec can be excluded within the full observed range of givinostat plasma concentration, up to approximately 745 ng/mL. There were no subjects with QTcF > 480 msec or ΔQTcF > 60 msec for the therapeutic dose group. Few abnormalities in ECG results were observed in the supratherapeutic dose group and moxifloxacin group only, none of these were considered clinically significant by the Investigator. No TEAEs related to ECGs abnormalities were reported in this study.

Osteoporosis/ osteopenia

Osteoporosis/ osteopenia was not a prespecified AESI for givinostat; however, a SMQ was conducted to examine the occurrence of these events, since several different fractures were reported as TEAEs and given that DMD itself is associated with an increased risk of bone fragility due to prolonged glucocorticoid therapy and progressive muscle weakness on bone strength.

TEAEs were more frequently reported in the givinostat group, i.e. 38 events in 30 patients (13.5%), as compared to placebo, i.e. 8 events in 4 patients (6.6%), while TEAEs per patient-year were similar in the two groups (0.07 TEAEs and 0.09 TEAEs PPY, respectively). The highest reporting of TEAEs was noted in Dose group A-B-C (27.5%), while TEAEs were reported in <10% of patients for the remaining dose levels. The TEAEs with the highest incidence derived from the SOC of injury, poisoning and procedural complications and were related to fractures (10.8% and 3.3% in the overall givinostat and placebo, respectively). The most frequently reported fractures for givinostat were femur fractures in 10 patients (12 events), followed by spinal fractures reported in 5 patients (5 events). No femur fracture and only a single spinal fracture occurred in the placebo group. Osteoporosis, osteopenia, and bone density decreased were less frequently reported and similar for the givinostat and placebo group.

2.6.8.3. Serious adverse events, deaths, and other significant events

No deaths were reported across all DMD studies.

Serious adverse events

The incidence of SAEs in the Group 2 - DMD studies pool (in study 48) was increased in the givinostat group (pooled data: 17.1%; study 48: 6.8%) as compared to placebo (3.3%). SAEs most frequently occurred in the A-B-C dose group (30.4%), followed by Dose A (12.5%), Dose B (12.1%), and Dose B-C (6.7%). Overall, the only SAEs reported in more than 1 patient in the givinostat group were femur fracture (8 patients, 3.6%), chest pain (3 patients, 1.4%), and back pain, rhabdomyolysis, tendinous contracture, platelet count decreased, and dehydration (each in 2 patients, 0.9%). The 2 patients in the placebo group both experienced an event of gastroenteritis.

SAEs with givinostat in the placebo-controlled *study 48* included 8 patients with 9 SAEs (6.8%) versus 2 patients in the placebo group (3.3%). The SAEs of rash and dizziness occurred in the same subject but on different occasions. None of these SAEs has been rated as related by the investigator.

SAEs with givinostat in the uncontrolled *study 43* included 1 SAE in Part 1 (platelet count decreased, leading to discontinuation), 2 SAEs during Part 2 of the study (lower limb fracture, rhabdomyolysis),

and a further 8 patients with 9 SAEs during the extensions of study 43 (Cushing's Syndrome, Cataracts (2 events), chest pain, femur fracture (2 events), platelet count decreased, haematuria, and hypertension). Except for the SAEs of platelet count decreased, none was rated related to givinostat.

Up to the data cut-off in *study 51*, there were 23 subjects (11.9%) with 26 SAEs. Slightly more SAEs were reported in the delayed givinostat group as compared to the continuous givinostat group and the naïve givinostat group (14.8% vs. 10.9% vs. 10%). The most frequently reported SOCs with SAEs in study 51 were injury, poisoning and procedural complications (4.6%) and the SAEs by PTs in this SOC are femoral fracture (6 subjects), femoral neck fracture, patella fracture, and wrist fracture (one subject each). 8 patients reported a SAE from the musculoskeletal and connective tissue disorders SOC with PTs of back pain and tendinous contracture (2 patients each), foot deformity, muscle contracture, rhabdomyolysis, and vertebral wedging (1 patient each). Dehydration has been reported in 2 subjects. All other reported SAEs have been reported in a single subject only and included appendicitis, infected cyst, rectal abscess, urinary tract infection, atrial fibrillation, and chest pain.

The SAE of atrial fibrillation occurred 475 days after initiation of IMP and was rated Grade 3 (severe or medically significant but not immediately life-threatening). ECG showed tachycardia at 200 bpm with an irregular rhythm, with no p-wave, and spontaneous decrease of heart rate to 120 bpm while givinostat was still administered. Sinus rhythm was found upon a new ECG. The patient was treated with antiarrhythmic medication for the SAE and givinostat was permanently discontinued. The patient had several concomitant medications at the time of the SAE, including steroids, perindopril for cardiomyopathy, and osteoporosis prevention medication. The medical history included vertebral fracture. The SAE was finally rated as related to givinostat and considered a SUSAR.

2.6.8.4. Laboratory findings

Haematology

For most haematology and coagulation parameters, small but no clinically relevant changes were seen during the clinical DMD studies in the overall givinostat group compared to placebo, including slight decreases from baseline for givinostat as compared to placebo for white cell lines, mainly leukocytes (up to Week 12) and neutrophils (up to Week 24).

White blood cell count decreased and leukopenia were reported as TEAEs in 5 and 3 patients on givinostat (2.3% and 1.4%) and in no patient on placebo. Anaemia and neutropenia were reported as TEAE in a single patient each on givinostat (and in none of the placebo-treated patients).

For haemoglobin, there was a median change (decrease) from baseline to EOT of -3.03 g/L for givinostat and 0.00 g/L for placebo (lowest at Month 3). For erythrocytes, there was a median change (decrease) from baseline to EOT of -0.57 x 10^{12}/L for givinostat and 0.01 x 10^{12}/L for placebo (lowest at Month 12).

For erythrocyte mean corpuscular haemoglobin (MCH), there was a median change (increase) from baseline to EOT of 3.00 pg for givinostat and 0.10 pg for placebo (highest around Month 6).

For erythrocyte mean corpuscular volume (MCV) a greater increase to the end of treatment was seen in the givinostat group (median change from baseline of 7.70 fL) compared to the placebo group (median change from baseline of 1.00 fL) (highest around Month 12).

TEAEs in line with decreases in white or red blood cell counts were rarely reported in the DMD studies pool.

Chemistry

Small but no clinically relevant changes were noted for most chemistry parameters during the clinical studies in the overall givinostat group compared to placebo. However, for alanine aminotransferase and alkaline phosphatase, a greater median change (*decrease*) from baseline to EOT was seen for givinostat as compared to placebo (givinostat: - 96.00 U/L for ALT and - 13.50 U/L for ALK; placebo: - 82.00 U/L for ALT and - 4.00 U/L for ALK).

A slight increase was seen from baseline to EOT in median *total bilirubin* and median *direct bilirubin* for givinostat (1.59 µmol/L and 0.15 µmol/L). Mean values for total and direct bilirubin remained within the reference range at all time points. Shift tables in study 48 for total bilirubin and direct bilirubin indicated shifts from normal at baseline to high post-baseline in less than 10% of subjects at each visit, which remained stable over the study. TEAEs in line with blood bilirubin increased and bilirubin conjugated increased were reported in 1.8% and 0.9% of patients in the overall givinostat group in the DMD studies. Hyperbilirubinemia was reported as TEAE in 0.9% of patients. However, these changes are not considered clinically relevant.

An *increase in creatinine* was observed during the studies, with a median change from baseline to EOT of 6.00 µmol/L in the givinostat group compared to no change in the placebo group. Based on *in vitro* studies, givinostat has the potential to inhibit the renal uptake transporter OCT2 for which creatinine is an endogenous substrate. Renal function parameter *Cystatin C* was also analysed, and the median change from baseline to EOT with givinostat was - 0.04 mg/L and 0.02 mg/L for placebo, which does not suggest an effect of givinostat on renal function.

An increase from baseline to EOT in median thyroid stimulating hormone (TSH; thyrotropin) was noted for givinostat compared to placebo (1.30 mU/L vs. 0.12 mU/L) and in median free thyroxine (fT4) (3.60 pmol/L vs. 0.39 pmol/L). This is in line with reporting of TEAEs of TSH increased in ≥5% of patients in the DMD studies. Mean values for thyrotropin appear to exceed the upper range of normal (up to 4 mU/L at certain time points). A slight decrease was seen in median free triiodothyronine (fT3) for givinostat compared to placebo (- 2.99 pmol/L and +0.50 pmol/L). Shift tables in study 48 for thyrotropin reported shifts from normal to high, and the percentage of patients with such shifts increased after 24 weeks of treatment up to 26.3% of subjects at Week 60. Shifts from normal to high for placebo-treated subjects was below 3.3%.

Troponin I was solely measured in study 51. Only 66 patients in the givinostat group had troponin levels assessed at baseline, and the median change from baseline to EOT was 0.00 µg/L. Increases in individual patients reported as TEAEs were not sustained or associated with clinical symptoms.

Vital signs, physical findings, and other observations related to safety

Small changes in vital signs (pulse rate, temperature, systolic blood pressure, and diastolic blood pressure) were reported in the DMD studies, which are not considered clinically relevant.

Small changes from baseline to the last available visit for *ECG parameters* of heart rate, QTcF, QTcB, QT interval, RR interval, PR interval, and QRS interval were observed during the DMD studies. The median change from baseline to EOT for QTcF interval was -7.00 msec for givinostat and 1.00 msec for placebo. The median change from baseline to EOT for heart rate was 4.00 bpm for givinostat and 2.00 bpm for placebo. Except for heart rate, greater decreases in mean and median values for ECG parameters were reported in the givinostat group compared to placebo, but the changes were small and not clinically relevant.

QT changes were assessed primarily based on QTcF formula. No patient had a change in QTcF interval of > 450 msec at the EOT visit or any post-baseline visit. Seven patients (3.2%) in the givinostat group and 0 patients in the placebo group had an increase from baseline in QTcF interval of ≥ 30 msec and < 60 msec at the EOT visit. No patient had a change in QTcF interval from baseline of ≥ 60 msec. A single subject on givinostat was reported with a QTcF value of >450 msec to ≤480 msec at Week 1,

but this was one of the value recorded of the triplicate measurement, the mean of the 3 ECG was 434.3 msec.

Shifts from an ECG normal at baseline to abnormal at the last available visit in DMD studies were more frequent with givinostat compared to placebo (20.7% vs. 13.1%). Less than ~15% of patients treated with givinostat reported a shift from normal at baseline to abnormal post-baseline in ECG at either post-baseline visit. There was a high number of missing ECGs at later time points (after Month 18). There were no shifts from normal at baseline to *clinically significant* abnormal ECG post-baseline in study 48, neither for givinostat nor for placebo. Shifts from normal at baseline to abnormal, not clinically significant post-baseline were in line with the pooled DMD studies.

Small median changes were seen during the DMD studies from baseline to EOT for echocardiography parameters of left ventricular ejection fraction (LVEF in %) and left ventricular end-systolic volume, but these were not clinically relevant.

Based on study 48, all pulmonary function parameters remained stable over time with the exception of peak expiratory flow (PEF), where an increase was observed in both treatment groups.

Results of the cognitive function test in study 48 (Raven coloured progressive matrices) revealed a similar mean (SD) change from baseline at EOS in both the givinostat and placebo groups.

In study 51, growth during long-term givinostat treatment was evaluated and an increase in mean body weight and height was noted after 12, 24, 36, and 48 months, respectively.

2.6.8.5. Safety in special populations

Intrinsic factors

Age: Analysis of the safety profile of givinostat was provided for children aged 6 to <12 years (givinostat 77.5%; placebo 80.3%) and adolescents aged ≥12 to <18 years (givinostat 22.5%; placebo 19.7%). The number of subjects with TEAEs were similar for both of the age groups (93% and 90%), but the incidence of TEAEs was higher in adolescents compared to children (5.64 TEAEs per patient-year in adolescents compared to 4.40 TEAEs per patient-year in children). The same relation was found for the number of subjects with SAEs, severe TEAEs, and ADRs were roughly similar, although, the number of events per patient-year (PY) was higher for adolescents as compared to children. Most of the frequent TEAEs by PT generally occurred at a similar incidence and in a similar number of patients in the two age subgroups. However, differences around 10% between groups were noted for nausea, thrombocytopenia and troponin increased that occurred more frequently in adolescents compared to children (nausea: 22% vs. 5.8%; thrombocytopenia: 28% vs. 15.1%; troponin increased: 14% vs. 5.2%), whereas nasopharyngitis, platelet count decreased and rash occurred more frequently in children as compared to adolescents (nasopharyngitis: 23.3% vs. 10%; platelet count decreased: 26.7% vs. 10%; rash: 9.9% vs. 0%).

29 of 38 patients on givinostat and 2 of 2 patients on placebo with *SAEs* were children, while the incidence of SAEs was similar. The main difference between the two age groups was in the injury, poisoning and procedural complications SOC (children 7.0% (0.03 TEAEs per PY) compared to adolescents 2% (0.02 TEAEs per PY)), basically regarding SAEs of femur fractures (4.1% vs. 2%).

Of the 9 patients who experienced *TEAEs that led to treatment discontinuation* (all in the givinostat group), 8 were children and 1 was an adolescent.

The number of patients who experienced at least one *adverse event of special interest* was higher in the givinostat group in children compared to adolescents (80.8% vs. 66.0%) but the number of AESI per PY was higher in the givinostat group in adolescents compared to children (1.94 TEAEs per PY vs.

1.39 TEAEs per PY). AESIs that occurred at a notably higher number of children in the givinostat group compared to adolescents were diarrhoea (41.3% vs. 34.0%), abdominal pain (24.4% vs. 18.0%), platelet count decreased (26.7% vs. 10.0%), and blood triglycerides increased (18.6% vs. 10.0%). AESIs that occurred at a notably higher number of adolescents in the givinostat group compared to children were nausea (22.0% vs. 5.8%), and thrombocytopenia (28.0% vs. 15.1%).

Differences in *laboratory evaluations* for *haematology* were small without clinically relevant differences between age groups. No difference was observed in median changes from baseline in platelet counts between children and adolescents in the overall givinostat group but the median decrease was more pronounced for Dose A in adolescents compared to children (-189.00 x 10^9/L vs. -110.50 x 10^9/L).

For the majority of *chemistry parameters* small but not clinically relevant changes have been noted for children and adolescents, some of them being reported for both treatment groups. No difference was noted for the median change from baseline in triglycerides between age groups in the overall givinostat group.

No consistent changes in *vital signs* or *ECG parameters* were seen that indicated an adverse change with givinostat treatment compared to placebo treatment in the two age groups. A similar low percentage of subjects in each age group in the givinostat group had an increase from baseline in QTcF interval of ≥30 msec and <60 msec at EOT. The percentage of patients shifting from an ECG normal at baseline to abnormal at the EOT visit was higher in children compared to adolescents for both the givinostat group (22.7% versus 14.0%) and the placebo group (14.3% versus 8.3%).

Other intrinsic factors have not been studied with givinostat in the DMD programme, including races, renal and hepatic impairment.

Extrinsic factors

No differential safety profile was reported for givinostat when administered in fasting and fed conditions in healthy volunteers (study DM/00/2357/04). In order to mitigate the bitter taste of the drug substance, the product information advises to take givinostat with food, which has likewise been applied in the clinical DMD studies.

Different regions have not been studied as extrinsic factor despite the fact that a substantial amount of study centres for study 48 (and also in study 51) was located in the US and Canada.

2.6.8.6. Safety related to drug-drug interactions and other interactions

In vitro data suggest that givinostat may have a potential for drug-drug interactions due to CYP3A-mediated metabolism and P-gp transport. Givinostat is a substrate for the P-gp transporter and may inhibit P-gp probably culminating into a clinical risk in vivo.

The *in vivo* DDI potential of givinostat was assessed in the healthy volunteer study 55: (1) givinostat serving as a potential inhibitor and inducer of CYP3A and P-gp activity (perpetrator) on the PK of midazolam (sensitive index CYP3A substrate) and dabigatran etexilate (P-gp substrate) and (2) the potential of givinostat as a DDI victim by determining the impact of clarithromycin (a clinical index inhibitor of P-gp) on the PK of givinostat. PK results indicate that givinostat is a weak *inhibitor* of CYP3A4 mainly due to intestinal CYP3A4 (based on evaluation of both, IV and oral midazolam). No inductive effect of CYP3A4 was seen after 14 days of givinostat administration. Contrasting *in vitro* evaluation of intestinal transporters, the bioavailability of dabigatran was found reduced after co-administration in study 55 after 3 days, which is however not compatible with induction of P-gp regarding the time course. Therefore, no firm conclusion on an inhibition and induction potential on P-gp can be derived based on these data.

Co-administration with the strong P-pg inhibitor clarithromycin caused an increase in the rate of exposure (C_{max}) of givinostat about 40% but had a minimal effect on the extent of exposure (AUC), which is in line with its function as a substrate for P-gp.

The reported TEAEs in the period of concomitant administration of givinostat were in line with the described safety profile of givinostat (mainly reports of thrombocytopenia and abdominal pain) or the concomitant drugs (e.g., somnolence for midazolam).

Given that patients in the DMD studies were all treated with corticosteroids, clinical safety of givinostat cannot be disentangled from the effects of the corticosteroids.

In the pooled analysis of non-oncology/ non-DMD studies, an analysis by concomitant use of steroids "yes/ no" was conducted. Use of concomitant steroids was not found to alter the PK of givinostat. Contrasting expectations, reporting of overall TEAEs, severe TEAEs, and AESI was found higher for the givinostat only group as compared to concomitant steroid treatment, while SAEs were more frequently reported in the group with concomitant steroids. In particular there was a higher reporting of diarrhoea and platelet counts decreased in patients not receiving concomitant steroids (17.0% [1.68 TEAEs per patient-year] and 19.1% [0.69 TEAEs per patient-year]) compared to those who were taking concomitant steroids (6.1% [0.33 TEAEs per patient-year] and 1.2% [0.24 TEAEs per patient-year]). However, a greater decrease in platelet counts was observed in the givinostat group in patients that did use concomitant steroids (median change from baseline of -109.00 x 10^9/L) compared to those that did not (median change from baseline of -83.00 x 10^9/L). Hypertriglyceridaemia was reported in more patients with givinostat alone as compared to concomitant steroids (7.1% vs. 0%).

