CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217865Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	NDA
Application numbers	217865
Priority or standard	PRIORITY
Submit dates	4/21/2023
Received dates	4/21/2023
PDUFA goal date	3/21/2024
Division/office	Division of Neurology I (DNI)
Review completion date	3/21/2024
Established/proper name	givinostat
(Proposed) proprietary name	Duvyzat
Pharmacologic class	Histone deacetylase inhibitor
Other product name(s)	Duvyzat (givinostat)
Applicant	ITALFARMACO SPA
Dosage form(s)/formulation(s)	Oral suspension
Dosing regimen	Weight-based dosing regimen given orally twice daily
Applicant-proposed indication(s)/ population(s)	Givinostat oral suspension is indicated for the treatment of Duchenne Muscular Dystrophy
SNOMED CT code for proposed indication disease term(s)1	76670001
Regulatory action	Approval
Approved dosage (if applicable)	Weight-based dosing regimen given orally twice daily
Approved indication(s)/ population(s) (if applicable)	Treatment of Duchenne Muscular Dystrophy in patients 6 years and older
SNOMED CT code for approved indication disease term(s)1	76670001

[1] For internal tracking purposes only.
Abbreviations: NDA, new drug application; PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

4SC	4-stair climb
6MWT	6-minute walk test
ADME	absorption, distribution, metabolism, and excretion
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BCRP	breast cancer resistance protein
BID	twice daily
CINRG	Cooperative International Neuromuscular Research Group
CL/F	apparent oral clearance
C_{max}	maximum plasma concentration
CYP	cytochrome P450
DDI	drug-drug interaction
DMD	Duchenne muscular dystrophy
EOS	end of study
E-R	exposure-response
ER	emergency room
FDA	Food and Drug Administration
FMQ	Food and Drug Administration Medical Dictionary for Regulatory Activities query
FVC	forced vital capacity
GD	gestational day
HDAC	histone deacetylase
IND	investigational new drug
ITT	intent-to-treat
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NDA	new drug application
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
OAT	organic anion transporter
OCP	Office of Clinical Pharmacology
OPQ	Office of Pharmaceutical Quality
PD	pharmacodynamic
PEF	peak expiratory flow
PI	Prescribing Information
PK	pharmacokinetic
PND	postnatal day
popPK	population pharmacokinetic
QT-IRT	Interdisciplinary Review Team for Cardiac Safety Studies

RFF	rise from floor
SAE	serious adverse event
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
VL MFF	vastus lateralis muscle fat fraction

I. Executive Summary

1. Summary of Regulatory Action

The Applicant (Italfarmaco SpA) submitted a new drug application (NDA) 217865 for givinostat. Givinostat is a zinc-dependent histone deacetylase (HDAC) class I and II inhibitor indicated for the treatment of Duchenne muscular dystrophy (DMD) in patients 6 years and older.

NDA 217865 was reviewed by a multidisciplinary review team, which did not identify any issues that preclude approval. Each discipline has recommended approval. The signatory authority for this application concurs with those recommendations and agrees that the benefit-risk assessment supports approval.

The submission contains efficacy and safety results from a single adequate and well-controlled Study DSC/14/2357/48 (referred to as Study 48 in the text). Study 48 was a double-blind, placebo-controlled, multicenter study in 179 male subjects aged 6 years and older with DMD who were also receiving stable doses of concomitant steroids as background therapy. The primary endpoint was the change from baseline to Month 18 in the time to climb four stairs (4SC), which is a functional assessment of motor function, and the primary analysis population included patients who met a prespecified range of baseline vastus lateralis muscle fat fraction (>5% and ≤30%) as determined by magnetic resonance spectroscopy (MRS).

In the primary analysis of the change from baseline to Month 18 in the time to climb four stairs (4SC), patients receiving givinostat demonstrated statistically significant less worsening (1.25 seconds) in the 4SC time ($p = 0.037$) compared to placebo (3.03 seconds). There was a treatment difference of -1.78 seconds, determined to be clinically meaningful as it demonstrates significantly less decline in the time to climb four stairs, a functional outcome that relies on muscle strength. The secondary endpoints all trended in favor of givinostat but were not statistically significant when adjusted for multiplicity. A key secondary efficacy endpoint, the Northstar Ambulatory Assessment (NSAA), which is a measure of physical function in ambulatory patients with DMD, showed nominally significant less decline at Month 18 compared to placebo ($p = 0.021$). There were some statistical concerns with the handling of missing data that limited the persuasiveness of the primary analysis results. Exposure-response analyses demonstrate a relationship between increasing cumulative exposure and improvement in both the 4SC and NSAA and further support the observed clinical benefits of givinostat in the studied population.

Additional data was considered for its ability to serve as confirmatory evidence. The percentage of fat fraction present in the vastus lateralis (VL) muscle of the thigh was measured using MRS as a secondary endpoint in Study 48. At 18 months, patients receiving givinostat demonstrated a smaller mean increase in the VL muscle fat fraction (VL MFF) compared to patients who received placebo. This pharmacodynamic effect of givinostat showing reduced loss of lean muscle mass is consistent with the purported mechanism of action of the drug and provides biologic plausibility that the drug is having its intended effect on the muscle, which helps to confirm the Study 48 findings of a treatment benefit in patients with DMD.

The Applicant also conducted a post hoc, exploratory analysis comparing patients in the open-label extension study with a natural history cohort of patients with DMD. The Applicant's analyses as well as supplemental analyses done by the clinical pharmacology review team demonstrated a benefit in the time to persistent loss of function compared to the natural history. Additionally, natural history data was used to confirm that the functional decline of the placebo group during the 18-month study was consistent with the rate of progression in the natural history of the disease.

Of note, the primary analysis population (Target Population) was an enriched population of subjects who met the prespecified range of baseline muscle fat fraction as determined by MRS. Subjects not meeting this criterion were included in the study (Off-Target Population); this subgroup had more variability at baseline and also indicated a somewhat more advanced disease population with nominally significant higher 4SC time at baseline. There are no other differences in the two populations or any expectations that the drug would work differently in the two populations. Although the Off-Target population was not powered to detect a statistically significant difference given the small sample size (n = 39), a treatment difference of -2.78 seconds in 4SC from baseline to Month 18 in 4SC time was observed in subjects treated with givinostat compared to placebo, indicating that all patients with DMD, regardless of baseline stage of disease, could have potential for treatment benefit with givinostat.

During the conduct of the study, a protocol amendment changed the initial starting dose to a lower dose regimen given a high rate of dosing reductions for adverse events, mainly related to thrombocytopenia. After initiating at a lower dose, subjects were allowed an additional dose reduction if needed. The variable dosing regimens raised concerns in the review for the ability to identify a safe and effective dose, given the small size of the study. Additionally, there were concerns that the frequent adverse events (e.g., diarrhea) and dose modifications could lead to functional unblinding that could introduce bias, especially with effort-dependent functional endpoints. Exposure-response analyses conducted by the clinical pharmacology review team support the overall persuasiveness of the study results, and also enabled the team to optimize the dosing regimen to find a safe and effective dose for labeling. Additionally, the confirmatory evidence helps to alleviate concerns regarding functional unblinding, since it demonstrates objective findings on the muscle, as well as a persistent treatment benefit on a stable dose beyond the double-blind treatment period.

The 2019 FDA draft guidance, *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products,* outlines the general requirements for the determination of substantial evidence of effectiveness, the situations in which a single adequate and well-controlled study could adequately support an effectiveness claim, and cites the ability of the Agency to also consider data from one adequate and well-controlled clinical investigation and confirmatory evidence to constitute substantial evidence of effectiveness.

In this situation, there is a single adequate and well-controlled study that won on a clinically relevant primary endpoint, with favorable trends on all secondary endpoints. Although it is a positive study that reached statistical significance on the prespecified primary endpoint, Study 48 is not statistically robust and is not capable of providing substantial evidence of effectiveness as a single study alone. In the context of this rare, progressive, and severely debilitating disease with unmet need, it is appropriate to rely on a single study plus confirmatory evidence. Thus, the single positive adequate and well-controlled study, along with confirmatory evidence of a pharmacodynamic effect on the muscle and the long-term comparison to a natural history cohort,

together demonstrate substantial evidence of effectiveness to support approval of givinostat for the treatment of DMD.

Based on the safety data submitted, there are risks associated with givinostat identified in Study 48, as well as known class effects with use of other HDAC inhibitors. Adverse events that occurred commonly (≥10% of givinostat-treated subjects) include diarrhea, abdominal pain, thrombocytopenia, nausea/vomiting, hypertriglyceridemia, and pyrexia. Thrombocytopenia is dose-related and led to dose reduction in 28% of patients treated with givinostat. There were no serious bleeding events related to thrombocytopenia; however, the study was small and many patients decreased the dose, limiting the overall number of subjects who remained on the highest dose for the whole duration of the study. A postmarketing requirement (PMR) will be issued to further characterize the incidence and severity of thrombocytopenia and serious bleeding events in the postmarket setting Additionally, enhanced pharmacovigilance will be requested for events of thrombocytopenia and bleeding events.

The Warnings and Precautions section of the prescribing information will include thrombocytopenia and other signs of myelosuppression, as well as hypertriglyceridemia and gastrointestinal disturbances. Hypertriglyceridemia occurred in 23% of subjects on givinostat versus 7% on placebo and resulted in discontinuation in 2% and dosage modification in 8% of subjects treated with givinostat. Diarrhea, nausea/vomiting, and abdominal pain, sometimes severe, occurred in givinostat treated subjects. Diarrhea occurred in 37% of patients treated with givinostat versus 20% on placebo. Dosage modification recommendations for thrombocytopenia, hypertriglyceridemia, and moderate or severe diarrhea will also be provided in labeling.

The Warnings and Precautions will also address QTc prolongation and recommend avoiding use of givinostat in patients at increased risk for ventricular arrhythmias, and to obtain ECGs at baseline and throughout treatment in patients with cardiac disease or on concomitant medications that increase the risk of QT prolongation.

Given the rarity of DMD and the severity of the disease, the overall benefit-risk is favorable as described in the benefit-risk framework below (Table 2). For detailed information supporting the basis for this approval, please refer to the detailed reviews included in this interdisciplinary assessment document and the product quality review.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	• Duchenne muscular dystrophy is a progressive, X-linked recessive muscle disorder that causes skeletal muscle weakness and leads to loss of ambulation, cardiac and respiratory failure, and death typically by 30 years of age. The disease affects approximately 1 in 3600 to 6000 male births. • The cause of the disease is a wide variety of mutations in the DMD gene that lead to insufficient or a complete absence of functional dystrophin protein.	DMD is a chronic, progressive neuromuscular disease that begins in childhood among boys and immobilizes children and young adults. Death usually results from cardiac and respiratory complications.
Current treatment options	• The standard of care for any patient with DMD is the use of medicines that reduce inflammation. The most commonly used medicines are corticosteroids, and the only FDA-approved corticosteroids for DMD are Emflaza (deflazacort) and the recently approved Agamree (vamorolone, approved November 2023). • Although corticosteroids help to manage muscle inflammation and pain, they do not prevent the eventual onset of disability and then death. Moreover, corticosteroids cause numerous adverse effects such as Cushing syndrome and adrenal crisis upon acute withdrawal, that limit their long term use and tolerability. • Other standards of care include physical rehabilitation, the use of mechanical braces, and surgical correction of muscle tendon contractures. • Eteplirsen, golodirsen, viltolarsen, and casimersen are targeted antisense oligonucleotide treatments approved using the accelerated approval pathway based on a small increase in dystrophin production in the subset of patients with DMD caused by mutations that are amenable to exon skipping for exons 51, 53, 53, and 44, respectively. These therapies increase dystrophin expression, but clinical benefit has not yet been established.	There remains unmet need for effective treatments for patients with DMD, particularly for patients who are unable to tolerate steroids and who don't have mutations amenable to gene-targeted therapies.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Elevidys (delandistrogene moxeparvovec-rokl) was recently FDA approved in June 2023 using the accelerated approval pathway based on the surrogate endpoint of increased expression of microdystrophin in young patients with DMD, aged 4 to 5 years. Clinical benefit has also not yet been established.	
Benefit	• The primary analysis in the single, double-blind, placebo-controlled pivotal study (Study 48) demonstrated a statistically significant difference from placebo, with less decline in the change from baseline to Month 18 in 4SC, a functional endpoint that measures the time to climb 4 stairs (p = 0.037). Secondary functional endpoints (NSAA, TTR, 6MWT) were not statistically significant, but trended toward the givinostat group. • There was a nominally significant finding of less decline from baseline to Month 18 on the NSAA, an outcome measure of physical function in ambulatory patients with DMD (p = 0.02) which was not statistically significant once adjusted for multiplicity. • Exposure-response analyses demonstrate a positive exposure-response between dose and improvement on the 4SC and NSAA. • A secondary endpoint in Study 48, the Vastus Lateral muscle fat fraction as measured by MRS, demonstrated less fat fraction after 18 Months of treatment in the givinostat arm compared to placebo. • Subjects treated with givinostat in the open-label extension study compared to a natural history cohort shows a benefit in delaying the time to loss of ambulation compared to natural history. • Pharmacometric analyses were able to optimize the dosing regimen to maximize potential benefit and minimize the risk of major adverse events.	Study 48 demonstrated a statistically significant treatment benefit of givinostat compared to placebo on the prespecified primary endpoint, the change from baseline to Month 18 in the 4SC. The secondary endpoints, including the NSAA, all favored treatment with givinostat, although they did not reach statistical significance. Exposure-response analyses provide further support for the observed benefits on the 4SC and NSAA. Confirmatory evidence comes from the findings of VL MFF as measured by MRS, which indicates that givinostat has a pharmacodynamic effect reduced loss of lean muscle mass which increases the biologic plausibility that the drug is having the intended treatment effect. Confirmatory evidence is also provided from an analysis of the open-label extension study of patients on givinostat compared to the natural history of patients with DMD which suggests improved clinical outcomes including delayed time to persistent loss of function for the givinostat population compared to patients receiving current standard of care therapies. Overall, the single positive study accompanied by confirmatory evidence of pharmacodynamic evidence of activity on the muscle and long-term comparison to natural history demostrating delayed loss of ambulation provides substantial evidence of effectiveness of a treatment benefit of givinostat in patients with DMD.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and risk management	• Thrombocytopenia and hypertriglyceridemia events were widespread in clinical trial subjects, leading to dosing reductions in approximately half of trial subjects within the first 3 months. • No serious clinical bleeding events occurred as a result of drug-induced thrombocytopenia. There were a few reports of contusion and hematoma, and adverse events of epistaxis reported in subjects receiving givinostat slightly more frequently than in subjects receiving placebo. Three subjects had epistaxis at the same time as recorded thrombocytopenia. • Overall, thrombocytopenia occurred in 33% of subjects treated with givinostat and no patients treated with placebo. • Adverse events of increased triglycerides occurred in 23% of subjects on givinostat compared to 7% on placebo. Hypertriglyceridemia resulted in discontinuation in 2% of subjects and dose modification in 8% of subjects treated with givinostat. • Diarrhea and vomiting, sometimes severe, was also frequent reported, along with abdominal pain. Diarrhea occurred in 37% of subjects treated with givinostat compared to 20% in the placebo group. • Most commonly observed (> 10%) of adverse events associated with the use of givinostat in clinical studies were diarrhea, nausea/vomiting, abdominal pain, thrombocytopenia, hypertriglyceridemia, and fever. • QTcF prolongation was detected in the thorough QT study at doses approximately 6-fold higher than the anticipated therapeutic exposures	Side effects of thrombocytopenia and hypertriglyceridemia are monitorable and manageable by dose adjustments. No clinically significant bleeding events were seen in the trial. Both thrombocytopenia and hypertriglyceridemia will be described in labeling in the Warnings and Precautions (Section 5) and in an associated Medication Guide. A Postmarketing Requirement will be issued to require a prospective, observational registry to characterize the frequency, incidence, and severity of thrombocytopenia and serious bleeding events in this patient population in the postmarket setting. Additionally, thrombocytopenia will be monitored with enhanced pharmacovigilance. Diarrhea and GI disturbances, including vomiting, were also seen frequency and resulted in discontinuations and dose modifications. This will also be described in the Warnings and Precautions Section of labeling. Per the Interdisciplinary Review Team for Cardiac Safety Studies, patients who are at risk for ventricular arrhythmias such as those with congenital long QT syndrome, coronary artery disease, electrolyte disturbance, and concomitant use of other medicinal products know to cause QT prolongation disturbance should avoid use of givinostat. This risk will be described in the Warnings and Precautions section of labeling.

Abbreviations: 4SC, 4-stair climb; 6MWT, 6 minute walk time; DMD, Duchenne Muscular Dystrophy; FDA, U.S. Food and Drug Administration; GI, gastrointestinal; MRS, magnetic resonance spectroscopy; NSAA, North Star Ambulatory Assessment; QT, interval from the beginning of the Q complex to the end of the T wave; QTc, heart-rate corrected QT interval; QTcF, heart-rate corrected QT interval by Fridericia's cube root formula; TTR, time to rise; TQT, thorough QT; VL MFF, vastus lateralis muscle fat fraction

2.2. Conclusions Regarding Benefit-Risk

Despite recent advances in treatments for DMD, there remains a significant unmet need for effective treatments, particularly for patients who are unable to tolerate steroids and who don't have mutations amenable to gene-targeted therapies.

Givinostat is an HDAC inhibitor, which targets the upregulation of follistatin thereby stimulating myogenesis and is intended to work directly on the muscle. There are other HDAC inhibitors that have been approved for treatment of cutaneous T-cell lymphoma (vorinostat, belinostat, and romidepsin) and for multiple myeloma (panobinostat). The class of drugs has been associated with significant toxicities, including severe diarrhea, nausea, and vomiting, myelosuppression including thrombocytopenia resulting in hemorrhage, hepatotoxicity, and cardiac arrhythmias.

The single adequate and well-controlled trial demonstrated a treatment benefit in a clinically meaningful endpoint, the 4SC, in patients treated with givinostat. After 18 months of treatment, patients treated with givinostat demonstrated statistically significant less worsening on the time to climb 4 stairs compared to patients receiving placebo. Additionally, all the secondary endpoints trended in favor of givinostat, and there was a nominally significant benefit with less worsening of the NSAA total score at 18 months compared to placebo. Exposure-response analyses demonstrated a correlation between cumulative exposure of givinostat with improvement in the clinical endpoints, supporting the robustness of the single study. The small size of the study, the protocol-driven dosing changes and amended starting dose mid-study, and statistical limitations regarding handling of missing data prevent Study 48 from serving as a single study capable of demonstrating substantial evidence of effectiveness on its own.

Confirmatory evidence is provided by a pharmacodynamic effect on VL MFF as measured by MRS indicating the drug is having its intended effect on the muscle, as well as findings of clinically meaningful delays in persistent loss of function among those treated with givinostat compared to the natural history of patients with DMD. Natural history analyses also confirmed that the amount of clinical decline on functional endpoints noted in the placebo arm in Study 48 is consistent with findings in the natural history studies.

Therefore, for this rare disease with unmet medical need, the single adequate and well-controlled study plus confirmatory evidence provide substantial evidence of effectiveness for givinostat in the treatment of DMD in patients 6 years and older.

There are identified safety concerns with use of givinostat, including thrombocytopenia, hypertriglyceridemia, gastrointestinal disturbances that can be severe, and a risk of QT prolongation at supratherapeutic doses. Thrombocytopenia is monitorable, and can be mitigated by dose adjustments. The risk will be described in the Warnings and Precautions section of the Prescribing Information and recommend monitoring blood counts during treatment. A PMR will also be issued to further characterize the incidence and severity of thrombocytopenia and serious bleeding risks in the postmarket setting. Enhanced pharmacovigilance for thrombocytopenia will be requested. The other identified risks can also be mitigated through inclusion in the Warnings and Precautions of the prescribing information and recommended monitoring for triglycerides. Overall, the safety profile of givinostat is acceptable to support approval despite identified safety concerns.

In conclusion, the provided substantial evidence of effectiveness with adequate characterization of the safety and proposed labeling, the benefit-risk of givinostat for DMD is favorable to support approval.

II. Interdisciplinary Assessment

3. Introduction

Duchenne muscular dystrophy is a progressive, X-linked, recessive muscle disorder that leads to loss of ambulation, cardiac and respiratory failure, and death typically by 30 years of age. It is characterized by mutations in the dystrophin gene that lead to progressive muscle fiber degeneration and weakness. The disease affects approximately 1 in 3600 to 6000 male births, with an estimated prevalence of 2 per 10,000 people in the United States.

Standard of care treatment has been generally limited to corticosteroids and supportive care. Deflazacort is a corticosteroid which is approved by the FDA for the broad DMD population but has numerous adverse effects, such as Cushing syndrome and adrenal crisis upon acute withdrawal, that limit its long-term use and tolerability. Recently, FDA also approved vamorolone (November 2023), a synthetic corticosteroid for the treatment of DMD in children 2 years of age and older.

Eteplirsen, golodirsen, viltolarsen, and casimersen are antisense oligonucleotides that are approved by FDA using the accelerated approval pathway based on a small increase in dystrophin production. Each of these treatments are approved in the subset of patients with DMD caused by mutations that are amenable to exon skipping. These therapies increase dystrophin expression, which has been found to be a surrogate endpoint that is reasonably likely to predict clinical benefit in patients with DMD, but clinical benefit has not yet been established. Additionally, Elevidys (delandistrogene moxeparvovec-rokl) was recently approved in June 2023 under accelerated approval based on the surrogate endpoint of increase in expression of microdystrophin in young patients, age 4 to 5 years, with DMD. There remains a significant unmet need for effective treatments for DMD, particularly for patients who are unable to tolerate steroids and who don't have mutations amenable to gene-targeted therapies.

Givinostat is a zinc-dependent histone deacetylase (HDAC) inhibitor (class I and II). There are prior approvals of other HDAC inhibitors for oncology indications (i.e., cutaneous T-cell lymphoma and multiple myeloma). Givinostat allows unraveling of DNA, binding to transcription factors, and synthesis of mRNA. It leads to the upregulation of follistatin which stimulates myogenesis. Givinostat is a non-steroidal, oral therapy and is indicated for a broad population of patients with DMD age 6 years and older and is not limited by genetic subtype.

A single, 18-month randomized, double-blind, placebo-controlled study of 179 male subjects with confirmed DMD evaluated clinical function using the change from baseline to Month 18 in 4SC time as the primary endpoint, and also evaluated multiple secondary endpoints at Month 18 including 6-minute-walk test (6MWT), time to rise-from-floor (RFF) and Northstar Ambulatory Assessment (NSAA). A secondary endpoint also examined the change from baseline to Month 12 and 18 in the vastus lateralis muscle fat fraction (VL MFF) assessed by magnetic resonance spectroscopy (MRS). An open-label extension study was also used to gather additional safety data, and was evaluated time to persistent loss of function (i.e., ambulation) compared to natural history data in similarly matched patients.

3.1. Review Issue List

3.1.1. Key Efficacy Review Issues

3.1.1.1. Robustness of Study 48

Determine the ability of Study 48 to serve as an adequate and well-controlled study that contributes to substantial evidence of givinostat in the treatment of patients with DMD and the ability of the exposure-response (E-R) analysis to strengthen the robustness of Study 48.

3.1.1.2. MRS Biomarker Data as Confirmatory Evidence of Effectiveness

Determine if the MRS Biomarker Data is adequate to contribute to confirmatory evidence, along with the single adequate and well-controlled Study 48, to provide substantial evidence of effectiveness of a treatment benefit of givinostat in patients with DMD.

3.1.1.3. Natural History as Confirmatory Evidence of Effectiveness

Determine if the natural history data are adequate to contribute to confirmatory evidence, along with the single adequate and well-controlled study, to provide substantial evidence of effectiveness of a treatment benefit of givinostat in patients with DMD.

3.1.2. Key Safety Review Issues

3.1.2.1. Thrombocytopenia

3.1.2.2. Hypertriglyceridemia

3.1.2.3. Gastrointestinal Disturbances

3.1.2.4. Prolonged QT

3.2. Approach to the Clinical Review

Efficacy and safety were assessed by evaluating the results from the randomized, placebo-controlled Study DSC/14/2357/48 (referred to as Study 48 in the text) and the open-label extension Study DSC/14/2357/51 (referred to as Study 51 in the text) in ambulant subjects ≥6 years of age with DMD who were on stable doses of corticosteroids. The efficacy assessment focused on Study 48. The Applicant submitted the following information as potential sources for confirmatory evidence: Study 51, Study 43 (phase 2 dose ranging study), mechanistic evidence from *Mdx* mouse models, and magnetic resonance imaging/magnetic resonance spectroscopy

(MRI/MRS) data of vastus lateralis muscle fat fraction (VL MFF). This data was reviewed and considered as part of the overall analysis of efficacy data.

The safety assessment was based on Study 48 with supportive evidence from Study 51 and was based on the Applicant's reports and data analyst and clinical reviewer analysis of the submitted data. The clinical reviewer acknowledges the data analyses of FDA Clinical Data Scientist Drs. William Quarles and DeAngelo McKinley.

Table 3. Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations[1] for Givinostat

Study/Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	No. of Centers and Countries
DSC/14/2357/48	Ambulant patients with Duchenne muscular dystrophy ages ≥ 6 years	Control type: Phase 3 Randomization: Randomized Blinding: Double-blind, placebo controlled Biomarkers: MR Fat Fraction. Innovative design features: N/A	Drug: Givinostat Dosage: 37.5 mg Number treated: 111 Duration (quantity and units): 19 mo	Primary: Mean change in 4SC before and after 18 mo of treatment compared to placebo Secondary: Mean change in time to rise from floor Mean change in 6MWT Mean change in NSAA Cumulative loss of function on NSAA Mean change of muscle strength (KE, EF) Mean change in VL MFF	Planned: 160 Actual: 179	Centers: 45 Countries: 11

Study/Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	No. of Centers and Countries
DSC/14/2357/51	Ambulant patients with Duchenne muscular dystrophy ages ≥ 6 years	Control type: Open label extension Randomization: N/A Blinding: N/A Biomarkers: N/A Innovative design features: N/A	Drug: Givinostat Dosage: weight-based dosing Number treated: 194 Duration (quantity and units): Up to 2 y	Primary: Safety and tolerability (VS, PE, weight, height, ECHO, ECG, AEs Secondary: PUL, MFM, PFT, QoL, Time to first occurrence of major disease milestone, 6MWT, NSAA, 10MWR, TTR, muscle strength, EK Total Score, Barthel Index Total Score	At least 100; 194 enrolled	Centers: 39 Countries: 11
DSC/14/2357/43	Ambulant males with Duchenne muscular dystrophy ages 7 to <11 years	Control type: Open-label Randomization: N/A Blinding: N/A Biomarkers: N/A Innovative design features: N/A	Drug: Givinostat Dosage: 25 mg BID, 37.5 mg BID Number treated: 19 Duration (quantity and units): 1 week (Part 1), up to 1 year (Part 2), additional 40 months (Part 3)	Primary: Change in biopsy MFA% Secondary: Changing in histological endpoints, 6MWT, NSAA, PUL	Approximately 20; 20 enrolled	Centers: 4 Countries: 1 (Italy)

Source: Reviewer
[1] Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and pharmacokinetic studies.
[2] If no randomization, then replace with "Actual Enrolled."
Abbreviations: 4SC, 4-stair climb; 6MWT, 6 minute walk time; 10MWR, 10 minute walk-run ; AE, adverse event; BID, twice daily; d, day; DB, double-blind; ECHO, echocardiogram; ECG, electrocardiogram; EF, ejection fraction ; EK, Egen Klassifikation; h, hour; KE, ; LTE, long-term extension; MC, multicenter; mo, month(s); MFM, Motor Function Measure ; mo, months; MR, magnetic resonance; N, number of subjects; N/A, not applicable; NCT, national clinical trial; NSAA, North Star Ambulatory Assessment; OL, open-label; PC, placebo-controlled; PE, physical exam ; PFT, pulmonary function test ; PG, parallel group; PUL, Performance of Upper Limb ; QoL, quality of life; R, randomized; TTR, time to rise; VS, versus; wk, week(s); y, year(s)

4. Patient Experience Data

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical Outcome Assessment Data Submitted in the Application		
☒	Patient-reported outcome	
☒	Observer-reported outcome	Section 6.2.1.4
☒	Clinician-reported outcome	
☒	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	Section 6.3.3
☐	Observational survey studies	
☒	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

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5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

Givinostat is an orally administered small molecule that is proposed to [redacted] (b) (4) [redacted] (b) (4). In vitro proof-of-concept studies, givinostat exhibited anti-inflammatory activity (i.e., suppression of inflammatory cytokines) in lipopolysaccharide-stimulated human peripheral blood mononuclear cells and lipopolysaccharide-pretreated Balb/c mice, and suppression of profibrotic genes in fibroblasts derived from patients with DMD. In in vivo studies in the mdx mouse model of DMD, which harbors a nonsense mutation in exon 23 of the dystrophin gene, givinostat delayed decline in treadmill performance, grip strength, muscular fibrosis, and adipocyte infiltration into muscle.

DRAFT

5.2. Clinical Pharmacology/Pharmacokinetics

Clinical pharmacology properties of givinostat were comprehensively evaluated and summarized in Table 5.

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
	Pharmacologic Activity
Established pharmacologic class	Histone deacetylase inhibitor
Mechanism of action	Givinostat is a histone deacetylase inhibitor that is proposed to [redacted] (b) (4)
Active moieties	Givinostat hydrochloride monohydrate
QT prolongation	QTcF prolongation was investigated in the thorough QT (TQT) study ITF/2357/54 with a therapeutic dose (100 mg) and a supertherapeutic dose (300 mg) which provided 6.6- fold coverage for the therapeutic C_{max} of givinostat at steady state in DMD patients (administered with a dose of 70 mg). The results did not suggest that a 100 mg dose is associated with significant QTc prolonging effect but did identify the presence of significant QTc prolonging effect for the 300 mg dose. The Applicant performed an exposure-response analysis, which showed that increased givinostat concentration was associated with an increase in QTc response. The peak effect on QTcF was observed at approximately 5 hours post dose and is consistent with QTc prolongation by metabolites ITF2374 and/or ITF2375. Avoiding use of givinostat is suggested in patients who are at increased risk for ventricular arrhythmias (including torsades de pointes) such as those with congenital long QT syndrome, coronary artery disease, electrolyte disturbance, concomitant use of other medicinal products known to cause QT prolongation disturbance. Refer to Section 7.7.4 Prolonged QT for more details.
	General Information
Bioanalysis	Validated HPLC/MS/MS methods were used to determine the concentrations of givinostat (ITF2357) and other major inactive metabolites including ITF2374, ITF2375, ITF2440, and ITF 2955 in human plasma, and urine (as applicable to individual studies). The bioanalytical methods and validation are acceptable.
Healthy subjects versus patients	Givinostat's PK is similar in healthy subjects and subjects with DMD

Characteristic	Drug Information
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	**Table 6. Summary Statistics of Steady State Metrics of Systemic Exposure (C_{max}, C_{min}, and AUC) of Study 48 Based on PK Simulation**

Dose Level	Parameter	Geometric Mean (25th, 75th Percentile)	Parameter	Geometric Mean (25th, 75th Percentile)	Parameter	Geometric Mean (25th, 75th Percentile)
Always A	AUC_{tau},ss	399 (332, 467)	C_{max},ss	59.7(50, 72.4)	C_{min},ss	13.1(10.4, 14.8)
A to B/C		302(250, 358)		41.8(34.8, 50.1)		10.8(9.2, 12.8)
Always B		271(226, 328)		37.1(29.8, 43.7)		9.87(7.5, 11.3)
B to C		237(208,282)		36.4(30.7, 46.4)		7.56(6.9, 8.3)

Source: ITAL-PMXGIVINOSTAT-3392 Modeling and Simulation Report
Abbreviations: AUC_{tau}, area under the curve during a dosing interval; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; PK, pharmacokinetic; ss, steady state

Range of effective dose(s) or exposure	In the phase 2 dose ranging study, three dose levels were studied at 25 mg BID, 37.5 mg BID, and 50 mg BID. The 50 mg BID dose was considered to be not tolerated. The phase 3 study used weight-tiered dosing and the highest dose level (Dose A) was targeted to achieve the exposure similar to that seen with 37.5 mg BID. As dose reduction was allowed in the phase 3 study, Dose B with exposure similar to that of the 25 mg BID and Dose C with a further dose reduction of 20% were also investigated.
Maximally tolerated dose or exposure	The maximally tolerated dose is 37.5 mg BID based on the phase 2 study results (see Study report DSC-11-2357-43 in Module 5.3.5.2).
Dose proportionality	The PK of givinostat is linear within the therapeutic dose range.
Accumulation	Accumulation of givinostat was about 1.0-1.7 for BID administration.
Time to achieve steady-state	Steady state is achieved within 5 to 7 days.
Bridge between to-be-marketed and clinical trial/study formulations	The to-be-marketed formulation (suspension) was used for the pivotal phase 3 clinical trial. No bridging is needed.
	Absorption
Bioavailability	Absolute bioavailability was not determined.
T_{max}	Achieved about 2 to 3 hours after administration

Characteristic	Drug Information
Food effect (fed/fasted)	Givinostat is recommended to be taken with food. Food effect was evaluated in a capsule formulation with a single dose of 100 mg. A high fat standard meal resulted in a small increase in givinostat exposure (about 40% increase in AUC and about 23% increase in C_{max}) and a delay in T_{max} of 2 to 3 hours.
Geometric mean least square ratio and 90% CI	Parameter Estimated GMR (90% CI) fed: fasted $AUC_{0-\infty}$ 1.40 (1.04-1.90) C_{max} 1.23 (0.79-1.93)
	Distribution
Volume of distribution	Apparent volume of distribution at steady state is 768 L based on popPK analysis.
Plasma protein binding	Approximately 96% bound to human plasma proteins and is slightly partitioned into red blood cells (blood to plasma ratio = 1.3).
Drug as substrate of transporters	Givinostat is a substrate of P-gp and BCRP.
	Elimination
Mass balance results	No dedicated mass balance study was conducted. Urinary excretion of givinostat and the main metabolites in humans has been evaluated in healthy volunteers after single and repeated doses of givinostat. Total recovery of givinostat and major metabolites in the urine was approximately 61% after a single dose of 100 mg. The percentage of givinostat (parent) recovered in urine was very low after single dose administration (<3% of the dose).
Clearance	Mean apparent clearance of givinostat after a 100 mg single dose is 151 L/hr.
Half-life	In plasma, givinostat displays bi-phasic elimination profile with a mean apparent elimination half-life of about 6 hours.
Metabolic pathway(s)	In vitro studies with human enzymatic preparations together with animal metabolism in vitro and in vivo showed that givinostat is extensively metabolized forming several metabolites. CYP450 and UGTs are not involved in the main metabolic reactions. 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%). The naphtalenic fragment ITF2440, formed by the oxidative cleavage of the carbamic bond, but none of the 15 human recombinant CYPs were involved. ITF2375 is formed by the hydrolysis of the hydroxamic acid into a carboxylic acid mainly in the blood. Human mitochondrial amidoxime reducing components (mARC) 1 and 2 were found to be responsible for the reduction of givinostat to the amide derivative ITF2374.

Characteristic	Drug Information
Primary excretion pathways (% dose)	See Metabolic pathway.
	Intrinsic Factors and Specific Populations
Body weight	The popPK analyses show that the PK of givinostat can be affected by body weight.
Age	The effect of age on the PK of givinostat was not evaluated as age is correlated with body weight.
Renal impairment	The pharmacokinetics and safety of givinostat has not been studied in patients with renal impairment. However, renal impairment is not expected to impact the exposure of givinostat because renal excretion is not a significant route of givinostat elimination
Hepatic impairment	The pharmacokinetics and safety of givinostat has not been studied in patients with hepatic impairment. Givinostat is highly metabolized and therefore liver involvement cannot be excluded. There is not enough data to provide recommendations in patients with hepatic impairment. A PMR will be issued to conduct a study in subjects with hepatic impairment.
	Drug Interaction Liability (Drug as Perpetrator)
Inhibition/induction of metabolism	Givinostat does not have significant inhibition and induction on hepatic CYP3A4 but does have weak inhibition on intestinal CYP3A4.
Inhibition/induction of transporter systems	A weak inhibition of the renal uptake transporter OCT2 by givinostat was seen in clinical trials by creatinine (OCT2 substate) measurements. In in vitro study, givinostat showed inhibition on P-gp transporter but the clinical DDI study did not indicate givinostat inhibit P-gp.

Source: Reviewer's table based on data from Sections 8.1, 8.2, 14.1 and 14.2
Abbreviations: AUC, area under the concentration-time curve; $AUC_{0-\infty}$, area under the concentration-time curve to infinity; AUC_{tau}, area under the curve during a dosing interval; BCRP, breast cancer resistance protein; BID, twice daily; CI, confidence interval; C_{max}, maximum plasma concentration; C_{trough}, trough concentration; CV, coefficient of variation; CYP, cytochrome P450; DDI, drug-drug interaction; DMD, Duchenne Muscular Dystrophy; GMR, geometric mean ratio; HDAC, histone deacetylase; HPLC, high performance liquid chromatography; ITF, givinostat; mARC, mitochondrial amidoxime reducing components; MS, mass spectrometry; MTD, maximum tolerated dose; P-gp, P glycoprotein; PK, pharmacokinetic; popPK, population pharmacokinetic; PMR, postmarketing requirement; QT, interval from the beginning of the Q complex to the end of the T wave; QTc, heart-rate corrected QT interval; QTcF, heart-rate corrected QT interval by Fridericia's cube root formula; T_{max}, time to maximum plasma concentration; TQT, thorough QT; UGT, Uridine 5'-diphospho-glucuronosyltransfe

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

6.1.1. Studied Dose Regimen in Study 48

The body-weight based dosages (see Table 7) Dose A, Dose B, and Dose C, were studied in the phase 3 trial (Study 48). The doses were taken with food twice daily (BID) in the study. Dose selection for givinostat in Study 48 was based on pharmacokinetic/pharmacodynamic (PK/PD) data from preclinical models (mdx mice), popPK/PD data from the phase 2 study (Study 43) and clinical safety and efficacy data from across the givinostat development program in other indications. In Study 43, doses of 37.5 mg BID and 25 mg BID were considered tolerated in children with DMD. In addition, the population pharmacokinetic (popPK) analysis indicated that body weight was a significant covariate for givinostat's clearance. Therefore, in the initial protocols (version <4) of Study 48, a body-weight tiered dosing (as defined as Dose A) was chosen as the starting dose to achieve similar area under the concentration-time curve (AUC) across body weight range to that obtained with 37.5 mg BID dose in the phase 2 study (Study 43). Dose reduction from Dose A to Dose B (a 33% dose reduction to achieve similar exposure as to that obtained with 25 mg BID dose in Study 43) was permitted in the protocol based on pre-specified criteria related to safety and tolerability, e.g., platelet count reduction, occurrence of moderate or severe diarrhea, elevated triglycerides levels.

With this dosing regimen, the interim safety analysis indicated that 50 to 60% of subjects receiving Dose A had to reduce to Dose B due to platelet count reduction, which triggered a major protocol amendment to change the dosing regimen during the study. In the revised protocol (version ≥4), the starting dose was changed to Dose B with an additional 20% dose reduction permitted (from Dose B to Dose C), if needed. The change of the dosing regimen in the middle of the study resulted in different starting doses among subjects who received givinostat during the duration of the study (38 subjects started with Dose A and 42 subjects started with Dose B). An overview of dosing history for individual subjects enrolled in Study 48 is present in Figure 1.

The review team aimed to evaluate the impact of dosing history (b) (4) (b) (4) The team (b) (4) evaluated a (b) (4) simplified dosing regimen for body weight-based dosing (b) (4)

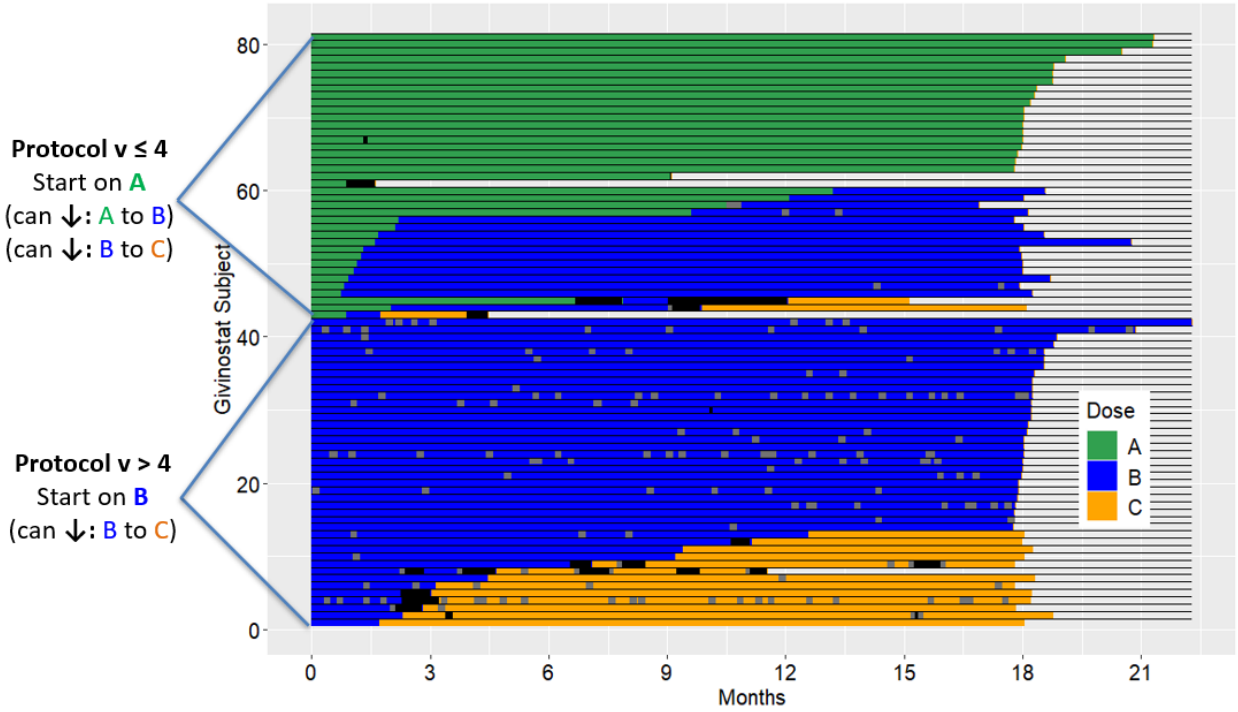
Reference ID: 5351242

Table 7. Studied Dosage in Study 48: Dose A (Starting Dose)

Weight (kg)	≥10 and <12.5	≥12.5 and <20	≥20 and <25	≥25 and <30	≥30 and <40	≥40 and <50	≥50 and <60	≥60 and <70	≥70
Dose A (mg)[1] BID	20	25	30	35	40	50	55	60	70
Oral suspension volume (mL)	2.0	2.5	3.0	3.5	4.0	5.0	5.5	6.0	7.0
Dose B (mg)[1] BID	13.3	16.7	20	23.3	26.7	33.3	36.7	40	46.7
Oral suspension volume (mL)	1.3	1.7	2.0	2.3	2.7	3.3	3.7	4	4.7
Dose C (mg)[1] BID	10.6	13.4	16	18.6	21.4	26.6	29.4	32	37.4
Oral suspension volume (mL)	1.1	1.3	1.6	1.9	2.1	2.7	2.9	3.2	3.7

Source: Clinical Study Report DSC/14/2357/48
[1] Givinostat hydrochloride monohydrate
Abbreviations: BID, twice daily

Figure 1. Dosing History Overview of Individual Subjects Who Received Givinostat in Study 48



Source: Reviewer's analysis
Reference to Section 14.5.2
Note: Each row represents a subject assigned to givinostat treatment in Study 48. Green, blue, and orange squares represent administration of Dose A, Dose B, and Dose C, respectively. Grey squares represent instances where the subject skipped one of the two daily doses. Black rectangles represent durations where subjects missed at least two administrations connectively (i.e. skipped both daily doses or dosing was temporarily paused). The end of a horizontal line segment indicates the time after which the subject permanently stopped receiving treatment in Study 48.

6.1.2. Optimization of the Dosing Regimen

Evaluation of the Starting Dose

Dosing in Study 48 started at Dose A (highest dose level) until protocol version 5 where the starting dose was reduced to Dose B due to safety and tolerability concerns (see Section 6.1.1).

The review team investigated the impact of change of dosing in the protocol during the study and evaluated whether the starting dose for the indicated patient population should be Dose A or Dose B. The team recommends starting with a dose similar to Dose A from Study 48 based on the following considerations:

- N = 19 (out of 81) subjects in Study 48 were able to remain on Dose A throughout the duration of Study 48. This suggests some patients may be able to tolerate the Dose A level. Further, E-R analyses indicate that a higher givinostat exposure tends to have better clinical outcome on clinical endpoints, especially for the secondary endpoint, North Star Ambulatory Assessment (NSAA) total score (see Section 6.3.1). This is suggestive of a potential benefit for Dose A.
- With an adequate monitoring plan, subjects unable to tolerate Dose A have the option to reduce to Dose B, and subsequently reduce to Dose C based on safety and tolerability. This approach can effectively mitigate the risk of thrombocytopenia and other adverse reactions when combining with appropriate safety monitoring as suggested in the label.
- A post-marketing requirement will be issued for further investigation of the safety of givinostat in patients with DMD in the post-market setting, which will provide more assurance on the safety of the proposed dosing scheme.

Evaluation of a Simplified Body-Weight-Based Dosing

The review team (b) (4) evaluated more simplified dosing regimens as described below. The review team noted that the (b) (4) body weight-based dosing was complex and there was a trend of higher exposure for subjects with higher body weight (Section 14.5.2). In addition, the dosing scheme used in Study 48 (b) (4) requires two syringes (e.g., a 5 mL syringe and a 1 mL syringe) for administration depending on the volume to be administered. For instance, the Dose "B" for a subject with body weight between 25 kg to 30 kg is 23.3 mg, which requires administration of a volume of 2.3 mL oral suspension. Administering a 2.3 mL volume requires a 5 mL syringe to administer the 2 mL portion and a 1 mL syringe to administer the remaining 0.3 mL.

A simplified dosing regimen was developed by the review team as presented in Table 8.

As studied in Study 48, givinostat should be taken orally twice daily with food. Dose A is recommended as the starting dose with permitted dose adjustment (to Dose B or further to Dose C) when meeting specified tolerability/safety criteria (see footnote of Table 8).

Table 8. Recommended Dosing Regimen for Givinostat

Dose	≥10 and <20 kg	≥20 and <40 kg	≥40 and <60 kg	≥60 kg
Dose A[1] dosage (volume) BID	25mg (2.5 mL)	35mg (3.5mL)	50mg(5mL)	60mg (6ml)
Dose B[2] dosage (volume) BID	20mg (2.0 mL)	25mg (2.5mL)	35mg (3.5 mL)	45mg (4.5mL)
Dose C dosage (volume) BID	15mg (1.5mL)	20mg (2.0mL)	30mg (3.0 mL)	40mg (4.0 mL)

Source: Duvyzat USPI
[1] Dosage interruption or modification is recommended based on adverse reaction and severity. When platelet count <150 x 10^9/L verified in two assessments one week apart; or moderate or severe diarrhea; or fasting triglycerides >300 mg/dL verified by two assessments one week apart; a dose reduction from Dose A to B is allowed. If the adverse reaction(s) persist after the first dosage modification, proceed to the second dosage modification (i.e., to Dose C)
[2] if the adverse reaction(s) persist after the second dosage modification, Duvyzat should be discontinued.
Abbreviations: BID, twice daily; USPI, United States Prescribing Information

Allometric scaling is applied in the popPK model to address the relationship between body weight and clearance. Though weight-based dosing was applied in Study 48, the givinostat exposure is higher at higher body weights than givinostat exposure at lower body weights (see Table 9). As such, an expected consequence is that subjects with higher body weights have higher thrombocytopenia risk (See Section 14.5.4).

An information request was sent to the Applicant requesting the Applicant conduct PK simulations as well as adaptive PK/PD simulations for platelet count and compare the regimen applied in Study 48 to an alternate dosing regimen designed to reduce the number of weight bins and reduce the number of syringes required for administration. The PK/PD simulations are adaptive based on platelet values. The platelet value for each virtual subject was simulated based on the simulated givinostat exposure levels. If platelet values <150 x 10^9 / L were predicted for a virtual subject at the 2-week assessment times, that virtual subject's subsequent dose was reduced (from A to B, or from B to C) as was done in Study 48. A detailed review of the results can be found in Section 14.5.4. Overall, the PK and PD simulations suggested by the FDA were used to evaluate the exposure of givinostat as well as the proportion of subjects that would require dose reduction for the (b) (4) simplified dosing (b) (4) (b) (4) (considering the need of dose adjustment based on simulated platelet values and the specified dose reduction criteria described in Table 8).

The Applicant's response to this information request includes three dosing regimens.

- The "Current" regimen: This is the dosing regimen used in Study 48.
- The "FDA Revised" regimen: This is an alternate dosing regimen provided by the FDA as a starting point for the Applicant's PK/PD simulations. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥40 kg (55 mg twice-daily for subjects 40 kg to <60 kg, and 70 mg twice-daily for subjects ≥60 kg) and a higher Dose B for subjects ≥40 kg (40 mg twice-daily for subjects 40 kg to <60 kg, and 50 mg twice-daily for subjects ≥60 kg).
- The "ITF Revised" regimen: Based on their initial analyses of the simulated PK and platelet count for the "Current" regimen and "FDA Revised" regimen, the Applicant (b) (4) a new regimen, named the "ITF Revised" regimen, which reduced the Dose A level for subjects ≥40 kg (compared to the "FDA Revised" regimen) to reduce the thrombocytopenia risk, i.e., Dose A changed from 55 to 50 mg twice-daily for patients within 40 kg to 60 kg and from 70 to 60 mg twice-daily for patients ≥60 kg. The "ITF Revised" regimen is similar to the

regimen presented in Table 8, but with a higher Dose B for subjects ≥40 kg (40 mg twice-daily for subjects 40 kg to <60 kg, and 50 mg twice-daily for subjects ≥60 kg).

The "FDA Revised" and "ITF Revised" regimens showed comparable exposures to the exposure of the studied dosing regimen in Study 48 ("Current") for each body weight bands (Table 9). It should be noted that the "FDA Revised" and "ITF Revised" regimens maintained the trend of higher exposure for higher body weight patients following the first administration. However, based on the predicted response to dose reduction criteria, subjects are expected to have comparable exposures across body weight at the final administration at steady state after final dose modifications based on platelet levels. Further, the "FDA Revised" and "ITF Revised" regimens are likely to provide comparable patterns of dose reduction and a minor improvement in thrombocytopenia risk over the "Current" regimen from Study 48 (Table 10 and Table 11). The simulated PK for each of the three regimens and the predicted proportion of subjects requiring dose reductions are presented in Table 9, Table 10, and Table 11 respectively and discussed in details below.

Table 9. Simulated Givinostat PK After Initial Dose and After Final Dose for Three Dosing Regimens by Weight Category

PK Parameter	Dosing Scenario	Regimen	10-20 kg	20-40 kg	40-60 kg	>60 kg	Unit
Mean AUC	First Administration (AUC_{0-12})	CURRENT	339	429	529	579	hr*ng/mL
		FDA REV	386	435	554	625	hr*ng/mL
		ITF REV	385	434	504	536	hr*ng/mL
	Final Administration (AUC_{tau})	CURRENT	327	368	390	384	hr*ng/mL
		FDA REV	368	376	410	423	hr*ng/mL
		ITF REV	367	377	404	417	hr*ng/mL
Mean C_{max}	First administration	CURRENT	45	61	78	91	ng/mL
		FDA REV	51	62	83	99	ng/mL
		ITF REV	51	62	76	85	ng/mL
	Final administration	CURRENT	43	53	59	60	ng/mL
		FDA REV	48	54	62	67	ng/mL
		ITF REV	49	54	61	66	ng/mL

Source: Reviewer's analysis
Abbreviations: AUC_{0-12}, area under the concentration-time curve from time 0 to 12 hours; AUC, area under the concentration-time curve; AUC_{tau}, area under the concentration-time curve for a dosing interval; C_{max}, maximum plasma concentration; FDA, U.S. Food and Drug Administration; hr, hour; ITF, givinostat; REV, revised

According to the PK simulations presented Table 9, compared to the "Current" regimen, the "FDA Revised" and "ITF Revised" regimens produce exposures within +14% to -7% for AUC and within +13% to -7% for the maximum plasma concentration (C_{max}) across the four weight groups.

The Applicant summarized the proportion of subjects in the simulation that required a dose reduction based on predicted platelet count by weight group and by dose regimen (see Table 10 and Table 11 below).

Table 10. Proportion of Virtual Subjects Requiring One Dose Reduction Due to Elevated Platelet Counts Stratified by Weight Group and by Dosing Regimen

Weight	Number of Dose Reductions	% of Subjects (1: Current)	% of Subjects (2: FDA Revised)	% of Subjects (3: ITF Revised)
10 – 20 kg	1	5.6	4.3	4.3
20 – 40 kg	1	15.2	13.5	13.5
40 – 60 kg	1	20.3	17.1	11.4
>60 kg	1	18.2	14.5	7.9

Source: sequence 0038, module 1, ir-dose-response-document.pdf, page 7.
The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg).
Abbreviations: FDA, U.S. Food and Drug Administration, ITF, givinostat

Table 11. Proportion of Virtual Subjects Requiring Two Dose Reductions Due to Elevated Platelet Counts Stratified by Weight Group and by Dosing Regimen

Weight	Number of Dose Reductions	% of Subjects (1: Current)	% of Subjects (2: FDA Revised)	% of Subjects (3: ITF Revised)
10 – 20 kg	2	3.3	7.0	7.0
20 – 40 kg	2	14.5	16.1	16.1
40 – 60 kg	2	35.9	42.0	40.7
> 60 kg	2	52.9	61.1	58.8

Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 7.
Note: The "1:Current" regimen is the dosing regimen used in Study 48. The "2:FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "3:ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg).
Abbreviations: FDA, U.S. Food and Drug Administration, ITF, givinostat

The adaptive simulations resulted in 41.5% of subjects undergoing a dose reduction which is consistent with the 47.3% (26 out of 55 subjects) of subjects in the overall population that started on Dose A and that had TEAEs leading to dose reduction in the givinostat group in Study 48. In addition, in the adaptive PK/PD simulations the dose reductions occurred within the first three months which is consistent with the reported observed mean time to platelet nadir of 92.6 days in subjects with a dose reduction due to platelet count decrease in Study 48.

The "ITF Revised" dosing regimen is expected to provide comparable efficacy to that observed in Study 48 based on comparable PK at each body-weight tier (see Table 9). In addition, the "ITF Revised" dosing regimen is likely to provide a minor improvement in thrombocytopenia risk over the "FDA Revised" regimen, e.g., for subjects ≥40 kg, the "ITF Revised" dosing regimen reduces the risk of dose reduction compared to "FDA Revised Regimen" by ~7% (i.e., 14.5% vs 7.9% requiring one dose reduction for FDA regimen vs ITF regimen; see Section 14.5.4.2).