2.6.8.7. Discontinuation due to adverse events

In the Group 2 – DMD studies pool, 9 patients (4.1%) experienced a total of 11 TEAEs that led to treatment discontinuation in the givinostat group compared to 0 patients in the placebo group (in study 48: 2.5% of patients on givinostat). The only TEAEs leading to treatment discontinuation that were reported in more than 1 patient in the givinostat group were blood triglycerides increased, hypertriglyceridaemia, and diarrhoea (all reported in 2 patients, 0.9%).

TEAEs leading to discontinuation from givinostat in study 51 were reported for 3 subjects, one subject in the givinostat group (nausea), and 2 subjects in the delayed givinostat group (atrial fibrillation and blood triglycerides increased).

2.6.8.8. Post marketing experience

N/A

2.6.9. Discussion on clinical safety

Safety database, doses and exposure

The main body of evidence regarding clinical safety for givinostat in the treatment of DMD includes patients aged 6 to 16 years at study entry and dosed in three clinical studies, which have been pooled in Group 2 – DMD studies for safety analysis (i.e. 20 patients in the open-label phase 2 study 43, 118 patients in the placebo-controlled study 48, and 84 patients in the ongoing open-label long-term study 51 [i.e. 54 patients on placebo during study 48 and 30 patients screened in study 48 without being treated]). While the focus should be set to the placebo-controlled experience of givinostat in study 48, pooling of the controlled and uncontrolled studies enables evaluation of the single dose groups by increasing the number of patients per dose group. While some uncontrolled data for adult patients are available for those who started treatment with givinostat in studies 43 or 48 and receive continuous treatment in study 51, patients <6 years of age have not been studied in the clinical DMD programme and this is meanwhile reflected in the restricted indication. Patients being non-ambulant at baseline did not start treatment with givinostat, but 34 patients lost ambulation in the givinostat group during the Group 2 DMD studies. The indication has meanwhile been further restricted to ambulant patients while patients, who become non ambulant during treatment with Duvyzat might continue treatment at the discretion of the treating physician, which is now reflected in section 4.2 of the SmPC.

Patients had to be on stable corticosteroid treatment for at least 6 months prior to entry in these studies. Thus, clinical efficacy and safety evaluation in the DMD programme only reflects the combination of givinostat and corticosteroids. The benefit-risk of givinostat monotherapy in DMD can therefore not be determined. This has been addressed by the applicant by restricting the indication of givinostat to patients with concomitant corticosteroid treatment.

Within the Group 2 safety pool, placebo-controlled data and all uncontrolled data are combined, which bears the risk of inflating the incidence of adverse events for givinostat over placebo. Upon request, the applicant reviewed the TEAEs occurring with givinostat primarily based on Study 48 with an incidence of ≥2% in the givinostat group and with a more than 2% difference to placebo representing a conservative approach. Based on the lack of a temporal relationship and biological plausibility, a number of TEAEs are not considered relevant ADRs for givinostat (i.e. contusion, arthropod bite, LDL increase, onychomycosis, and upper respiratory tract infection). As requested, grouping of preferred terms has been applied without diluting frequency of the included ADRs: thrombocytopenia (refers to platelet count decreased and thrombocytopenia; frequency 'very common'), hypertriglyceridaemia (refers to hypertriglyceridaemia and blood triglycerides increased; frequency 'very common'), diarrhoea (refers to diarrhoea and faeces soft; frequency 'very common'), blood thyroid stimulating hormone increased (refers to thyroid function test abnormal and blood thyroid stimulating hormone increased; frequency 'common'), and abdominal pain (refers to abdominal pain and abdominal pain upper; frequency 'very common'). Section 4.8 in the SmPC and the respective section in the PL have been revised in line with the review of ADRs.

Long-term safety evaluation currently relies on data from patients continuously treated with givinostat in one of the parent studies 43 or 48 with further open-label treatment in study 51. Duration of study 51 is envisaged up to the marketing authorisation of givinostat (or if based on a decision taken by the Sponsor and/or the Competent Authorities based on safety and/or efficacy reasons for the DMD population). The cut-off date for the MAA submission is 31 December 2021. Results from the 5th interim analysis of study 51 with a data cut-off 31 December 2023 have been provided in the responses.

Additional safety pools have been generated and high-level summaries of the results have been provided for different givinostat development programmes, including for example Non-oncology/non-

DMD indications (Group 3), and Haematological malignancies (Group 5). However, these data are not further discussed in depth given that all other indications for which givinostat is currently evaluated apply higher doses than those in DMD hampering comparison of safety.

The **doses of givinostat** relevant for establishing the safety profile derived from information from the mdx mouse model and PK-PD analysis suggesting a daily exposure of 300 ng*h/mL as the target for efficacy. During study 43 Part 1, the starting dose was 25 mg b.i.d (for body weight 20 kg to 49 kg), while 37.5 mg b.i.d (for body weight ≥ 50 kg) was not used since all patients were less than 50 kg. A scheduled dose increase to 50 mg b.i.d triggered a stopping criterion (i.e. platelet count decreases), thus, an intermediate dose of 37.5 mg b.i.d. was chosen to treat patients during part 2 of the study. However, at this dose, some patients additionally required dose reduction to 25 mg b.i.d due to platelet count decreased to below 150 × 10^9/L within the third month of treatment (12 of 19 patients; 63.2%).

In addition, revision of the popPK model with data from study 43 accounts for the significant effect of patient's weight on givinostat clearance, i.e. the apparent clearance increases with body weight. Thus, 9 weight bands between 10 kg and ≥70 kg body weight have been defined for dosing in study 48. The conversion of doses applied during study 43 (no weight-based dosing) to dose levels A and B with different weight bands defined in study 48 appears reasonable.

Dose A in study 48 simulates an exposure related to a dose of 37.5 mg b.i.d. in study 43, which can be reduced to Dose B, which simulates exposures of the 25 mg b.i.d dose in study 43. This was done under Protocol version ≤4.0 in study 48 (treatment regimen 1).

Blinded safety review during study 48 indicated ~50-60% of patients with starting Dose A had to reduce doses due to platelet count decreases within 8 weeks of treatment requiring further dose reduction to be implemented in protocol version >4.0 (treatment regimen 2): patients were started on Dose B (one-third reduction from Dose A) and were allowed to reduce a further 20% to Dose C (still aiming to provide an effective exposure).

The dose regimen proposed for treatment of DMD patients in the product label, i.e. a combination of the three dose levels A, B, and C, has not been pre-specified in any of the clinical studies. The differences in dosing schedules between studies and the various dose modifications add challenges to the assessment of clinical safety of givinostat in the target population.

Based on the originally presented pharmacometric modelling and simulation data, the incidence of thrombocytopenia is predicted to be lower in subjects with lower body weight (receiving lower doses) as compared to those with higher body weight receiving higher doses. For patients ≥ 60 kg to < 70 kg, the incidence of thrombocytopenia >Grade 1 was predicted to be 17%, 6%, and 2% in the worst-case scenario for the highest dose levels of Doses A, B, and C, respectively. The risk for thrombocytopenia >Grade 1 for the highest Dose level A for patients with a body weight 50 – 60 kg and 40 – 50 kg can be calculated as 11% and 9%, respectively.

The applicant presented new PK/PD simulations to predict the risk for thrombocytopenia (all doses combined) based on the revised dosing regimen with 4 (instead of 9) weight bands (10-<20 kg, 20-<40 kg, 40- <60 kg, and >60 kg), which aimed at lower starting doses leading to lower exposures of givinostat for patients with higher body weights (40-<60 kg and >60 kg).

As a result, a small decrease in the predicted risk for thrombocytopenia >Grade 1 is noted for the revised dose regimen with 4 instead of 9 weight bands, e.g., for patients with a BW between 40 and 60 kg and for a BW >60 kg.

For patients in the newly proposed weight bands 10-<20 kg and 20-<40 kg, exposures were found slightly increased, while these are not considered to affect clinical safety outcomes. The safety

evaluation for givinostat is based on the exposure of n=222 patients treated for a median of 23.57 months in DMD studies and includes n=186 patients, who still participate in the ongoing study 51.

The median duration of exposure to placebo for 61 subjects is 18.14 months. The majority of patients in the DMD studies belonged to the age group 6 to <12 years (givinostat 77.5%; placebo 80.3%) while the remainder were adolescent patients ≥12 years to <18 years.

The overall disposition based on the different dose levels achieved during treatment in the Group 2 studies is summarised below:

- 14.4% (32 of 222) of patients received givinostat at **Dose A**; the updated median exposure to this dose was 30.72 months and discontinuations at this dose were highest (15.6%).
- 41% (91 of 222) of the overall patients were treated with **Dose B** and having the lowest number of discontinuations [1%]; median updated exposure was 17.58 months.
- 20.3% (45 of 222) of patients received givinostat at **Dose A-B** with a median treatment exposure of 42.94 months
- 10.8% (24 of 222) of patients of all givinostat-treated patients were treated at **Dose A-B-C** and had a median treatment exposure of 37.11 months.
- 13.5% (30 of 222) of patients received givinostat at **Dose B-C** with a median updated treatment exposure of 16.59 months.

9 of 222 patients in all DMD studies discontinued from givinostat treatment due to a TEAE. Five of them also discontinued from the study (due to platelet count decreased, diarrhoea, elevated triglycerides (2), and hypertriglyceridaemia). Treatment interruptions occurred during the studies and are likewise recommended in the product information in case of adverse events like diarrhoea, platelet count decreases, and increased triglycerides prior to dose modification depending on the severity of these events. 30.2% of the overall givinostat group (67 of 222 patients) had at least 1 treatment interruption. The majority of patients (41 of 67; 61%) had only one interruption followed by 14 of 67 patients (21%) with 2 interruptions. The number of patients with at least one dose interruption was highest for the A-B-C group, followed by the B-C group and the A-B group. There was also a higher number of treatment interruptions at Dose A-B-C and these were - as expected - also of longer duration. Drug interruption most frequently occurred in the context of hypertriglyceridaemia/ blood triglycerides increased, mainly observed at dose level A-B-C, which is reasonable given that patients had to reduce their dose in case of these events. Upon request, the applicant reasonably explained that treatment interruptions with a median duration of 29 days are not expected to affect efficacy of givinostat when compared the median treatment duration of almost 24 months in the overall givinostat group.

The overall exposure to givinostat appears sufficiently large given the rarity of the disease. 187 of 222 patients (84%) had an overall exposure to givinostat of more than 12 months, and 105 of 222 patients (47%) had an overall exposure to givinostat of more than 24 months. The exposure to givinostat for more than 12 months and 24 months, respectively, was highest at Dose B and B-C, while exposure to givinostat was lower in these two groups for subsequent months given that Dose B was first introduced with a later protocol amendment in study 48. Between 9.4 and 25% of patients from Dose groups A, A-B, and A-B-C had a cumulative exposure to givinostat between 54 and 60 months.

Demographics

Patient demographics as well as medical history and concomitant medication are in support of an ambulatory target population, with a median age around 10 years, median time since diagnosis of

6 years, and the median time since first corticosteroid initiation being 4 years. Three patients became non-ambulatory while being treated with placebo in the parent studies. All except one patient received concomitant corticosteroids and half of the patient in each group received cardiac comedication (ACE inhibitors).

Most common adverse events

Reporting of TEAEs was similar for patients treated with givinostat and placebo, ranging between 83.5% on Dose B to 100% of patients on Dose A-B-C. TEAEs per patient-year were highest in the dose group B-C due to the lower median exposure to givinostat in this group (16.59 months vs. 23.57 months overall) leading to an overestimation of the TEAEs given that TEAEs often tend to occur shortly after drug initiation. In study 48, the reporting of TEAEs under protocol versions ≤4 (higher starting doses) was higher as compared to protocol versions >4 (lower starting doses). In study 51, which includes patients with continuous givinostat treatment from parent studies 43 or 48 (referred to as "givinostat group"), a median duration of exposure to givinostat of ~500 days is added, which is almost the same duration of exposure as in study 48. However, no increased reporting of TEAEs was noted.

TEAEs were more frequently reported for givinostat as compared to placebo for the SOCs GI disorders, investigations, Injury, poisoning and procedural complications, General disorders and administration site conditions, Respiratory, thoracic and mediastinal disorders, Skin and subcutaneous tissue disorders, Blood and lymphatic system disorders, Metabolism and nutrition disorders, and Endocrine disorders. Overall, a relation to givinostat could be demonstrated for a majority of TEAEs from the investigations SOC, GI disorders SOC, Blood and lymphatic system disorders SOC and for the Metabolism and nutrition disorders SOC.

TEAEs that occurred at a notably higher incidence in the overall givinostat group compared to the placebo group were diarrhoea (39.6% vs. 18%), vomiting (28.4% vs. 13.1%), abdominal pain (23% vs. 14.8%), platelet count decreased (23% vs. 0%), blood triglycerides increased (16.7% vs. 4.9%), thrombocytopenia (18% vs. 0%), decreased appetite (8.1% vs. 0%), hypertriglyceridaemia (9% vs. 1.6%), fatigue (9.5% vs. 0%), hypothyroidism (5.4% vs. 0%), arthralgia (10.4% vs. 1.6%), pyrexia (20.7% vs. 8.2%), and rash (7.7% vs. 1.6%). Of these, a relation to dose based on a higher reporting at Dose A and A-B-C as compared to Dose B and B-C can be established for diarrhoea, vomiting, platelet count decreased, thrombocytopenia, decreased appetite, fatigue, hypothyroidism, arthralgia, and pyrexia.

A decreased reporting of the majority of these events was noted with continuous treatment in study 51 compared to the pooled studies and compared to the placebo-controlled study 48, which might be explained by the fact that a majority of TEAEs occurred early during treatment or as a consequence of dose reductions. Nevertheless, similar rates of TEAEs are noted for blood triglycerides increased, hypertriglyceridaemia, and TEAEs probably related to the underlying disease progression (musculoskeletal disorders TEAEs). An increased reporting of troponin increased in the givinostat group as compared to placebo in the Group 2 pool (7.2% vs. 0%) does not reflect a clear signal of concern given that troponin was only measured during study 51, where all patients receive givinostat. From interim results of troponin I during study 51, patients from the delayed givinostat group (those with placebo during study 48) presented with high mean troponin values at baseline in line with literature reports that troponin increases occur to a variable extent in DMD patients (Sheybani et al., 2022; Spurni et al., 2021). No trend on mean troponin increases over time is evident for patients treated with givinostat during study 51.

Most TEAEs were mild to moderate in severity. In the overall givinostat group, at least one TEAE of mild (moderate) severity was reported by 92% (42.3%) of patients. 16.7% of patients reported at least one TEAE of severe intensity. Reporting of moderate and severe TEAEs was generally highest in

the dose reduction groups A-B, A-B-C, and B-C. Severe TEAEs were almost exclusively reported in subjects on givinostat (16.7%) and the types of TEAEs were basically in line with common AEs. The majority of severe TEAEs occurred at Dose B and A-B-C (16.5% and 24.6%). Of note, platelet count decreased was only reported as severe in the higher dose groups A and A-B-C, while blood triglycerides increased was exclusively reported as severe in the lower Dose groups B and B-C. Reporting of severe TEAEs was more frequent with longer treatment duration in study 51 (data cut-off 31 Dec 2023) as compared to study 48 (18.8% vs. 4.2%), due to an increased reporting of severe TEAEs in the Musculoskeletal and connective tissue disorders SOC (4.8% vs. 0%) and injury, poisoning and procedural complications SOC (8.7% vs. 0%). The increase in severe TEAEs with longer givinostat treatment rather appears to be a consequence of the progressing disease than a drug effect.

The first dose reduction occurred within a median time of 105 days and a second dose reduction became necessary after a median time 516.5 days in approximately one-third of patients who had a first dose reduction in the dose group A-B-C. Dose reductions were in 95% driven by platelet decreased/ thrombocytopenia or increased triglycerides/ hypertriglyceridemia. The two remaining cases were abdominal pain (one case, moderate) and diarrhoea (one case, moderate). Reporting of diarrhoea, abdominal pain, platelet count decreased, and thrombocytopenia could be reduced by ~50%. In contrast, the frequency of blood triglycerides increased/ hypertriglyceridaemia remained similar after dose reduction. Upon request, the applicant provided a summary of the reasons for the absence of an effect of dose reductions on efficacy. From the pharmacometrics analyses results in study 48, simulation of exposures at Dose levels A, B, C at different weight bands as well as for the clinical endpoint results at different doses it can be concluded that exposures to givinostat sufficiently exceed the preclinically determined target exposure for efficacy (i.e. a daily AUC of 300 ng*h/ml), even with the simplified dosing regimen using 4 instead of 9 weight bands.

No further dose reduction was stipulated after reaching Dose C, questioning the consequences for further treatment, including possible treatment interruptions and permanent treatment stop. Half of the patients in Dose group B-C (14 of 30 patients) had temporary or permanent interruption of givinostat following a TEAE. Ten patients had one or more temporary dose interruption, mainly due to triglycerides increased/ hypertriglyceridaemia, while 4 patients had to be permanently withdrawn from givinostat treatment, all of them due to a TEAE of triglycerides increased/ hypertriglyceridaemia. The applicant assumes that less patients (<1.8%) will be affected from permanent discontinuation in clinical practise given that dietary measures are recommended in section 4.4 of the SmPC, which was not in place during the clinical studies with givinostat.