It was also noted that the optimized Dose B values in the "ITF Revised" regimen for subjects ≥40 kg (i.e., 40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥60 kg) were slightly higher than the Dose B studied in Study 48. Though weight-based dosing was applied in Study 48, the resulting givinostat exposure was generally higher at higher body weights. The higher exposure of givinostat results in higher thrombocytopenia risk based on the PK/PD model (see Section 14.5.2). Therefore, a further reduction of Dose B in "ITF Revised" regimen for subjects ≥40 kg was recommended to further mitigate thrombocytopenia risk. The review team recommends a reduction in Dose B for subjects ≥40 kg to 35 mg twice-daily for

subjects weighing 40 kg to <60 kg, and to 45 mg twice-daily for subjects weighing ≥60 kg. The final recommendation on dosing regimen is presented in Table 8.

6.2. Clinical Studies/Trials Intended to Demonstrate Efficacy

6.2.1. Study 48

6.2.1.1. Design, Study 48

Study 48 was a multicenter, multinational, randomized, double-blind, placebo-controlled, phase 3 study of givinostat in ambulant subjects ≥6 years of age with DMD who were on stable doses of concomitant corticosteroid therapy. A total of 110 subjects were planned to be randomized into the Target Population (subjects with baseline VL MFF assessed by MRS in the range of >5% and ≤30%). Up to 50 subjects (about 35% of the Overall Population) with baseline VL MFF outside of the target range were planned to be recruited into the study (Off-Target population).

The primary objective of Study 48 was to establish the effect of givinostat versus placebo administered chronically over 18 months to slow disease progression in ambulant DMD subjects.

The secondary objectives were to assess the safety and tolerability of givinostat versus placebo, to evaluate the PK profile of givinostat administered chronically, and to evaluate the impact on quality of life and activities of daily living on givinostat versus placebo administered chronically in DMD subjects.

All patients were on stable doses of background therapy with corticosteroids. At randomization, subjects were stratified for their steroid dosing regimen in 4 strata:

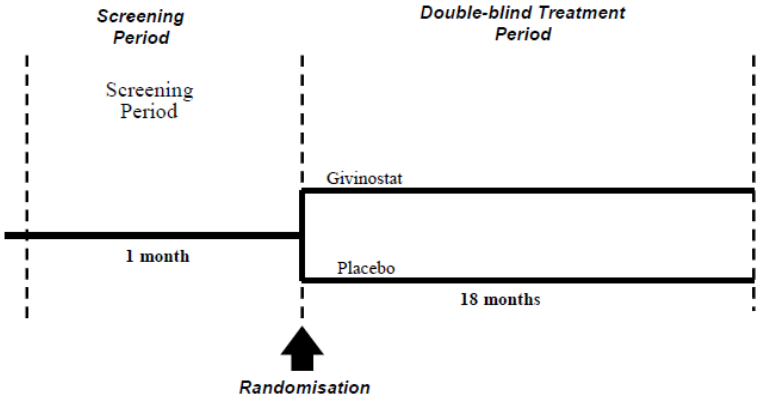
- Deflazacort daily regimen
- Deflazacort intermittent regimen
- Other steroids daily regimen
- Other steroids intermittent regimen

The planned study duration was 19 months. The study comprised of 2 phases:

1. Screening period: starting 4 weeks before randomization.
2. Treatment period: 18 months of treatment.

The study design is shown in Figure 2.

Figure 2. Study Design Schema for Study 48



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Study 48 Endpoints

Primary Endpoint

The primary endpoint of this Study was mean change in time to climb four stairs (i.e., 4-stair climb [4SC]) before and after 18 months of treatment of givinostat versus placebo.

Key Secondary Endpoints

All Subjects

1. Mean change in time to rise from floor (RFF) before and after 18 months of treatment of givinostat versus placebo
2. Mean change in 6 Meter Walk Test (6MWT) before and after 18 months of treatment with givinostat versus placebo
3. Mean change of 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

Magnetic Resonance Cohort

1. Mean change in VL MFF comparing MRS before and after 12 months of treatment of givinostat versus placebo
2. Mean change in 4SC time before and after 12 months of treatment of givinostat versus placebo
3. Mean change in time to RFF before and after 12 months of treatment of givinostat versus placebo
4. Mean change in 6MWT before and after 12 months of treatment of givinostat versus placebo
5. Mean change in muscle strength evaluated by knee extension before and after 12 months of treatment of givinostat versus placebo
6. Mean change in fat fraction of vastus lateralis muscles comparing the MRS before and after 18 months of treatment of givinostat versus placebo

Safety Endpoints

1. Number of subjects experiencing treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) (Baseline through end of study [EOS]).
2. Type, incidence, and severity of TEAEs and SAEs (Baseline through EOS)
3. Changes from Baseline to EOS of:
   a. Vital signs and clinical laboratory tests (blood chemistry and hematology)
   b. Respiratory function evaluated by forced vital capacity, forced expiratory volume at 1 second (FEV1), and peak expiratory flow
   c. Cardiac function evaluated by electrocardiogram and echocardiogram
   d. Cognitive function evaluated by the Raven colored progressive matrices
   e. Weight, height, and body mass index (BMI)
4. Evaluation of acceptability/palatability of the oral suspension

Pharmacokinetic Endpoints

1. Description of the PK of givinostat and its major metabolites ITF2374 and ITF2375 in the subject population.
2. Identification of the relevant demographic and pathophysiological covariates influencing the PK of givinostat.

Exploratory Endpoints

1. Mean changes before and after 12 months of treatment of givinostat versus placebo of
   a. Time to 4SC
   b. Time to RFF
   c. 6MWT
   d. Muscle strength
2. Mean changes before and after 12 and 18 months of treatment of givinostat as compared to placebo of:
   a. Time to walk/run 10 meters
   b. Physical function as measured by NSAA
   c. PODCI scores
3. Mean changes before and after 18 months of treatment of givinostat versus placebo of:
   a. Percent-predicted 6MWT (using equation of Geiger)
   b. MRI cohort: MRI parameters (fat fraction of thigh muscles, CSA of vastus lateralis and other thigh muscles)
4. Time to 10% persistent worsening in 6MWT (Baseline through EOS)
5. Proportion of subjects with ≥10% worsening in 6MWT at EOS
6. Time to loss of standing (Baseline through EOS)
7. Proportion of subjects who lose ambulation during the Study
8. PK/PD analyses; relationships between metrics of exposure and efficacy/safety endpoints of givinostat

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6.2.1.2. Eligibility Criteria, Study 48

Key Inclusion Criteria

9. Ambulant male ages ≥6 years at randomization with DMD characteristic clinical symptoms or signs already present at screening
10. DMD diagnosis confirmed by genetic testing
11. Able to complete 2 4SC screening assessments; the results of these tests must be within ±1 second of each other
12. Have a mean of 2 screening 4SC assessments ≤8 seconds
13. Have time to RFF of <10 seconds at screening
14. Have manual muscle testing of quadriceps at screening ≥ Grade 3
15. Are eligible according to the protocol-specified functional algorithm predictive of VL MFF that should be in the range of >10% but <30%
16. Have used systemic corticosteroids for a minimum of 6 months immediately prior to the start of study treatment, with no significant change in dosage or dosing regimen (excluding changes related to body weight change) for a minimum of 6 months immediately prior to start of study treatment and reasonable expectations that dosage and dosing regimen will not change significantly for the duration of the study
17. Willing to use adequate contraception

Key Exclusion Criteria

18. Exposure to another investigational drug within 3 months prior to the start of the study treatment
19. Exposure to idebenone within 3 months prior to the start of study treatment
20. Exposure to any dystrophin restoration product within 6 months prior to the start of study treatment
21. Use of any pharmacologic treatment, other than corticosteroids, that might have had an effect on muscle strength or function whin 3 months prior to the start of study treatment
22. Surgery that might have an effect on muscle strength or function whin 3 months before study entry or planned surgery at any time during the study
23. Ankle joint contractures due to a fixed loss of ≥10 degrees of dorsiflexion from plantigrade (normal = 20 degrees)
24. Change in contracture treatment such as serial casting, contracture control devices, night splints, stretching exercises within 3 months prior to enrollment, or expected need for such intervention during the Study
25. Presence of other clinically significant disease which could affect assessment
26. Abnormal platelet count, symptomatic cardiomyopathy or heart failure, liver disease, inadequate renal function, hepatitis B, hepatitis C, HIV, baseline long QT

6.2.1.3. Statistical Analysis Plan, Study 48

In a December 16, 2016, response to FDA's study May Proceed letter, the Applicant agreed to include North Star Ambulatory Assessment (NSAA) as a key secondary endpoint. They also stated in their response that since all key secondary endpoints are of importance and clinical relevance in DMD, the Applicant does not consider it appropriate to pre-specify what would be an essentially arbitrary hierarchy for the purposes of statistical analysis of the secondary

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endpoints. Therefore, the Applicant proposed that formal statistical testing of key secondary endpoints would be performed only if the primary endpoint was met and, should this occur, a Hochberg (Hochberg 1988) closed test procedure would be used to control the type I error amongst the key secondary endpoints.

Two interim analyses had been planned (Protocol Amendment 7.0 dated August 29, 2019).The initial blinded interim analysis of Study 48 was performed in January 2020. The analysis concluded that futility was not met and the trial should continue, and thus a pre-planned sample size re-estimation was performed. Based on this re-estimation, the Applicant reduced the target population sample size from 192 (protocol version 7.0) to 102 (allowing for an estimated drop-out rate of 8%, a total of 110 male ambulant subjects were to be randomized into the target population). Note that the original sample size was calculated to provide 90% power and a 1-sided alpha of 2.5% to detect a true difference between givinostat and placebo in the target population in 4SC change from baseline to Month 18 of 3 seconds, assuming a common SD of 6 seconds. The estimated SD of 6 was based on publicly available phase 3 study data on ataluren and drisapersen in subjects with DMD in addition to internal Italfarmaco data.

The SAP also noted that if the IDMC indicated that the Study should proceed at the initial interim futility analysis, a blinded sample size re-estimation was to be conducted for VL MFF and 4SC in the target population as follows: To maintain the blind on the primary endpoint, the within treatment SD for the change from baseline was to be estimated as $\hat{\sigma}_{within} = \sqrt{\hat{\sigma}^2_{overall} - (\Delta/2)^2}$ where $\hat{\sigma}_{overall}$ is the overall SD for the change from baseline in 4SC based on the n = 50 subjects in the interim and Δ is the originally hypothesized treatment effect, i.e., Δ = 3 seconds. Dependent upon the blinded SD estimate, the sample size may have been decreased or increased to maintain 90% power. Any increase in sample size for 4SC was to be limited to 1.5 times maximum of the initial target population sample size, i.e., to a maximum of 1.5 x 192 = 288 subjects. For a 20% increase in the SD, this degree of increase in sample size was expected to maintain 90% power, and for a 40% increase in SD power was expected to be maintained at 80%.

In the type C meeting minutes dated May 11, 2020, the FDA responded that “We strongly advise against decreasing the sample size following the interim analysis, as this will inflate the type I error rate.”

Protocol version 7 specified the analysis of the primary efficacy endpoint as follows: The change from baseline to 18 months in 4SC was to be evaluated in the target population. The data was to be analyzed using an Analysis of Covariance (ANCOVA) model with the change from baseline to 18 months in 4SC as the dependent variable and with terms for baseline 4SC value as a covariate and randomized treatment, concomitant steroid use, and age at baseline as independent class variables. Least squares means will be estimated for givinostat and placebo. The treatment effect, being the difference in Lsmeans, will be presented along with the associated 95% confidence interval and the p-value.

The final version of the statistical analysis plan V6, was finalized on April 13, 2022, and stated “Additional covariates will also be included for the following baseline values where appropriate: 4SC, time to rise from floor, time to run/walk 10 meters, distance walked in 6 minutes (for the analysis of percent predicted 6MWT, this will be replaced with baseline percent predicted distance walked in 6 minutes).”

Furthermore, the need for a possible log transformation of these variables (endpoints) was to be investigated prior to unblinding by assessing the ANCOVA model residuals without a treatment term (note: this statistical analysis plan statement raises the issue of a possible transformation of the outcome variable but does not provide sufficient details for a fully objective assessment of the need for a transformation).

Missing Data Handling

*For the efficacy analyses, any missing data for continuous values for a subject will be classified as follows:

1. The subject has missing data due to reasons other than being non-ambulatory or physically unable to perform the test/assessment
2. The subject has missing data due to being either non-ambulatory or otherwise
3. The subject is physically unable to perform the test/assessment

Any missing values classified as 1 will be imputed using the mean of all non-missing values for the respective measurement and timepoint across all subjects randomized to the respective treatment and in the respective concomitant steroid stratum and Group (i.e., Target population or Off-Target population). Any missing values classified as 2 will be imputed by setting the value to zero or to twice the maximum non-missing value recorded across all subjects depending on the directionality of the test.

In all cases, imputation of any data classified as 1 will be completed first, followed by imputation of any data classified as 2. Note that where the endpoint to be analyzed is a change from baseline, any imputation will be applied to the observed values prior to finding the corresponding change from baseline.

The National Academy of Science’s report on the prevention and treatment of missing data in clinical trials discourages the use of such single value imputation methods for handling missing data. Similarly, page 9 of the EMA guideline on missing data states "A potential disadvantage of single imputation methods is that these methods risk biasing the standard error downwards by ignoring the uncertainty of imputed values. Therefore, the confidence intervals for the treatment effect calculated using single imputation methods may be too narrow and give an artificial impression of precision that does not really exist. This possibility should be addressed when results from these analyses are presented." A possible approach for handling a subject unable to perform the test would be a single value imputation for that patient only, together with a more appropriate approach for subjects with missing data not related to the inability to perform the test, e.g., MMRM of all observed post-baseline 4SC data.

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Tipping Point Sensitivity Analysis

In addition to the above methods for handling missing data, if the treatment effect on the primary analysis is significant, a 'tipping point' sensitivity analysis of the primary outcome will be performed. This analysis assesses the impact of missingness across a continuum of assumptions until the 'tipping point' is identified. This is the point at which the assumption regarding the effect of the treatment in subjects with missing data nullifies or reverses the treatment effect obtained in the primary analysis. Implementation of this sensitivity analysis mimics standard multiple imputation and is described below:

27. For any Class 1 missing 4SC values, use the mean and SD of all non-missing values for the respective treatment group to generate the imputed values from a normal distribution. Note that any Class 2 missing 4SC values should continue to be imputed with twice the maximum non-missing value recorded across all subjects as applicable per the above rules.
28. Implement Step 1 10 times so that 10 complete datasets are produced. For each of these datasets, find the change from baseline to 18 months in 4SC values for each subject.
29. Analyze the 10 complete datasets; this should be performed with the same model as the primary analysis.
30. Combine the results from the 10 data sets using Rubin's method via PROC MIANALYZE for inference.
31. Repeat Steps 1 to 4 but now with an appropriate small shift (+δ) applied to the individual subject values for the change from baseline in 4SC in the givinostat treatment group (but not to the placebo group). This shift is applied after step 2, i.e., after the generation of the datasets including any imputation, and after finding the change from baseline values but prior to the analysis of these. The directionality of the shift will be such that this reduces the treatment difference between givinostat and placebo.
32. Display the estimated treatment effect and associated 2-sided CIs vs degree of shift.
33. Repeat Steps 5 and 6, increasing the shift each time as +2 δ, +3 δ,…,+(treatment effect seen in the primary analysis).
34. Identify the tipping point where $p>0.025$.

The key secondary efficacy endpoints (time to RFF, 6MWT, muscle strength evaluated by knee extension, muscle strength evaluated by elbow flexion, physical function as measured by total NSAA score, physical function as measured by cumulative loss of function on the NSAA, VL MFF as assessed by MRS). Each of these endpoints will be analyzed and the resulting 1- sided p-values will be ordered in descending order, from least to most significant. The highest p-value will then be compared with 0.025, the specified level of significance for the primary endpoint at the final analysis for this study, and if it is >0.025, the subsequent p-value will be considered in relation to 0.025/2 = 0.0125. As soon as the i^{th} endpoint has a corresponding p-value <0.025/i, the treatment effects for that endpoint and all of the endpoints following it are considered to be significant. A table will be presented summarizing the p-values and associated adjusted 2-sided CIs from each of the key secondary efficacy endpoints with significance indicated according to the above approach.

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6.2.1.4. Results of Analyses, Study 48

Disposition of Subjects

A total of 359 subjects were screened, of whom 179 (49.9%) subjects were randomized. Thirty-six (10%) subjects passed screening but were not randomized, and 144 (40.1%) subjects failed screening. The Overall Population (Safety Population) includes all subjects randomized and treated in Study 48. The Target Population includes all subjects with baseline VL MFF assessed by MRS in the range of >5% and ≤30%. The Off-Target Population includes those subjects whose baseline VL MFF fell outside the target range.

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 RFF 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).

* Note: Of the 79 subjects who failed inclusion criterion #8, 1 subject (Subject (b) (6)) was enrolled under protocol version >4.0, but an incorrect inclusion criterion was used to record screen failure. This subject was defined as Off-Target based on MRI results. Therefore, "Other – screening failure because of Off-Target" should be used instead of inclusion criterion #8.

In the Overall Population, a total of 118 subjects were enrolled in the givinostat group, of whom 111 (94.1%) subjects completed the study; and 61 subjects were enrolled in the placebo group, of whom 59 (96.7%) subjects completed the study. A summary of subject disposition for the Overall Population is presented in Table 12.

Table 12. Subject Disposition, Overall Population Study 48

Disposition Outcome	Givinostat N=118 n (%)	Placebo N=61 n (%)	Overall (N=179) n (%)
Patients randomized	118 (100)	61 (100)	179 (100)
Safety population	118 (100)	61 (100)	179 (100)
Discontinued study	7 (5.9)	2 (3.3)	9 (5.0)
Adverse event	3 (2.5)	0	3 (1.7)
Withdrawal by subject	4 (3.4)	2 (3.3)	6 (3.4)

Source: ds.xpt and adsl.xpt; Software: R
Duration is 18-month double-blind treatment period.
Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients in specified population or group; NA, not applicable

A summary of subject disposition for the Target Population is provided in Table 13. A total of 81 subjects were enrolled in the givinostat group, of whom 77 (95.1%) subjects completed the study. 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.

Table 13. Subject Disposition, Target Population, Study 48

Disposition Outcome	Givinostat N=81 n (%)	Placebo N=39 n (%)	Overall N=120 n (%)
Patients randomized	81 (100)	39 (100)	120 (100)
Safety population	81 (100)	39 (100)	120 (100)
Discontinued study	4 (4.9)	2 (5.1)	6 (5.0)
Adverse event	1 (1.2)	0	1 (0.8)
Withdrawal by subject	3 (3.7)	2 (5.1)	5 (4.2)

Source: ds.xpt and adsl.xpt; Software: R
Duration is 18-month double-blind treatment period.
Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients in specified population or group; NA, not applicable

Demographics in Overall Population

A summary of demographics for the Overall Population (Safety Population) is presented in Table 14.

The demographic characteristics were well balanced for the Overall Population across the givinostat and placebo groups. The mean age of subjects in the givinostat group was 9.8 years (range, 6.3 to 15.9 years); the mean age of subjects in the placebo group was 10.0 years (range, 6.1 to 14.4 years). The majority of subjects in both groups were White and not Hispanic or Latino. The mean weight of subjects in the givinostat group was 31.2 kg (range, 17.9 to 64.1 kg); the mean weight of subjects in the placebo group was 32.6 kg (range, 18.9 to 66.0 kg). The mean BMI of subjects in the givinostat and placebo groups was 19.7 kg/m2 and 19.9 kg/m2, respectively.

Table 14. Baseline Demographic Characteristics, Overall Population, Study 48

Characteristic	Givinostat N=118	Placebo N=61
Sex, n (%)		
Male	118 (100)	61 (100)
Age, years		
Mean (SD)	9.8 (2)	10 (2.1)
Median (min, max)	9.8 (6.3, 15.9)	9.9 (6.1, 14.4)
Age group, years, n (%)		
Adolescent (≥12 to <18)	18 (15.3)	12 (19.7)
Children (6 to <12)	100 (84.7)	49 (80.3)
Race, n (%)		
Asian	4 (3.4)	2 (3.3)
Black or African American	3 (2.5)	0
Other	5 (4.2)	2 (3.3)
White	106 (89.8)	57 (93.4)
Ethnicity, n (%)		
Hispanic or Latino	9 (7.6)	3 (4.9)
Not Hispanic or Latino	109 (92.4)	58 (95.1)
Country of participation, n (%)		
Germany	13 (11.0)	2 (3.3)
Spain	17 (14.4)	6 (9.8)
Italy	24 (20.3)	13 (21.3)
United States	24 (20.3)	19 (31.1)
Others	40 (33.9)	21 (34.4)

Characteristic	Givinostat N=118	Placebo N=61
Is in United States, n (%)		
United States	24 (20.3)	19 (31.1)
Non-United States	94 (79.7)	42 (68.9)
Height, cm		
Mean (SD)	125.1 (7.9)	126.7 (9.8)
Median (min, max)	125.0 (109, 151)	127.0 (112, 163)
Weight, kg		
Mean (SD)	31.2 (8.9)	32.6 (10.8)
Median (min, max)	29.7 (17.9, 64,1)	30.0 (18.9, 66)
Body Mass Index, (kg/m^2)		
Mean (SD)	19.7 (4.1)	19.9 (4.4)
Median (min, max)	18.5 (12.4, 30.6)	19.5 (13.3, 31.4)

Source: adsl.xpt; Software: R
Abbreviations: N, number of patients in treatment group; n, number of patients with given characteristic; SD, standard deviation

A summary of the baseline disease characteristics, including diagnosis of DMD and corticosteroids treatment for the Overall Population is presented in Table 15. The baseline disease characteristics were generally balanced for the Overall Population across the givinostat and placebo groups. The mean time since diagnosis in the givinostat and placebo groups was 5.45 and 5.62 years, respectively. The type of DMD mutation for most of the subjects in both groups was deletion (70.3% in the givinostat group and 65.6% in the placebo group). The mean time since steroid initiation to the date of signing the ICF in the givinostat and placebo groups was 3.62 and 3.97 years, respectively. The steroid deflazacort was used by most of the subjects in both groups (77.1% in the givinostat group and 73.8% in the placebo group). Most of the subjects in both groups (83.9% in the givinostat group and 78.7% in the placebo group) were receiving steroids daily.

Table 15. Diagnosis of Duchenne Muscular Dystrophy, Overall Population, Study 48

Characteristic	Givinostat N=119	Placebo N=61
Time Since Diagnosis[1]		
Mean (SD)	5.5 (2.6)	5.6 (2.7)
Median (min, max)	5.4 (0.1, 11.4)	5.3 (0.5, 12.3)
DMD mutation, n (%)		
Deletion	83 (70.3)	40 (65.6)
Duplication	17 (14.4)	14 (23.0)
Point mutation	18 (15.3)	7 (11.5)
Time Since Steroid initiation (years)[2]		
Mean (SD)	3.62 (2.1)	3.97 (2.1)
Median (min, max)	3.4 (0.4, 8.9)	4.0 (0.5, 9.2)

Characteristic	Givinostat N=119	Placebo N=61
Type of Steroid, n (%)		
Prednisone	20 (16.9)	15 (24.6)
Deflazacort	91 (77.1)	45 (73.8)
Other	7 (5.9)	1 (1.6)
Steroid Schedule, n(%)		
Daily	99 (83.9)	48 (78.7)
Intermittent	19 (16.1)	13 (21.3)
Every other day	1 (0.8)	4 (6.6)
10 days ON- 5 days OFF	1 (0.8)	1 (1.6)
10 days ON- 10 days OFF	14 (11.9)	4 (6.6)
Only during weekend	3 (2.5)	4 (6.6)
20 days ON – 10 days OFF	0	0
Other	0	0

Abbreviations: DMD, Duchenne Muscular Dystrophy; N, number of subjects in the analysis set; n, number of subjects meeting the criterion; SD, standard deviation. Note: (%) = n/N x 100 for categorical variables.
[1] Time since diagnosis was calculated in years as the date of informed consent – date of diagnosis +1
[2.] Time since steroid initiation was calculated in years as the date of informed consent – start date of initial steroid usage +1
Source: page 107-108 of Applicant's study report and verified by reviewer

Demographics in Target Population

A summary of demographics for the Target Population in the safety analysis set is presented in. Table 16. The Target Population includes all subjects with baseline VL MFF assessed by MRS in the range of >5% and ≤30%, and makes up the primary analysis population.

The demographic characteristics were well balanced for the Target Population across the givinostat and placebo groups. The mean age of subjects in the givinostat group was 9.63 years (range, 6.3 to 14.2 years); the mean age of subjects in the placebo group was 9.76 years (range, 6.1 to 13.5 years). The majority of subjects in both groups were White and not Hispanic or Latino. The mean weight of subjects in the givinostat group was 30.2 kg (range, 17.9 to 45.5 kg); the mean weight of subjects in the placebo group was 31.8 kg (range, 18.9 to 56.7 kg). The mean body mass index of subjects in the givinostat and placebo groups was 19.5 kg/m2 and 20.1 kg/m2, respectively.

Table 16. Baseline Demographics, Target Population, Study 48

Characteristic	Givinostat N=81	Placebo N=39
Sex, n (%)		
Male	81 (100)	39 (100)
Age, years		
Mean (SD)	9.6 (2)	9.8 (2)
Median (min, max)	9.8 (6.3, 14.2)	9.6 (6.1, 13.5)
Age group, years, n (%)		
Adolescent (≥12 to <18)	9 (11.1)	7 (17.9)
Children (6 to <12)	72 (88.9)	32 (82.1)
Race, n (%)		
Asian	3 (3.7)	1 (2.6)
Black or African American	0	0
Other	4 (4.9)	2 (5.1)
White	74 (91.4)	36 (92.3)

Characteristic	Givinostat N=81	Placebo N=39
Ethnicity, n (%)		
Hispanic or Latino	6 (7.4)	3 (7.7)
Not Hispanic or Latino	75 (92.6)	36 (92.3)
Country of participation, n (%)		
Canada	7 (8.6)	5 (12.8)
Germany	9 (11.1)	1 (2.6)
Spain	13 (16.0)	4 (10.3)
Italy	17 (21.0)	9 (23.1)
United States	17 (21.0)	11 (28.2)
Others	18 (22.2)	9 (23.1)
Is in United States, n (%)		
United States	17 (21.0)	11 (28.2)
Non-United States	64 (79.0)	28 (71.8)
Height, cm		
Mean (SD)	124.1 (7.3)	124.8 (7.6)
Median (min, max)	125 (109, 145)	125 (112, 146)
Weight, kg		
Mean (SD)	30.2 (7.1)	31.8 (9.6)
Median (min, max)	29.5 (17.9, 45.5)	30.0 (18.9, 56.7)
Body Mass Index, (kg/m^2)		
Mean (SD)	19.5 (9.3)	20.1 (4.5)
Median (min, max)	18.5 (12.4, 30.6)	19.5 (13.3, 31.3)

Abbreviations: N, number of subjects in the analysis set, n, number of subjects meeting the criterion; SD, standard deviation
Note: (%) – n/N x100 for categorical variables
Source: page 106 of Applicant's study report and verified by reviewer

A summary of the baseline disease characteristics, including diagnosis of DMD and corticosteroids treatment for the Target Population in the safety analysis set is presented in Table 16. The baseline disease characteristics were generally balanced for the Target Population across the givinostat and placebo groups. The mean time since diagnosis in the givinostat and placebo groups was 5.70 and 5.60 years, respectively. The type of DMD mutation for most of the subjects in both groups was deletion (71.6% in the givinostat group and 71.8% in the placebo group). The mean time since steroid initiation to the date of signing the ICF in the givinostat and placebo groups was 3.71 and 3.76 years, respectively. The steroid deflazacort was used by most of the subjects in both groups (81.5% in the givinostat group and 74.4% in the placebo group). Most of the subjects in both groups (85.2% in the givinostat group and 79.5% in the placebo group) were receiving steroids daily.

Table 17. Diagnosis of Duchenne Muscular Dystrophy, Target Population, Study 48

Characteristic	Givinostat N=81	Placebo N=39
Time Since Diagnosis[1]		
Mean (SD)	5.7 (2.5)	5.6 (2.9)
Median (min, max)	5.5 (0.4, 10.6)	5.3 (0.5, 12.3)
DMD mutation, n (%)		
Deletion	58 (71.6)	28 (71.8)
Duplication	13 (16.0)	9 (23.1)
Point mutation	10 (12.3)	2 (5.1)
Time Since Steroid initiation (years)[2]		
Mean (SD)	3.71 (2.1)	3.76 (2.1)
Median (min, max)	3.4 (0.5, 8.9)	3.6 (0.5, 8.2)

Characteristic	Givinostat N=81	Placebo N=39
Type of steroid, n (%)		
Prednisone	10 (12.3)	10 (25.6)
Deflazacort	66 (82.5)	29 (74.4)
Other	5 (6.2)	0
Steroid Schedule, n(%)		
Daily	69 (85.2)	31 (79.5)
Intermittent	12 (14.8)	8 (20.9)
Every other day	1 (1.2)	2 (5.1)
10 days ON- 5 days OFF	1 (1.2)	1 (2.6)
10 days ON- 10 days OFF	8 (9.9)	2 (5.1)
Only during weekend	2 (2.5)	3 (7.7)
20 days ON – 10 days OFF	0	0
Other	0	0

Abbreviations: DMD, Duchenne Muscular Dystrophy; N, number of subjects in the analysis set; n, number of subjects meeting the criterion; SD, standard deviation. Note: (%) = n/N x 100 for categorical variables.
[1] Time since diagnosis was calculated in years as the date of informed consent – date of diagnosis +1
[2.] Time since steroid initiation was calculated in years as the date of informed consent – start date of initial steroid usage +1
Source: Source: page 108-109 of Applicant's study report and verified by reviewer

Analysis of Primary Endpoint

At baseline, in the Target population, the mean 4-stair climb time was 3.48 (SD = 1.16) for placebo (n = 39) and 3.39 (SD= 1.16) for givinostat (n=81).

The Applicant's analysis of change from baseline to Month 18 in 4SC time (seconds) is presented in Table 18 for the Target Population in the intent-to-treat (ITT) analysis set. The treatment effect was demonstrated for 4SC time by a statistically significant difference in the generalized least square mean (GLSmean) ratio of givinostat versus placebo (GLSmean ratio [givinostat/placebo]: 0.86 seconds; p-value: 0.035) for change from baseline at 18 months. When the analysis was performed on non-log-transformed data, the results demonstrated a 1.78-second slower decline to perform this functional task in the givinostat group (p-value: 0.037).

Table 18. Analysis of Time (Seconds) to Climb 4 Standard Stairs, Change From Baseline at 18 Months (ITT Analysis Set- Target Population)

Statistics	Givinostat (N=81)	Placebo (N=39)
Log transformation applied		
Number of subjects included in analysis, n (%)	81 (100.0)	39 (100.0)
GLSmean (log scale SE)	1.27 (0.040)	1.48 (0.058)
95% CI for GLSmean	1.172, 1.372	1.317, 1.657
GLSmean ratio (givinostat/placebo) (log scale SE)	0.86 (0.071)	
95% CI for GLSmean ratio	0.745, 0.989	
P-value	0.0345	
No log transformation applied		
Number of subjects included in analysis, n (%)	81 (100.0)	39 (100.0)
LS mean	1.25	3.03
95% CI for LS mean	0.311, 2.181	1.666, 4.394
Difference in LS means (givinostat-placebo)	-1.78	
95% CI for difference in LS means	-3.462, -0.106	
P-value	0.0374	

Abbreviations: 4SC, climb 4 stairs; CI, confidence interval; EOS, end of study; GLSmean, generalised least square mean; ITT, Intent-to-Treat population; LS, least square; N, number of subjects in the analysis set; n, number of subjects meeting the criterion; 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.
Source: Applicant Study report page 120 and verified by review team based on submitted dataset \\CDSESUB1\evsprod\NDA217865\0003\m5\datasets\dsc-14-2357-48\analysis\adam\datasets\adft.xpt.

This Applicant analysis involved 12 class 1 imputations and 1 Class 2 imputation for Month 18 4SC data. The Applicant based the imputations on the mean Month 18 4SC time, not the mean Month 18 4SC change from baseline, and the Applicant's imputations were not adjusted for the subject's baseline score or age or other baseline functional scores in the analysis model.

Table 19 breaks down the Month 18 4SC time endpoint used in the analysis by whether it was observed or imputed. The review team identified one problem with this imputation method: the standard deviation of the imputed values is less than the standard deviation for observed data, although the former is truly more uncertain. Underestimating the true variability like this can increase the type I error/ false positive rate. In addition, the mean of the imputations for the Class 1 Month 18 missing values is slightly more favorable for givinostat than placebo compared to the corresponding means for observed data. Since the imputed data is really unknown and this assumes that dropouts are exchangeable with completers this may have caused bias in the primary analysis.

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Table 19. Summary Statistics for Observed and Imputed Month 18 4-Stair Climb Data

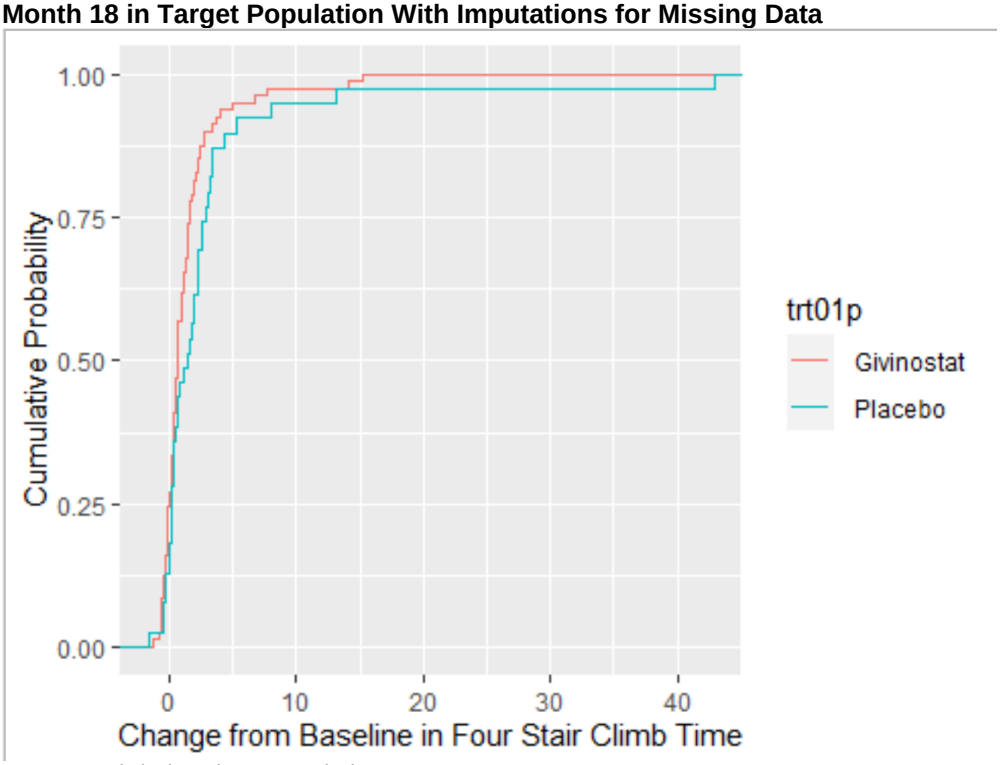
Month 18 Imputed or Observed	Treatment	N	Mean	SD	Min	Max
Imputed class1	Givinostat	8	5.16	1.19	4.30	6.60
Imputed class1	Placebo	4	6.05	1.81	4.90	8.70
Imputed class2 (missing but unable to perform test)	Givinostat	0	N/A	N/A	N/A	N/A
Imputed class2 (missing but unable to perform test)	Placebo	1	49.0	.	49.00	49.00
Observed	Givinostat	73	4.65	3.30	1.20	20.10
Observed	Placebo	34	5.15	3.36	1.60	18.70

Source: Statistical Reviewer's analysis
Abbreviations: max, maximum; min, minimum; N, number of subjects; N/A, not available; SD, standard deviation

Excluding Class 1 (missing but no indication that subject was unable to perform the test) imputations, which involved a suboptimal single imputed value as if it was actually observed 4SC data for patients with missing Month 18 4SC (thus underestimating the variability), the estimated difference for the untransformed 4SC is -1.77 (SE = 0.947), p = 0.065 (N = 108).

A missing data handling method requiring less stringent assumptions (relative to single value imputation for missing data) in order to be valid is MMRM of all observed data (note: here the single value imputation for the Class 2 missing data is also included since otherwise it would be informatively missing) which includes dropouts in the analysis by means of analyzing all post-baseline assessments, i.e., including earlier available assessments while still acknowledging their Month 18 assessment is missing. This approach gives an estimated difference of -1.64 (SE = 0.94), p = 0.083 (N = 119: note that 1 subject with a class 1 imputation in the Applicant's analysis had no post-baseline 4SC assessment after Week 4). Due to possible excessive influence of Class 2 missing data which involves a somewhat arbitrary imputed value (twice the maximum observed 4SC time across all subjects and visits) on the distribution of 4SC for a subject unable to perform the 4SC test, a MMRM analysis based on ranks of 4SC time may have better statistical properties for the analysis of 4SC times. A rank MMRM ANCOVA for 4SC time in the Target population yields a difference in mean ranks at Month 18 of -6.1 (SE = 4.2), p = 0.147.

Figure 3. Empirical Cumulative Distribution Plot for Change From Baseline in 4-Stair Climb Time at Month 18 in Target Population With Imputations for Missing Data



Source: Statistical Reviewer's analysis
Note: One placebo subject was unable to perform the test at month 18 and so by the analysis plan twice the maximum observed value in the study (42.9 secs) was imputed for this subject; There were 4 other placebo and 8 Givinostat imputations for subjects with missing data and no indication it was because they could not perform the test
Abbreviations: trt, treatment

The interim blinded re-estimation of standard deviation of 4SC concluded that the standard deviation based on the first 50 target patients to complete 12 months was 3 rather than the 6 hypothesized in the original sample size calculation. The final statistical analysis plan dated 2/11/22 states that N = 102 would be sufficient for 90% power and further it states with allowance for a small dropout rate, 110 patients will be randomized in the Target population. The final estimate of SD based on the Applicant's method for blinded re-estimation would be 4. The subject that was unable to perform the Month 18 assessment was enrolled after the interim analysis was completed. This subject's Class 2 imputation for Month 18 4SC has considerable influence on the 4SC standard deviation estimation as well as the analysis as a whole. This Class 2 imputation for the single (placebo) subject unable to perform the Month 18 test also influences differences in the group variances, which the Applicant's analysis assumes are equal. Furthermore, it has been shown in the statistical literature that with an unequal randomization ratio, as in this trial, and unequal group variances, the type I error is inflated when a method assuming equal group variances such as the Applicant's method is used (Hayes 2000). Note that this issue is also relevant to the applicant's post hoc permutation test that simultaneously investigates the endpoint data across all primary and key secondary endpoints.

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Sensitivity Analyses

Tipping Point Analysis

The results of the tipping point analysis for the primary outcome at 18 months using the ITT analysis set – Target Population are provided in Table 20 for the Target Population in the ITT analysis set.

Table 20. Tipping Point Sensitivity Analysis of Time (Seconds) to Climb 4 Standard Stairs, Change From Baseline at 18 Months (Intent-to-Treat Analysis Set- Target Population)

Approach 1: Standard

log scale shift	GLSmean ratio (givinostat/placebo) (log scale SE)	95% CI for GLSmean ratio	p-value
0.000	0.87 (0.085)	(0.738, 1.035)	0.1172
0.010	0.87 (0.078)	(0.749, 1.019)	0.0843
0.020	0.88 (0.081)	(0.753, 1.035)	0.1237
0.030	0.89 (0.077)	(0.765, 1.034)	0.1263
0.039	0.89 (0.081)	(0.760, 1.045)	0.1572
0.049	0.92 (0.082)	(0.780, 1.076)	0.2833
0.058	0.92 (0.080)	(0.783, 1.071)	0.2681
0.068	0.94 (0.081)	(0.804, 1.107)	0.4721
0.077	0.95 (0.080)	(0.808, 1.107)	0.4860
0.086	0.94 (0.080)	(0.802, 1.101)	0.4404
0.095	0.96 (0.084)	(0.812, 1.131)	0.6115
0.104	0.95 (0.081)	(0.813, 1.120)	0.5640
0.113	0.97 (0.085)	(0.818, 1.142)	0.6868
0.122	0.98 (0.079)	(0.836, 1.141)	0.7641
0.131	0.98 (0.079)	(0.841, 1.147)	0.8217
0.140	0.99 (0.077)	(0.850, 1.151)	0.8901
0.148	1.02 (0.080)	(0.873, 1.194)	0.7956
0.157	1.02 (0.076)	(0.877, 1.182)	0.8134
0.166	1.01 (0.079)	(0.867, 1.183)	0.8722
0.174	1.04 (0.082)	(0.888, 1.224)	0.6107
0.182	1.05 (0.080)	(0.894, 1.225)	0.5698

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.
A tipping point analysis was performed as described in section 4.4.4.3 of the SAP. The tipping point occurs where p>0.05.
For each value of the shift, LS Means, CIs, and p-values were obtained for each of 10 datasets from an analysis of covariance (ANCOVA) model on change from baseline in 4SC at Month 18 with baseline values for: 4SC, time to rise from floor, time to run/walk 10 metres, 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. Results from the 10 data sets were then combined using Rubins methods via PROC MIANALYZE for inference.
Data were log transformed prior to calculating change from baseline and the results from the analysis were then back transformed for presentation. GLSmean change from baseline should therefore be interpreted as a rate change (EOS/baseline).

Source: Page 242 of Table-figures.pdf
Abbreviations: 4SC, 4-stair climb; CI, confidence interval; EOS, end of study; GLS, generalized least squares; LS, least squares; SAP, statistical analysis plan; SE, standard error

The results considering no shift showed a treatment effect of 0.87 (GLSmean ratio [givinostat/placebo]) for both Tipping Point analysis approaches, similar to that obtained in the primary 4SC analysis (0.86; GLSmean ratio [givinostat/placebo]). 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. However, the 95% CI was slightly larger for the Tipping point analyses (primary analysis: 0.745, 0.989; Tipping Point analysis, Approach 1: 0.738, 1.035; Tipping Point analysis, Approach 2: 0.745, 1.008). As a result of increased variability, the primary endpoint p-value with zero shift were 0.117 and 0.064, respectively.

In summary, the tipping point analysis indicates that when the uncertainty of the missing data was treated as recommended in regulatory guidance documents on missing data (that were available prior to the start of the Study), the result was no longer statistically significant, even when missing outcomes from patients discontinuing from the Study were not assumed to be

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systematically different from completers' outcomes. The fact that the zero-shift part of the tipping point analysis is not significant also illustrates the inadequacy of the Applicant's primary missing data handling method.

Time to Rise From the Floor

The mean time to RFF changed from 5.57 to 14.43 seconds in the givinostat group and 5.59 to 19.17 seconds in the placebo group from baseline through Month 18. The mean changes from baseline in time to RFF at EOS (Week 72) were 8.86 seconds and 13.59 seconds in the givinostat and placebo groups, respectively.

An analysis of time (seconds) to rise from the floor by change from baseline at 18 months is presented in Table 21 for the Target Population in the ITT analysis set. Difference in LS means (givinostat-placebo) time (seconds) to rise from the floor was not statistically significant (p-value: 0.304) for change from baseline at 18 months; however, the findings did show a numerically less worsening in the subjects treated with givinostat.

Table 21. Analysis of Time (Seconds) to Rise From the Floor, Change From Baseline at 18 Months (ITT Analysis Set- Target Population)

Statistics	Givinostat (N=81)	Placebo (N=39)
Number of subjects included in analysis, n (%)	81 (100.0)	39 (100.0)
LS mean	9.33	12.61
95% CI for LS mean	5.821, 12.838	7.491, 17.724
Difference in LS means (givinostat-placebo)	-3.28	
95% CI for difference in LS means	-9.573, 3.018	
P-value	0.3044	

Abbreviations: 4SC, climb 4 stairs; CI, confidence interval; ITT, Intent-to-Treat population; LS, least square; N, number of subjects in the analysis set; n, number of subjects meeting the criterion.
Notes: Baseline was defined 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 time to rise from the floor at Month 18 with baseline values for: 4SC, time to rise from floor, time to run/walk 10 metres, 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.
Source: Page 126 of Applicant's study report

6-Minute Walk Test

The mean distance walked (meters) at the end of the 6-minute walk test (6MWT) ranged from 365.11 to 403.31 meters in the givinostat group and 344.83 to 399.64 meters in the placebo group from baseline through EOS.

The mean changes from baseline in distance walked (meters) at the end of the 6MWT at EOS (Week 72) were -38.20 meters and -48.86 meters in the givinostat and placebo groups, respectively. Overall, the change in 6MWT results over time was small for both treatment groups.

An analysis of distance walked (meters) at the end of the 6MWT by change from baseline at 18 months is presented in Table 22 for the Target Population in the ITT analysis set. Difference in LS means (givinostat-placebo) of distance walked (meters) at the end of the 6MWT was not

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statistically significant (p-value: 0.372) for change from baseline at 18 months; however, there was numerically less worsening in the distance walked in subjects treated with givinostat.

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Table 22. Analysis of Distance Walked (Meters) at End of 6MWT, Change From Baseline at 18 Months (ITT Analysis Set- Target Population)

Statistics	Givinostat (N=81)	Placebo (N=39)
Number of subjects included in analysis, n (%)	81 (100.0)	39 (100.0)
LS mean	-38.43	-48.38
95% CI for LS mean	-50.704, -26.153	-66.288, -30.482
Difference in LS means (givinostat-placebo)	9.96	
95% CI for difference in LS means	-12.071, 31.983	
P-value	0.3723	

Abbreviations: 4SC, climb 4 stairs; 6MWT, 6-minute walking test; CI, confidence interval; ITT, Intent-to-Treat population; LS, least square; N, number of subjects in the analysis set; n, number of subjects meeting the criterion.
Notes: Baseline was defined as the last non-missing value recorded prior to or on the date of first study treatment. Subjects were included in the analysis regardless of whether they stopped the test prematurely, using the distance walked at the end of the test.
LS means, CIs, and p-values were obtained from an analysis of covariance model on change from baseline in distance walked at the end of the 6MWT at Month 18 with baseline values for: 4SC, time to rise from floor, time to run/walk 10 metres, 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.
Source: Page 129 of Applicant's study report

North Star Ambulatory Assessment

Mean (SD) total NSAA scores at baseline were 24.44 (5.094) and 24.67 (4.787) in the givinostat and placebo groups, respectively, and decreased (worsened) throughout the treatment period. Mean (SD) total NSAA scores at Month 18 were 21.89 (6.481) and 19.87 (6.974) in the givinostat and placebo groups, respectively. Mean (SD) change from baseline NSAA total score were -1.32 (4.248) at Week 48, -1.46 (4.402) at Week 60, and -2.56 (4.450) at EOS for the givinostat group, whereas mean (SD) change from baseline NSAA total score were -3.41 (3.837) at Week 48, -5.49 (5.675) at Week 60, and -4.79 (4.652) at EOS for the placebo group.

An analysis of total NSAA score by change from baseline at 18 months is presented in Table 23 for the Target Population in the ITT analysis set. At 18 months, the LS mean (95% CI) change from baseline in the NSAA total score was -2.66 (-3.563, -1.759) in the givinostat group and -4.58 (-5.891, -3.260) in the placebo group, with an LS mean difference (givinostat-placebo) of 1.91 (p-value: 0.0209). There was nominally significant less worsening on the NSAA for the treatment group compared to placebo. These results were also less sensitive to missing data handling than the primary analysis results.

Table 23. Analysis of Total NSAA Score, Change From Baseline at 18 Months (ITT Analysis Set-Target Population)

Statistics	Givinostat (N=81)	Placebo (N=39)
Number of subjects included in analysis, n (%)	81 (100.0)	39 (100.0)
LS mean	-2.66	-4.58
95% CI for LS mean	-3.563, -1.759	-5.891, -3.260
Difference in LS means (givinostat-placebo)	1.91	
95% CI for difference in LS means	0.295, 3.533	
P-value	0.0209	

Abbreviations: 4SC, climb 4 stairs; ITT, Intent-to-Treat population; CI, confidence interval; LS, least square; N, number of subjects in the analysis set; n, number of subjects meeting the criterion; NSAA, North Star Ambulatory Assessment.
Notes: Baseline was defined 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 total NSAA score at Month 18 with baseline values for total NSAA score, 4SC, time to rise from floor, time to run/walk 10 metres, distance walked in 6 minutes, and rederived age at first dose fitted as covariates, with concomitant steroid use and treatment group as independent classification factors.
Source: Page 131 of Applicant's study report

Analysis of cumulative loss of function on the NSAA over 18 months of treatment is presented in Table 24 for the Target Population in the ITT analysis set. The treatment effect was demonstrated with a ratio (givinostat/placebo) of cumulative failures of 0.61 (p-value: 0.020).

Table 24. Analysis of Cumulative Loss of Function on the NSAA Over 18 Months of Treatment (ITT Analysis Set- Target Population)

Statistics	Givinostat (N=81)	Placebo (N=39)
Number of subjects included in analysis, n (%)	81 (100.0)	39 (100.0)
Estimated cumulative failures	3.42	5.56
95% CI for estimated cumulative failures	2.692, 4.334	4.002, 7.715
Ratio of cumulative failures (givinostat / placebo)	0.61	
95% CI for ratio of cumulative failures	0.408, 0.927	
P-value	0.0202	

Abbreviations: 4SC, 4-stair climb; CI, confidence interval; ITT, Intent-to-Treat population; N, number of subjects in the analysis set; n, number of subjects meeting the criterion; NSAA, North Star Ambulatory Assessment.
Notes: Baseline was defined as the last nonmissing value recorded prior to or on the date of first study treatment. At each postbaseline visit, failure to perform each of the 17 items of the NSAA was determined as a score transition from 2 or 1 at baseline to 0 at the respective visit. Cumulative postbaseline items failed was the total number of items failed across all postbaseline visits. Baseline total items failed was the total number of items with a score of 0 at baseline. For subjects who withdraw early, the Early Withdrawal visit itself was excluded from the cumulative count. Subjects were excluded if all items at baseline were scored zero. Estimated cumulative failures, ratio of cumulative failures, CIs, and p-values were obtained from a negative binomial regression on the subject cumulative number of failures across all postbaseline visits. Total failed items at baseline, baseline values for 4SC, time to rise from floor, time to run/walk 10 metres, distance walked in 6 minutes, and rederived age at first dose (in years and months) were included as independent covariates in the model, with randomised treatment group and concomitant steroid use included as independent classification factors. A lower ratio indicates a greater reduction in cumulative loss of function across 18 months for givinostat compared with placebo.
Source: Page 134 of Applicant's study report

Description and Analysis of Fat Fraction of Vastus Lateralis Muscle

Fat infiltration in the vastus lateralis (VL) muscle of the thigh is a characteristic of disease progression in patients with DMD and was measured using a noninvasive objective imaging

method called MRS to assess the efficacy of givinostat versus placebo in the magnetic resonance (MR) cohort, i.e., those randomized Target population subjects with at least one post-baseline MRI/MRS assessment. An analysis of VL MFF assessed by MRS (%) by change from baseline at 18 months is presented in Table 25 for the Target Population in the MR cohort. Results indicated that treatment with givinostat had less increase in fat fraction by approximately 30% (LS mean difference [givinostat-placebo]: -2.92%; p-value: 0.0354) after 18 months.

Table 25. Analysis of Fat Fraction of Vastus Lateralis Muscle Assessed by MRS (%), Change From Baseline at 18 Months (Magnetic Resonance Cohort- Target Population)

Statistics	Givinostat (N=77)	Placebo (N=37)
Number of subjects included in analysis, n (%)	77 (100.0)	37 (100.0)
LS mean	7.63	10.56
95% CI for LS mean	6.098, 9.172	8.331, 12.783
Difference in LS means (givinostat-placebo)	-2.92	
95% CI for difference in LS means	-5.641, -0.204	
P-value	0.0354	

Abbreviations: CI, confidence interval; LS, least square; MRS, magnetic resonance spectroscopy; N, number of subjects in the analysis set; n, number of subjects meeting the criterion; VL MFF, vastus lateralis muscle fat fraction.
Notes: Baseline was defined as the last nonmissing 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 VL MFF at Month 18 with baseline VL MFF and rederived age at first dose fitted as covariates, with concomitant steroid use and treatment group as independent classification factors.
Source: Page 139 of Applicant's study report

The analysis of VL MFF data at Month 18 in the MR set is sensitive to missing data handling despite there only being a very small proportion having missing data. Similar to the primary analysis, the applicant used single value imputation for missing data which relies on a very strong assumption for validity and is likely to underestimate the true variability. The Applicant's imputed values do not appear to agree with the analysis model prediction for the subjects who are missing Month18 VL MFF or the observed pattern of the earlier data for the 2 individual subjects with missing Month 18 VL MFF. The reason seems to be that the Applicant's imputation is based on the mean final VL MFF value, irrespective of baseline, and not the final mean change from baseline. The baseline for the subject who dropped out of the givinostat arm is in the 95th percentile, i.e., nearly the highest baseline score observed, which makes imputing with the average final VL MFF quite optimistic since higher baseline predicts a higher final VL MFF score. This also conflicts with the pattern of observed VL MFF for this subject: at Week 48 the VL MFF had increased from baseline by 10, but the Month 18 imputed value is a decrease of 1.3, likely due to the use of the average final VL MFF for a subject with a baseline in the 95th percentile and the imputation method not accounting for the baseline. In fact, if the imputation was based on the mean change at Month 18 in completers from the same treatment group and concomitant steroid therapy stratum, the VL MFF analysis at Month 18 is no longer nominally significant (-2.43 [95% C.I.: -5.06 ,0.21], p = 0.071). The analysis results based on completers only is an estimated difference of -2.17 (SE = 1.39), p = 0.122 (N = 112). The Month 18 difference based on MMRM, which uses all observed post-baseline data without imputation and which is valid under a weaker assumption regarding the missing data, i.e., missing at random, is -2.54 (SE = 1.40), p = 0.073 (N = 114). Therefore, it appears that the VL MFF data

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would not be nominally significant at Month 18 based on a method that makes missing data assumptions more in line with regulatory guidances on missing data handling. See Section 6.3.2 or further analysis of VL MFF as a pharmacodynamic biomarker.

Hochberg Analysis of Secondary Endpoints

Summary of Applicant p-values and adjusted CIs for the treatment effect on each key secondary efficacy endpoint at 18 months following the Hochberg procedure is presented in Table 26 for the Target Population in the ITT analysis set. Key secondary endpoints did not reach formal statistical significance based on the pre-specified Hochberg analysis.