No death occurred in the clinical DMD studies up to the original data cut-off 31 Dec 2021. However, there were two deaths reported during study 51 as per the data cut off 31 December 2023 of the 5th interim analysis. Both death cases involved consequences related to accidents and occurred in the delayed givinostat group. One patient (003-0063) had a post-traumatic thoracic haemorrhage resulting in cardiorespiratory arrest and death after fall from a wheelchair. While the patient's platelet counts have not been reported, the Investigator assessed this event as unrelated to givinostat. Patient 201-0172 died following a road traffic accident resulting in diffused intracranial haemorrhage including parenchymal contusion and epidural with cranial fracture, pulmonary contusion, and broken hip. While there were no information on any laboratory parameters prior to this event, the narrative suggests that disseminated intravascular coagulation took place in the patient after the traumatic injury (thrombocytopenia, D Dimers increased, aPTT prolonged). The investigator rated this SAE as not related to givinostat.

Serious adverse events summarised by the Assessor in the absence of a detailed discussion by the applicant do not point towards an unexpected safety issue with givinostat. **SAEs** in the pooled DMD studies were more frequently reported with givinostat compared to placebo (17.1% [0.08 events per PY] vs. 3.3% [0.02 events per PY]), and highest in the dose group A-B-C (30.4% of patients with SAEs

treated in this group), while there was no difference between groups A and B (12.5% and 12.1%, respectively). The only SAEs reported in more than 1 patient in the givinostat group were femur fracture (8 patients, 3.6%), chest pain (3 patients, 1.4%), and back pain, rhabdomyolysis, tendinous contracture, platelet count decreased, and dehydration (each in 2 patients, 0.9%). Longer treatment with givinostat does not appear to alter the reporting of SAEs in general. However, SAEs of fractures were reported for 13 patients in the givinostat group vs. none on placebo, and most of them occurred during study 51 (mainly femur fractures); these patients had longer treatment durations, were older and probably more progressed in their disease. Only three SAEs were rated as related/ possibly related, including two SAEs of platelet count decreased and atrial fibrillation. For two SAEs in a single patient in study 48 (rash and dizziness), a possible contribution of givinostat cannot be excluded while rash and dizziness are both listed as adverse drug reactions for givinostat with "common" frequency in section 4.8 of the SmPC. For some of the reported SAEs, the underlying disease as well as the corticosteroid comedication could be an alternative explanation (e.g. for SAEs related to fractures, muscular and connective tissue disorders SOC, and Cushing's syndrome).

Nine subjects on givinostat versus none on placebo **discontinued** treatment due to eleven TEAEs, with the lowest number reported in the Dose B group. The events were in line with the AESI, i.e. GI disorders (diarrhoea, nausea, and abdominal pain), blood triglycerides increased, hypertriglyceridaemia, and platelet count decreased. As per the data cut-off 31 Dec 2023 of the 5th interim analysis, 9 TEAE in total led to discontinuation during study 51 (blood triglycerides increased [n=3], atrial fibrillation [n=2], abdominal pain, nausea, road traffic accident, and thoracic haemorrhage).

Adverse events considered as adverse drug reactions

Placebo-controlled data (from study 48) with an incidence of ≥2% in the givinostat group and with a more than 2% difference to placebo were the basis for inclusion and frequency categorisation of ADRs taking into account a temporal relationship and biological plausibility. As a result, *thrombocytopenia, diarrhoea, vomiting, abdominal pain, pyrexia, and hypertriglyceridaemia* were rated as "very common" ADRs. *Constipation, fatigue, gastroenteritis, blood thyroid stimulating hormone increased, decreased appetite, myalgia, arthralgia, muscular weakness, dizziness, anxiety, erythema, rash, and haematoma* were rated as "common" ADRs. Grouping of preferred terms has been applied without diluting frequency of the included ADRs: thrombocytopenia (refers to platelet count decreased and thrombocytopenia; frequency 'very common'), hypertriglyceridaemia (refers to hypertriglyceridaemia and blood triglycerides increased; frequency 'very common'), diarrhoea (refers to diarrhoea and faeces soft; frequency 'very common'), blood thyroid stimulating hormone increased (refers to thyroid function test abnormal and blood thyroid stimulating hormone increased; frequency 'common'), and abdominal pain (refers to abdominal pain and abdominal pain upper; frequency 'very common').

Adverse events of special interest

Thrombocytopenia/ platelet count decrease, hypertriglyceridaemia/ triglyceride increase, gastrointestinal disturbances: diarrhoea, nausea, vomiting and abdominal pain, and QT prolongation were identified as AESIs for givinostat for Group 2 – DMD studies evaluation. In addition, the SMQs of haematopoietic thrombocytopenia, osteoporosis/ osteopenia and triglycerides increased (analysed using selected specific terms such as blood triglycerides abnormal, blood triglycerides increased and hypertriglyceridaemia) have been taken into consideration. At least 1 AESI was reported by 77.5% of patients treated with givinostat.

Thrombocytopenia/ platelet count decreased

Thrombocytopenia is a well-known dose-limiting side effect with HDAC inhibitors (for example for panobinostat, romidepsin, and vorinostat), for which the mechanism remains controversial.

AESI of platelet count decreased and thrombocytopenia (i.e. clinically significant platelet abnormalities) were reported in 23% and 18% of patients on givinostat, respectively (none on placebo). A SMQ analysis of haematopoietic thrombocytopenia (lacking double counting of patients with platelet count decreased and thrombocytopenia) revealed 157 events in 85 (38.3%) patients. The highest incidence was reported for Dose groups A-B (86.7%), followed by A-B-C (79.2%) and B-C (46.7%), reflecting that a substantial number of patients (64 of 85 patients; 75%) was in need for dose reductions due to thrombocytopenia/ platelet count decreases.

A dose-relation can be observed for Dose A compared to B (18.8% vs. 7.7% of patients in these groups) and for Dose A-B compared to B-C (86.7% vs. 46.7). Long-term treatment with givinostat in study 51 did not lead to an increased reporting of these events. In the givinostat overall group (n=222), for almost all patients, the AESI of thrombocytopenia/ platelet count decreased was rated as related to study drug, was mild in 88% of patients with at least one AESI. 21% of patients had an event of moderate severity, and severe AESI have been reported for 3 patients (3.5%), two of which also being reported as SAEs, both in study 43, where no weight-adjusted dosing has been applied. In one patient treated with 50 mg b.i.d, this SAE was a life-threatening (Grade 4) with a platelet count nadir of 20 x 10^9/L leading to treatment discontinuation. No concomitant bleeding events were reported. The second SAE of platelet count decreased occurred in a subject after weight-adjusted increase in givinostat dose. Based on the revised dosing regimen, platelet counts $\leq$ 50 x 10^9/L are not expected and are thus not defined as a threshold for permanent treatment stop; however, it cannot be fully ruled out, especially when patients are treated with concomitant medication known to lower platelet counts.

The median time to onset of an AESI ranged between 29 and 33 days after initiation of treatment in the three dose reduction groups. In Dose groups A and B, where no dose reduction occurred, the median time to onset was much higher (i.e. 353.5 days at Dose A and 252.0 days for Dose B).

Mean/ median platelet counts decreased over the first 6 months of treatment with givinostat by ~40% from baseline with the steepest decrease within the first two weeks of treatment (-30% irrespective of dose level) and remained stable thereafter up to EOT. There were no further decreases based on continuous long-term treatment with givinostat in study 51. The median time to nadir for platelets was 88 days in the overall givinostat group (highest in Dose A group [133 days], followed by Dose B [114 days], Dose B-C [87.5 days], and Dose A-B-C [60.5 days]. The incidence in shifts from normal at baseline to abnormal (low) post-baseline was highest around Week 12 based on study 48 data. The median time to recovery in study 48 was 27 days (with min and max being 8 and 512 days), which is approx. 4 weeks.

Temporary dose interruptions for thrombocytopenia/ platelet count decreased were solely reported in the open-label long-term study 51 (in N=4 patients). Treatment was resumed in all of them.

Concomitant medications known to increase the risk for thrombocytopenia and/or the risk for bleeding used in patients in the DMD studies were NSAIDs (~20% of patients), followed by cephalosporins, and H2-anatgonists (ranitidine). Mean changes in platelet counts in patients with (N=57) and without (N=165) concomitant medication known to lower platelet counts based on Group 2 DMD studies (enoxaparin, enoxaparin sodium, furosemide, ketorolac, ibuprofen, ketoprofen, benzydamine hydrochloride, ranitidine, cefixime, cefdinir, and ceftriaxone sodium) were similar. However, it remains unknown, how concomitantly administered antithrombotic medication affects platelet counts with givinostat. This has been adequately reflected in section 4.5 of the SmPC.

The risk minimisation measures have been further revised during the procedure. The recommendations provided for sections 4.2 and 4.4 are acceptable, i.e. the need for a baseline blood count, the recommendation not to start givinostat if platelet count is < 150 × 10^9/L, to interrupt treatment based on the severity of adverse reactions prior to dosage modification, and additional monitoring in case of

dose increases due to weight gain to avoid the risk for severe platelet count decreases.

While the originally proposed monitoring scheme in the SmPC (*'every 2 weeks for the first 2 months of treatment, on the third month, on the sixth month and then every 6 months'*) generally accounts for the timely changes in platelet counts in DMD studies based on the median changes from baseline and the time to nadir, a clear dose-related effect of givinostat on platelet counts has been observed. The extent of the effect is not fully characterised given that there were various dose changes in Study 48 against the background of the small overall number of subjects enrolled in Study 48 and in each of the dose groups. Therefore, a tighter monitoring has been recommended and agreed on after the first 2 months of treatment: "*every 2 weeks for the first 2 months of treatment, at month 3, and then every 3 months thereafter*".

In summary, platelet count decreases/ thrombocytopenia with givinostat generally appear to be manageable with cautiously defined monitoring and dose reduction. Haemorrhagic disorders are an important potential risk included in the RMP for givinostat. In addition, the risk will be further addressed by means of a PASS.

Hypertriglyceridaemia/ triglyceride increase

Increased triglyceride values have been reported in nonclinical studies with givinostat in animals treated with the highest dose and associated with histopathological finding of bile duct hyperplasia. Moreover, there is emerging evidence that muscular dystrophies themselves are associated with an altered lipid metabolism in the early phase of the disease, while it remains uncertain whether these changes are primary or secondary to muscular dystrophy (White et al., 2020; Srivastava et al., 2017). Clinically, effects of givinostat on lipids have first been observed during study 53, a phase 2 study in Becker muscular dystrophy.

AESI of blood triglycerides increased/ hypertriglyceridaemia were more frequently reported with givinostat compared to placebo (25.2% [56 patients] vs. 6.6% [4 patients]). The highest incidence of triglyceride increased/ hypertriglyceridaemia AESI was reported for Dose groups A-B-C, followed by B-C and A-B, which appears reasonable since dose reduction in case of triglyceride increases was prespecified in the clinical studies. In the givinostat overall group, events were rated as related to study drug in a majority of patients, were mild in 77% of patients with at least one AESI, and of moderate severity in 23% of patients. Severe events have been reported in 5 patients (9%). In 6 of the 56 patients (11%) with at least one AESI of triglyceride increased/ hypertriglyceridaemia, the event led to dose reduction (2 patients in Dose group A-B, 4 patients in group A-B-C). Triglycerides increased/ hypertriglyceridaemia in Group 2 DMD studies led to temporary drug interruption in 30 patients (some patients had more than 1 TEAE leading to dose interruption), mainly those from group A-B-C and B-C.

No SAEs have been reported but 3 patients permanently discontinued givinostat due to an AESI given that triglycerides did not recover with treatment interruption and dose reduction. All of them had increased baseline triglycerides probably contributing to the increased values post-baseline necessitating treatment discontinuation, while medical history and concomitant medication remains inconclusive. As per the data cut-off 31 Dec 2023, two additional patients discontinued givinostat in study 51 due to blood triglycerides increased.

Upon request, an analysis of TEAEs in 18-months intervals from treatment start has been provided. TEAEs of *blood triglycerides increased* were more frequently reported in dose group B-C (33.3%) as compared to other dose groups (<16%), which might be attributed to 14 patients with such TEAEs in dose group B-C (from 30 patients in total). These patients had predisposing factors, like higher median BMI and triglyceride values at baseline as compared to the overall givinostat-treated group. Half of these patients (n=7) had baseline triglycerides >ULN (1.71 mmol/L).

The median time to onset ranged between 73.5 and 621.5 days after initiation of treatment in the different dose groups. The median time to recovery for patients, whose maximum triglyceride value was > ULN, calculated as the time from the maximum value > ULN to the first value within the normal range, was approximately 100 days after temporary dose interruption, based on the data collected in study 48.

Triglyceride values started to increase in the first 4 weeks and were found highest at Month 3 and 6 but always remained within Grade 1 (>150 mg/dL-300 mg/dL [1.71 mmol/L-3.42 mmol/L] according to CTCAE v.5.0). No progressive increases in triglycerides (despite some fluctuations) were noted with continuous treatment in study 51. Hypertriglyceridaemia (including blood triglycerides increased) is a very common ADR in section 4.8 of the SmPC. Upon request, additional risk minimisation measures/ wording have been implemented in the SmPC section 4.2 and 4.4, including the need for a baseline monitoring for triglycerides, a conservative threshold for triggering dose reduction for persisting triglyceride values of > 300 mg/dL (Grade 2 as per the CTCAE criteria), and regular monitoring of triglycerides (at least on the third and the sixth month, and then every 6 months), which is acceptable. Patients with a known risk for hypertriglyceridaemia or a pre-specified triglyceride cut-off are not excluded from treatment. The applicant clarified that these patients can be handled with the same recommended minimisation measures. With regard to concomitant medication known to be associated with hypertriglyceridaemia and potentially pancreatitis, triglyceride levels in patients with such medication in the clinical DMD studies have been provided upon request. Baseline mean values were only slightly higher in patients concomitantly treated with such medications as compared to those without (1.58 mmol/L vs. 1.44 mmol/L). However, the mean changes at any postbaseline time points were clearly higher in the concomitantly treated group. A warning has been requested based on the more pronounced increases of triglycerides with concomitant medication, which has been added in section 4.5. Overall, the risk regarding triglyceride increases appears manageable with monitoring and treatment interruption/ dose reduction, if triglycerides remain elevated. Severe consequences of elevated triglycerides have not been observed, e.g., acute pancreatitis or cardiovascular events, for which Grade 3 (> 500 mg/dL) and especially Grade 4 hypertriglyceridaemia (values > 1000 mg/dL) is (highly) predictive. Clinical consequences of hypertriglyceridaemia (e.g., pancreatitis, coronary artery disease, hepatic steatosis) has been included in the RMP as important potential risk for givinostat.

Gastrointestinal disturbances: diarrhoea, nausea, vomiting and abdominal pain

GI disorders, like nausea, vomiting and diarrhoea, which may require the use of corrective medications as well as fluid and electrolyte supplementation, have been reported following treatment with the class of HDACis (Shah 2019). In a majority of patients, GI events were mild to moderate in severity and were reported as SAE in two patients. The most frequently reported GI event with givinostat was diarrhoea (~40% of patients). Three patients discontinued givinostat due to either abdominal pain, nausea or diarrhoea. A majority of diarrhoea events in study 48 were mild in severity, characterised by a stool frequency of <4 stools/day (affecting 39 patients). Three and one patient had moderate (4 to 6 stools/day) or severe diarrhoea (≥7 stools/day), respectively. Two patients required corrective fluids, but electrolytes were always within normal limits. In contrast, half of the placebo-treated patients had diarrhoea, all of them being mild in severity. The incidence of GI disorders TEAEs, specifically diarrhoea and abdominal pain, is related to dose and can be mitigated by dose reduction, which is specified for moderate to severe diarrhoea in section 4.2 (more than 4 stools per day; ≥Grade 2 according to CTCAE v.5.0). The dose reduction scheme is the same as for platelet count decreases. Diarrhoea, vomiting and constipation are "very common" ADRs in section 4.8 of the SmPC. If abnormalities persist, givinostat needs to be discontinued. In the RMP, 'dehydration' has been included as potential risk following diarrhoea events. Dehydration due to vomiting or diarrhoea can trigger electrolyte disturbances (hypokalaemia) and corrective treatment needs to be considered.

QT prolongation

HDAC inhibitors carry the risk for variable effects on ECG waveform and prolongation of the heart rate corrected QT (QTc) interval as a consequence of direct hERG channel inhibition or through complex transcriptional changes of genes required for ion channel trafficking (Subramanian et al., 2010; Stan et al., 2016). The potential effect on the cardiovascular system was investigated in preclinical studies, but an impact of givinostat on cardiovascular function at therapeutic human doses seems unlikely (IC_{50} of givinostat for hERG channel inhibition translates into an approximately 74-fold safety margin). The results of the TQT study 54 do not indicate QT prolongation with givinostat therapeutic doses to exceed the regulatory threshold of concern (10 msec), and results are corroborated by the lack of AESI related to QT prolongation. Moreover, vital signs do not indicate clinically significant changes from baseline that would contribute to an increased risk of cardiac events.

However, one patient was reported with a severe and medically significant SAE of atrial fibrillation in study 51, rated as possibly related to givinostat. The patient was already treated for hypertrophic cardiomyopathy (heart failure prevention) for several years as per his narrative, which - if symptomatic - should have been an exclusion criterion under the previous study protocol 48. A contributing effect of givinostat to the event in this patient with underlying cardiomyopathy can thus not be ruled out. A second patient in study 51 was reported with a SAE of atrial fibrillation in the givinostat group as per the data cut-off 31 Dec 2023, which was moderate in intensity and led to discontinuation of givinostat. The Investigator rated this SAE as unknown to be related to givinostat. It needs to be considered that this patient had many concomitant medications at the time of the event and an influenza vaccination one months prior to the onset of the event.