Table 26. 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 for treatment effect	Adjusted CI for treatment effect	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
Total NSAA	0.0209	-0.282, 4.110	0.0083	No
Cumulative loss of function	0.0202	0.350, 1.081	0.0071	No

Abbreviations: ITT, Intent-to-Treat population; 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: Page 139 of Applicant's study report

6.2.1.5. Subgroup Analyses, Study 48

Age at baseline in the Target population of Study 48 ranged from 6 to 14 years with a median of 9.7 years. Subgroup analyses of Month 18 4SC by age ≤9.7 versus age >9.7 suggested possible inconsistency across age subgroups (Table 27). The difference between age subgroups was less prominent with the Applicant's age grouping based on age ≤8 versus age >8, but there were only about 25 % subjects with age ≤ 8. Regardless of the split chosen, there appears to be a trend towards less treatment effect on 4SC at Month 18 for higher ages, although it should be noted that the Study was not powered to detect differences between age groups. All subjects in Study 48 were male and 92% were White with less than 3% (and ≤2% placebo subjects) in any other Race group, so differences in how givinostat worked by Gender and Race subgroups could not be determined.

Table 27. Subgroup Analyses for Primary Endpoint by Age Group- Target Population

Age Subgroup	Treatment Group	Baseline Mean (SD)	Month 18 LS Mean 4-Stair Climb (95% C.I.)	Treatment Group LS Mean Difference (95% C.I.)
Age ≤ 9.7	Givinostat (N=40)	3.72(1.3)	3.96 (1.92,6.00)	-3.62 (-6.82,-0.42)
	Placebo (N=20)	3.90(1.2)	7.58 (4.91,10.24)	
Age > 9.7	Givinostat (N=41)	3.06(0.9)	4.58 (2.65,6.51)	0.24 (-1.13,1.61)
	Placebo (N=19)	3.04(1.0)	4.34 (2.23,6.44)	

Source: Statistical Reviewer's analysis
Abbreviations: CI, confidence interval; LS, least squares; N, number of subjects in treatment arm; SD, standard deviation

The treatment difference on the 4SC at Month 18 was numerically favoring placebo in the US subgroup, but this could be a subgroup sample size issue (the US accounted for only 23% of the Target study population). See Table 28.

Table 28. Subgroup Analyses for Month 18 4-Stair Climb by U.S. and non-U.S.- Target Population

Country	Treatment Group	Baseline Mean (SD)	Month 18 LS Mean 4-Stair Climb (95% C.I.)	Treatment Group LS Mean Difference (95% C.I.)
U.S.	Givinostat (N=17)	3.12(0.8)	6.07 (4.00, 8.13)	0.53 (-1.67, 2.73)
U.S.	Placebo (N=11)	3.57(0.7)	5.54 (3.38, 7.69)	0.53 (-1.67, 2.73)
Non-U.S.	Givinostat (N=64)	3.46(1.2)	3.13 (1.45, 4.80)	-2.59 (-4.65, -0.52)
Non-U.S.	Placebo (N=28)	3.44(1.3)	5.71 (3.69, 7.74)	-2.59 (-4.65, -0.52)

Source: Statistical Reviewer's analysis
Abbreviations: CI, confidence interval; LS, least squares; N, number of subjects in treatment arm; SD, standard deviation; U.S., United States

Off-Target Subgroup

Table 29 shows summary statistics for 4SC time in the Off-Target population/subgroup as compared to the Target population/subgroup that was designated as the population for the primary analysis. The baseline 4SC time was nominally significantly higher in the Off-Target population than in the Target population, indicating a somewhat more advanced disease population. The table shows that the distribution of Month 18 4SC time is also different within both treatment groups between Target and Off-Target groups. In particular, the Month 18 standard deviation is roughly twice as large in the Off-Target population and the mean 4SC is also bigger than in the corresponding Target subgroup that was designated primary, likely with the expectation of such differences.

In addition, the proportion of subjects unable to perform the 4SC test at Month 18 was higher in the Off-Target population: in the Off-Target subgroup 2 (9%) placebo and 1 (3%) givinostat subject were unable to perform the Month 18 4-stair climb, whereas in the Target subgroup 1(3%) and 0(0%) were unable to perform the test. Because of these distributional differences between strata, a pooled analysis of the Off-Target and Target populations would be questionable unless these differences were addressed.

The Off-Target subgroup has a smaller sample size and greater variability, so the subgroup specific analysis of the treatment difference in this non-primary subgroup is not nominally significant despite a larger estimated treatment difference: -2.78 (SE = 2.60), p = 0.290 (or p = 0.177 based on log transformed 4SC analysis). The estimated treatment difference indicates that all patients with DMD, even those not meeting the Target population criteria, could have potential for treatment benefit with givinostat compared to placebo.

Table 29.Table Summary Statistics for Four Stair Climb time in Off Target and On Target Strata

	Givinostat			Placebo			Total N
Population group	N	Baseline 4SC Mean (SD)	Month 18 4SC Mean (SD)	N	Baseline 4SC Mean (SD)	Month 18 4SC Mean (SD)	
Off-Target	37	3.9 (1.4)	6.6(7.9)	22	3.7(1.6)	9.8(13.3)	59
Target	81	3.4 (1.1)	4.7(3.2)	39	3.5(1.3)	6.4(7.7)	120

Source: Statistical Reviewer's Analysis
Note: Off-Target-16% givinostat and 14% placebo month 18 4SC were missing so had to be imputed;
Note: Target- 10% givinostat and 13% placebo were missing so had to be imputed
Abbreviations: 4SC, 4-stair climb; N, number of subjects in treatment arm; SD, standard deviation

6.3. Key Efficacy Review Issues

6.3.1. Robustness of Study 48

Issue

Determine the ability of Study 48 to serve as an adequate and well-controlled study that contributes to substantial evidence of givinostat in the treatment of patients with DMD and the ability of the exposure-response (E-R) analysis to strengthen the robustness of Study 48.

Background

In Study 48, subjects were randomized in a double-blind manner to a flexible dosing regimen, initially starting with a dose as high as possible (Dose A) based on the phase 2 trial and allowing a dose reduction based on emerging toxicity (Treatment Regimen 1). The protocol was later amended to Treatment Regimen 2, which used a lower starting dose (Dose B) and again allowed dose reduction based on emerging toxicity (Dose C). The impact of the dose modifications on the interpretability of the study results and ability to determine a safe and effective dose was unclear at the time of NDA submission. There were additional concerns identified prior to submission and during the review regarding the possibility of functional unblinding due to the frequent GI-related adverse events and the thrombocytopenia resulting in dose modifications. Given the use of effort-dependent outcome measures, there was a concern on how the potential unblinding would impact the robustness of the study results.

Additionally, it was unclear if the available data from Study 48 were robust enough to support effectiveness of givinostat in patients with DMD given the statistical limitations outlined above (see Section 6.2.1.4) and the limited understanding of the dose and exposure that would be expected to see a treatment effect.

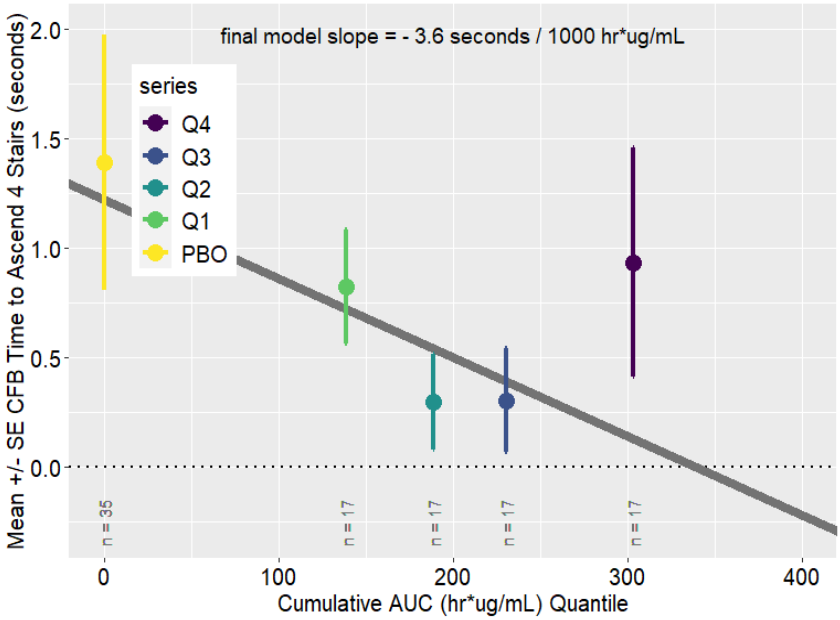
Assessment

In order to support the robustness of the Study 48 results and identify the appropriate dose(s) for labeling, E-R analyses were conducted by the clinical pharmacology review team to evaluate the relationship between givinostat AUC and change from baseline (in efficacy endpoints (4SC, NSAA total score, and 6MWT) from Study 48. Cumulative AUC (through the beginning of the dosing to the end of study), was utilized as the PK metric in the E-R analyses for efficacy. If the analyses support a relationship between increasing cumulative givinostat AUC and improvement in a clinical endpoint, such a result supports efficacy findings for givinostat in treatment of DMD patients. Note that a reduction in change from baseline in 4SC is consistent with improvement. For change from baseline in NSAA total score and 6MWT, an increase is consistent with improvements in both of these endpoints.

The cumulative AUC for givinostat plasma concentration profiles were generated for each subject, based on individual PK parameters and dosing history that accounts for dose changes and missed doses during the study. These exposure-response analyses accounted for concomitant steroid use and baseline value for each endpoint of interest. In addition to the Month 18 timepoint used in the primary efficacy analyses, the Month 12 timepoint was also assessed due to the variations in dosing history across patients (different timing of dose reductions, instances

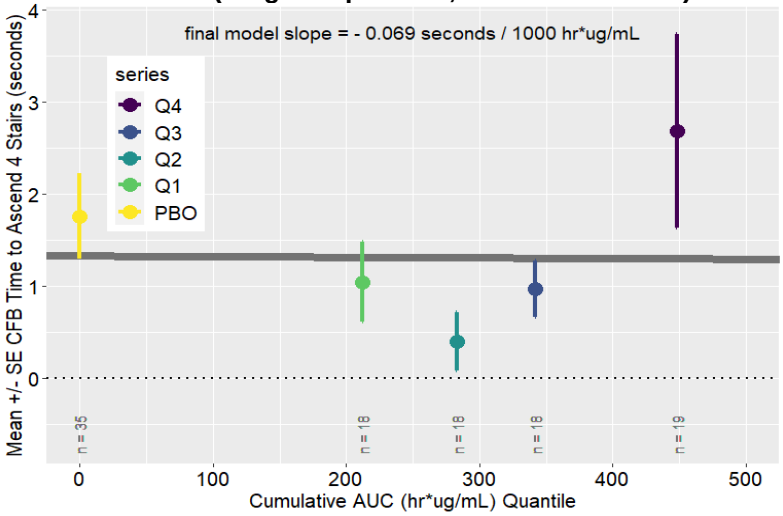
where one daily dose is missed, and temporary dose pausing). The final E-R model relating cumulative AUC to change from baseline in 4SC, NSAA total, and 6MWT at Month 12 and Month 18 are presented in Figure 4 through Figure 9.

Figure 4. Relationship Between the Month 12 4SC Change From Baseline and Month 12 Cumulative AUC (Target Population, Placebo Included)



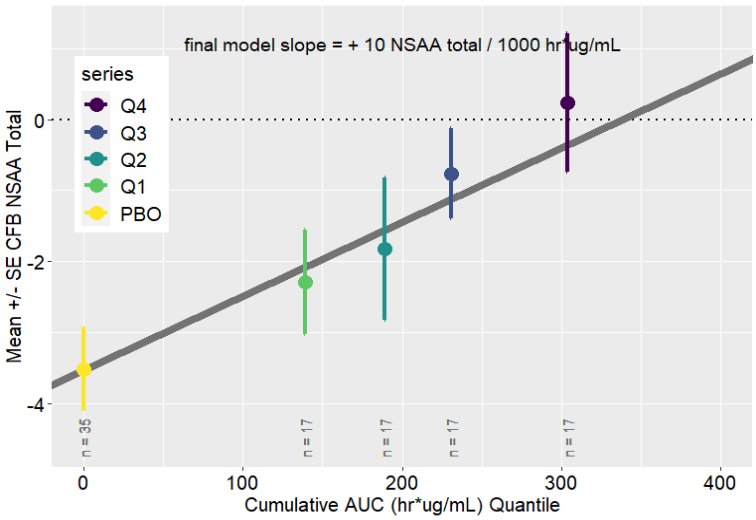
Source: Reviewer's analyses
Note: A reduction in 4SC represents benefit. The dots and vertical line segments represent the mean ± standard error of efficacy within the quantile of cumulative AUC. The placebo group is presented at a location of 0 as each subject in the placebo group was assigned a value of 0 cumulative AUC. After adjusting for covariates, the slope of cumulative AUC as a predictor in the final model is listed at the top of the plot. The dark sloped line represents a relationship of cumulative AUC to efficacy in the final model for a subject with the median baseline efficacy value and the weighted average of each concomitant medication. The units for the slope of cumulative AUC are points (seconds) / (1000 hr*ug/mL).
Abbreviations: 4SC, 4-stair climb; AUC, area under the concentration-time curve; CFB, change from baseline; hr, hour; PBO, placebo; Q, quartile; SE, standard error

Figure 5. Relationship Between the Month 18 4SC Change From Baseline and Month 18 Cumulative AUC (Target Population, Placebo Included)



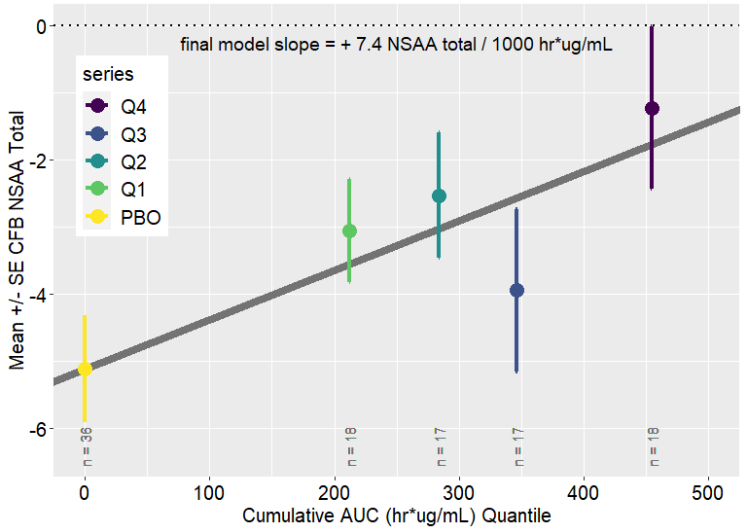
Source: Reviewer's analyses
Note: A reduction in 4SC represents benefit. The dots and vertical line segments represent the mean ± standard error of efficacy within the quantile of cumulative AUC. The placebo group is presented at a location of 0 as each subject in the placebo group was assigned a value of 0 cumulative AUC. After adjusting for covariates, the slope of cumulative AUC as a predictor in the final model is listed at the top of the plot. The dark sloped line represents a relationship of cumulative AUC to efficacy in the final model for a subject with the median baseline efficacy value and the weighted average of each concomitant medication. The units for the slope of cumulative AUC are points (seconds) / (1000 hr*ug/mL).
Abbreviations: 4SC, 4-stair climb; AUC, area under the concentration-time curve; CFB, change from baseline; hr, hour; PBO, placebo; Q, quartile; SE, standard error

Figure 6. Relationship Between the Month 12 NSAA Total Score Change From Baseline and Month 12 Cumulative AUC (Target Population, Placebo Included)



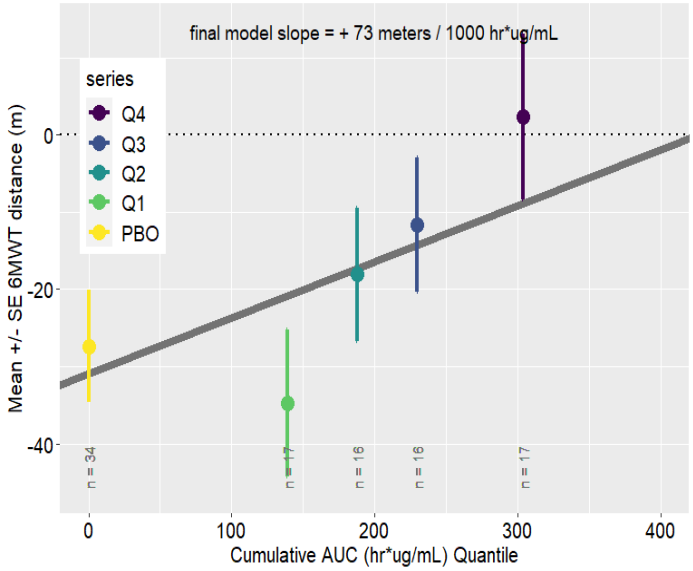
Source: Reviewer's analyses
An increase in NSAA total represents benefit. The dots and vertical line segments represent the mean ± standard error of efficacy within the quantile of cumulative AUC. The placebo group is presented at a location of 0 as each subject in the placebo group was assigned a value of 0 cumulative AUC. After adjusting for covariates, the slope of cumulative AUC as a predictor in the final model is listed at the top of the plot. The dark sloped line represents a relationship of cumulative AUC to efficacy in the final model for a subject with the median baseline efficacy value and the weighted average of each concomitant medication. The units for the slope of cumulative AUC are points (NSAA total) / (1000 hr*ug/mL).
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; NSAA, North Star Ambulance Assessment; PBO, placebo; Q, quartile; SE, standard error

Figure 7. Relationship Between the Month 18 NSAA Total Score Change From Baseline and Month 18 Cumulative AUC (Target Population, Placebo Included)



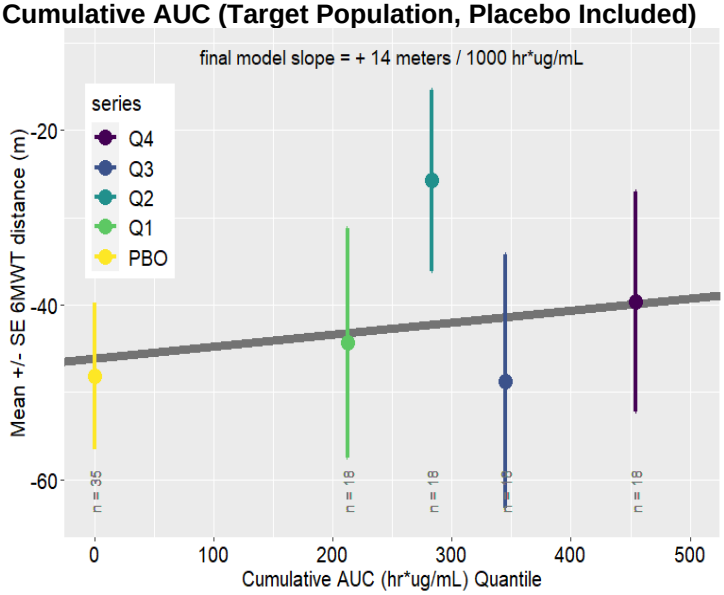
Source: Reviewer's analyses
Note: An increase in NSAA total represents benefit. The dots and vertical line segments represent the mean ± standard error of efficacy within the quantile of cumulative AUC. The placebo group is presented at a location of 0 as each subject in the placebo group was assigned a value of 0 cumulative AUC. After adjusting for covariates, the slope of cumulative AUC as a predictor in the final model is listed at the top of the plot. The dark sloped line represents a relationship of cumulative AUC to efficacy in the final model for a subject with the median baseline efficacy value and the weighted average of each concomitant medication. The units for the slope of cumulative AUC are points (NSAA total) / (1000 hr*ug/mL).
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; NSAA, North Star Ambulance Assessment; PBO, placebo; Q, quartile; SE, standard error

Figure 8. Relationship Between the Month 12 6MWT Change From Baseline and Month 12 Cumulative AUC (Target Population, Placebo Included)



Source: Reviewer's analyses
An increase in the 6-minute walk test distance represents benefit. The dots and vertical line segments represent the mean ± standard error of efficacy within the quantile of cumulative AUC. The placebo group is presented at a location of 0 as each subject in the placebo group was assigned a value of 0 cumulative AUC. After adjusting for covariates, the slope of cumulative AUC as a predictor in the final model is listed at the top of the plot. The dark sloped line represents a relationship of cumulative AUC to efficacy in the final model for a subject with the median baseline efficacy value and the weighted average of each concomitant medication. The units for the slope of cumulative AUC are points (meters) / (1000 hr*ug/mL).
Abbreviations: 6MWT, 6 minute walk test; AUC, area under the concentration-time curve; hr, hour; PBO, placebo; Q, quartile; SE, standard error

Figure 9. Relationship Between the Month 18 6MWT Change From Baseline and Month 18 Cumulative AUC (Target Population, Placebo Included)



Source: Reviewer's analyses
Note: An increase in the 6-minute walk test distance represents benefit. The dots and vertical line segments represent the mean ± standard error of efficacy within the quantile of cumulative AUC. The placebo group is presented at a location of 0 as each subject in the placebo group was assigned a value of 0 cumulative AUC. After adjusting for covariates, the slope of cumulative AUC as a predictor in the final model is listed at the top of the plot. The dark sloped line represents a relationship of cumulative AUC to efficacy in the final model for a subject with the median baseline efficacy value and the weighted average of each concomitant medication. The units for the slope of cumulative AUC are points (meters) / (1000 hr*ug/mL).
Abbreviations: 6MWT, 6 minute walk test, AUC, area under the concentration-time curve; PBO, placebo; Q, quartile; SE, standard error

Conclusion

Overall, for subjects that were in the ITT Analysis Set - Target Population in Study 48:

- The E-R analyses support a relationship of improving NSAA Total Score with increasing cumulative givinostat AUC at Month 12 as well as at Month 18 across the range of cumulative givinostat AUC values observed in Study 48.
- The E-R analyses support a relationship of improving 4SC and 6MWT distance with increasing cumulative givinostat AUC at Month 12 across the range of cumulative givinostat AUC values observed in Study 48. Though the E-R analyses for 4SC and 6MWT with cumulative givinostat AUC do not support a robust exposure-response relationship for these endpoints at Month 18, the Month 18 estimates for 4SC and 6MWT are trending in a direction that favors givinostat. Please refer to Section 14.5.5 for additional details.

These E-R analyses support the positive findings on the primary analysis in Study 48 to increase the overall persuasiveness of study results.

Furthermore, these exposure-response analyses, in addition to the findings of the MRS biomarker data and natural history comparisons (Section 6.3.2 and 6.3.3) help to alleviate the concerns regarding any potential bias that could have been introduced through functional unblinding, since the MRS data is an objective, non-effort dependent outcome, and the findings in the open-label extension demonstrate persistent treatment benefit on a stable dose. Study 48 is a single, positive, adequate and well-controlled study; however, the findings are not robust enough for the study to

serve as a single study capable of providing substantial evidence of effectiveness alone, and confirmatory evidence is needed.

6.3.2. MRS Biomarker Data as Confirmatory Evidence of Effectiveness

Issue

Determine if the MRS Biomarker Data is adequate to contribute to confirmatory evidence, along with the single adequate and well-controlled Study 48, to provide substantial evidence of effectiveness of a treatment benefit of givinostat in patients with DMD.

Background

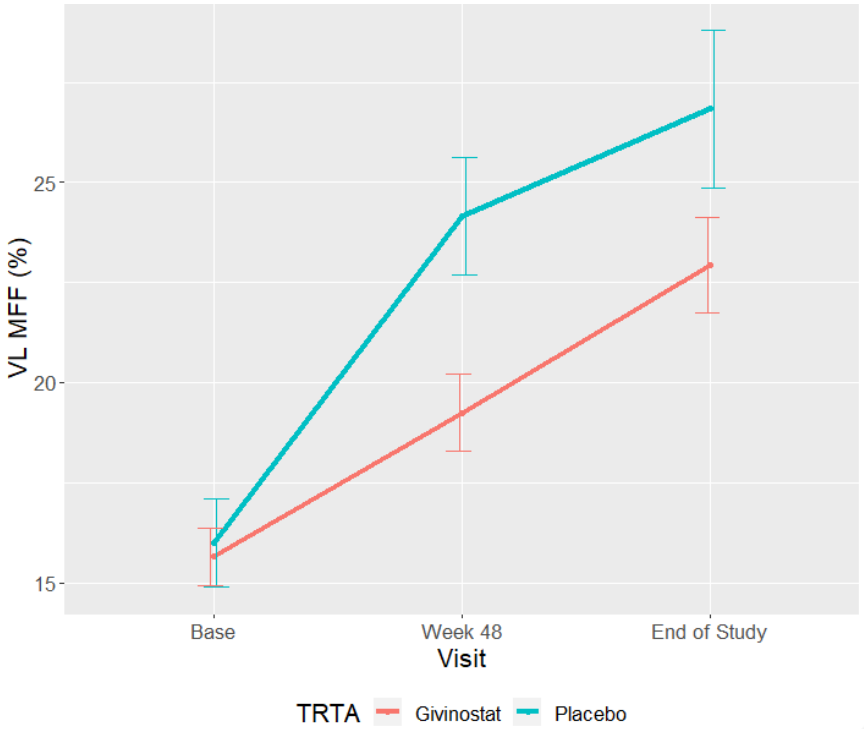
Repetitive muscle damage in DMD patients leads to muscle fiber death with replacement by fat and connective tissues. Fat infiltration in the vastus lateralis muscle of the thigh is considered as a characteristic of disease progression in patients with DMD (Barnard et al. 2020). Vastus lateralis muscle fat fraction has been shown to be closely related to functional performance and predictive of functional decline in published studies (Forbes et al. 2014; Mankodi et al. 2016; Kim et al. 2023)

There is an observed difference in fat fraction as measured by MRS between givinostat and the placebo in Study 48. The Applicant has proposed this difference as one piece of evidence to provide confirmatory evidence of efficacy. The biomarker results were fully analyzed by the clinical pharmacology review team. The MR imaging techniques were also reviewed by Dr. Daniel Krainak from the Center for Devices and Radiological Health (CDRH).

Assessment

In Study 48, VL MFF (expressed as a percentage) was included as one of secondary endpoints to assess the efficacy of givinostat, which was measured by MRS at baseline, Week 48, and EOS (approximately at Month 18). The longitudinal change of VL MFF for subjects in the Target population is presented in Figure 10. The observed VL MFF and the change from baseline for subjects in the target population taking givinostat and placebo are summarized in Table 30.

Figure 10. VL MFF Measures (Mean and SE) for Target Population With Givinostat and Placebo Treatment



Source: Reviewer's Analysis
Abbreviations: SE, standard error; TRTA, treatment arm; VL MFF, vastus lateralis muscle fat fraction

Table 30. Summary of Fat Fraction of Vastus Lateralis Muscle Assessed by MRS (%) and Change From Baseline Over Time (MR Cohort- Target Population)

	Mean (SD)			
	Givinostat (N = 77)		Placebo (N = 37)	
Visit	Observed Value	CFB	Observed Value	CFB
Baseline	15.45 (6.371)		15.95 (6.655)	
Week 48	19.25 (8.425)	3.79 (5.638)	24.15 (8.994)	8.20 (5.874)
EOS	22.93 (10.363)	7.48 (7.099)	26.84 (12.034)	10.89 (8.674)

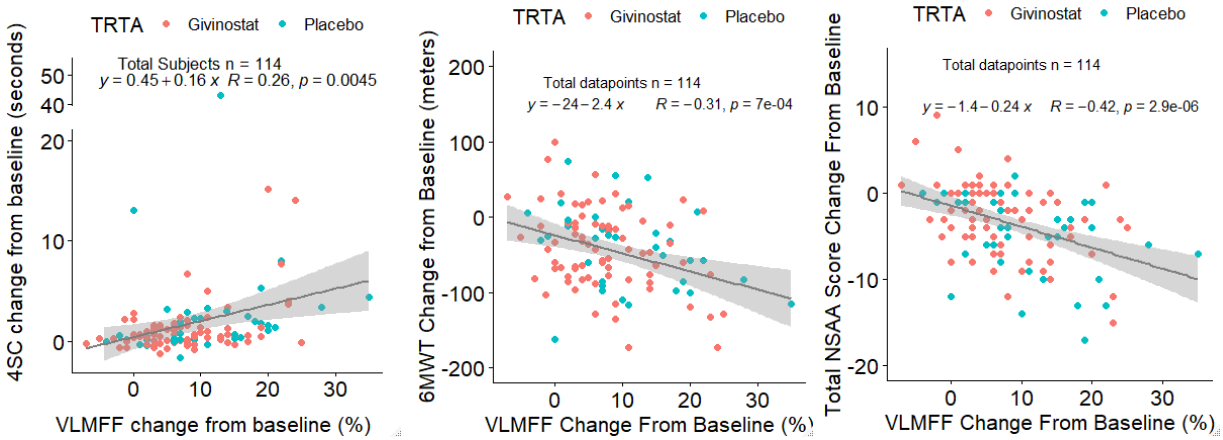
Source: Study 48 CSR, Table 35 Summary of Fat Fraction of Vastus Lateralis Muscle Assessed by MRS (%) and Change From Baseline Over Time (Magnetic Resonance Cohort –Target Population)
Note: Baseline was defined as the last non-missing value recorded prior to or on the date of first study treatment. At each time point, only patients with a value at both baseline and that time point were included in the CFB column.
Abbreviations: CFB, change from baseline; CSR, clinical study report; EOS, end of study; MR, magnetic resonance; MRS, magnetic resonance spectroscopy; N, number of patients in the analysis set; SD, standard deviation.

Based on the results, VL MFF increased with time in subjects with DMD in both the placebo and givinostat treatment arms, indicating continued disease progression. However, it appeared that the givinostat group showed smaller increase of VL MFF from baseline compared to placebo at both time points: Week 48 and EOS. At the end of the study, the mean VL MFF change from baseline for givinostat was 7.48%, compared to 10.89% for the placebo, with a nominal p-value of 0.035 for the difference. Although the difference did not reach formal statistical significance based on the Hochberg analysis (see Section 6.2.1.4), this result of VL MFF change is considered supportive of a treatment effect of givinostat given the clear trend in a difference between givinostat and placebo over multiple time points.

The relationship between VL MFF change from baseline and the changes of key efficacy endpoints from baseline to end of the study was explored. Correlations between VL MFF and the key efficacy endpoints (4SC, 6MWD and Total NSAA) are displayed in Figure 11. Based on these results, VL MFF change from baseline correlates with the change of major efficacy endpoints from baseline in general, e.g., smaller VL MFF change from baseline is associated with better performance in the efficacy endpoint measures. The trend of correlations is not affected by data imputation (i.e., whether or not the imputed results on VL MFF and imputed results on efficacy outcome measures were included in the analysis).

Figure 11. Correlations Between VL MFF Change From Baseline and the Change of Efficacy Endpoints at the End of Study 48

A: 4SC B: 6MWD C: Total NSAA score



Source: Reviewer's analysis on Study 48
Abbreviations: 4SC, 4-stair climb; 6MWD, 6 minute walk distance; NSAA, North Star Ambulatory Assessment; TRTA, treatment arm; VL MFF, vastus lateralis muscle fat fraction

Per Dr. Krainak's review of the MR spectroscopy data measurements, his analysis suggested that the change in muscle morphology appears to be a measurable observed difference between groups.

Conclusion

In conclusion, the increase of VL MFF from baseline in the subjects treated with givinostat was found to be smaller than the placebo group, and VL MFF change from baseline is associated with the key efficacy endpoints (4SC, 6MWD and Total NSAA). The VL MFF data from Study 48 demonstrate a pharmacodynamic effect on the muscle indicating that the drug is having the intended target engagement resulting in downstream benefit to the muscle. This pharmacodynamic evidence is capable of contributing to the confirmatory evidence of effectiveness of givinostat in patients with DMD.

Reference ID: 5351242

6.3.3. Natural History Data as Confirmatory Evidence of Effectiveness

Issue

Determine if the proposed natural history data are adequate to contribute to confirmatory evidence, along with the single adequate and well-controlled study, to provide substantial evidence of effectiveness of a treatment benefit of givinostat in patients with DMD.

Background

The Applicant submitted a comparison to natural history as part of their proposed sources of confirmatory evidence to demonstrate substantial evidence of effectiveness. Please refer to the review by Dr Danielle Abraham, PhD, MPH, Division of Epidemiology I, Office of Surveillance and Epidemiology for a detailed discussion on the limitations of the natural history data analysis [NDA 217865 Epidemiology Review, 09/19/2023, Addendum 02/23/2024]. The data was reviewed further to explore the interpretability of the results in light of these limitations.

In the natural history comparison, the Applicant included data from subjects treated with givinostat in Study 43, 48 and 51. The Applicant initially considered seven distinct natural history studies:

35. Magnetic Resonance Imaging and Biomarkers for Muscular Dystrophy (ImagingDMD)
36. Cooperative International Neuromuscular Research Group (CINRG)
37. Cincinnati Children's Hospital Medical Center
38. Leuven
39. North Star UK
40. DMD Italian Group
41. iMDEX

The Applicant selected ImagingDMD and CINRG for the analyses, which included a higher proportion of patients who met the Study 48 eligibility criteria. The details of the ImagingDMD and CINRG studies are provided below:

- ImagingDMD: An ongoing, observational, prospective, US multicenter natural history (NH) study. 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 anthropometric measurements, medical history, treatment history, functional tests and MR measurements of the thigh and lower leg.
- CINRG: An ongoing observational, prospective, longitudinal, international multicenter NH study. Patients with DMD were enrolled from 2006 to 2016; patients ages 2 to 28 years were enrolled from 2006 to 2009 and additional patients ages 4 to 8 years were enrolled from 2012 to 2016. Patients were assessed at baseline and months 3, 6, 9, and 12 (ambulatory), or months 6 and 12 (non-ambulatory). Long-term follow-up visits were at Months 18, 24, and annually thereafter. At each visit, historical and current use of glucocorticoid therapy was documented, and functional and pulmonary function tests were performed.

Reference ID: 5351242

Assessment

There were 345 patients included in the integrated analysis of long-term efficacy with natural history data; 148 were exposed to givinostat and 197 were untreated natural history controls. For the givinostat group, the median number of months of follow-up in the study was 25.82 (minimum = 2.9, maximum = 50.1). For the controls, the median number of months of follow-up was 36.61 (minimum 2.5, maximum 110.6). For the givinostat group, the number of subjects who received Dose A, Dose B, Dose A-B-C, and Dose B-C throughout the Studies, are 18, 69, 37, and 24, respectively.

The demographics and baseline characteristics of the study sample are shown in Table 31.

Table 31. Demographic and Baseline Characteristics of Study Sample

	Givinostat (n=148)		Controls (n=197)*	
	n (%)	Mean (SD)	n (%)	Mean (SD)
Age (years)		9.92 (2.08)		7.87 (1.66)
Age at last assessment (years)		12.25 (2.22)		11.74 (3.62)
Race				
Asian	6 (4.1)		13 (6.6)	
Black/African American	4 (2.7)		1 (0.5)	
White	128 (86.5)		111 (56.3)	
Other	10 (6.8)		7 (3.6)	
Unknown	0 (0.0)		3 (1.5)	
Missing	0 (0.0)		62 (31.5)	
Height (cm)		125.23 (8.33)		118.17 (8.35)
Weight (kg)		31.46 (9.01)		26.11 (8.14)
BMI (kg/m^2)		19.79 (4.08)		18.32 (3.52)
Time since diagnosis (years)		5.74 (2.69)		4.37 (1.88)
Time since first corticosteroids initiation (years)		3.80 (2.23)		2.10 (1.56)
Use of corticosteroids at baseline				
Deflazacort	118 (79.7)		94 (47.7)	
Other	30 (20.3)		101 (51.3)	
Baseline time to rise from floor (seconds)		6.62 (6.71)		4.97 (1.75)
Baseline time to climb 4 stairs (seconds)		3.58 (1.25)		3.58 (1.21)
Baseline time to walk/run 10 meters (seconds)		8.28 (33.60)		5.38 (1.58)
Baseline distance walked at 6 minutes (meters)		395.94 (71.03)		389.22 (56.26)

Abbreviations: SD, Standard Deviation; BMI, Body Mass Index
*Noted instances where sample size is reduced for controls: Height n=193; Weight n=195; BMI n=191; time since diagnosis n=62; time since corticosteroids n=192; use of corticosteroids at baseline n=195; baseline time to walk/run 10 meters n=196; baseline distance walked at 6 minutes n=58

Source: Table 1 in Dr Abraham's OSE review [NDA 217865 Epidemiology Review in DARRTS, 09/19/2023]
Abbreviations: OSE, Office of Surveillance and Epidemiology

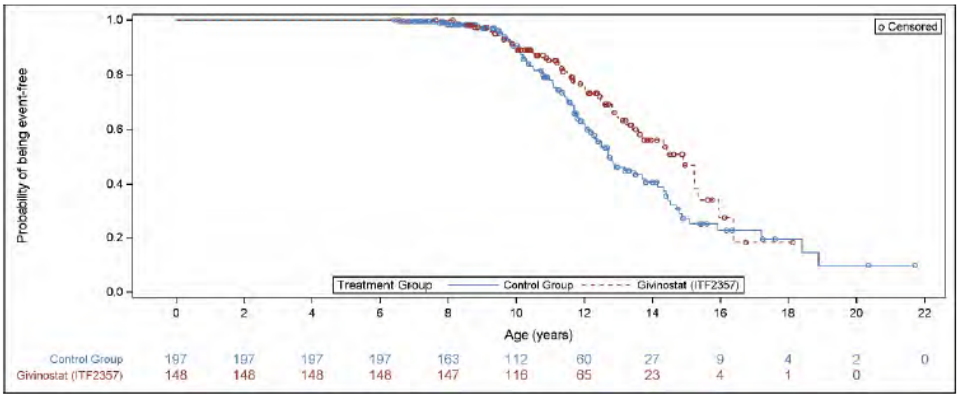
The Applicant planned a comparison between givinostat treated and natural history controls using propensity score matching. Matching was not further pursued for comparative analysis because it resulted in the loss of 20 subjects from the givinostat treated group and the standardized mean difference of the logit score and its variance ratio were not within defined thresholds.

Figure 12 shows time to persistent loss of rise from floor, persistent loss of 4SC, and persistent loss of ambulation using crude (unmatched) groups. Per Applicant:

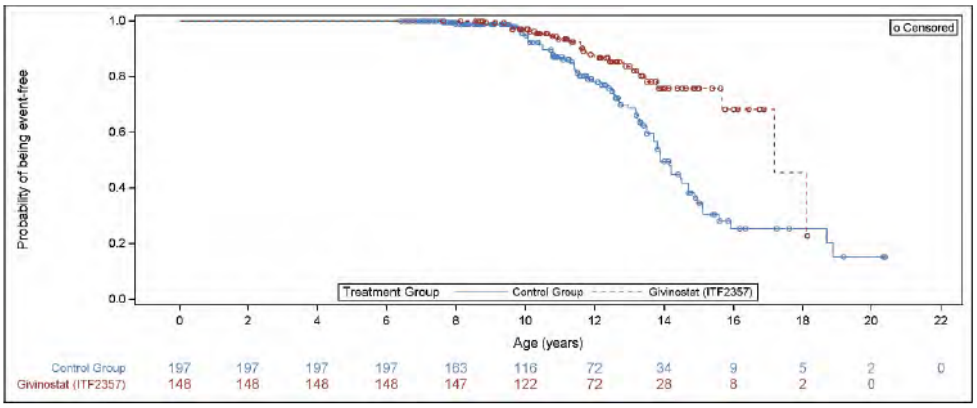
- Median age at persistent loss of rise from floor was delayed by 2.2 years (Hazard Ratio [95% CI]: 0.68 [0.47, 0.97]; p-value: 0.034).
- Median age at persistent loss of 4 standard stairs climb (4SC) was delayed by 3.3 years (Hazard Ratio [95% CI] 0.42 [0.26, 0.67]; p-value: < 0.001).
- Median age at persistent loss of ambulation was delayed by 2.7 years (Hazard Ratio [95% CI] 0.48 [0.27, 0.86]; p-value: 0.014).

Figure 12. Kaplan-Meier Curves Showing Persistent Loss of Rise From Floor, Persistent Loss of 4SC, and Persistent Loss of Ambulation Between Givinostat and Natural History Control Group.

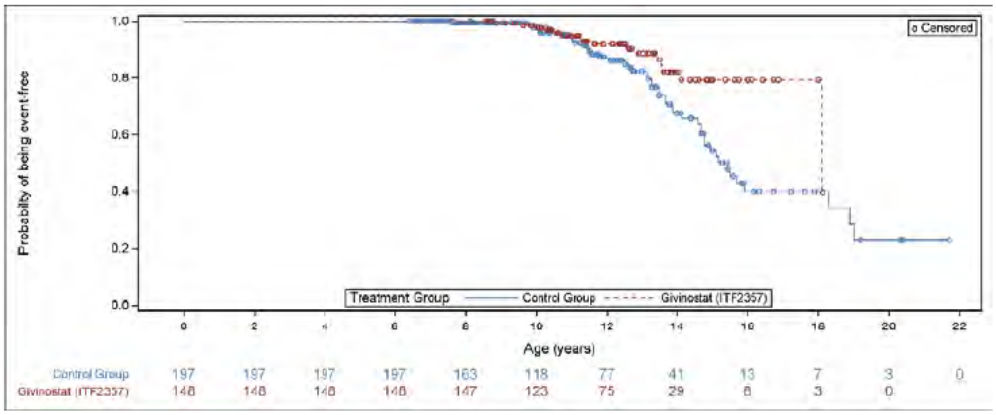
Persistent Loss of Rise From Floor



Persistent Loss of 4SC



Persistent Loss of Ambulation



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.

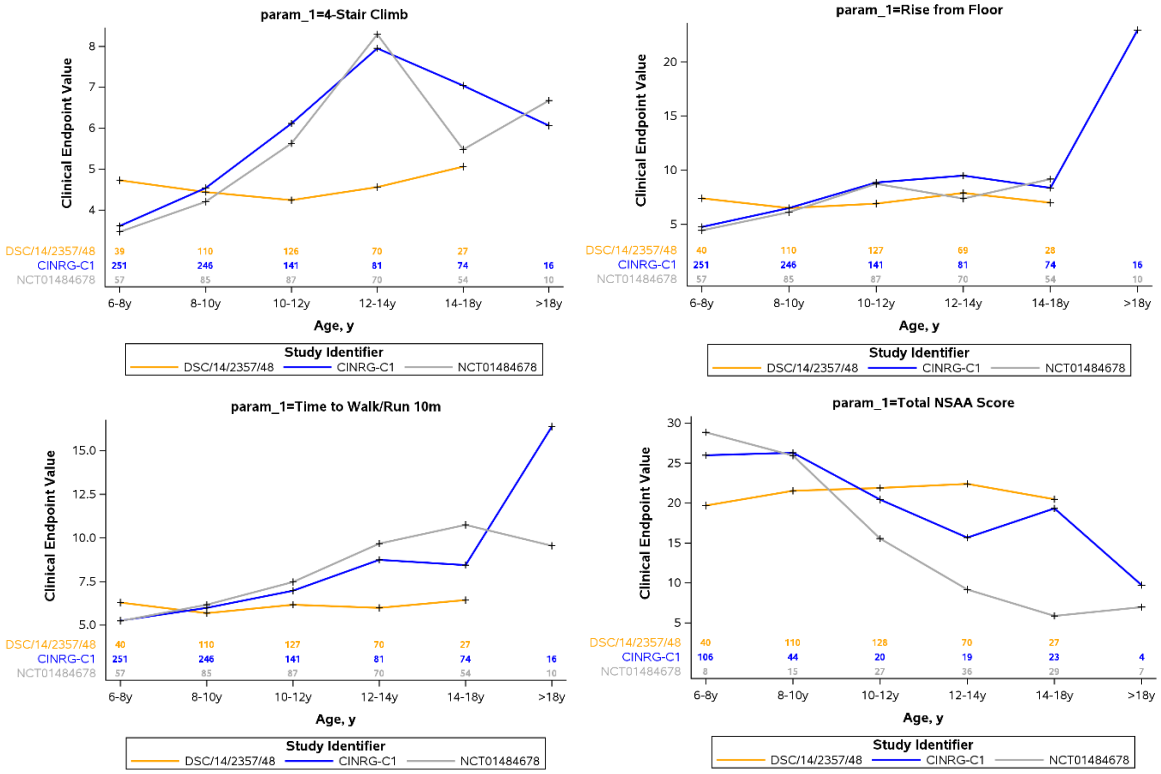
Circled values represent censored results.

Source: Figure 15 on page 99 in sum-clin-efficacy.pdf

The Office of Clinical Pharmacology reviewer (Dr. Bhattaram) explored if the disease progression was similar between subjects in the placebo group of Study 48 and those subjects in the natural history studies. If similar, then comparisons between givinostat-treated subjects and those in natural history studies would be reasonable to help quantify the treatment effect. Figure

13 shows that there are differences in disease progression between subjects in Study 48 placebo group and natural history studies for 4SC but the rise time from floor (RFF) progression looks similar.

Figure 13. Comparison of Clinical Endpoint Progression by Age Groups in Study 48 and Natural History Studies (CINRG-C, NCT01484678)



Source: Reviewer's analysis
Abbreviations: NSAA, North Star Ambulatory Assessment; y, years

The Applicant states that these differences are likely due to:

- Duration of Follow-ups (Placebo arm of Study 48, CINRG, iDMD) are different by treatment groups. For subjects in the Placebo arm of Study 48, mean duration of exposure was 512± 43.1 days (mean ± SD) corresponding to approximately 73 weeks, while for subjects in the Natural History control arm, the mean duration of follow up was 202± 140.6 weeks (mean ± SD).
- In Study 48, recording of the Time Function Tests was also dependent on the grade assigned by the assessor. In particular, if the grade was equal to 2 for the Time to Rise (i.e., Assisted Gower's – requires furniture for assist in rising from supine to full upright posture) or for the 10- Meter walk (i.e., Unable to walk independently but can walk with knee-ankle foot orthoses or support from a person), the time to perform the test was not collected. It is not possible to know if the same instructions were used in the natural history studies, since the grade was not always collected nor shared with the Applicant.

- The frequency of assessments differed between Study 48 and the natural history studies. In Study 48, time function tests were planned every 3 months for a total of 7 measurements during the 18 months of follow-up. In the natural history studies function tests were performed mostly every 12 months.

Considering the above-mentioned reasons, the reviewer conducted further analysis to explore the effect of givinostat on loss of ambulation.

Loss of ambulation is defined as:

Figure 14. Loss of Ambulation

Loss of ambulation

Loss of ambulation is defined as satisfying both the following criteria at the same visit:

- Subject is unable to perform the 6MWT due to physical inability.
- Subject is unable to complete the 10-metre walk/run test in 30 seconds or less without any support or devices (10-metre walk/run test grading ≤2).

Clinical Trial studies

For study DSC/14/2357/48:

- A subject is considered as unable to perform the 6MWT due to physical inability if missing 6MWT values were imputed as Class 2 missing values, as per study definition.
- A subject is unable to complete the 10-metre walk/run test in 30 seconds or less if fastest time to walk 10 meters is > 30 or 10 meter walk qualitative grade ≤ 2.
- Single study analysis data will be considered for these evaluations, i.e. imputed values where necessary.

For study DSC/14/2357/51:

If subject is ambulant at study baseline (i.e. no date of loss of ambulation collected at baseline):

- A subject is considered as unable to perform the 6MWT due to physical inability if missing 6MWT values were imputed as Class 2 missing values, as per study definition.
- A subject is unable to complete the 10-metre walk/run test in 30 seconds or less if fastest time to walk 10 meters is > 30 or 10 meter walk qualitative grade ≤ 2.
- Single study analysis data will be considered for these evaluations, i.e. imputed values where necessary.

If subject is non-ambulant at study baseline (i.e. date of loss of ambulation collected at baseline), loss of ambulation will be defined looking at study DSC/14/2357/51 eCRF collected variable. This will automatically exclude the possibility to check that information for persistency.

Natural History studies

For CINRG:

Due to lack of grading collection for 10-metre walk/run test, loss of ambulation will be defined as Ambulatory Status equal to 2 (i.e. non-ambulatory).

For iDMD:

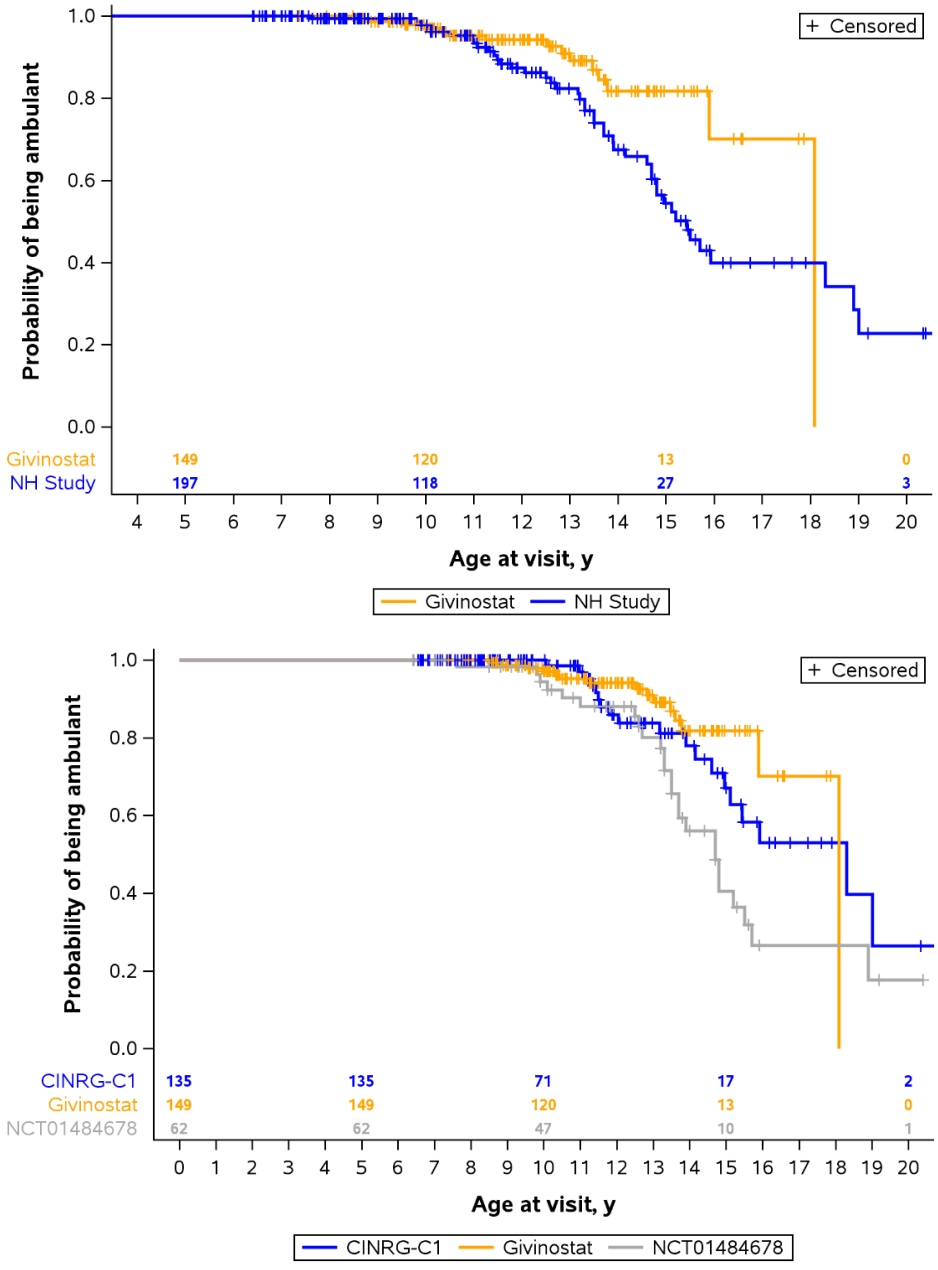
- A subject is considered as unable to perform the 6MWT due to physical inability if 6-minute walk total distance is equal to 0 = lost ability to perform.
- A subject is unable to complete the 10-metre walk/run test in 30 seconds or less if fastest time to walk 10 meters is > 30 or 10 meter walk qualitative grade ≤ 2.

Source: Text from Applicant document
Abbreviations: 6MWT, 6 minute walk test; CINRG, Cooperative International Neuromuscular Research Group; iDMD, imaging Duchenne Muscular Dystrophy

Reference ID: 5351242

Figure 15 shows the time to first reported loss of ambulation in givinostat and natural history control groups (combined and by each individual study). Natural history control group was selected on the basis of age, steroid use, and baseline 4-stair climb. The data suggest that there is a trend towards delayed loss of ambulation in givinostat treated group when compared to natural history controls.

Figure 15. Kaplan-Meier Plot of Age at First Reported Loss of Ambulation



Source: Reviewer's analysis
Abbreviations: CINRG-C1, Cooperative International Neuromuscular Research Group; NCT, National Clinical Trial; NH, natural history

It is believed that patients with certain mutations may progress more slowly or retain the ability to ambulate for a longer duration. Therefore, the distribution of mutations was considered in the above analysis. Table 32 shows that the delayed loss of ambulation in givinostat treated group is not due to imbalances in the type of mutations.

Table 32. Summary of Mutation Types and Exon Skippable Groups in Givinostat Treated Group and Natural History Data

ITT SET AND NATURAL HISTORY DATA

	Givinostat (N=118)	Placebo (N=61)	CINRG (N=135)	iDMD (N=62)	NH Control Group (N=197)
Mutation Type (n (%))					
Deletion	83 (70.3%)	41 (67.2%)	90 (66.7%)	42 (67.7%)	132 (67.0%)
Exon 8 Skippable	4 (4.8%)	2 (4.9%)	2 (2.2%)	1 (2.4%)	3 (2.3%)
Exon 20 Skippable	0	0	2 (2.2%)	1 (2.4%)	3 (2.3%)
Exon 44 Skippable	11 (13.3%)	9 (22.0%)	14 (15.6%)	5 (11.9%)	19 (14.4%)
Exon 45 Skippable	9 (10.8%)	2 (4.9%)	16 (17.8%)	6 (14.3%)	22 (16.7%)
Exon 46 Skippable	0	0	0	0	0
Exon 51 Skippable	4 (4.8%)	0	13 (14.4%)	7 (16.7%)	20 (15.2%)
Exon 52 Skippable	6 (7.2%)	5 (12.2%)	3 (3.3%)	2 (4.8%)	5 (3.8%)
Exon 53 Skippable	3 (3.6%)	1 (2.4%)	12 (13.3%)	5 (11.9%)	17 (12.9%)
Other	46 (55.4%)	22 (53.7%)	28 (31.1%)	15 (35.7%)	43 (32.6%)
Duplication	17 (14.4%)	13 (21.3%)	12 (8.9%)	8 (12.9%)	20 (10.2%)
Point mutation	18 (15.3%)	7 (11.5%)	NA	0	0
Other	0	0	22 (16.3%)	11 (17.7%)	33 (16.8%)
Missing	0	0	11 (8.1%)	1 (1.6%)	12 (6.1%)
Total	118 (100.0%)	61 (100.0%)	135 (100.0%)	62 (100.0%)	197 (100.0%)

Note 1: For Mutation Type percentages are based on the number of subjects (N), while for Exon Skippable percentages are based on number of subjects with deletion mutation.
Note 2: For CINRG no information on point mutation is available, when no large deletion/duplication was indicated this was categorized as Other mutation type.
Note 3: Control Group is given by NH Data (iDMD and CIRNG).
Note 4: For iDMD, when Point Mutation/Other was indicated this was categorized as Other mutation type.
NA= Not Applicable.