No further clinical data is available to confirm the absence of an effect on cardiac conduction in patients with cardiomyopathy. Upon request, cautious wording has been proposed in sections 4.2 and 4.4 of the SmPC to recommend a baseline ECG, and ECGs during givinostat treatment, and as clinically indicated if patients with underlying cardiac disease or concomitant medication known to prolong the QTc interval are to be treated. Moreover, sections 4.2 and 4.4 inform on withholding givinostat in case of a QTc interval of > 500 msec or the change from baseline being > 60 msec. In the light of the absence of a clinically relevant QTcF effect in the TQT study at therapeutic doses of givinostat, this might be sufficient. In addition, section 4.7 informs on the potential risk with givinostat overdose and the need for cardiac monitoring and supportive care. Upon request, the warning in section 4.4 has been revised by adding examples for cardiac diseases and underlying conditions setting the patient at risk for cardiac conduction abnormalities. A summary of cardiac abnormalities in 37 patients with concomitant medication with known or possible risk for TdP (i.e. for sevoflurane, amiodarone hydrochloride, sotalol, ondansetron, fluconazole, aripiprazole, risperidone, azithromycin, clarithromycin, ciprofloxacin, famotidine, and propofol) has been provided. As a result, the incidence of events from the cardiac disorders SOC was notably increased in those patients with concomitant medication as compared to those patients without (29.7% vs. 5.9%) despite the absence of a clear pattern of cardiac-related events. A warning in section 4.5 of the SmPC has been added. Overdose and use with drugs known to prolong the QTc interval is also included in the RMP.

Osteoporosis/ osteopenia

Nonclinical data do not point towards a risk of givinostat to elicit adverse effects on the bone. However, TEAEs related to a SMQ of osteoporosis/ osteopenia were reported at the expense of givinostat (13.5% vs. 6.6% for placebo), which mainly derived from disproportionate reporting of fractures (10.8% vs. 3.3% for placebo) reported as SAEs in a majority of patients. Upon request, the study 48 TEAEs between givinostat and placebo have been compared resulting in an increased incidence for placebo-treated patients as compared to the givinostat group based on the 18-month treatment duration (6.6% vs. 1.7%). The most frequently reported fractures based on Group 2 DMD studies were femur fractures

(10 patients with 12 events on givinostat and none on placebo). Leg fractures, and specifically femur fractures, are often the first fractures in DMD patients with a mean age around 11.3 years (James et al., 2015). Narratives for fracture SAEs indicate that almost all patients were between 10 to 17 years at the time of the events. The randomisation on the one hand and the longer exposure to givinostat as compared to placebo on the other hand (median treatment duration difference of more than 6 months) coupled with a corresponding increased risk of fracture in older DMD patients could explain the disproportionate reporting. Moreover, the disease progression itself as well as steroid use could have contributed to these events.

Laboratory parameters

For most **haematology and coagulation parameters** (except for platelet count decreases) small but not clinically relevant changes were seen in DMD studies for givinostat as compared to placebo. However, nonclinical data suggest that the effect of givinostat in the bone marrow also involves the erythroid lineage. Median haemoglobin and erythrocyte (RBC) values remained within the normal reference ranges after an initial decline within the first 12 months and a plateau was reached thereafter. Givinostat (but not placebo) clearly increased MCH and - more pronounced – MCV. 60% of all givinostat-treated patients had at least one abnormality in MCV rated as macrocytosis; however, in most of them, haemoglobin values were normal (macrocytosis without anaemia). A single TEAE of MCV increase has been recorded in study 51. While it is agreed with the applicant that obviously no clinical signs with regard to red blood cell homeostasis have been reported in the clinical DMD studies with givinostat (and likewise no corrective treatment like transfusions), the SmPC has been amended upon request to be more explicit with regard to myelosuppression with givinostat.

The following noteworthy observations have been made for **chemistry parameters***:*

Nonclinical data revealed increases in liver enzymes, bilirubin, urea and triglycerides in monkeys given the highest doses in both, the 13- and 39-week study. These changes were considered related to bile duct hyperplasia, which is in line with hepatic biochemistry findings and histopathological examination confirming that the **liver** is a target organ for givinostat. The observed changes in ALT, AST and ALK, i.e. slight decreases from baseline, are difficult to interpret given that these enzymes are generally increased in DMD. In contrast, there was an increase in direct and total bilirubin corroborated by some shifts from normal at baseline to high post-baseline for givinostat but not for placebo. Few of these changes have been reported as TEAEs.

Renal function seems not to be adversely affected by givinostat based on Cystatin C values during study 48 and 51 contrasting an increase in serum creatinine with givinostat. Givinostat has been identified as mild OCT2 inhibitor *in vitro* for which creatinine is a substrate.

Givinostat seems to affect **thyroid function** in DMD patients, i.e. increases in TSH, which resulted in reporting of TEAEs of blood thyroid stimulating hormone increased and hypothyroidism. Shifts from normal to high TSH were highest at Month 12 and less shifts occurred thereafter. A relation to dose is apparent. Shifts from normal to high of fT3 and fT4 at Month 12 were overall lower than shifts for thyrotropin and appear lower with higher doses. Half of the patients treated with givinostat in the DMD studies had an increase in TSH (compared to 8.2% on placebo) with a majority of them still presenting with normal fT3 and fT4 levels. While blood thyroid stimulating hormone increased also is rated a common ADR for givinostat, the incidence of hypothyroidism in study 48 was 1.7% for givinostat and 0 for placebo. Nevertheless, it was confirmed to be a common adverse reaction with long-term givinostat treatment in study 51 (6.3% overall as per the interim cut-off 31 December 2023). The increase in TSH could be a consequence of inhibition of class I HDAC1/2 by givinostat while modifications of the acetylation state of histones in the promoter region of TSHα and TSHβ are crucial for the activation and repression of the two subunits (Wang et al, 2009; Sasaki et al. 1999).

Vital signs:

The DMD population is generally known to be susceptible to ECG changes (Thrush et al. 2009). Cardiovascular complications remain the main cause of death. Sinus tachycardia and short PR interval are characteristic ECG abnormalities in children with DMD, while QTc prolongation can also occur (Tang et al., 2022). Givinostat did not elicit cardiovascular adverse effects in a safety pharmacology study in the dog and in oral toxicity studies in dogs and monkeys in the preclinical programme. No significant changes in vital signs or **ECG changes** have been noted in the DMD studies. The median change from baseline to any post-baseline time point and EOT in heart rate was higher for givinostat than for placebo (EOT: 4.00 bpm for givinostat and 2.00 bpm for placebo). Median changes (decreases) from baseline to any post-baseline time points in the QTcF interval were more frequent with givinostat as compared to placebo, which supports the absence of a clinically significant QTc prolongation at therapeutic doses. Nevertheless, an imbalance in TEAEs from the cardiac disorders SOC (e.g., palpitations, tachycardia) was noted for givinostat compared to placebo (9.9% vs. 1.6%). Although, the Group 2 pool depicts longer treatment duration for givinostat as compared to placebo, disparity for the incidence of events from cardiac disorders SOC between givinostat and placebo was also evident in study 48 (6.8% vs. 1.6%). However, no single PT with an increased incidence could be identified. An additional analysis by age at onset of cardiac disorders TEAEs in the Group 2 DMD studies revealed that patients in the givinostat group were slightly older as compared to placebo patients (median 11 vs. 10 years). In summary, there is no clear signal on an increased risk for cardiac disorders TEAEs. There were no subjects with increases from baseline in QTcF of >60 msec except for a single subject at Month 40 during study 51 as per the data cut-off 31 Dec 2023. A maximum QTcF increase from baseline of >30 - <60 msec was found sporadically with givinostat (less than 5% of subjects per visit up to Month 36). Up to the data cut-off, no subject had an absolute QTcF of >500 msec.

Intrinsic/ extrinsic factors:

Evaluation of the safety profile in children aged 6 to <12 years (accounting for 77.5% of the givinostat-treated patients) and adolescents aged ≥12 to <18 years (22.5%), revealed some differences. Children were found to be more susceptible compared to adolescents for the AESI diarrhoea, abdominal pain, platelet count decreased, and blood triglycerides increased. However, the differential reporting of TEAEs/ AESI of platelet count decreased/ thrombocytopenia and blood triglycerides increased was not corroborated by different median changes from baseline in laboratory values, which were similar for both age groups. Nevertheless, and contributing to the results from pharmacometric modelling data, adolescents were found to present with higher decreases in platelet counts at the highest Dose A (no differences noted for the lower dose levels) as compared to children, supporting the observed weight/ age effect on platelet counts. Although, a minority of patients was aged ≥12 to <18 years, physical growth (height, weight, BMI) and pubertal development (Tanner stages) is of high relevance, especially since DMD patients already have delayed growth and development. A delay in growth might also be triggered by the high incidence of gastrointestinal complaints in patients treated with givinostat resulting in diarrhoea. Data from study 48 indicate that persistent diarrhoea (defined as an episode of diarrhoea that lasts at least 14 days) did not affect growth and development when comparing 19 patients with persistent diarrhoea with givinostat-treated patients without persistent diarrhoea. One subject experienced a growth retardation during the study in givinostat group. Tanner stages have not been collected hampering any conclusions on pubertal development. One subject experienced a growth retardation during the study in givinostat group.

Race has not been studied as intrinsic factor given that almost all subjects were White. However, based on the pharmacokinetic properties of givinostat, racial differences are not expected.

Givinostat has not been studied in patients with renal or hepatic impairment, and no different dosing is proposed. Treatment of givinostat in patients with renal or hepatic impairment has been deleted from

the list of missing information in the RMP given that information in the product information is sufficient.

The safety of givinostat appears independent from its administration in the fasted or fed state, while the product information recommends administration with food due to the bitter taste of the drug.

Approximately one-third of patients in the clinical DMD studies were enrolled from the US and Canada, while two-thirds of patients were enrolled and treated in Europe and Israel. However, based on similarities in the incidence in DMD, demographic data, standard of care in the US/Canada and in the EU (including stable corticosteroid treatment), natural history of the disease, and pharmacokinetic properties of givinostat, applicability from the US/Canadian to the EU DMD population for givinostat is sufficiently justified.

The potential for clinically significant **drug-drug interactions** with givinostat as perpetrator (inhibitor and inducer of CYP3A and P-gp activity) or victim drug (inhibition of P-gp) reveals a weak potential for CYP3A4 inhibition (midazolam), while induction of CYP3A4 was observed in vitro but not in vivo. With regard to the possible activity on P-gp, a firm conclusion on the potential for givinostat on the inhibition / induction of P-gp transporter by coadministration of dabigatran etexilate cannot be derived. Co-administration of P-gp transporter inhibitor clarithromycin was found to elicit a 40% increase in c_{max} of givinostat and T_{max} was found 1 hour earlier (indicating a more rapid absorption), while the overall amount of drug absorbed remains the same, i.e. AUC_{0-t} and $AUC_{0-\infty}$ fulfilled bioequivalence criteria. It cannot be ruled out that givinostat concentrations increase when co-administered with p-gp transporter inhibitors, and - in a worst-case scenario - lead to clinically relevant QT prolongation. Upon request, simulated calculations for givinostat plasma concentrations in a worst-case scenario, i.e. when coadministered with p-gp transporter inhibitors, has been provided. For this scenario it is assumed that C_{max} increases about 40%, which might be relevant for C_{max} – depending TEAEs (e.g. QTc prolongation). Simulations demonstrate that median givinostat plasma concentrations in this scenario at all possible weight bands still remain far below the clinical threshold for concern of QTc prolongation of > 10 msec. The safety margins regarding median C_{max} concentrations have been calculated as being 6- to 11-fold lower than the threshold of 745 ng/ml, when a relevant QTc prolonging effect is expected to occur. The potential of givinostat to inhibit the renal transporter OCT2 is included in section 4.5 of the SmPC warranting caution in case of concomitant administration with substrates of the OCT2 transporter.

Importantly, the safety profile of givinostat in DMD cannot be disentangled from any possible effects of the stable corticosteroid regimen that has been applied throughout the clinical DMD studies. From the pooled analysis of non-oncology/ non-DMD (Group 3) studies by concomitant steroid treatment "yes/no" it could be deduced that the most relevant AESI, i.e. platelet count decreased, diarrhoea, and hypertriglyceridaemia were more frequently reported with givinostat alone as compared to the combination. However, the use of steroids in the studies included in Group 3 was variable, e.g., a majority of patients with Crohn's disease received steroids concomitantly with givinostat, while only a single subject with Becker muscular dystrophy (BMD) did so.

Two-third of patients with concomitant steroids derived from study 06 in Crohn's disease (56 of 82 patients). Taking into account that one of the most important symptoms of Crohn's disease is diarrhoea, the decreased incidence of diarrhoea in patients with concomitant steroids could in parts be related to the efficacy outcome (reduction of diarrhoea). However, given that the reason for premature stop of the clinical programme for Crohn's disease was 'lack of efficacy', this hypothesis cannot be verified. Study 53 in BMD was the only study in the Group 3 pool where triglycerides have been measured. Given that only a single patient received concomitant steroids in study 53, comparison for the outcome of hypertriglyceridaemia between patients with and without steroids is impossible.

As proposed by Grodzielski et al. (2023), steroids can increase thrombopoiesis. This is a reasonable explanation for lower platelet counts in patients treated with givinostat monotherapy. The applicant

provided an indirect comparison for platelet count decreases observed in study 53 (BMD; all but two patients received givinostat monotherapy) and in study 48 (DMD; all patients received steroids in addition to givinostat). While the percent mean change in platelet counts from baseline to Months 3 and 6 was roughly comparable in the two studies, the absolute mean values were lower at each of the time points in study 53. Moreover, the incidence of thrombocytopenia (aggregated term also including platelet count decreased) was almost twice as high in study 53 as compared to study 48 (58.8% vs. 32.2%). The following limitations need to be taken into account for this indirect comparison: patients from study 53 were low in number (N=34, who received givinostat in monotherapy) contrasting the number of patients in study 48 (N=118); patients in study 53 were older (mean age 37.4 years) than those in study 48 (mean age 9.84 years); the mean daily givinostat doses applied in study 53 were higher as compared to study 48; patients in study 48 were requested to be on a stable steroid dose for at least 6 months while patients in study 53 were almost exclusively steroid-naïve. As a consequence, higher mean baseline platelet counts were found in study 48 as compared to study 53.

To conclude, givinostat without corticosteroids could lead to an even more pronounced decrease in platelet counts as observed in the DMD programme, where all patients received stable steroid doses. In this context, the adequacy of the proposed RMMs for givinostat regarding thrombocytopenia/ platelet count decreases cannot be judged for patients not treated with steroids. The applicant therefore accepted to limit the indication for givinostat to patients *with concomitant corticosteroid treatment*.

In summary, addition of clarifying wording has been added in section 5.1. stating that all patients were on a stable dose of corticosteroids throughout studies 48 and 51.

The safety data package is considered comprehensive for a rare disease. As of 31 December 2023, the 5th interim data cut-off for the clinical follow-up study 51, a total of 207 participants were exposed to givinostat for a mean duration of 1195.7 days (range: 26 to 2259 days). 136 subjects reached 36 months, 61 subjects reached 48 months, and 16 subjects reached 72 months year post-treatment safety follow-up in this study. No new safety signals were detected. Drug related TEAEs were manageable with appropriate monitoring and dose adjustments allowed per study protocol. The length of safety follow-up is considered appropriate.

2.6.10. Conclusions on clinical safety

The safety database with a total of 222 patients treated with givinostat at different dose levels (A, B, A-B, A-B-C, and B-C) reflecting doses between 10.6 mg b.i.d (referring to the lowest dose C and the lowest body weight of ≥10 and <12.5 kg) and 70 mg b.i.d (referring to the highest dose A and the highest body weight of ≥70 kg), and a median duration of exposure of 24.90 months in the DMD clinical programme up to the initial data cut-off 31 December 2021 is considered acceptable when considering that DMD affects 1 in 3600 – 9300 live male births. 187 of 222 patients (84%) have been exposed to givinostat for more than 12 months and 105 of 222 patients (47%) had an exposure of more than 24 months as of the updated safety data cut-off (31 December 2021). Updated long-term safety data from study 51 up to 31 December 2023 have been provided within this procedure, and results are in support of givinostat's safety profile.

Upon revision, the following ADRs have been identified for givinostat based on the incidence in the placebo-controlled study 48: thrombocytopenia (32.2%; including thrombocytopenia and platelet count decreased), diarrhoea (38.1%; including diarrhoea and faeces soft), vomiting (28.8%), abdominal pain (33.9%; including abdominal pain and abdominal pain upper), pyrexia (12.7%), hypertriglyceridaemia (22.9%; including hypertriglyceridaemia and blood triglyceride increased), constipation (6.8%), fatigue (7.6%), gastroenteritis (7.6%), blood thyroid stimulating hormone increased (6.8%; including thyroid

function test abnormal and blood thyroid stimulating hormone increased),decreased appetite (6.8%), myalgia (9.3%), arthralgia (8.5%), muscular weakness (3.4%), dizziness (4.2%), anxiety (2.5%), erythema (4.2%), rash (9.3%), and haematoma (4.2%). The main safety issues identified for givinostat are thrombocytopenia, gastrointestinal disturbances (diarrhoea, bearing the risk for dehydration and electrolyte disturbances), and an increase in triglycerides.

The risk for thrombocytopenia and diarrhoea is considered to be effectively reduced by reducing the dose, while mitigation of increases in triglycerides additionally required interruption of dosing in some cases in the clinical studies.

In summary, the safety issues identified for givinostat are thought to be manageable with the proposed risk minimisation measures.

Although, clinical data is not available to confirm the absence of an effect on cardiac conduction in patients with cardiomyopathy, givinostat was not found to prolong the QT interval at therapeutic doses and significant findings on other ECG parameters were absent. Additional cautious wording with regard to the paragraph on QTc prolongation in section 4.4 has been introduced.

Nevertheless, clinical safety has insufficiently been evaluated for:

- patients younger than 6 years,
- non-ambulatory patients; while the 34 patients, who lost ambulation during treatment with givinostat did not show an altered efficacy or a worsened safety profile, initiation of givinostat in these patients has not been evaluated. Nevertheless, non-ambulant patients will be further evaluated in the ongoing PIP study 5 (DSC/14/2357/50), and
- DMD patients without concomitant steroid treatment, i.e. in monotherapy. In non-DMD studies (Group 3 data), concomitant treatment with corticosteroids seemed to have mitigated some of the main adverse effects of givinostat, i.e. TEAEs of diarrhoea, hypertriglyceridaemia, and thrombocytopenia were found more frequently reported with givinostat alone as compared to the combination, which suggest the safety profile in monotherapy to be different in general (and potentially worse with regard to the risk of thrombocytopenia) than in the combination with corticosteroids.

The lack of clinical data for givinostat in the aforementioned patients, i.e. under the age of 6 years, those being non-ambulatory prior to treatment initiation with givinostat, and those without concomitant corticosteroid treatment has been addressed in the course of this procedure and the applicant agreed to restrict the indication accordingly.

Overall, the safety data package is considered comprehensive.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Haemorrhagic disorders

Summary of safety concerns	
	Clinical consequences of hypertriglyceridaemia (e.g., pancreatitis, coronary artery disease, hepatic steatosis) Dehydration with potential electrolyte imbalance
Missing information	Long-term safety

2.7.2. Pharmacovigilance plan

Safety data will be collected in observational PAES study as outlined in section 2.7.3.

2.7.3. Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Haemorrhagic disorders (*potential*)	Routine risk minimisation measures: *SmPC sections 4.2 and 4.4 where advice is given on dose adjustment and monitoring blood platelet counts* *PL sections 2 and 4* Additional risk minimisation measures: no risk minimisation measures	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: observational PAES study
Clinical consequences of hypertriglyceridaemia (e.g., pancreatitis, coronary artery disease, hepatic steatosis) (*potential*)	Routine risk minimisation measures: *SmPC sections 4.2 and 4.4 where advice is given on dose interruption and adjustment and monitoring blood triglycerides* *PL sections 2 and 4* Additional risk minimisation measures: no risk minimisation measures	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: observational PAES study
Dehydration with potential electrolyte imbalance (*potential*)	Routine risk minimisation measures: *SmPC sections 4.2 and 4.4 where advice is given on dose adjustment in case of severe diarrhoea* *PL sections 2 and 4*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: observational PAES study

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Additional risk minimisation measures: no risk minimisation measures	
Long-term safety (*missing information*)	Routine risk minimisation measures: Additional risk minimisation measures: no risk minimisation measures	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: observational PAES study

2.7.1. Conclusion

The CHMP considers that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the European birth date based on UK approval of 20 December 2024. The new EURD list entry will therefore use this date to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Duvyzat (givinostat) is included in the additional monitoring list as it includes new active substance.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic context

3.1.1. Disease or condition

Duchenne muscular dystrophy (DMD) is a rare, disabling, progressive and ultimately fatal X-linked recessive neuromuscular disorder caused by mutations in the gene for dystrophin. Functional dystrophin is critical for the structural stability of myofibers in skeletal, diaphragm and cardiac muscle and is also of importance for smooth muscles. The disease primarily affects males with a birth incidence of 1 in 3600 – 9300 males (Mah *et al*., 2014). DMD is characterised by a progressive degeneration of skeletal muscles, with symptoms that manifest early, at around 3 years, causing loss of ambulation before the age of 12, followed by cardiac complications (e.g. dilated cardiomyopathy and arrhythmia) and respiratory disorders, including chronic respiratory failure (Birnkrant et al., 2018).

The current application of givinostat is for the treatment of Duchenne muscular dystrophy (DMD) and includes ambulant patients aged 6 years and older, and with concomitant corticosteroid treatment.

3.1.2. Available therapies and unmet medical need

There are no approved treatments to cure or stop the ultimately fatal progression of DMD.

Even with the introduction in the 1990s of assisted ventilation in the later stages of the disease, the mean age of survival (for those ventilated patients who do not develop early and severe cardiomyopathy) is still only 24 years.

Supportive care (e.g., physiotherapy) and glucocorticoids remain the mainstays of DMD treatment and should continue after loss of ambulation (Birnkrant *et al*., 2018). Although, glucocorticoid treatment recommended by pertinent treatment guidelines is well-established, i.e. prednisone and deflazacort, only one of them is licensed for the treatment of DMD in Europe. Moreover, uncertainty exists regarding the most adequate corticosteroid dosing regimen and duration of treatment for DMD.

Agamree (active substance: vamorolone) received marketing authorisation for treatment of Duchenne muscular dystrophy in patients aged 4 years and older on 14 December 2023. Vamorolone is a synthetic corticosteroid analogue that structurally diverges from other members of the class of glucocorticoid agents. It reduces inflammation by blocking the production of cytokines. The structural peculiarity of vamorolone further entails that it acts as mineralocorticoid receptor antagonist, which contrasts the agonistic MR activity of other GCs. In addition, vamorolone is not a substrate for 11-β HSD enzymes, thereby potentially reducing the risk for typical GC side effects (muscle atrophy, bone loss, insulin resistance, hypertension and weight gain).

There is still an unmet medical need for (mutation-independent) treatments with an acceptable benefit-risk profile.

3.1.3. Main clinical studies

For the main evidence of efficacy, a single phase 3, multicentre, randomised, double-blind, parallel group, placebo (overall n = 61; target population n = 39) controlled study of 18 months treatment duration to assess the efficacy and safety of givinostat, (overall n = 118; target population n = 81) in ambulant boys with Duchenne muscular dystrophy (DMD) has been submitted. The initial dose

suggested for the study was chosen to achieve similar exposure as the 37.5 mg b.i.d. dose in the phase 2 proof-of concept study 43 targeting a daily exposure of 300 ng*h/mL.

The study included boys between 6 and 16 years of age on stable corticosteroid treatment with a diagnosis of DMD confirmed by genetic testing. Included subjects had to be able at screening to complete two 4-stairs climb (4SC) assessments with results within ±1 second of each other and a mean of the 2 4SC assessments of ≤ 8 seconds, be able to complete the time to rise from floor test between ≥ 3 and < 10 seconds and had manual muscle testing of quadriceps of grade ≥ 3. Additionally, the Target Population had to have a vastus lateralis muscle fat fraction (VL MFF) (MRS) > 5% to ≤30%.

3.2. Favourable effects

The primary endpoint was the mean change in time to climb 4 stairs (4SC) before and after 18 months of treatment of givinostat versus placebo. While the time to 4SC increased in the givinostat group from 3.39 (SD 1.162) to 4.70 (SD 3.154) seconds it increased even more in the placebo group from 3.48 (SD 11.158) to 6.37 (SD 7.698) seconds. The GLS mean (log scale SE) was 1.27 (0.040) and 1.48 (0.058) for givinostat and placebo, respectively, with a GLS mean ratio (95% CI) of 0.86 (0.071); (p = 0.0374). In the analysis performed on non-log-transformed data, the LS mean was 1.25 and 3.03 seconds for givinostat and placebo, respectively, with a difference in LS means of - 1.78 seconds in favour of givinostat (p=0.0374). A pre-specified supportive analysis of 4SC velocity (stairs /second) showed a difference in LS means for 4SC velocity of 0.09 stairs/second (p = 0.1696), corresponding to a treatment effect of 0.0225 tasks/sec.

A supportive analysis to assess shifts in grade for functional adaption of 4SC ascent from baseline to 18 months as well as a sensitivity analysis with and without adjustment for MRS VL MFF were in line with the results of the primary analysis. Sensitivity analyses demonstrated that the COVID-19 pandemic did not affect the results of the primary analysis at month 18.

Efficacy results for the primary endpoint, the key secondary endpoints and further velocity endpoints for the "off-target" and the "overall population" were in line with those of the target population showing a positive trend in the givinostat group when compared to placebo. With regard to the primary endpoint time to 4SC, results in the overall population were consistent with those of the target population [treatment effect 0.84 (95% CI: 0.733, 0.961) and 0.86 (95% CI: 0.745, 0.989), respectively].

Difference in seconds in 4SC based on non-log transformed data and results in 4SC velocity (task/seconds) are in the same range as published data on minimum clinical important differences (MCID) and could be interpreted as justifying the clinical relevance of the observed effects.

With respect to the selected key secondary endpoints, the LS mean (95% CI) change from baseline in the NSAA total score for givinostat and placebo was - 2.66 (-3.563, -1.759) and -4.58 (-5.891, -3.260), respectively, representing less decrease under givinostat treatment when compared against placebo. The LS means difference in the NSAA total score of 1.91 was in favour of givinostat (p = 0.0209). The same related to the ratio of cumulative loss of function on the NSAA scores (givinostat/placebo) of 0.61 (p = 0.0202).

With respect to changes in vastus lateralis muscle fat fraction for the Target Population in the MR cohort, there was less increase in fat fraction under givinostat with a LS mean (95% CI) in change from baseline at 18 months for givinostat and placebo of 7.63 (6.098, 9.172) and 10.56 (8.331, 12.783), respectively. The LSM difference for givinostat against placebo was -2.92 (p = 0.0354).

Fat Fraction of Thigh Muscles by Magnetic Resonance Imaging**:** An analysis of change from Baseline in

fat fraction (%) in 5 thigh muscles (VL, biceps femoris long head, semitendinosus, quadriceps and hamstrings) as measured by MRI at 18 months was performed. Difference in LS means (givinostat-placebo) in all 5 thigh muscles were: VL: -3.95% [p-value: 0.0033]; biceps femoris long head: -4.44% [p-value: 0.0235]; semitendinosus: -4.43% [p-value: 0.0009]; quadriceps: -4.19% [p-value: 0.0009]; hamstrings: -4.38% [p-value: 0.0026]).

Time to rise from floor (TTR) increased (worsened) over time in both the givinostat group (from 5.57 to 14.43 seconds) and in the placebo group (from 5.59 to 19.17 seconds). Mean (SD) change from Baseline in time to rise from floor at EOS was lower in the givinostat group in the givinostat group (8.86 seconds) compared to placebo (13.59 seconds). Analysis of change from baseline in mean time to rise from floor provided a LS mean difference of -3.28 seconds in favour of givinostat (p = 0.3044), Time to rise from floor velocity decreased from baseline to EOS in the givinostat group from 0.198 to 0.151 rises/sec and in the placebo arm from 0.198 to 0.118 rises/sec with mean changes of -0.047 rises/sec and -0.080 rises/sec for givinostat and placebo, respectively. LS mean change in time to rise from floor velocity from baseline to 18 months was – 0.05 rises/sec in the givinostat group and – 0.08 rises/sec in the placebo group. The LSM difference for givinostat from placebo was 0.03 rises/sec (p = 0.0124).

Time to 10MWR increased (worsened) over time in both the givinostat group (from 5.17 seconds at Baseline to 6.04 seconds at EOS) and the placebo group (from 5.18 seconds at Baseline to 7.99 seconds at EOS). Mean (SD) change from Baseline in time to 10MWR at EOS was lower in the givinostat group (0.63 [1.489] seconds) compared with placebo (2.81 [8.214] seconds). Analysis of change from Baseline in mean time to 10MWR at 18 months provided a LS mean difference of -2.35 seconds (p-value: 0.0140).

Subgroup analyses by baseline age 6 to 7 years of age and above 7 years as well as subgroup analyses by treatment regimen were in line with those in all patients in the target population showing a positive trend in the givinostat group when compared to the placebo group for the primary and the key secondary endpoints.

An analysis of covariance (ANCOVA) was performed by adding class factor for 'pre' and 'post' protocol amendment along with an interaction term between dosing schema and randomised treatment. The interaction between randomised treatment and treatment regimen was non-significant.

In order to evaluate whether the givinostat treatment effect of the primary endpoint was disproportionately driven by the results of Dose A, patients on givinostat were grouped into those who had stayed on Dose A for the full study (Group A) and those patients in Treatment Regimen 1 who reduced the dose, together with patients in Treatment Regimen 2 (Group Not A). The results show that givinostat treatment effect versus placebo was similar in Groups A and Not A.

The applicant showed that patients in study 48 with treatment 1 achieved comparable exposures compared to patients with treatment 2. With both treatment regimens, the overall exposures were higher than the predicted threshold level for efficacy that had been established based on preclinical results and pharmacometrics analyses, i.e., a target minimum daily exposure of 300 ng*h/mL. Moreover, the applicant could demonstrate by simulation that exposures across the 9 different weight bands used in study 48, were also comparable. Likewise, exposures were found comparable when patients had to reduce their dose due to side effects.

With respect to modified dosing during the study, the applicant could sufficiently justify, that efficacy results are not affected. The new proposed dosing regimen is also overall agreed with.

With respect to the primary endpoint of the phase 2 study DSC/11/2357/43, change from baseline in Muscle Fibre Area (MFA), the mean MFA% at baseline was 51.003% and increased to 64.909% at EoS (End of Part 2) with a mean change from baseline until months 12 of 13.906%.

Cross-sectional area (CSA) of the muscle fibres increased from mean (SD) 1191.087 μm^2 (400.9813) at baseline to 2056.356 μm^2 (781.3925) at EOS with a mean change from baseline of 865.269 μm^2 (555.3543). There were observed decreases between baseline and EOS visit in mean (SD) percentages of total fibrosis (-12.640 ± 4.6493), perimysial fibrosis (-7.585 ± 6.4779), endomysial fibrosis (-5.056 ± 6.2531), fatty replacement (-0.302 ± 0.27569, and necrosis (-0.964 ± 0.6260). The mean (SD) number of hypercontracted fibres decreased from 1.977 (0.7139) at baseline to 0.773 (0.5429) at EOS with a mean change from baseline of -1.204 (0.6621). An increase could also be observed in regenerative fibres with no depletion in the pool of satellite cells.

3.3. Uncertainties and limitations about favourable effects

The evidence for efficacy was generated in one pivotal study. Post-hoc analyses for the primary, key secondary and velocity endpoints to address uncertainties with respect to the primary analysis using more conservative missing data handling strategies failed to show statistical significance. Overall, the point estimates became smaller and/or the confidence interval became wider. Thus, there are uncertainties whether efficacy of Duvyzat for the treatment of patients with DMD has been robustly shown as the statistical significance of the estimated treatment effects could not be reassured.

With the answers and during an Oral Explanation the applicant provided further arguments to justify the evidence of efficacy based on statistical aspects as well as on clinical data. Overall, the applicant could plausibly refute that the J2R approach is of highest relevance for regulatory decision making compared to the other analysis performed in the context of a disease modifying treatment where it is not expected that the effect will completely be lost after stopping treatment.

The applicant provided additional analyses of the Study 48 data to further illustrate that the level of uncertainty in the efficacy of givinostat is actually low and acceptable, particularly in the context of a treatment for a serious and life-threatening rare disease with substantial unmet medical need. However, the still existing uncertainties with respect to robustness of the overall dataset could not sufficiently been solved.

With respect to long-term effects of givinostat the provided post-hoc analyses for DMD disease milestones can be interpreted as a delay of 1.7 to 3.3 years in progression for loss of function milestones for givinostat compared to natural history data. However, these data can be considered only supportive of the results obtained in the 18 months placebo-controlled pivotal study, which by default is designed to produce the most valid results.

Results for subgroup analyses based on the final doses received by patients during the study, i.e. Dose A, B and C numerically favoured givinostat when compared against placebo. Although, the results across the final dose groups indicate no dose-response relationship, the effect sizes under Dose B were generally smaller than for Dose A and/or Dose C. While exposures at Dose B are within the range of exposures at Doses A and C, this issue is not further pursued. It also needs to be considered, that the study was not powered to evaluate differences in givinostat dose regimens while patients were not randomised to Doses A, B or C.

3.4. Unfavourable effects

Givinostat was demonstrated to potently inhibit the ubiquitously expressed human class I as well as class IIa and class IIB HDAC enzymes *in vitro*, which are predominant in heart, skeletal muscle and brain (McKinsey *et al*., 2001). The HDAC inhibition of givinostat mediates anti-inflammatory but no immunosuppressant effects.

The target organs for givinostat toxicities identified from non-clinical studies comprise white and red

blood cell lineages with related findings in spleen, lymphatic and bone marrow tissues as well as the liver.

Well known clinical toxicities of HDAC inhibitors comprise Grade 1 to 2 gastrointestinal disorders, constitutional and haematological TEAEs, as well as risks for hepatic and cardiac abnormalities (Bondarev et al. 2020).

The safety of givinostat in the proposed dose range between 10.6 mg b.i.d (referring to the lowest Dose C and the lowest body weight of ≥10 and <12.5 kg) and 70 mg b.i.d (referring to the highest Dose A and the highest body weight of ≥70 kg) has been evaluated in three clinical studies with 222 paediatric male subjects in the age range 6 to 16 years at study entry, with all of them having received a stable corticosteroid treatment regimen. No safety data are available for subjects <6 years of age. The median duration of treatment exposure in the studies was 23.57 months for givinostat (and 18.14 months for placebo). 187 of 222 patients (84%) have been exposed to givinostat for more than 12 months and 105 of 222 patients (47%) had an exposure of more than 24 months. Long-term safety is further being evaluated in the ongoing open-label study 51 with the latest cut-off date 31 December 2023. 14.4%, 41%, 20.3%, 10.8%, and 13.5% of all givinostat-treated patients were treated at Dose levels A, B, A-B, A-B-C, or B-C.

Two deaths were reported during study 51 as per the data cut off 31 December 2023. Both involved fatalities related to accidents and occurred in the delayed givinostat group. Both were rated as not related to givinostat. During the pooled DMD studies (Group 2), nine patients discontinued givinostat due to diarrhoea, abdominal pain, nausea, blood triglycerides increased, platelet count decreased, hypertriglyceridaemia, and atrial fibrillation). Reporting of SAEs was higher with givinostat compared to placebo (17.1% vs. 3.3%).

TEAEs with a notably higher incidence in the overall givinostat group compared to the placebo group were diarrhoea (39.6% vs. 18%), vomiting (28.4% vs. 13.1%), abdominal pain (23% vs. 14.8%), platelet count decreased (23% vs. 0%), blood triglycerides increased (16.7% vs. 4.9%), thrombocytopenia (18% vs. 0%), decreased appetite (8.1% vs. 0%), hypertriglyceridaemia (9% vs. 1.6%), fatigue (9.5% vs. 0%), hypothyroidism (5.4% vs. 0%), arthralgia (10.4% vs. 1.6%), pyrexia (20.7% vs. 8.2%), and rash (7.7% vs. 1.6%).