Source: Table 14.1.2.4.3 submitted by applicant on 01/12/2024
Abbreviations: CIRNG, Cooperative International Neuromuscular Research Group; iDMD, imaging Duchenne muscular dystrophy; N, number of subjects in treatment arm; n, number of subjects with mutation; NH, national history

Conclusion

The integrated analysis of long-term efficacy with natural history data indicated clinically meaningful delays in persistent loss of function among those treated with givinostat, compared to external controls. Additionally, the natural history data confirmed that the amount of deterioration in the functional endpoints in the placebo arm is consistent with findings in the natural history studies.

As detailed in OSE's review ([NDA 217865 Epidemiology Review, 9/19/2023, Addendum 2/23/2024]), there are limitations to the external control analysis using natural history data, including the heterogeneity of DMD progression, lack of baseline comparability between givinostat treated subjects and natural history controls, potential for residual confounding, enrichment of the pivotal trial sample, differences in index date and follow-up, and use of effort-based outcomes that make the externally controlled study results difficult to interpret.

However, the overall results of the natural history comparison do suggest a treatment benefit in delay of persistent loss of function compared to two natural history cohorts, which is able to confirm the clinically meaningful treatment benefit observed in the single adequate and well-controlled pivotal Study 48. Both the Division of Epidemiology I and the Office of Clinical Pharmacology agreed that, for this rare disease indication, the natural history control analyses, despite the limitations as described above, are capable of contributing to the confirmatory evidence of effectiveness of givinostat in the treatment of patients with DMD.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The nonclinical studies submitted to the NDA consisted of single dose (rat and monkey) and repeat dose (rat, dog, and monkey) general toxicity studies, a complete battery of genetic toxicology and reproductive and developmental toxicology studies, and a juvenile animal toxicology study (rat). All general toxicity and reproductive and development toxicology studies were GLP-compliant and conducted using daily oral administration.

In a 4-week study in beagle dog, doses higher than 30 mg/kg were not tolerated due to gastrointestinal (GI) toxicity consisting of vomiting and loose stools. Based on the GI effects, toxicity testing in dogs was discontinued. In rat, the primary toxicity findings consisted of decreases in platelet counts at the mid and high doses (40 and 160 mg/kg) and decreases in total WBC and bone marrow myeloid series at the high dose in a 13-week study. Similar findings were not observed in a 26-week study in rat; reductions in total white blood cells (WBC) were observed at the mid and high doses (30 and 90 mg/kg) at the end of the dosing period, but were not present after an 8-week recovery period. In monkey, primary toxicity consisted of diffuse bile duct hyperplasia and reduced thymus size and thymic involution at the high dose (30 mg/kg) in a 13-week study. At the same high dose in a 39-week monkey study, findings consisted of transient gait abnormalities and decreased grip strength, increases in ALT and bilirubin, bile duct hyperplasia, and reduced bone marrow cellularity; reduced thymus weight was seen at all doses (0, 5, 12, and 30 mg/kg). The bile duct and bone marrow findings did not completely resolve over 4-week recovery period.

In a fertility and early embryonic development study in rat (0, 40, 80, and 160 mg/kg), oral administration of givinostat prior to and throughout mating in male and female rats and continuing to gestation day 7 in females resulted in no adverse effects on fertility. However, there was an increase in corpora lutea at mid and high doses and in pre- and postimplantation loss at all doses.

In an embryofetal development study in rat (0, 40, 80, and 160 mg/kg), oral administration of givinostat throughout organogenesis resulted in reduced fetal body weight at the high dose and increases in skeletal variations (rib and thoracic vertebral abnormalities, and incomplete vertebral ossification) and visceral variations (partially undescended thymus and rudimentary or absent kidney papilla) at the mid and high doses.

In an embryofetal development study in rabbit (0, 40, 80, and 160 mg/kg), oral administration of givinostat throughout organogenesis resulted in maternal death at the highest dose tested, resulting in too few fetuses to evaluate. No adverse effects on embryofetal development were observed at the low and mid doses.

In a pre and postnatal development study in rat (0, 40, 80, and 160 mg/kg), oral administration of givinostat throughout pregnancy and lactation resulted in embryofetal mortality, stillbirths, and offspring mortality at the highest dose tested. When offspring were tested postweaning (postnatal day 49), adverse effects on behavior (decreased open field activity) were observed at all doses.

Reference ID: 5351242

In a study in juvenile male and female rats, givinostat was administered orally at doses of 0, 10, 20, and 40 mg/kg on postnatal days (PND) 7 to 27, doses of 0, 15, 30, and 60 mg/kg on PNDs 28-48, and doses of 0, 15, 45, and 90 mg/kg on PNDs 49-92. Adverse effects on behavior (increased locomotor activity and decreased auditory startle prepulse inhibition) were observed at the high dose at the end of the dosing period. Adverse effects on locomotor activity (but not prepulse inhibition) were observed at the end of the recovery period primarily at the mid and high doses. Persistent decreases in bone density were observed at all doses tested.

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

HDAC inhibitors have been approved for several oncologic indications, including for the treatment of cutaneous T-cell lymphoma (vorinostat, belinostat, and romidepsin) and for multiple myeloma (panobinostat). HDAC inhibitor-induced thrombocytopenia is a known dose-limiting toxicity of the class of drugs. Thrombocytopenia is generally dose-related, asymptomatic, and reversible upon treatment discontinuation (Subramanian et al. 2010). In addition to thrombocytopenia, neutropenia and anemia may also be seen; however, these effects are generally transient and reversible. Nausea and vomiting have also been observed with the use of HDAC inhibitors, and fatigue is a commonly seen constitutional side effect. Cardiac effects may include electrocardiogram changes and QT interval prolongation, though the clinical significance of these changes is uncertain. Metabolic side effects previously observed with other HDAC inhibitors include elevated transaminases, hyperbilirubinemia, hypoalbuminemia, hypocalcemia, hypokalemia, and hypophosphatemia. Less frequent reported side effects are elevated creatinine, hyperuricemia, elevated lactate dehydrogenase, hypertriglyceridemia, and hyperglycemia.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

Not applicable. Givinostat is a new molecular entity. It is not approved in other regions of the world.

7.3.1. Adverse Events Identified in Postmarket Experiences

Not applicable. Givinostat is a new molecular entity. It is not approved in other regions of the world.

7.3.2. Expectations on Safety

The following adverse events of special interest were identified: thrombocytopenia, hypertriglyceridemia, gastrointestinal disturbances (diarrhea, nausea, vomiting, abdominal pain),

and QT prolongation. These have been identified as adverse reactions in the clinical trials and will be described in Warnings and Precautions Section of the Prescribing Information. A postmarking requirement will be imposed to characterize the incidence, frequency, and severity of thrombocytopenia and of serious events of bleeding in patients with DMD exposed to givinostat. The Applicant will also be requested to conduct enhanced pharmacovigilance for thrombocytopenia.

Givinostat was administered with food in the clinical studies and it will be recommended to be taken with food in the prescribing information (see the discussion on food effect in Sections 8.2 and 14.1). In vitro data showed that givinostat may have a potential for direct drug-drug interaction (DDI) due to cytochrome P450 (CYP3A)-mediated metabolism and P-gp transport (see Section 8.2).

The effects of givinostat on pregnancy and lactation have not been studied; however, as DMD is primarily a disease of young male patients, use in pregnant patients would be expected to occur infrequently.

No cases of overdose have been reported with givinostat in the clinical studies to date. No specific antidote exists. No cases of abuse have been reported to date. The data collected from studies with givinostat showed little evidence indicative of abuse potential (refer to CSS review, September 14, 2023). No significant structural similarities were identified comparing givinostat to known drugs of abuse. Withdrawal and rebound effects have not been studied.

7.3.3. Additional Safety Issues From Other Disciplines

Not applicable

7.4. FDA Approach to the Safety Review

Clinical study data were provided by the clinical data scientist support team and independently analyzed using JMP software. All safety assessments and conclusions are those of the clinical review team unless otherwise specified. No major data quality or integrity issues were identified that would preclude a safety review of this NDA. There were no major identified issues with respect to recording, coding, and categorizing AEs. The Applicant's translation of verbatim terms to Medical Dictionary for Regulatory Activities Version 24.1 preferred terms of events reported in Study 48 was reviewed and found to be acceptable.

Table 33 lists the preferred terms for common adverse events that were combined together for purposes of the key safety analyses to avoid splitting of similar terms.

Table 33. Common Adverse Events Terms Combined for Safety Analyses

Preferred Term	Replaced Terms
Abdominal pain	Abdominal pain, Abdominal pain upper, Abdominal discomfort
Hypertriglyceridemia	Blood triglycerides increased, hypertriglyceridemia
Thrombocytopenia	Platelet count decreased, thrombocytopenia
Thyroid changes	Hypothyroidism, Blood thyroid stimulating hormone increased, thyroid disorder, thyroid function test abnormal, hyperthyroidism, blood thyroid stimulating hormone decreased
Hypothyroidism	Hypothyroidism, Blood thyroid stimulating hormone increased

Preferred Term	Replaced Terms
Vomiting/nausea	Nausea, vomiting, procedural nausea
Diarrhea	Diarrhea, diarrhea hemorrhagic

Source: Clinical Reviewer's Analysis of Preferred Terms

Data from the completed double blind, placebo-controlled Study 48 and the open-label long-term extension Study 51 formed the basis of the clinical safety evaluation, as well as some supportive data from the early Phase 2 study, Study 43. Safety data from Study 48 will provide the primary description of safety, with Study 51 providing supporting data.

A summary of the pivotal study design can be found in Section 3.2 and Section 6.2.1

7.5. Adequacy of the Clinical Safety Database

In total, 222 subjects aged 6 years and older with DMD have been exposed to givinostat, including 210 treated for ≥6 months, 187 subjects treated for ≥12 months and 105 treated for ≥24 months. The overall number of exposed subjects in this indication includes 118 subjects in Study 48 and 222 patients overall with DMD.

Of note, the protocol amendment that resulted in a change to the starting dose as well as the frequent dose reductions throughout the study make it more challenging to determine safety of each dose and in particular the highest dose proposed for labeling. Approximately half of the subjects in Study 48 initially had dose reductions in response to adverse events within their first three months on givinostat. Then, the weight-based starting doses were reduced for all subjects starting after protocol amendment 4. Some subjects remained on their starting dose throughout the duration of the study, while others had one or even two dose reductions. Dosing changes due to thrombocytopenia and hypertriglyceridemia complicated the assessment of the adequacy of the safety database.

This was discussed with the Applicant in a teleconference on August 25, 2022, at which point it was clear that the dosing regimen modifications would limit the ability to interpret the data to identify a safe and effective dose.

DMD is a rare, serious, and life-threatening disease; therefore, the safety database and overall subject exposure was determined to be adequate.

Table 34. Duration of Exposure, Safety Population, Study 48

Parameter	Givinostat N=118	Placebo N=61
Duration of treatment, weeks		
Mean (SD)	70.9 (11.1)	73.1 (6.2)
Median (Q1, Q3)	72.4 (71.7, 73.1)	72.4 (72, 74)
Min, max	3.4, 89.1	33.1, 85.1
Total exposure (person years)	160	86

Parameter	Givinostat N=118	Placebo N=61
Patients treated, by duration, n (%)		
<4 weeks	1 (0.8)	0
≥4 to <8 weeks	0	0
≥8 to <12 weeks	0	0
≥12 to <24 weeks	1 (0.8)	0
≥24 to <36 weeks	1 (0.8)	1 (1.6)
≥36 to <48 weeks	3 (2.5)	0
≥48 to <60 weeks	1 (0.8)	0
≥60 to <72 weeks	30 (25.4)	12 (19.7)
≥72 to <76 weeks	69 (58.5)	39 (63.9)
≥76 weeks	12 (10.2)	9 (14.8)

Source: adex.xpt and adsl.xpt; Software: R
Note: Duration is 18-month double-blind treatment period.
Abbreviations: max, maximum; min, minimum; N, number of subjects in treatment arm; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

7.6. Safety Results

7.6.1. Safety Results, Study 48

7.6.1.1. Overview of Treatment-Emergent Adverse Events Summary, Study 48

Results of the FDA analysis of adverse events for the placebo-controlled Study 48 are shown in Table 35. The proportion of subjects experiencing AEs was 95% in the givinostat group and 93% in the placebo group. The proportion experiencing SAES was 7% in the givinostat group and 3% in the placebo group. Permanent discontinuation due to AEs occurred in 4% of the givinostat group and 0% of the placebo group. Dose modification due to AEs occurred in 43% of the givinostat group and 8% of the placebo group. There were no deaths.

Table 35. Overview of Adverse Events, Safety Population, Study 48

Event Category	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
SAE	8 (6.8)	2 (3.3)	3.5 (-2.9, 9.9)
SAEs with fatal outcome	0	0	0 (0, 0)
Life-threatening SAEs	0	0	0 (0, 0)
AE leading to permanent discontinuation of study drug	4 (3.4)	0	3.4 (0.1, 6.7)[1]
AE leading to dose modification of study drug	50 (42.4)	5 (8.2)	34.2 (22.9, 45.4)[1]
AE leading to interruption of study drug	16 (13.6)	4 (6.6)	7.0 (-1.8, 15.8)
AE leading to reduction of study drug	42 (35.6)	1 (1.6)	34.0 (24.7, 43.2)[1]
AE leading to dose delay of study drug	0	0	0 (0, 0)
Other	0	0	0 (0, 0)

Event Category	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Any AE	112 (94.9)	57 (93.4)	1.5 (-5.9, 8.8)
Severe and worse	5 (4.2)	1 (1.6)	2.6 (-2.2, 7.4)
Moderate	37 (31.4)	17 (27.9)	3.5 (-10.5, 17.5)
Mild	70 (59.3)	39 (63.9)	-4.6 (-19.6, 10.3)

Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Severity as assessed by the investigator.
Note: The Applicant reported 1 less AE leading to permanent discontinuation of study drug, because they did not include subject (b) (6) who permanently discontinued the study drug after experiencing a hypertriglyceridemia AE on Day 420. The subject continued to participate until the end of the study, but discontinued drug consumption after Day 420.
Note: The Applicant reported 1 more moderate AE because the Applicant counted an AE (Pain in extremity) for subject (b) (6) that began on May 24, 2021 which is 2 months after the subject completed the study on March 18, 2021.
Abbreviations: AE, adverse event; CI, confidence interval; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with at least one event; SAE, serious adverse event

7.6.1.2. Deaths, Study 48

There were no deaths in Study 48.

7.6.1.3. Serious Treatment-Emergent Adverse Events, Study 48

Results of the FDA analysis of SAEs for the placebo-controlled Study 48 are shown in Table 36 The proportion of subjects experiencing SAEs was 7% in the givinostat group and 3% in the placebo group. No individual SAE occurred in more than one subject in the givinostat group; gastroenteritis occurred in 2 patients on placebo, and 1 patient each on treatment had gastroenteritis and viral gastroenteritis.

Table 36. Subjects With Serious Adverse Events by System Organ Class and Preferred Term, Safety Population, Study 48

System Organ Class Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Any SAE	8 (6.8)	2 (3.3)	3.5 (-2.9, 9.9)
Gastrointestinal disorders (SOC)	2 (1.7)	0	1.7 (-0.6, 4.0)
Abdominal pain	1 (0.8)	0	0.8 (-0.8, 2.5)
Vomiting	1 (0.8)	0	0.8 (-0.8, 2.5)
General disorders and administration site conditions (SOC)	1 (0.8)	0	0.8 (-0.8, 2.5)
Chest pain	1 (0.8)	0	0.8 (-0.8, 2.5)
Infections and infestations (SOC)	3 (2.5)	2 (3.3)	-0.7 (-6.0, 4.6)
Gastroenteritis viral	1 (0.8)	0	0.8 (-0.8, 2.5)
Influenza	1 (0.8)	0	0.8 (-0.8, 2.5)
Gastroenteritis	1 (0.8)	2 (3.3)	-2.4 (-7.2, 2.3)
Injury, poisoning and procedural complications (SOC)	1 (0.8)	0	0.8 (-0.8, 2.5)
Spinal fracture	1 (0.8)	0	0.8 (-0.8, 2.5)
Nervous system disorders (SOC)	1 (0.8)	0	0.8 (-0.8, 2.5)
Dizziness	1 (0.8)	0	0.8 (-0.8, 2.5)
Skin and subcutaneous tissue disorders (SOC)	1 (0.8)	0	0.8 (-0.8, 2.5)
Rash	1 (0.8)	0	0.8 (-0.8, 2.5)

Source: adae.xpt; Software: R
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: AE, adverse event; CI, confidence interval; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event; SAE, serious adverse event; SOC, system organ class

Table 37 shows SAEs grouped using the Food and Drug Administration Medical Query ([FMQ] Narrow). Viral infection is the only SAE that occurred approximately 2% more frequently in the givinostat group compared to placebo.

Table 37. Subjects With Serious Adverse Events by System Organ Class and FDA Medical Query (Narrow), Safety Population, Study 48

System Organ Class FMQ (Narrow)	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Gastrointestinal disorders (SOC)			
Abdominal pain	1 (0.8)	0	0.8 (-0.8, 2.5)
Vomiting	1 (0.8)	0	0.8 (-0.8, 2.5)
General disorders and administration site conditions (SOC)			
Dizziness	1 (0.8)	0	0.8 (-0.8, 2.5)
Infections and infestations (SOC)			
Viral infection	2 (1.7)	0	1.7 (-0.6, 4.0)
Musculoskeletal and connective tissue disorders (SOC)			
Fracture	1 (0.8)	0	0.8 (-0.8, 2.5)
Skin and subcutaneous tissue disorders (SOC)			
Rash	1 (0.8)	0	0.8 (-0.8, 2.5)

Source: adae.xpt; Software: R
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
Note: Some preferred terms are not included in any FDA medical query. Those preferred terms are not shown or counted in this table.
Note: For specific preferred terms under each FMQ, see the table "Serious Adverse Events by System Organ Class, FDA Medical Query (Narrow) and Preferred Term…"
Abbreviations: AE, adverse event; CI, confidence interval; FDA, Food and Drug Administration; FMQ, FDA medical query; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event; PT, preferred term; SOC, system organ class

Serious adverse events, as identified by the Applicant, are further described in detail below, none of which were reason for study discontinuation:

Gastrointestinal Events

Several subjects in Study 48 experienced SAEs involving abdominal symptoms (gastroenteritis, vomiting, abdominal pain) as described below. None of these SAEs appear to be related to givinostat.

- Subject (b) (6) an 8-year-old-male taking 40 mg of givinostat daily, had severe viral gastroenteritis on Study Day 14. This was accompanied by nausea, emesis, and elevated temperature and the subject was briefly hospitalized. Abnormal laboratory results included elevated white blood cell and neutrophil counts. He was given hydrocortisone, ondansetron, and paracetamol. The gastrointestinal symptoms resolved on Study Day 15, though he continued to have intermittent fevers for the next few days. The subject missed 2 days of his givinostat dose due to gastrointestinal symptoms. There were no further dose interruptions, and the subject completed the study on Day 506.

- Subject (b) (6) a 12-year-old male received givinostat 100 mg daily. He had severe vomiting on Study Day 267 accompanied by fever. He received zoledronic acid and paracetamol. He was hospitalized that day, and had the following notable abnormal laboratory results: low sodium 128 mmol/L, low potassium 3.1 mmol/L, and low chloride 91 mmol/L, elevated C-Reactive Protein (CRP), and ketones found on urine dipstick. He was given intravenous glucose and potassium gluconate. The event resolved after a 3-day hospitalization. This subject also experienced thrombocytopenia beginning on Day 16 that was not an SAE but led to dose reduction and hypertriglyceridemia beginning on Day 249 that led to temporary interruption and eventual dose reduction. The subject completed the study on Day 536.
- Subject (b) (6) a 12-year-old male receiving 80.0 mg givinostat daily experienced mild abdominal pain on Day 113 that resolved on the same day, and he had an SAE of abdominal pain of moderate intensity of Study Day 142. At that time, he was hospitalized several days for abdominal pain and fever. Abnormal laboratory results included increased neutrophils, white blood count, and CRP. There was some concern for acute appendicitis, but the event resolved on Day 144 after receiving sodium chloride. This patient also had a dose reduction due to hypertriglyceridemia and competed the study on Day 502.
- Subject (b) (6) a 6-year-old-male receiving 33.4 mg givinostat daily, was hospitalized on Study Day 30 due to vomiting and an SAE of gastroenteritis. Givinostat was interrupted because of the event, the event resolved on Day 30, and the subject was discharged after receiving IV rehydration. The event did not recur after givinostat was restarted.

Spinal fracture

Subject (b) (6) a 10-year-old male on 70.0 mg of givinostat daily had a SAE of vertebral fracture on Study Day 343 after a fall. He was hospitalized and had a CT which demonstrated T5-T9 collapsing. He received paracetamol and a corset. This was moderate in severity and did not affect medication administration. It resolved with sequelae and was felt to be unrelated to study treatment. Of note, this subject did reduce his dose for other reasons later in the study. In general, patients with DMD have a high fracture burden due to progressive myopathy and steroid-induced osteoporosis (Phung et al. 2023). There was a greater incidence of vertebral fractures in the control group compared to the givinostat group.

Chest Pain

Subject (b) (6) an 8-year-old male on 80 mg of givinostat daily experienced moderate chest pain which resolved. A few days later, the chest pain returned with increasing intensity, characterized as severe and reported as an SAE. He presented to the hospital where he was found to be weaker and required breaks after short periods of exertion. He had a normal physical examination, labs, and ECG. The dose was not changed. The symptoms did not appear related to study treatment, but were a grade 3 in severity, medically significant but not immediately life-threatening This subject also had dose reductions due to thrombocytopenia.

Rash

Subject (b) (6) an 11-year-old male taking 70 mg of givinostat daily had intermittent mild rashes on Days 5 and 28 that resolved on the same day, as well as reduced platelet count beginning on Day 20 for which the givinostat dose was reduced to 46.6 mg daily. On Study Day 35, the subject was hospitalized with an SAE of erythematous rash with concomitant headache and runny nose and mild hepatomegaly. Abnormal laboratory results included fibrinogen of 1.62 g/L (normal 1.9-4.2 g/L), creatinine of 19 umol/L (normal 22-83 umol/L), AST 229 U/L (normal 11-36 U/L), ALT 485 U/L (normal 6-43 U/L), and platelet count of 115 x 10^9 U/L (normal 140-100 x 10^9 U/L). He was in the hospital for 1 day, no treatment was reported for the event, and the event was considered resolved on Day 56. It was mild, self-resolving, and the dosage was not changed. This same subject experienced associated dizziness with headache (see below). He competed the study on Day 533, and his platelet count was still decreasing.

Dizziness

Subject (b) (6) an 11-year-old male taking 46.6 mg of givinostat experienced an SAE of dizziness on Study Day 377, 2 days after experiencing AEs of dizziness and headache after his last givinostat dose. He was admitted to the hospital where his heart rate was transiently 122 bpm with no arrhythmia, and his troponin level was increased with normal cardiac evaluation. Viremia and panic attack were both suspected, but not confirmed. The SAE was moderate and was considered resolved on Day 379, and the dosage was not changed. This same subject also experienced an SAE of rash, as referenced above.

Influenza

Subject (b) (6) a 12-year-old male, had an SAE of influenza on Study Day 241 which lasted for 4 days. He was taking 66.6 mg of givinostat which was temporarily stopped. He was seen in the ER with poor oral intake and no urine output and was hospitalized. Symptoms resolved with pharmacologic intervention including Tamiflu, Tylenol, Motrin, and hydrocortisone stress dosing.

Other designated medical events identified in Study 48 included rhabdomyolysis seen in 2 subjects in the givinostat group and none in the placebo group. These events were mild and self-resolving. Hemorrhage (defined as contusion, hematoma, and epistaxis) was seen in 22% of the givinostat group compared to 13% in the placebo group (see Table 38 below). Only the event of epistaxis was attributed by the Applicant as related to givinostat. Hepatic injury was evident in 2% of the givinostat population compared to none in the placebo group. Additional information regarding hepatic injury can be found under Drug-Induced Liver Injury in Section 7.6.1.6 below.

Table 38. Subjects With Hemorrhage, Safety Population, Study 48

System Organ Class FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Vascular disorders (SOC)			
Hemorrhage (FMQ)	26 (22.0)	8 (13.1)	8.9 (-2.4, 20.2)
Contusion	11 (9.3)	3 (4.9)	4.4 (-3.1, 12.0)
Hematoma	5 (4.2)	0	4.2 (0.6, 7.9)[1]
Epistaxis	7 (5.9)	5 (8.2)	-2.3 (-10.4, 5.8)

Source: Clinical Reviewer Created Table from adae.xpt
[1] Indicates that 95% confidence interval excludes zero.
Abbreviations: CI, confidence interval; FMQ, FDA medical query; N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

7.6.1.4. Adverse Events and FDA Medical Queries Leading to Treatment Discontinuation, Study 48

Results of the FDA analysis of AEs leading to treatment discontinuation for the placebo-controlled Study 48 are shown in Table 39. The proportion of subjects experiencing AEs leading to treatment discontinuation was 3% in the givinostat group and 0% in the placebo group. There were a total four AEs leading to discontinuation, each with a frequency of 1-2% in the givinostat group and 0% in the placebo group.

Table 39 shows AEs leading to treatment discontinuation grouped by FDA Medical Query (Narrow).

Table 39. Patients With Adverse Events Leading to Treatment Discontinuation by System Organ Class and FDA Medical Query (Narrow), Safety Population, Trial DSC/14/2357/48

System Organ Class FMQ (Narrow)	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Any AE leading to discontinuation	4 (3.4)	0	3.4 (0.1, 6.7)[1]
Gastrointestinal disorders (SOC)			
Diarrhea	2 (1.7)	0	1.7 (-0.6, 4.0)
Abdominal pain	1 (0.8)	0	0.8 (-0.8, 2.5)
Metabolism and nutrition disorders (SOC)			
Hypertriglyceridemia	2 (1.7)	0	1.7 (-0.6, 4.0)

Source: adae.xpt; Software: R
[1] Indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
Note: For specific preferred terms under each FMQ, see the table "Adverse Events Leading to Discontinuation by System Organ Class, FDA Medical Query (Narrow) and Preferred Term…"
Note: Some preferred terms are not included in any FDA medical query. Those preferred terms are not shown or counted in this table.
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event; PT, preferred term; SOC, system organ class

Table 40 shows a listing of subjects with adverse events leading to treatment discontinuation.

Table 40. Listing of Subjects With Adverse Events Leading to Treatment Discontinuation From Study Drug, Safety Population, Study 48

Study Arm	Patient ID	Dosage	MedDRA Preferred Term	Verbatim Term	SAE	AE Study Day of Onset/Stop	Study Day of Last Dose of Study Drug	Study Day of D/C	Investigator's Assessment of Relatedness
Givinostat	(b) (6)	10 mg/mL	Hypertriglyceridemia	Hypertriglyceridemia	N	307, 323	310	310	Related
Givinostat		10 mg/mL	Diarrhea	Diarrhea	N	205, 232	223	223	Related
Givinostat		10 mg/mL	Blood triglycerides increased	Elevated triglycerides	N	348, NA	351	351	Related
Givinostat		10 mg/mL	Abdominal pain	Abdominal pain	N	450, 542	473	473	Related
Givinostat		10 mg/mL	Diarrhea	Diarrhea (1 stool per day)	N	13, 542	473	473	Related

Source: adae.xpt; Software: R
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.
Note: Duration is 18-month double-blind treatment period.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; D/C, discontinuation; ID, identifier; IMP, investigational medicinal product; MedDRA, Medical Dictionary for Regulatory Activities; N, no; NA, not available; SAE, serious adverse event

Selected narratives for discontinuations are provided below.