Most TEAEs were mild to moderate in severity, while severe TEAEs were almost exclusively reported in subjects on givinostat and the types of TEAEs were basically in line with the common TEAEs.

Adverse drug reactions (ADRs) with givinostat were based on the placebo-controlled data from study 48 taking into account TEAEs in ≥2% of givinostat-treated patients, a frequency >2% compared to the placebo group, and a causal plausibility. *Thrombocytopenia, diarrhoea, vomiting, abdominal pain, pyrexia, and hypertriglyceridaemia* were rated as "very common" ADRs. *Constipation, fatigue, gastroenteritis, blood thyroid stimulating hormone increased, decreased appetite, myalgia, arthralgia, muscular weakness, dizziness, anxiety, erythema, rash, and haematoma* were rated as "common" ADRs.

Thrombocytopenia/ platelet count decrease, Hypertriglyceridemia/ triglyceride increase, Gastrointestinal disturbances (diarrhoea, nausea, vomiting and abdominal pain), and QT prolongation were identified as **AESI** for givinostat. In addition, SMQs of haematopoietic thrombocytopenia, osteoporosis/ osteopenia and triglycerides increased have been conducted.

Givinostat dose-dependently affected haematological parameters in rodents as evidenced by decreased counts of platelets with concomitantly increased megakaryocytes in spleen and bone marrow.

AESI of **platelet count decreased and thrombocytopenia** were reported in 23% and 18% of patients on givinostat, respectively (none on placebo), and based on SMQ analysis (lacking double

counting of patients with either TEAE), 38.3% patients were reported. The majority of events was recorded in dose groups A-B-C and B-C, which included patients with dose reductions due to emerging safety issues with givinostat. Long-term treatment in study 51 did not lead to an increase in these events. Platelet count decreased was reported as SAE in two patients: in one patient treated with 50 mg b.i.d in study 43, this SAE was a life-threatening (Grade 4) with a platelet count nadir of 20 x 10^9/L that led to treatment discontinuation. No concomitant bleeding events were reported. However, *haemorrhagic disorders* is included as an important potential risk in the RMP, which will be further addressed by mean of a PASS study post-marketing. TEAEs of platelet count decreased and thrombocytopenia were rated severe in 1.4% and 0% of patients. Thrombocytopenia is a defined ADR in section 4.8 of the SmPC. Platelet counts decreased over the first 6 months of treatment with givinostat by ~40% from baseline, with the steepest decrease within the first two weeks of treatment (- 30% irrespective of dose level), and plateauing thereafter. Further decreases based on continuous treatment with givinostat during study 51 appear to be absent. Shifts from normal platelets at baseline to abnormal (low) platelets post-baseline were highest around Week 12 (study 48 data). Based on pharmacometric modelling and simulation data, patients with a higher body weight have a higher risk for low platelet counts given the negative correlation between body weight and baseline platelet counts. The incidence of thrombocytopenia is predicted to be lower in subjects with lower body weight (receiving lower dose levels).

An increase in hepatic biochemistry parameters (liver enzymes activities, higher bilirubin, urea and triglycerides values) was observed in animals and these were associated with the histopathological finding of bile duct hyperplasia. AESI of blood triglycerides increased and **hypertriglyceridaemia** (also including the PT blood triglycerides abnormal) were reported in a higher number of patients on givinostat compared to placebo: 25.2% and 6.6%. TEAEs were mainly mild to moderate in severity, and severe for 5 subjects. No clear relation to dose could be determined based on the reporting of events in Dose A-B-C and B-C groups (31.9% vs. 46.7%). No SAEs were reported but 3 patients permanently discontinued givinostat due to an AESI given that triglycerides did not recover with treatment interruptions and dose reductions. In these patients, triglyceride values peaked between 5.08 mmol/L (450 mg/dL) and 5.38 mmol/L (476 mg/dL), i.e. Grade 2. Triglyceride values generally started to increase in the first 4 weeks of treatment and were found highest at Month 3 and 6 but always remained within Grade 1 according to CTCAE criteria. The median time to maximum triglyceride levels was shorter for givinostat (221.0 days) compared to placebo (255.0 days). No further progressive increases (despite some fluctuations) in triglycerides were noted with continuous treatment in study 51. Two patients in the (continuous) givinostat group in study 51 reduced their dose due to triglyceride increases. Overall, triglyceride increases were reversible with interruption/ discontinuation of givinostat treatment. Hypertriglyceridaemia is a defined ADR in section 4.8 of the SmPC. TEAEs in line with clinical consequences of elevated triglyceride levels, i.e. acute pancreatitis, coronary artery disease, and hepatic steatosis have not been observed in the clinical DMD studies; however, the risk will be addressed in a PASS post-marketing.

GI disturbances, including diarrhoea, nausea, vomiting and abdominal pain, were reported in 60.8% of patients in the givinostat group and 41.0% of patients in the placebo group, with the most frequently TEAE being **diarrhoea** (39.6% vs. 18%). In a majority of patients, GI events were mild to moderate in severity, characterised by a stool frequency of either <4 stools/day or 4-6 stools/day. One patient had severe diarrhoea (≥7 stools/day). Two SAEs were reported (abdominal pain and vomiting). Two patients required corrective fluids, but electrolytes were always within normal limits. Three patients had to discontinue givinostat due to a related TEAE of either abdominal pain, nausea or diarrhoea. The incidence of GI disorders TEAEs, specifically diarrhoea and abdominal pain, was found to be dose-related and could be mitigated by dose reduction. Dose reduction is specified for moderate to severe diarrhoea in section 4.2 of the SmPC (more than 4 stools per day; ≥Grade 2 according to CTCAE v.5.0). Diarrhoea, vomiting and constipation are "very common" ADRs in section 4.8 of the

SmPC. If abnormalities persist after dose reductions, givinostat needs to be discontinued.

The potential effect of givinostat on the cardiovascular system was investigated in nonclinical studies. Givinostat was shown to inhibit hERG K^+ currents with an IC_{50} value of 1.4 µM *in vitro*, whereas lower inhibition was determined for its metabolites. Taking into account the highest achievable plasma concentration with the maximum recommended human starting dose of 70 mg bid givinostat for DMD patients ≥70 kg and the fact that about 95 % of givinostat is bound to plasma proteins, the IC_{50} of givinostat for hERG channel inhibition translates into an approximately 74-fold safety margin. Therefore, an impact of givinostat on cardiovascular function at therapeutic human doses seems unlikely. No changes on ECG parameters, heart rate, arterial and ventricular systolic pressure were evident during cardiovascular safety assessment of anaesthetised Beagle dogs as well as in repeat-dose toxicity studies in dogs and monkeys. Givinostat was not found to prolong the **QT interval** when administered at a therapeutic dose (100 mg) in the thorough QT study 54 in healthy volunteers. The highest mean effect on ΔΔQTcF was 5.5 msec (90% CI: 1.99 to 9.01) compared to 13.6 msec (90% CI: 10.15 to 17.05) for a supratherapeutic dose of 300 mg. Based on the E_{max} model, a QT effect (ΔΔQTcF) exceeding 10 msec can be excluded within the full observed range of givinostat plasma concentration, up to ~745 ng/mL. No AESI related to QT prolongation was reported in the DMD studies. Small changes from baseline to the last available visit for *ECG parameters* were observed. The median change from baseline to EOT for the QTcF interval was - 7.00 msec for givinostat and 1.00 msec for placebo. 3.2% of patients on givinostat compared to no patient on placebo had an increase from baseline in QTcF interval of ≥ 30 msec and < 60 msec at the EOT visit. No patient had a change in QTcF interval from baseline of ≥ 60 msec or a value >500 msec at either time point. The percentage of patients shifting from a normal ECG at baseline to an abnormal ECG at the last available visit was higher for givinostat compared to placebo (20.7% vs. 13.1%). Based on study 48 controlled data, ECG shifts from normal at baseline to *clinically significant* abnormal post-baseline were absent.

Nonclinical data do not point towards a risk of givinostat to elicit adverse effects on the bone. However, 13.5% and 6.6% of patients in the givinostat and placebo group reported TEAEs in the SMQ of **osteoporosis/ osteopenia**, which derived from a disproportionate reporting of fractures (10.8% for givinostat vs. 3.3% for placebo), while TEAEs per patient-year were similar. In a majority of patients fractures were reported as SAEs. The most frequently reported fracture for givinostat was femur fracture in 10 patients (12 events) versus none on placebo. Spinal fractures were reported in 5 patients (5 events), and a single event occurred in the placebo group.

Nonclinical data suggest that the effect of givinostat in the bone marrow impacts white blood cells homeostasis (lower lymphocytes and neutrophils) and also red blood cell homeostasis (lower haemoglobin, haematocrit and mean corpuscular haemoglobin concentration), which is correlated with the observed histopathological changes consisting in atrophy of lymphoid organs. Decreased WBC counts and leukopenia were reported as TEAEs in 5 and 3 patients on givinostat (2.3% and 1.4%) and in no patient on placebo in the DMD studies. Anaemia and neutropenia were reported as TEAE in a single patient each on givinostat (and in no patient on placebo). There were slight median decreases from baseline for givinostat as compared to placebo for white cell lines, mainly leukocytes (up to Week 12) and neutrophils (up to Week 24) as well as for red cell lines, mainly haemoglobin (median change from baseline to EOT of -3.03 g/L for givinostat and 0.00 g/L for placebo) and erythrocytes. Erythrocyte indices MCH and MCV were found to increase, with a plateau reached between 6 to 12 months. The SmPC recommends routine monitoring of haematological effects.

Nonclinical data revealed increases in liver enzymes, bilirubin, urea and triglycerides in monkeys given the highest doses in both, the 13 and 39 week study. These changes were considered related to bile duct hyperplasia, which is in line with hepatic biochemistry findings and histopathological examination confirming that the **liver** is a target organ for givinostat. The observed changes in liver parameters in DMD patients, e.g., increase in direct and total bilirubin corroborated by some shifts from normal at

baseline to high post-baseline for givinostat but not for placebo, are not considered of clinical concern.

Givinostat seems to affect **thyroid function** in DMD patients but not in animals, i.e. thyrotropin (TSH) was found to increase with givinostat but not with placebo (median: 1.30 mU/L vs. 0.12 mU/L), which likewise resulted in reporting of TEAEs of blood thyroid stimulating hormone increased, which is a common ADR in section 4.8 of the SmPC for givinostat.

No relevant changes in other laboratory parameters, including electrolytes, have been noted.

The potential for clinically significant **drug-drug interactions** with givinostat as perpetrator (inhibitor and inducer of CYP3A and P-gp activity) or victim drug (inhibition of P-gp) reveals a weak potential for CYP3A4 inhibition (midazolam), while induction of CYP3A4 was observed in vitro but not in vivo. A firm conclusion on the potential for givinostat on the inhibition/ induction of P-gp transporter by coadministration of dabigatran etexilate cannot be derived. Co-administration with the strong P-pg inhibitor clarithromycin caused an increase in the rate of exposure (C_{max}) of givinostat about 40% but had a minimal effect on the extent of exposure (AUC). The potential of givinostat to inhibit the renal transporter OCT2, for which creatinine is a substrate, is included in section 4.5 of the SmPC warranting caution in case of concomitant administration with substrates of the OCT2 transporter.

3.5. Uncertainties and limitations about unfavourable effects

No safety data are available for patients aged <6 years, and this population has been excluded from the indication. The maximum age was 17.5 years for the continuous givinostat group at study entry for study 51. Therefore, only few uncontrolled data are available for patients 18 years and older. A post-hoc comparison of the safety profile separating children 6 to <12 years and adolescents ≥12 to <18 years based on data in the DMD studies pool revealed some quantitative differences in AESI, i.e. slightly higher incidences for diarrhoea, abdominal pain, platelet count decreased, and blood triglycerides increased in children compared to adolescents. The clinical relevance of these findings remains uncertain.

Moreover, no efficacy and safety data is available for patients starting on givinostat despite being non-ambulatory at baseline, while safety of givinostat remains unaltered in patients who lose ambulation during treatment. Thus, the B/R in patients initiated with givinostat while being in the non-ambulatory phase of their disease has not been determined leading to a restriction of the indication.

In addition, patients had to be on stable corticosteroid treatment for at least 6 months prior to entry and throughout the DMD studies. Thus, clinical safety data solely reflect the combination of givinostat and corticosteroids. Uncertainty derives from the unexpected finding of clinical safety profile differences in the pooled analysis of non-oncology/ non-DMD (Group 3) studies with and without concomitant administration of steroids, which revealed a higher reporting of overall TEAEs, severe TEAEs, and AESI for the givinostat only group as compared to givinostat plus steroid group, and also for the most relevant TEAEs identified in DMD studies, i.e. platelet count decreased, diarrhoea, and hypertriglyceridaemia. As a consequence, section 5.1 of the SmPC has been amended by adding information that all patients were on a stable dose of corticosteroids throughout studies 48 and 51. Moreover, the benefit-risk of givinostat in the absence of concomitant corticosteroid treatment in DMD cannot be assessed based on the available data, which has also been added for clarity. An indirect comparison for platelet count decreases observed in study 53 (BMD; almost all patients received givinostat monotherapy) and in study 48 (DMD; all patients received steroids in addition to givinostat) at 3 months and 6 months, respectively has been requested to further inform on the adequacy of the aforementioned wording in section 5.1. As a result, corticosteroids might have contributed to the increased platelet levels in patients with DMD versus those with BMD. In order to account for this uncertainty regarding the clinical consequences of more pronounced decreases in platelet counts

without concomitant corticosteroids, the indication has been agreed to be further restricted to patients with concomitant corticosteroid treatment.

The safety profile of givinostat has not been described for different races (90% of patients included in the DMD studies pool were White) and different regions (~32% of patients in study 48 were non-European patients). However, no difference is expected for different ethnicities based on the pharmacokinetic profile of givinostat. Moreover, applicability from the US/Canadian to the EU DMD population for givinostat has been justified by similarities in the incidence in DMD, demographic data, standard of care in the US/Canada and in the EU, natural history of the disease, and pharmacokinetics.

Treatment interruptions were reported for 67 of 22 patients (30.2%) in the Group 2 DMD studies. The majority of patients (41 of 67; 61%) had only one interruption followed by 14 and 6 of 67 patients (21% and 9%) with 2 or 3 interruptions. Drug interruption most frequently occurred in the context of hypertriglyceridaemia/ blood triglycerides increased, which was mainly observed at dose level A-B-C. This is reasonable given that patients had to reduce their dose in case of these events.

A number of uncertainties in the analysis and presentation of AESI platelet count data/ thrombocytopenia have been raised during the procedure. A summary of relatedness, severity, dose reductions, time to onset, and time to recovery has been provided and added to the product information for clarity. The monitoring scheme as well as absence of restrictions on baseline platelet counts in patients being eligible for givinostat treatment in the product information deviates from the prerequisites in the clinical studies. However, it could be clarified that - based on the revised dosing regimen - platelet counts $\leq 50 \times 10^9$/L are not expected and are thus not defined as a threshold for permanent treatment stop; moreover, routine monitoring of platelet counts has been revised to include more frequent measures during the first 3 months (every 2 weeks for the first 2 months of treatment, then at month 3), followed by every 3 months thereafter.

Based on the originally presented pharmacometric modelling and simulation data, the incidence of thrombocytopenia is predicted to be lower in subjects with lower body weight (receiving lower doses) as compared to those with higher body weight receiving higher doses. New PK/PD simulations have been presented to predict the risk for thrombocytopenia (all doses combined) based on a simplified dosing regimen with 4 (instead of 9) weight bands, which aimed at lower starting doses for higher body weights and the use of only one oral syringe for the administration. As a result, a small decrease in the predicted risk for thrombocytopenia >Grade 1 is noted for the revised dose regimen, e.g., for patients with a BW >60 kg. Thus, a more frequent platelet count monitoring based on higher exposures in heavier patients previously observed for the 9 weight bands regimen appears dispensable.

Hypertriglyceridaemia with givinostat appears manageable with implementation of a baseline monitoring, dose reductions based on a conservative triglyceride threshold of > 300 mg/dL (Grade 2 as per the CTCAE criteria), and treatment interruptions, while remaining uncertainties on the presentation of triglyceride data have been addressed and added to sections 4.4 and 4.8 of the SmPC. Specific situations, e.g., pre-existing high triglyceride levels and concomitant medication known to increase the risk for clinical consequences of hypertriglyceridaemia have been addressed.

Uncertainty remains with regard to the clinical consequences of vomiting and diarrhoea, i.e. dehydration that could lead to electrolyte disturbances, which can then be the trigger for cardiac abnormalities. Details with regard to corrective measures and occurrence of electrolyte disturbances have been specified in section 4.4 of the SmPC.

Givinostat has not been studied in patients with symptomatic cardiomyopathy. However, two SAEs of atrial fibrillation have been reported in study 51 up to 31 Dec 2023, one of which was rated as related to study drug. Although, an impact of givinostat on cardiovascular function at therapeutic human doses

seems unlikely, the risk cannot be fully excluded for patients with a medical history of cardiovascular impairment, baseline abnormalities or familial predisposition, as well as for conditions leading to electrolyte disturbances and with concomitant medications known to prolong the QTc interval.

With regard to the effects of givinostat on thyroid function in DMD patients, i.e. increases in TSH and mean fT4, with mean decreases in fT3, as well as TEAEs in line with TSH increased and hypothyroidism, animal data do not point towards an effect, and thyroid function has not been assessed in other indications for givinostat. The increase in TSH could be a consequence of inhibition of class I HDAC1/2 by givinostat while modifications of the acetylation state of histones in the promoter region of TSHα and TSHβ are crucial for the activation and repression of the two subunits. Regulation of TSHβ as the specific subunit of TSH could be more important for determining TSH levels as compared to TSHα and inhibition of HDAC1/2 by givinostat could lead to the slight increase in TSH.

Uncertainty has been raised regarding physical growth (height, weight, BMI) including pubertal development. It could be clarified that persistent diarrhoea did not affect BMI in patients treated with givinostat. However, no conclusion can be made for pubertal development by means of Tanner staging given that it has not been evaluated per study protocols.

3.6. Effects Table

Table 22 Effects Table for givinostat, indicated for the treatment of Duchenne muscular dystrophy (DMD) (data cut-off: 31 December 2021).

Effect	Short Description	Unit	Givinostat	Placebo/ comparator	Uncertainties/ Strength of evidence	References
Favourable Effects						
Change from baseline in time to 4SC at 18 months	Primary endpoint	Log scale analysis: GLS mean change from baseline (SE) GLS mean ratio (SE) 95%CI	 1.27 (0.040) 0.86 (0.071) 0.745;0.989	1.48 (0.058)	Pre-specified primary analysis using single imputation methods for handling missing data not the most appropriate considering that it underestimates variability and makes the questionable assumption that patients with missing data (other than due to being non-ambulatory or physically unable to have the assessment) will benefit from treatment similarly to patients with available measurements. Superiority of givinostat against placebo P = 0.0345 (statistical significant)	(1)
Change from baseline in NSAA total score at 18 months	Key secondary endpoint	LSM change from baseline LSM difference 95%CI	-2.66 1.91 0.295;3.533	-4.58	Superiority of givinostat against placebo P = 0.0209 (nominal statistical significant)	(1)
Change from baseline in Cumulative loss of function on the NSAA at month 18	Key secondary endpoint	Estimated cumulative failures Ratio of cumulative failures 95%CI	3.42 0.61 0.408;0.927	5.56	Superiority of givinostat against placebo P = 0.0202 (nominal statistical significant)	(1)
Change from baseline in Time to rise from floor (seconds) at 18 months	Key secondary endpoint	LSM change from baseline LSM difference 95%CI	9.33 -3.28 -9.573;3.018	12.61	Superiority of givinostat against placebo P = 0.3044	(1)

Effect	Short Description	Unit	Givinostat	Placebo/ comparator	Uncertainties/ Strength of evidence	References
6MWT distance	Key secondary endpoint	LSM change from baseline LSM difference 95%CI	-38.43 9.96 -12.071;31.983	-48.38	Superiority of givinostat against placebo P = 0.3723	(1)
Change from baseline in Muscle strength – overall knee extension at 18 months	Key secondary endpoint	LSM change from baseline LSM difference 95%CI	-0.32 0.19 -0.030; 0.401	-0.50	Superiority of givinostat against placebo P = 0.0902	(1)
Change from baseline in Muscle strength – overall elbow flexion at 18 months	Key secondary endpoint	LSM change from baseline LSM difference 95%CI	-0.10 0.09 -0.041;0.213	-0.19	Superiority of givinostat against placebo P = 0.1818	(1)
Change from baseline in VL MFF at 18 months	Key secondary endpoint	LSM change from baseline LSM difference 95%CI	7.63 -2.92 -5.641;-0.204	10.56	Superiority of givinostat against placebo P = 0.0354	(1)

Unfavourable Effects						
Thrombocytopenia*	Number of patients with TEAEs	%	38.3 (overall givinostat) [18.8 (Dose A) 7.7 (Dose B) 86.7 (Dose A-B) 79.2 (Dose A-B-C) 46.7 (Dose B-C)]	0	Higher incidence with givinostat monotherapy compared to combination with corticosteroids based on non-DMD studies Uncertainty regarding an increased risk for thrombocytopenia >Grade 1 in patients with higher body weight based on simulations has been addressed by the new dose regimen reducing the starting Dose A and Dose B.	SMQ Analysis of (2), (3), (4), (5)
Triglyceride increase**	Number of patients with TEAEs	%	25.2 (overall givinostat) [21.9 (Dose A) 14.3 (Dose B) 22.2 (Dose A-B) 50 (Dose A-B-C) 46.7 (Dose B-C)]	6.6	Higher incidence with givinostat monotherapy compared to combination with corticosteroids based on non-DMD studies remains uncertain	Grouping of PTs of (2), (3) (4)
GI disorders - Diarrhoea	Number of patients with TEAEs	%	60.8 (overall givinostat) [71.9 (Dose A) 47.3 (Dose B) 78.3 (Dose A-B-C) 50.0 (Dose B-C)] 39.6 (overall givinostat) [59.4 (Dose A) 25.3 (Dose B) 48.9 (Dose A-B) 70.8 (Dose A-B-C) 23.3 (Dose B-C)]	41.0 18.0	Higher incidence with givinostat monotherapy compared to combination with corticosteroids from non-DMD studies Uncertainties regarding clinical consequences of diarrhoea (e.g. dehydration leading to electrolyte disturbances)	(2), (3), (4)

QT prolongation	ΔΔQTcF, LS mean placebo-corrected (90% CI), 5 hours post-dose	msec	100 mg: 5.5 (1.99 to 9.01) 300 mg: 13.6 (10.15 to 17.05)	400 mg Moxiflox.: 12.1 (8.66 to 15.62)	No AESI reported in the DMD studies. Uncertainty remains regarding pre-existing conditions increasing the risk for ECG abnormalities, concomitant medication, and patients with cardiomyopathy Two SAEs of atrial fibrillation in study 51.	(3), (6)
Osteoporosis/ osteopenia - Fractures	Number of patients with TEAEs	%	13.5 10.8	6.6 3.3	Lack of relevant preclinical findings; no mechanistic rationale.	SMQ Analysis of (2)
Hypothyroidism Blood thyroid stimulating hormone increased	Number of patients with TEAEs	%	1.7 5.4 5.4	0 0 1.6	Mechanistic rationale hypothetical; thyroid changes have not been evaluated in preclinical studies; hypothyroidism more frequently reported in study 51	(1) (2) (2)

Abbreviations: SMQ (Standardised MedDRA queries),
Notes: (1) placebo controlled study DSC/14/2357/48; (2) Group 2 – DMD studies pool (including studies 43, 48, and ongoing study 51); (3) TQT study ITF/2357/54; (3) pooled analysis of non-oncology/ non-DMD studies; (5) ITAL-PMX-GIVINOSTAT-3392; (6) open-label long-term study DSC/14/2357/51
* including PTs platelet count decreased and thrombocytopenia
** including PTs 'blood triglycerides increased', 'hypertriglyceridaemia', and 'blood triglycerides abnormal'

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Efficacy:

In the pivotal study DSC/14/2357/48, givinostat demonstrated in comparison to placebo in the primary pre-specified analysis a statistically significant treatment effect on the time to climb 4 standard stairs (4SC) after 18 months of treatment in an enriched so called "target population" e.g., patients with VL MFF (MRS) > 5% to ≤ 30%, ambulant DMD population between 6 and 16 years of age on corticosteroid treatment. The GLS mean ratio of 0.86 on the log scale analysis represents a 14% less increase in the time to 4SC in favour of givinostat. Post hoc analyses in the overall population and the off-target population were in line with those of the target population showing a positive trend in the givinostat group when compared to placebo.

The chosen primary analysis was not considered the most appropriate one. Moreover, the effect was not fully supported by the provided sensitivity analyses, i.e. a tipping point sensitivity analysis, or the time to 4SC velocity as well as additional analyses using more conservative missing data handling strategies (i.e. the jump-to-reference multiple imputation approach). Nevertheless, the jump-to-reference multiple imputation approach may be too conservative and reduces the power to detect a potentially effective therapy in the context of disease modifying treatment. Additional analyses, i.e., a permutation-based analysis, probability densities and a Bayesian analysis provided supportive information. These analyses indicate that even under the conservative J2R condition, the probability of observing the results seen on the primary and key secondary endpoints would be extremely low if givinostat would be ineffective, respectively, the observed totality of evidence for givinostat is unlikely due to chance alone.

Overall, the effect sizes were small but consistently showed a smaller deterioration compared to placebo after 18 months.

Given the described statistical uncertainties, the size and significance of the estimated treatment effects need to be reassured in an additional, specific obligations study (SOB 1).

Although, the applicant could not confirm that the effect of a 14% less increase in the time to 4SC test for the primary endpoint in the primary analysis (log scale analysis) represents a clinically meaningful effect per se, as a MCID has not been established and is highly population dependent, further analyses and approaches, i.e. difference in seconds in 4SC based on non-log transformed data and results in 4SC velocity (task/seconds) are in the same range as MCIDs of the published data and are considered appropriate to justify the clinical relevance of the observed effects.

The primary endpoint is based on a single timed-function test. Given the overall heterogeneous patient population of DMD patients and taken the intended indication into consideration, it needs to be supported by effects on further relevant functional outcome parameters.

The NSAA total score, presently considered the preferred efficacy endpoint by experts in the field, showed less reduction under givinostat in comparison to placebo (effect size of 1.91 on the NSAA total score; cumulative loss of function on the NSAA score: The ratio of NSAA cumulative loss of function (givinostat/placebo), obtained from a negative binomial regression on the subject's cumulative number of failures across all post-baseline visits, was 0.61). Overall, the effects achieved on the NSAA score over 18 months of treatment can be considered clinically relevant as they are in the range of published data.

An analysis on VL fat fraction (FF) showed a delay in fat infiltration by approximately 30% after 18 months of treatment with givinostat compared to placebo. Although, FF of the vastus lateralis is currently not accepted as a validated surrogate parameter in clinical DMD trials, it could serve as a relevant pharmacodynamic parameter. Results for the key secondary endpoint VL MFF as obtained with MRS can be considered supportive for the results of the functional parameters. Moreover, results for vastus lateralis fat fraction as assessed by MRS were supported by results of further exploratory endpoints, i.e., the fat fraction of 5 thigh muscles (VL, biceps femoris long head, semitendinosus, quadriceps and hamstrings) measured by Dixon MRI at 18 months.

All of these three key endpoints reached nominally statistical significance.

The time to rise from floor (TTR) in seconds increased in both treatment arms with mean changes from baseline through EOS of 8.86 seconds and 13.59 seconds with givinostat and placebo, respectively (LS mean 9.33 and 12.61 seconds, respectively). Although, the LS mean difference of -3.28 seconds in favour of givinostat was not nominally statistically significant (p = 0.3044), the observed effect size cannot be considered irrelevant. Using the TTR velocity (rises/sec) is supportive to address the increased variability caused by patients who were unable to perform the TTR due to disease progression. LS mean change in time to rise from floor velocity from baseline to 18 months was – 0.05 rises/sec in the givinostat group and – 0.08 rises/sec in the placebo group with a LS Mean difference for givinostat from placebo of 0.03 rises/sec that reached nominal statistical significance (p = 0.0124). Referring to the former defined MCID for TTR velocity of -0.023 rises/sec by Duong 2021 (i.e. annual decline in TTR velocity of 0.023 rises/sec), the average decrease in TTR velocity under givinostat as compared to placebo is greater than the MCID described by Duong.

Overall, knee extensor and elbow flexor muscle strength slightly decreased with a smaller decrease in the givinostat group compared to placebo over 18 months. Although, muscle strength and motor function are related, their relationship is not linear. Therefore, although not statistically compelling, results on muscle strength are considered in support of the results on functional outcome parameters.

In summary, based on the chosen primary and key secondary endpoints in the pivotal trial, which included motor function, pharmacodynamic parameters and muscle strength, and taking into account on the one hand the lack of well-established MCIDs for several outcome parameters as well as on the other hand the difficulties in interpreting former published MCIDs, the observed effects for givinostat against placebo over 18 months of treatment indicate clinically relevant treatment effects in the patient population studied.

Overall, the clinical relevance of the difference in TFTs is difficult to interpret as a clear cut off value has not formally been established.

Generally, it is acknowledged that no improvement compared to baseline could be achieved with givinostat, but there was a slowing of the deterioration compared to placebo confirmed by small but consistent effects in relevant endpoints.

Dosing of givinostat was modified several times during the study. Overall, the applicant could sufficiently justify, that based on the different treatment regimens implemented in study 48, there was no substantial variability in exposures that might have affected the outcome on efficacy parameters.

A new revised dosing regimen was proposed for givinostat, because higher than expected exposures in patients with higher bodyweights had to be addressed.

In the revised dosing regimen, the weight bands were reduced from 9 weight bands to 4 weight bands. For patients with lower bodyweights, the width of the weight bands is considered to be rather broad. It is acknowledged that a dose regimen using only one syringe instead of two syringes was preferred. In this context the applicant provided simulations for the resulting exposure (AUC0-12, Cmax) after using

dosing scheme based on 4, 6 or 9 weight bands. As modelling predicts similar exposures for all dosing schemes, the applicant prefers using only 4 weight bands. The dosing scheme was revised regarding the lower weight cut-off from 10 kg bodyweight to 15 kg bodyweight. This is agreed as the indication includes paediatric patients 6 years of age and older.

In the uncontrolled, phase 2, proof-of concept study, a significant improvement in histological muscle parameters based on the primary and further structural secondary endpoints from baseline to month 12 could be observed; however, the morphological effects could not compellingly be transferred into effects on functional outcome parameters, i.e. there were consistent declines in the 6MWT distance and the total NSAA score until week 52, while the PUL at least remained almost stable until week 36. However, in principle, the observed changes need to be put into relation to treatment naïve patients for interpretation of the obtained results. However, a placebo control group has not been included in the study.

With respect to persistency of the effects, neither continuity (no clear trend over time) of the treatment effects observed at EOS in Study 48 nor consistency with respect to the difference between the givinostat and delayed givinostat group across all analysed endpoints could be identified in study 51. The results of the provided analyses are generally difficult to interpret, and no meaningful conclusions can be drawn, e.g., given the small number of included subjects.

The integrated analysis of long-term efficacy (ISE) based on the data from clinical studies, i.e. studies 48 and 51, and the Natural History Studies Imaging DMD and CINRG using a propensity score matching approach can be interpreted for DMD disease milestones, e.g. persistent rise from floor >10 seconds (1.7 years), persistent loss of rise from floor (2 years), persistent 10MWR > 10 seconds (3.3 years), persistent loss of 4SC (3.3 years), persistent loss of ambulation (2.9 years) in progression for the mentioned disease milestones under givinostat compared to natural history. Therefore, they can be considered supportive of the results obtained in the 18 months placebo-controlled pivotal study. However, as the matching procedure was selected post-hoc, and it cannot be fully ensured that the results are not biased by other confounders, uncertainties remain with respect to the plausibility of the results. Overall, the results of the ISE analysis cannot be used as conclusive evidence for the efficacy of givinostat. Additional long-term data need to be generated in a PAES study (SOB 2).

Safety

Overall, the main safety issues that have been identified during treatment with givinostat in the clinical DMD studies do not per se point towards an unacceptably high risk, while these need to be weighed against the lack of robust and clinically relevant effects in the studied patient population. Based on the placebo-controlled experience in study 48, a majority of TEAEs were mild to moderate in severity, serious in single patients only without specific pattern, and rarely led to discontinuation of treatment.

Around 50% of the patients studied in the DMD programme were exposed to givinostat for at least 24 months as of the safety data cut-off, which is considered to constitute a sufficiently long-term safety database taking into account the nature of the condition. Further long-term safety data from study 51 have been made available within the procedure and are in support of the safety profile of givinostat. So far, TEAEs were not found to increase with long-term givinostat treatment, except those, which are likely to be explained by progression of the underlying disease (e.g., fractures and TEAEs from the musculoskeletal and connective tissue disorders).

No formal dose-response study has been conducted. The study 48 protocol amendment that resulted in a change of the starting dose from Dose A to B as well as numerous dose reductions throughout studies 48 and 51 due to thrombocytopenia, hypertriglyceridaemia, and diarrhoea complicate safety assessment of each dose, especially for the highest dose proposed in the posology. Approximately half of the subjects in Study 48 initially treated with givinostat at Dose A had dose reductions following

adverse events within the first three months. The reduction of weight-based starting dose to Dose B after protocol amendment 4 in study 48 led to a higher number of patients remaining at Dose B, while a subset had to reduce to Dose C. In summary, while some subjects remained on their starting doses, others had one or even two dose reductions. From a safety perspective, it appears reasonable to start weight-based treatment with givinostat at the highest possible dose (Dose A) and - in case of predefined tolerability issues - to reduce to Dose B (one-third reduction), which has been applied in 20.3% of all patients, and – if necessary - to Dose C (further decrease of 20% from Dose B); this has been applied in 10.8% of patients. This proceeding is expected to be applied in clinical practice. Revision of the originally proposed dose regimen with 9 weight bands to 4 weight bands for simplified dosing further safeguards against the higher risk for thrombocytopenia in patients with higher body weight, who will now receive slightly lower starting doses (and also a lower reduced Dose B). At the same time, reduction of weight bands slightly increases the exposure in the weight groups 10 to 20 kg and 20 to 40 kg, while this is not considered clinically relevant and far below 20%.

The overall limited sample size and several dose changes (reductions and interruptions) in study 48 challenged the assessment of clinical relevance and robustness of the efficacy results, especially in light of the finally proposed dosing regimen (A-B-C). Overall, the applicant could sufficiently justify, that based on the different treatment regimens implemented in study 48, there was no substantial variability in exposures that might have affected the outcome on efficacy parameters. In this context, while a minority of patients is expected to remain on Dose A (i.e. only 14.4% of patients in DMD studies did not have dose reductions on Dose A), the majority of patients is expected to be treated with Dose B (i.e., 41% of patients in DMD studies were started and remained on this dose), a dose for which the main tolerability and safety issues, i.e. diarrhoea, thrombocytopenia, and hypertriglyceridaemia have reported to decrease. While overall data are not fully conclusive regarding a clear dose relationship for hypertriglyceridaemia and dose reductions might not be sufficient for all patients, the risk for permanent treatment stop due to hypertriglyceridaemia in Group 2 DMD studies was low (4 patients, 1.8% of the overall givinostat population). At the same time, these patients were predisposed for high triglyceride levels due to their baseline abnormalities.

The identified AESI for givinostat are generally considered manageable with the proposed measures, including dose reductions and temporary dose interruptions based on routine monitoring. Clinical consequences related to thrombocytopenia (haemorrhages), hypertriglyceridaemia (acute pancreatitis, coronary artery disease, hepatic steatosis), or diarrhoea (electrolyte disturbances following dehydration) have not been observed in the DMD studies, which supports the adequacy of the risk minimisation measures, and these outcomes will be additionally followed post-marketing by means of a PAES study. Uncertainties with regard to the risk for concomitant treatment known to decrease platelet counts, to increase triglycerides, or to increase the QTc interval have been addressed but need further revision in the product information. Despite this, initiation of treatment with givinostat in patients with several baseline conditions (e.g. with cardiac medical history or comedication) or in patients with laboratory abnormalities is adequately labelled.

Other uncertainties (i.e. small thyroid effects, increased incidence of fractures, and DDIs) might be considered acceptable taking into account the limitation of alternative treatments in DMD.

Extrapolation of the aforementioned safety profile could be justified for

- patients becoming non-ambulatory during treatment with givinostat; no differential safety profile derived from 34 patients prior and after losing ambulation in DMD studies and is also not expected, and
- patients with cardiomyopathy, despite clinical data is not available to confirm the absence of an effect on cardiac conduction in these patients. Givinostat was not found to prolong the QT interval at therapeutic doses and significant findings on other ECG parameters were absent. As

risk minimisation measure, ECG monitoring is proposed in the labelling.

3.7.2. Balance of benefits and risks

Based on the pre-specified primary analysis, efficacy of givinostat has been demonstrated in ambulatory, corticosteroid treated DMD patients, aged 6 years and older and those who become non ambulant during treatment with givinostat. However, the pre-specified primary analysis using single imputation methods for handling missing data, used in the one pivotal study DSC/14/2357/48 is not considered the most robust considering that it underestimates variability and makes the questionable assumption that patients with missing data (other than due to being non-ambulatory or physically unable to have the assessment) will benefit from treatment similarly to patients with available measurements. As per CHMP request at Day 120, the applicant has provided results from an analysis using jump- to-reference multiple imputation of missing data which, however, might be too conservative to base confirmatory decisions for disease modifying treatments, since this method reduces the power to detect a potentially effective treatment. Although, additional analyses of the Study 48 data indicate that the level of uncertainty in the efficacy of givinostat is low and might be acceptable, particularly in the context of a treatment for a serious and life-threatening rare disease with substantial unmet medical need, these analyses can only be considered supportive. The benefit/risk balance of Duvyzat for the treatment of Duchenne muscular dystrophy (DMD) in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment is considered positive. However, taking into consideration the uncertainties in the treatment effect estimates in these more relevant analyses, (i.e., lack of statistical robustness of the primary endpoint, and the absence of statistical significance shown for secondary endpoints) and the uncertainties of the long-term effects with respect to efficacy , the CHMP does not consider the available data to be comprehensive to support a full MAA as initially requested by the applicant, but considered a CMA to be the most appropriate approach. The applicant agreed with the CMA with the imposition of the following two specific obligations (SOBs):

- replication of the results in a smaller RCT combined with Study 48 in a meta-analysis in ambulatory, corticosteroid-treated DMD patients within a reasonable timeframe to confirm the positive benefit-risk balance of Duvyzat
- PAES to evaluate the long-term effectiveness and safety of givinostat in a "real-world" setting

Data from the external comparison of givinostat against natural history data can only be interpreted to a limited extent for methodological reasons and although generally supportive for the maintenance of effect cannot overrule the uncertainties from the pivotal study.

With respect to dosing, comparable exposures across different treatment groups were shown by the applicant using simulations based on population PK modelling and a revised dose regimen was proposed.

The indication has been restricted to ambulant, corticosteroid-treated DMD children from 6 years and above. A decision regarding continuation of treatment in subjects who become non-ambulant during treatment with Duvyzat should be at the discretion of the treating physician based on the overall benefit and risk assessment, which has been adequately reflected in section 4.2 of the SmPC.

The short-term safety as well as available long-term experience of givinostat concomitantly administered with corticosteroids in the studied DMD population, which includes patients 6 years and older, does not present with major findings that would at present preclude treatment solely from a clinical safety perspective. It is agreed that there were no AESI related to QT prolongation reported

while patients with symptomatic cardiomyopathy were excluded. The potential impact of givinostat on the cardiac conduction in patients with cardiomyopathy (regardless of the symptoms) has been discussed and cautious measures have been proposed.

Adverse drug effects are generally considered to be manageable provided that appropriate monitoring recommendations and labelling in the product information will be applied, while post-marketing measures (e.g. routine pharmacovigilance and a PAES study) safeguard for any clinical consequences of AESI that have not been detected during the clinical studies.

3.7.3. Additional considerations on the benefit-risk balance

1. Quality of evidence (including feasibility considerations)

Despite the rarity of the disease, a randomised placebo-controlled trial was performed. The sample size of 120 subjects in the target population (and 59 additional subjects in the Off-Target population) is in principle considered sufficient, taking into consideration the prevalence of this orphan condition. The pivotal study was adequately designed and aimed to demonstrate superiority over placebo in the mean change in time to 4SC before and after 18 months of treatment of givinostat versus placebo. Moreover, there is an ongoing long-term extension study.

Based on the **pre-specified primary analysis**, the study showed a statistically significant effect. However, the chosen analysis was not considered robust enough and post-hoc analyses for the primary, key secondary, and velocity endpoints, conducted to address uncertainties using more conservative missing data handling strategies, failed to show statistical significance. The absence of replication further increases uncertainty regarding the established efficacy.

2. Efficacy: precision of effect size

There was a slowing of the deterioration compared to placebo confirmed by small but consistent effects in relevant endpoints. Post hoc analyses in the overall population and the off-target population were in line with those of the target population showing a positive trend in the givinostat group when compared to placebo. All secondary endpoints pointed into the same direction.

3. Efficacy: clinical meaningfulness of the endpoint

Although, the applicant could not confirm that the effect of a 14% less increase in the time to 4SC test for the primary endpoint in the primary analysis (log scale analysis) represents a clinically meaningful effect per se, as a MCID has not been established and is highly population dependent, further analyses and approaches, i.e. difference in seconds in 4SC based on non-log transformed data and results in 4SC velocity (task/seconds) are in the same range as MCIDs of the published data and are considered appropriate to justify the clinical relevance of the observed effects.

4. Efficacy: duration of efficacy

DMD is a very heterogeneous disease with variable progression. The treatment duration of the study was 18 months that is considered adequate to provide clinically relevant (and not merely statistically significant) effect sizes on function, i.e. on the primary and secondary endpoints.

Follow-up in the open-label long-term extension study 51 is still ongoing. However, more robust data on the long-term effects of efficacy and safety are needed.

5. Safety: exposure

The safety evaluation for givinostat is based on the exposure of 222 patients treated for a median of 24.90 months in the DMD studies and includes 187 patients, who still participate in the open-label

long-term study 51 as per the data cut-off 31 December 2021. Overall, the exposure of patients in the clinical DMD programme reflects the rareness of the disease.

6. Safety: length of follow-up

As of 31 December 2023, the 5th interim data cut-off for the clinical follow-up study 51, a total of 207 participants were exposed to givinostat for a mean duration of 1195.7 days (range: 26 to 2259 days). 136 subjects reached 36 months, 61 subjects reached 48 months, and 16 subjects reached 72 months year post-treatment safety follow-up in this study. No new safety signals were detected. Drug related TEAEs were manageable with appropriate monitoring and dose adjustments allowed per study protocol. The length of safety follow-up is considered appropriate.

7. Target population vs study population

The primary analysis population was an enriched the so called "target population" e.g., patients with VL MFF (MRS) > 5% to ≤ 30%, ambulant DMD population between 6 and 16 years of age on corticosteroid treatment. There were additionally patients included from the "off-target population", defined as subjects with a baseline VL MFF in the range ≤ 5% or >30%.

Overall, the study population is considered representative for the intended indication.

8. Pharmacological rationale

The pharmacological rationale for treatment of human DMD is regarded plausible and sufficiently supported by non-clinical data: The interplay of histone acetylases and histone deacetylases (HDACs) normally directs muscle development by control of gene expression and transcription factor activity. This balance is disturbed in dystrophic muscle because the inactivation of HDACs is impaired. By specific inhibition of HDACs, givinostat dose-dependently improved muscle mass and mitochondrial biogenesis, reduced fibrosis and fat infiltration and temporarily improved functional outcome in two mouse models of human DMD. Nevertheless, other HDAC inhibitors revealed similar improvements in mouse models.

Furthermore, an analysis on VL fat fraction (FF) as assessed by MRS showed a delay in fat infiltration by approximately 30% after 18 months of treatment with givinostat compared to placebo. Although VLFF FF is currently not accepted as a validated surrogate parameter in clinical DMD trials, it could serve as a relevant pharmacodynamic parameter. Results for the key secondary endpoint VL MFF as obtained with MRS can be considered supportive for the results of the functional parameters.

9. Natural history/ course of the disease

The natural history of the disease is well described. Two natural history studies were included which are considered supportive to contextualize the effect of treatment.

In conclusion, at present the data are not considered comprehensive to justify a full MA and additional studies need to be conducted as SOBs.

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was proposed by the CHMP during the assessment, after having consulted the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment of a seriously debilitating and life-threatening disease. In addition, the product is designated as an orphan medicinal product.

Furthermore, the CHMP considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data. Duchenne Muscular Dystrophy is a rare disease but it is still considered feasible to conduct randomised clinical trials in the regions where Duvyzat is not already on the market or expected to be launched shortly. The small RCT will be complemented by real world data on the loss of ambulation in patients receiving givinostat. Data collected in these studies is expected to address the current uncertainties with regards to givinostat efficacy.

The following specific obligations are imposed:

SOB#1: A randomised, placebo-controlled trial in ambulant patients

SOB#2: PAES to evaluate the long-term effectiveness and safety of givinostat in a "real-world" setting. A matched concurrent untreated cohort – in addition to the Published Natural History Results (McDonald CM, 2018) - should be foreseen in the study protocol.

Details of the study protocols are recommended to be agreed in a scientific advice procedure.

- The third requirement for CMA is that unmet medical need to be fulfilled.

  As other medicinal products for the treatment of DMD are authorised in EU, it should be justified that Duvyzat provides a major therapeutic advantage over each existing authorised medicinal products in an overlapping indication. Prednisone is available and widely used in the European Union but is not specifically indicated for DMD. On the other hand, deflazacort was recently approved for "treatment of Duchenne muscular dystrophy (DMD) in patients 2 years and older" across five EU/EEA countries via the mutual recognition procedure.

The pivotal study DSC/14/2357/48 of this CMA is a Phase 3, Randomised, Double Blind, Placebo Controlled, Multicentre Study to Evaluate the Efficacy and Safety of Givinostat in Ambulant Patients with Duchenne Muscular Dystrophy. However, it should be noted that inclusion criteria required that subjects had used systemic corticosteroids for a minimum of 6 months immediately prior to start of study treatment, with no significant changes in corticosteroid type or dosage or dosing regimen. Hence, this pivotal trial is actually designed to measure causal effects (efficacy) of Duvyzat on top of corticosteroids. Thus, the observed differences in the time-function tests and NSAA as main efficacy endpoints observed in the pivotal trial in favor of Duvyzat could be in principle considered as meaningful improvements of morbidity of the disease and thus, supporting that Duvyzat provides MTA in addition to corticosteroid therapy.

Finally, a marketing authorisation was granted for the medicinal product Agamree (active substance: vamorolone) on 14 December 2023. It is acknowledged that none of the subjects enrolled in the pivotal trial received Vamorolone as this medicinal product was no authorised at the time of the design of the pivotal study. However, Vamorolone is a synthetic corticosteroid that mainly differentiates in the potential of the typical GC side effects adverse events (Agamree EPAR). While the pivotal study supporting the MA was not designed to formally evaluate efficacy of Agamree versus Prednisone, it was acknowledged that overall improvements seen with vamorolone 2 mg/kg were slightly smaller than those seen with prednisone (Agamree EPAR). Ultimately, the conclusion is that efficacy was not substantially different between Agamree and Prednisone as expected for the shared mechanism of action. Consequently, it is expected that the about conclusion from the pivotal trial can be extrapolated to Agamree. Accordingly, it is agreed that Duvyzat could also provide a MTA versus Agamree.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. The safety profile of Duvyzat is relatively mild. In the

view of DMD being a progressive disease, early initiation of treatment is essential to slow the decline in ambulation.

Involvement of patient representatives

Patients' perception of benefits and risks of a medicine may be different from the view of medical experts. That is why EMA engages with patients and their representatives at multiple stages of its activities and the added value of including their perspectives in the evaluation of medicines has been well demonstrated. To that effect, CHMP invited World Duchenne Organisation to share patients' perspectives on behalf of its patient/carer members with respect to givinostat in the treatment of Duchenne Muscular Dystrophy. The following points are noted from the response from World Duchenne Organisation (WDO) and are summarised below:

Duchenne muscular dystrophy, the disease

WDO reflected on the epidemiology and pathology of DMD, including delayed diagnosis, the substantial decline in function occurring even before 7 years of age in individuals with DMD, wheelchair – dependent age between 10 to 12 years, followed by deterioration in upper limb function, limiting ability for self-care such as feeding and dressing. Besides the muscle damage and loss of muscle strength and motor functions, comorbidities are noted in DMD, such as ADHD, ADD, OCD and ASS.

Standards of care (care considerations) - changing life expectancy

WDO noted that life expectancy has improved over the last 2 decades, not due to new medicines but as a result of better (standards of) care, including the (off-label) use of steroids (different regimens), the use of (preventive) cardiac medication, non-invasive or invasive ventilation, and the use of the so-called cough-assist, a mechanical insufflation-exsufflation device extending life expectancy in some cases to even 30 and 40 years of age. Still, a considerable percentage of children with DMD die in their early teens. Health Care Professionals have been involved in the implementation of these Standards of Care. In addition to the DMD diagnosis and management papers of Birnkrant et al. (2018), focussing on children and adolescents, care considerations for adult men with DMD (Quinlivan et al. 2021) have now been developed.

Burden of care

WDO noted that DMD results in an extremely high burden of care, especially for the parents, which increases over time, and negatively affects their HRQoL, contributes to a decline in psychological well-being, and places an increased financial burden on the family.

Unmet need

Despite several innovative medicines for rare diseases gaining worldwide approval in recent years, for Duchenne a high unmet medical need persists. In the EU, one drug received conditional approval in 2014 and one received approval in October 2023. These treatments and steroids have formally demonstrated clinical efficacy in DMD. There is a considerable discrepancy between the number of compounds that seem promising in early-stage development and the number that proceed to phase 2/3 studies, despite positive early results, or signals of efficacy only in secondary endpoints. Another concern is the fact that several drugs in the pipeline and/or approved by the FDA are mutation specific, further limiting the group of patients eligible for clinical trials and those who may ultimately benefit from the product.

Givinostat

The expectations of the Duchenne community regarding givinostat are very high. The understanding is that the givinostat phase 3 RCT has met its primary endpoint (for the first time in DMD history), and secondary and exploratory endpoints show consistent results. In addition, givinostat appears to have a

good safety profile and its mechanism of action is not mutation specific. WDO, however, notes that studies did not include the non-ambulant DMD population, which represent the majority of DMD patients with a high unmet need as there is little in the pipeline for this population.

Patient preferences

Several patient preference studies have been conducted in DMD. A study showed that caregivers are willing to accept considerable risk involved with a non-curative treatment if it could slow disease progression, even if there were no increase in life-expectancy (Peay et al., 2014).

3.8. Conclusions

The overall benefit/risk balance of Duvyzat is positive, subject to the conditions stated in section 'Recommendations'.

Divergent position(s) are appended to this report.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP is of the opinion that Duvyzat is not similar to Translarna and Agamree within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the benefit-risk balance of Duvyzat is favourable in the following indication(s):

> Duvyzat is indicated for the treatment of Duchenne muscular dystrophy (DMD) in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment.

The CHMP therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.
- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
In order to confirm the efficacy and safety of givinostat in the treatment of Duchenne Muscular Dystrophy in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment, the MAH should conduct and submit the results of a randomised, double blind, placebo-controlled study in ambulant patients with Duchenne Muscular Dystrophy, according to an agreed protocol.	31 July 2033
In order to confirm the long-term efficacy and safety of givinostat in the treatment of Duchenne Muscular Dystrophy in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment, the MAH should conduct and submit the final results of a non-interventional study based on data from sites and/or patient registries, according to an agreed protocol.	December 2037

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that Givinostat is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

Refer to Appendix on new active substance (NAS).

Divergent position(s)

Divergent position(s) to the majority recommendation are appended to this report.

5. Appendices

5.1. Divergent position(s) to the majority recommendation

The undersigned members of the CHMP did not agree with the CHMP's majority opinion recommending granting of a conditional Marketing Authorisation for Duvyzat indicated for treatment of Duchenne muscular dystrophy (DMD) in ambulant patients, aged 6 years and older, and with concomitant corticosteroid treatment.

The strength of evidence for the efficacy of givinostat in Duchenne's muscular dystrophy is insufficient.

Notably, the primary analysis, which is borderline statistically significant, used single imputation within the treatment group for missing data. This single imputation approach generally underestimates variability. Moreover, using data from the same treatment group is valid only under the assumption that the treatment effect among subjects discontinuing the study would have been the same as for subjects remaining in the study had they not discontinued. Applying reasonable sensitivity analyses indeed resulted in the loss of statistical significance implying that the results are not robust.

In addition to this, the effect sizes of the primary as well as relevant secondary endpoints were generally below the limits of what is considered clinically relevant.

Furthermore, the mechanistic rationale is considered weak as the proposed mechanism of action and the demonstrated pharmacodynamic effects of givinostat do not provide reassurance for clinical efficacy.

In summary, efficacy has not been established. Moreover, the toxicity of givinostat is non-negligible. The B/R balance is negative.

Daniela Philadelphy

Bruno Delafont

Blanka Hirschlerova

Tomas Radimersky

Thalia Marie Estrup Blicher

Outi Mäki-Ikola

Jean-Michel Race

Jayne Crowe

Elita Poplavska

Peter Mol

Kristina Dunder