Gastrointestinal Events

- Subject (b) (6) was a 7-year-old male on 60.0 mg daily of givinostat. He experienced diarrhea on Days 205 and 223, and permanently discontinued the drug on Study Day 223 because of diarrhea. He was withdrawn from the study on Day 281.
- Subject (b) (6) was a 9-year-old-boy receiving givinostat 70.0 mg daily. He experienced intermittent gastrointestinal symptoms including an AE of diarrhea on Day 13 and the AE of abdominal pain on an unspecified date around day 443 that were considered resolved on Day 542. On Study Day 473, the medication was permanently discontinued due to abdominal pain. He completed the study on day 542.

Hypertriglyceridemia

- Subject (b) (6) an 8-year-old-male receiving 66.6 mg givinostat daily experienced multiple isolated episodes of hypertriglyceridemia throughout the study. The dose was temporarily interrupted because of events on Days 58-78 (triglycerides as high as 383.5 mg/dL) and Days 105-127 (triglycerides as high as 454.87 mg/dL) with dose reduction in each case, temporarily interrupted on Days 176-208 and 253-272 with triglycerides as high as 344.5 mg/dL, and permanently stopped after event on Day 307-323 with triglycerides as high as 325.66 mg/dL) . The medication was permanently discontinued on Study Day 311because of hypertriglyceridemia.
- Subject (b) (6) was an 8-year-old male taking givinostat 53.4 mg daily. He had several adverse events of hypertriglyceridemia leading on Days 92-152 with triglycerides as high as 408.85 mg/dL for which dose was temporarily stopped and then restarted at a lower dose, and beginning on day 348 with triglycerides as high as 449.56 mg/dL for which the dose was permanently stopped. He withdrew from the study on Day 364 when his triglyceride level was slightly improved but still elevated.

Table 41 shows a listing of adverse events leading to dose modification by System Organ Class and FDA Medical Query (Narrow). These include thrombocytopenia (28%), abdominal pain and diarrhea (each 1%), and lipid disorders (hypertriglyceridemia, 8%). Only 1 subject in the placebo group modified the medication dose, due to lipid disorder.

Table 41. Patients With Adverse Events Leading to Dose Modification by System Organ Class and FDA Medical Query (Narrow), Safety Population, Study 48

System Organ Class FMQ (Narrow)	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Blood and lymphatic system disorders (SOC)			
Thrombocytopenia	33 (28.0)	0	28.0 (19.9, 36.1)[1]
Gastrointestinal disorders (SOC)			
Abdominal pain	1 (0.8)	0	0.8 (-0.8, 2.5)
Diarrhea	1 (0.8)	0	0.8 (-0.8, 2.5)
Metabolism and nutrition disorders (SOC)			
Hypertriglyceridemia	9 (7.6)	1 (1.6)	6.0 (0.2, 11.7)[1]

Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

7.6.1.5. Treatment-Emergent Adverse Events, Study 48

Table 42 shows the TEAEs that occurred in at least 2% of subjects in the givinostat group and occurred with more frequency than in the placebo group. The most common TEAEs were diarrhea, abdominal pain, thrombocytopenia, and nausea/vomiting.

The long-term safety data are consistent with the TEAEs in the pivotal study; no new safety signals appeared.

Table 42. Subjects With Common Adverse Events Occurring at ≥2% Frequency, Safety Population, Study 48 and More Frequently Than in Placebo

Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Any AE	112 (94.9)	57 (93.4)	1.5 (-5.9, 8.8)
Diarrhea	43 (36.4)	11 (18)	18.4 (5.4, 31.4)[1]
Abdominal pain	40 (33.9)	15 (24.6)	9.3 (-5.2, 22.4)
Thrombocytopenia	39 (33.1)	0	33.1 (24.6, 41.5)[1]
Vomiting/nausea	38 (32.2)	11 (18.0)	14.2 (1.4, 27.0)[1]
Hypertriglyceridemia	27 (22.9)	4 (6.6)	16.3 (6.5, 26.1)[1]
Pyrexia	15 (12.7)	5 (8.2)	4.5 (-4.6, 13.7)
Contusion	11 (9.3)	3 (4.9)	4.4 (-3.1, 12.0)
Myalgia	11 (9.3)	2 (3.3)	6.0 (-0.8, 12.9)
Rash	11 (9.3)	1 (1.6)	7.7 (1.5, 13.8)[1]
Arthralgia	10 (8.5)	1 (1.6)	6.8 (0.9, 12.8)[1]
Thyroid changes	10 (8.5)	2 (3.3)	5.2 (-1.5, 11.9)
Fatigue	9 (7.6)	0	7.6 (2.8, 12.4)[1]
Gastroenteritis	9 (7.6)	3 (4.9)	2.7 (-4.5, 9.9)
Constipation	8 (6.8)	1 (1.6)	5.1 (-0.4, 10.7)
Decreased appetite	8 (6.8)	0	6.8 (2.2, 11.3)[1]
Ligament sprain	8 (6.8)	3 (4.9)	1.9 (-5.2, 8.9)
Oropharyngeal pain	7 (5.9)	1 (1.6)	4.3 (-1.0, 9.6)
Dizziness	5 (4.2)	0	4.2 (0.6, 7.9)[1]

Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Erythema	5 (4.2)	0	4.2 (0.6, 7.9)[1]
Hematoma	5 (4.2)	0	4.2 (0.6, 7.9)[1]
Limb injury	5 (4.2)	2 (3.3)	1.0 (-4.8, 6.7)
Ear pain	4 (3.4)	1 (1.6)	1.8 (-2.8, 6.3)
Fungal skin infection	4 (3.4)	1 (1.6)	1.8 (-2.8, 6.3)
Muscular weakness	4 (3.4)	0	3.4 (0.1, 6.7)[1]
Pharyngitis	4 (3.4)	0	3.4 (0.1, 6.7)[1]
Viral infection	4 (3.4)	1 (1.6)	1.8 (-2.8, 6.3)
Weight decreased	4 (3.4)	1 (1.6)	1.8 (-2.8, 6.3)
Arthropod bite	3 (2.5)	0	2.5 (-0.3, 5.4)
Bronchitis	3 (2.5)	1 (1.6)	0.9 (-3.4, 5.2)
Feces soft	3 (2.5)	0	2.5 (-0.3, 5.4)
Hypercholesterolemia	3 (2.5)	0	2.5 (-0.3, 5.4)
Low density lipoprotein increased	3 (2.5)	0	2.5 (-0.3, 5.4)
Onychomycosis	3 (2.5)	0	2.5 (-0.3, 5.4)
Respiratory tract infection	3 (2.5)	0	2.5 (-0.3, 5.4)

Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Abdominal pain replaces: Abdominal pain, Abdominal pain upper, Abdominal discomfort
Hypertriglyceridemia replaces: Blood triglycerides increased, hypertriglyceridemia
Note: Thrombocytopenia replaces: Platelet count decreased, Thrombocytopenia
Note: Thyroid change replaces: Hypothyroidism, Blood thyroid stimulating hormone increased, Thyroid disorder, Thyroid function test abnormal, Hyperthyroidism, Blood thyroid stimulating hormone decreased
Vomiting/nausea replaces: Nausea, Vomiting, Procedural nausea
Abbreviations: AE, adverse event; CI, confidence interval; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event

TEAEs of hypothyroidism and/or thyroid stimulating hormone (TSH) increased occurred in 5% (6/118) of givinostat treated subjects and in 2% (1/61) of placebo subjects. Laboratory shifts from low to high or from normal to high TSH at any point during the study occurred in 52% (61/118) of givinostat treated subjects and in 8% (5/61) of placebo subjects. However, there were no clinically significant TSH laboratory abnormalities except for those few that were reported as TEAEs.

Table 43 shows TEAEs grouped using the FDA Medical Query (Narrow). Multiple TEAEs had risk difference 95% confidence intervals that did not include zero, indicating higher proportions in the givinostat group: diarrhea, thrombocytopenia, lipid disorder, vomiting, arthralgia, decreased appetite, and dizziness.

Table 43. Subjects With Adverse Events by System Organ Class and FDA Medical Query (Narrow), Safety Population, Study 48

System Organ Class FMQ (Narrow)	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Blood and lymphatic system disorders (SOC)			
Thrombocytopenia	39 (33.1)	0	33.1 (24.6, 41.5)[1]
Leukopenia	1 (0.8)	0	0.8 (-0.8, 2.5)

System Organ Class FMQ (Narrow)	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Cardiac disorders (SOC)			
Arrhythmia	2 (1.7)	0	1.7 (-0.6, 4.0)
Cardiac conduction disturbance	2 (1.7)	0	1.7 (-0.6, 4.0)
Tachycardia	2 (1.7)	0	1.7 (-0.6, 4.0)
Palpitations	2 (1.7)	1 (1.6)	0.1 (-3.9, 4.0)
Ear and labyrinth disorders (SOC)			
Vertigo	1 (0.8)	0	0.8 (-0.8, 2.5)
Endocrine disorders (SOC)			
Hyperglycemia	1 (0.8)	0	0.8 (-0.8, 2.5)
Gastrointestinal disorders (SOC)			
Diarrhea	44 (37.3)	12 (19.7)	17.6 (4.4, 30.9)[1]
Vomiting	34 (28.8)	8 (13.1)	15.7 (3.9, 27.5)[1]
Abdominal pain	40 (33.9)	15 (24.6)	9.3 (-4.5, 23.1)
Constipation	8 (6.8)	1 (1.6)	5.1 (-0.4, 10.7)
Nausea	9 (7.6)	4 (6.6)	1.1 (-6.8, 8.9)
Dyspepsia	20 (16.9)	10 (16.4)	0.6 (-10.9, 12.1)
General disorders and administration site conditions (SOC)			
Fatigue	12 (10.2)	2 (3.3)	6.9 (-0.2, 13.9)
Decreased appetite	8 (6.8)	0	6.8 (2.2, 11.3)[1]
Pyrexia	17 (14.4)	5 (8.2)	6.2 (-3.1, 15.6)
Dizziness	6 (5.1)	0	5.1 (1.1, 9.0)[1]
Volume depletion	1 (0.8)	0	0.8 (-0.8, 2.5)
Local administration reaction	0	1 (1.6)	-1.6 (-4.8, 1.5)
Fall	15 (12.7)	13 (21.3)	-8.6 (-20.5, 3.3)
Hepatobiliary disorders (SOC)			
Hepatic injury	2 (1.7)	0	1.7 (-0.6, 4.0)
Infections and infestations (SOC)			
Fungal infection	6 (5.1)	1 (1.6)	3.4 (-1.6, 8.5)
Viral infection	18 (15.3)	9 (14.8)	0.5 (-10.5, 11.5)
Bacterial infection	3 (2.5)	3 (4.9)	-2.4 (-8.5, 3.7)
Nasopharyngitis	43 (36.4)	26 (42.6)	-6.2 (-21.3, 9.0)
Metabolism and nutrition disorders (SOC)			
Lipid disorder	27 (22.9)	4 (6.6)	16.3 (6.5, 26.1)[1]
Hypertriglyceridemia/blood triglycerides increased	27 (22.9)	4 (6.6)	16.3 (5.3, 26.0)
Blood cholesterol increased/hypercholesterolemia	4 (3.4)	0	3.4 (-2.6, 8.4)
LDL increased	3 (2.5)	0	2.5 (-0.3, 5.4)
HDL decreased	1 (0.8)	0	0.8 (-0.8, 2.5)
Musculoskeletal and connective tissue disorders (SOC)			
Arthralgia	10 (8.5)	1 (1.6)	6.8 (0.9, 12.8)[1]
Myalgia	11 (9.3)	3 (4.9)	4.4 (-3.1, 12.0)
Rhabdomyolysis	2 (1.7)	0	1.7 (-0.6, 4.0)
Tendinopathy	2 (1.7)	0	1.7 (-0.6, 4.0)
Fracture	6 (5.1)	5 (8.2)	-3.1 (-11.1, 4.8)
Osteoporosis	0	3 (4.9)	-4.9 (-10.3, 0.5)
Back pain	6 (5.1)	8 (13.1)	-8.0 (-17.4, 1.3)
Nervous system disorders (SOC)			
Somnolence	1 (0.8)	0	0.8 (-0.8, 2.5)
Paresthesia	0	1 (1.6)	-1.6 (-4.8, 1.5)
Headache	27 (22.9)	15 (24.6)	-1.7 (-14.9, 11.5)
Psychiatric disorders (SOC)			
Anxiety	3 (2.5)	1 (1.6)	0.9 (-3.4, 5.2)

System Organ Class FMQ (Narrow)	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Parasomnia	1 (0.8)	0	0.8 (-0.8, 2.5)
Insomnia	1 (0.8)	2 (3.3)	-2.4 (-7.2, 2.3)
Renal and urinary disorders (SOC)			
Renal & urinary tract infection	2 (1.7)	0	1.7 (-0.6, 4.0)
Respiratory, thoracic and mediastinal disorders (SOC)			
Dyspnea	2 (1.7)	0	1.7 (-0.6, 4.0)
Bronchospasm	1 (0.8)	0	0.8 (-0.8, 2.5)
Cough	14 (11.9)	9 (14.8)	-2.9 (-13.5, 7.8)
Skin and subcutaneous tissue disorders (SOC)			
Rash	18 (15.3)	6 (9.8)	5.4 (-4.5, 15.3)
Erythema	6 (5.1)	2 (3.3)	1.8 (-4.2, 7.8)
Alopecia	2 (1.7)	0	1.7 (-0.6, 4.0)
Pruritus	0	1 (1.6)	-1.6 (-4.8, 1.5)
Urticaria	0	1 (1.6)	-1.6 (-4.8, 1.5)
Vascular disorders (SOC)			
Hemorrhage	26 (22.0)	8 (13.1)	8.9 (-2.4, 20.2)

Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
Note: For specific preferred terms under each FMQ, see the table “Adverse Events by System Organ Class, FDA Medical Query (Narrow) and Preferred Term…”
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event; PT, preferred term; SOC, system organ class

FMQ

- FMQ Diarrhea contains: Diarrhea, Diarrhea hemorrhagic, Dysentery
- FMQ Nausea contains: Nausea, Procedural nausea
- FMQ Abdominal Pain contains: Abdominal pain, Abdominal pain upper, Abdominal discomfort
- FMQ Vomiting contains: Vomiting

7.6.1.6. Laboratory Findings, Study 48

Chemistry

Results of the FDA analysis of chemistry laboratory value abnormalities for Study 48 are shown in Table 44. The safety population consisted of all subjects who received givinostat or placebo. Overall, there were no clinically significant patterns or trends observed in abnormalities in blood chemistry. There were more cases of low potassium (23% givinostat versus 7% placebo), high chloride (38% givinostat versus 16% placebo), low calcium (3% givinostat versus 0% placebo), low total protein (64% givinostat versus 46% placebo), high amylase (6% givinostat versus 3% placebo), and high blood urea nitrogen (30% givinostat versus 2% placebo).

Although notable more in the givinostat treatment group, the reports of low potassium were not clinically significant. In one case (subject (b) (6)), hypokalemia occurred within 1 week of diarrhea symptoms, but no reports of hypokalemia occurred at the same time as a reported AE of diarrhea. No arrhythmias were associated with hypokalemia.

It was noted that 40% of givinostat subjects with elevated BUN also had nausea and vomiting; therefore, it is possible that dehydration may have resulted in elevated BUN. There does not appear to indicate a signal for renal injury. Hepatic assessments are further discussed later in this section under Drug-Induced Liver Injury.

Table 44. Subjects With One or More Chemistry Analyte Values With Elevated or Low Values Meeting Specified Levels, Safety Population, Study 48

Laboratory Parameter	Givinostat N=118 n/Nw (%)	Placebo N=61 n/Nw (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Sodium, low (mEq/L)			
Level 1 (<132)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 2 (<130)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (<125)	0/118 (0)	0/61 (0)	0 (0, 0)
Sodium, high (mEq/L)			
Level 1 (>150)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 2 (>155)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (>160)	0/118 (0)	0/61 (0)	0 (0, 0)
Potassium, low (mEq/L)			
Level 1 (<3.6)	23/118 (19.5)	3/61 (4.9)	14.6 (5.6, 23.5)[1]
Level 2 (<3.4)	4/118 (3.4)	1/61 (1.6)	1.8 (-2.8, 6.3)
Level 3 (<3)	0/118 (0)	0/61 (0)	0 (0, 0)
Potassium, high (mEq/L)			
Level 1 (>5.5)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 2 (>6)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (>6.5)	0/118 (0)	0/61 (0)	0 (0, 0)
Chloride, low (mEq/L)			
Level 1 (<95)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 2 (<88)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (<80)	0/118 (0)	0/61 (0)	0 (0, 0)
Chloride, high (mEq/L)			
Level 1 (>108)	45/118 (38.1)	10/61 (16.4)	21.7 (9.0, 34.5)[1]
Level 2 (>112)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (>115)	0/118 (0)	0/61 (0)	0 (0, 0)
Bicarbonate, low (mEq/L)			
Missing	NA	NA	NA
Bicarbonate, high (mEq/L)			
Missing	NA	NA	NA
Glucose, low (mg/dL)			
Level 1 (<70)	29/115 (25.2)	20/54 (37.0)	-11.8 (-26.9, 3.3)
Level 2 (<54)	2/115 (1.7)	3/54 (5.6)	-3.8 (-10.4, 2.7)
Level 3 (<40)	0/115 (0)	0/54 (0)	0 (0, 0)
Glucose, fasting, high (mg/dL)			
Missing	NA	NA	NA
Glucose, random, high (mg/dL)			
Level 2 (≥200)	0/115 (0)	0/54 (0)	0 (0, 0)
Level 3 (>250)	0/115 (0)	0/54 (0)	0 (0, 0)

Laboratory Parameter	Givinostat N=118 n/Nw (%)	Placebo N=61 n/Nw (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Calcium, low (mg/dL)			
Level 1 (<8.4)	4/118 (3.4)	0/61 (0)	3.4 (0.1, 6.7)[1]
Level 2 (<8)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (<7.5)	0/118 (0)	0/61 (0)	0 (0, 0)
Calcium, high (mg/dL)			
Level 1 (>10.5)	1/118 (0.8)	2/61 (3.3)	-2.4 (-7.2, 2.3)
Level 2 (>11)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (>12)	0/118 (0)	0/61 (0)	0 (0, 0)
Magnesium, low (mg/dL)			
Missing	NA	NA	NA
Magnesium, high (mg/dL)			
Missing	NA	NA	NA
Phosphate, low (mg/dL)			
Missing	NA	NA	NA
Protein, total, low (g/dL)			
Level 1 (<6)	70/118 (59.3)	28/61 (45.9)	13.4 (-1.9, 28.7)
Level 2 (<5.4)	5/118 (4.2)	0/61 (0)	4.2 (0.6, 7.9)[1]
Level 3 (<5)	0/118 (0)	0/61 (0)	0 (0, 0)
Albumin, low (g/dL)			
Level 1 (<3.1)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 2 (<2.5)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (<2)	0/118 (0)	0/61 (0)	0 (0, 0)
CPK, high (U/L)			
Level 1 (>3X ULN)	118/118 (100)	61/61 (100)	0 (0, 0)
Level 2 (>5X ULN)	118/118 (100)	61/61 (100)	0 (0, 0)
Level 3 (>10X ULN)	118/118 (100)	61/61 (100)	0 (0, 0)
Amylase, high (U/L)			
Level 1 (>1.1X ULN)	6/118 (5.1)	2/61 (3.3)	1.8 (-4.2, 7.8)
Level 2 (>1.5X ULN)	1/118 (0.8)	0/61 (0)	0.8 (-0.8, 2.5)
Level 3 (>3X ULN)	0/118 (0)	0/61 (0)	0 (0, 0)
Lipase, high (U/L)			
Missing	NA	NA	NA
Blood urea nitrogen, high (mg/dL)			
Level 1 (>23)	29/118 (24.6)	1/61 (1.6)	22.9 (14.5, 31.3)[1]
Level 2 (>27)	7/118 (5.9)	0/61 (0)	5.9 (1.7, 10.2)[1]
Level 3 (>31)	1/118 (0.8)	0/61 (0)	0.8 (-0.8, 2.5)

Source: adlb.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note that glucose values for hyperglycemia do not follow a nested format like the other labs. Level 1 corresponds to the diagnosis of prediabetes and is not inclusive of Level 2 and 3. Level 2 corresponds to the diagnosis of diabetes. Level 3 represents significant hyperglycemia that may indicate need for insulin or increased risk for diabetic ketoacidosis or other complications.
Note: Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; CPK, creatine phosphokinase; N, number of patients in treatment arm; n, number of patients meeting criteria; NA, not applicable; N_w, number of patients with data; ULN, upper limit of normal

Kidney Function

Results of the FDA analysis of kidney function values for Study 48 are shown in Table 45. There were more cases of elevated creatinine (46% givinostat versus 12% placebo) and decreased eGFR (6% givinostat versus 2% placebo) in the treatment group, although no significant safety signals were identified. Elevated creatinine levels are difficult to assess in the DMD population where these values are elevated at baseline due to their underlying muscle disorder.

Table 45. Subjects With One or More Kidney Function Analyte Values Exceeding Specified Levels, Safety Population, Study 48

Laboratory Parameter	Givinostat N=118 n/N_w (%)	Placebo N=61 n/N_w (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Creatinine, high (mg/dL)			
Level 1 (≥1.5X Baseline)	39/118 (33.1)	4/61 (6.6)	26.5 (16.0, 37.0)[1]
Level 2 (≥2X Baseline)	14/118 (11.9)	3/61 (4.9)	6.9 (-1.0, 14.9)
Level 3 (≥3X Baseline)	1/118 (0.8)	0/61 (0)	0.8 (-0.8, 2.5)
eGFR, low (ml/min/1.73 m2)			
Level 1 (≥25% decrease)	7/113 (6.2)	1/59 (1.7)	4.5 (-1.0, 10.0)
Level 2 (≥50% decrease)	0/113 (0)	0/59 (0)	0 (0, 0)
Level 3 (≥75% decrease)	0/113 (0)	0/59 (0)	0 (0, 0)

Source: adlb.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data

Lipid Assessment

Results of the FDA analysis of lipid analytes for Study 48 are shown in Table 46. Laboratory analyses show an increase in triglycerides in the givinostat group compared to placebo-treated subjects. This will be discussed in more detail in Section 7.7.2.

Table 46. Subjects With One or More Lipids Analyte Values Exceeding Specified Levels, Safety Population, Study 48

Laboratory Parameter	Givinostat N=118 n/Nw (%)	Placebo N=61 n/Nw (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Cholesterol, total, high (mg/dL)			
Level 1 (>200)	32/118 (27.1)	21/61 (34.4)	-7.3 (-21.7, 7.1)
Level 2 (>210)	24/118 (20.3)	17/61 (27.9)	-7.5 (-20.9, 5.9)
Level 3 (>225)	12/118 (10.2)	6/61 (9.8)	0.3 (-8.9, 9.6)
HDL, low (mg/dL)			
Level 1 (<40)	23/118 (19.5)	11/61 (18.0)	1.5 (-10.5, 13.5)
Level 2 (<30)	0/118 (0)	2/61 (3.3)	-3.3 (-7.7, 1.2)
Level 3 (<20)	0/118 (0)	0/61 (0)	0 (0, 0)
LDL, high (mg/dL)			
Level 1 (>130)	41/118 (34.7)	30/61 (49.2)	-14.4 (-29.6, 0.8)
Level 2 (>160)	13/118 (11.0)	5/61 (8.2)	2.8 (-6.1, 11.7)
Level 3 (>190)	3/118 (2.5)	1/61 (1.6)	0.9 (-3.4, 5.2)

Laboratory Parameter	Givinostat N=118 n/Nw (%)	Placebo N=61 n/Nw (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Triglycerides, high (mg/dL)			
Level 1 (>150)	109/118 (92.4)	42/61 (68.9)	23.5 (11.0, 36.1)[1]
Level 2 (>300)	42/118 (35.6)	5/61 (8.2)	27.4 (16.4, 38.4)[1]
Level 3 (>500)	1/118 (0.8)	1/61 (1.6)	-0.8 (-4.4, 2.8)

Source: adlb.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data

Hematology

Results of the FDA analysis of hematology analysis for Study 48 are shown in Table 47. Of note, there more subjects with decreases in hemoglobin observed in the treatment group (27% givinostat versus 5% placebo >1.5g/dL decrease from baseline, 8% givinostat versus 2% placebo >2g/dL decrease from baseline). However, TEAEs of anemia were not observed, and only 3 subjects decreased to levels below the lower limit of normal. The hemoglobin decreases were not clinically significant.

Thrombocytopenia is an identified safety signal and is discussed further in Section 7.7.1 and in Table 47 below. There were no major bleeding events associated with thrombocytopenia, and this will be monitored closely. Decreased neutrophil count was also seen more frequently in the givinostat group (36% givinostat compared to 12% placebo).

Table 47. Subjects With One or More Hematology Analyte Values Exceeding Specified Levels, Safety Population, Study 48

Level	Givinostat N=118 n/Nw (%)	Placebo N=61 n/Nw (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Complete blood count			
WBC, low (10^3 cells/uL)			
Level 1 (<3.5)	3/118 (2.5)	0/61 (0)	2.5 (-0.3, 5.4)
Level 2 (<3)	1/118 (0.8)	0/61 (0)	0.8 (-0.8, 2.5)
Level 3 (<1)	0/118 (0)	0/61 (0)	0 (0, 0)
WBC, high (10^3 cells/uL)			
Level 1 (>10.8)	75/118 (63.6)	43/61 (70.5)	-6.9 (-21.3, 7.4)
Level 2 (>13)	48/118 (40.7)	26/61 (42.6)	-1.9 (-17.2, 13.3)
Level 3 (>15)	23/118 (19.5)	11/61 (18.0)	1.5 (-10.5, 13.5)
Hemoglobin, low (g/dL)			
Level 2 (>1.5 g/dL dec. from Baseline)	32/118 (27.1)	3/61 (4.9)	22.2 (12.5, 31.9)[1]
Level 3 (>2 g/dL dec. from Baseline)	9/118 (7.6)	1/61 (1.6)	6.0 (0.2, 11.7)[1]
Hemoglobin, high (g/dL)			
Level 2 (>2 g/dL inc. from Baseline)	5/118 (4.2)	3/61 (4.9)	-0.7 (-7.2, 5.9)
Level 3 (>3 g/dL inc. from Baseline)	0/118 (0)	0/61 (0)	0 (0, 0)
Platelets, low (10^3 cells/uL)			
Level 1 (<140)	40/118 (33.9)	0/61 (0)	33.9 (25.4, 42.4)[1]
Level 2 (<125)	20/118 (16.9)	0/61 (0)	16.9 (10.2, 23.7)[1]
Level 3 (<100)	6/118 (5.1)	0/61 (0)	5.1 (1.1, 9.0)[1]

Level	Givinostat N=118 n/Nw (%)	Placebo N=61 n/Nw (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
WBC differential			
Lymphocytes, low (10^3 cells/uL)			
Level 1 (<1)	9/118 (7.6)	5/61 (8.2)	-0.6 (-9.0, 7.8)
Level 2 (<0.75)	5/118 (4.2)	0/61 (0)	4.2 (0.6, 7.9)[1]
Level 3 (<0.5)	0/118 (0)	0/61 (0)	0 (0, 0)
Lymphocytes, high (10^3 cells/uL)			
Level 1 (>4)	96/118 (81.4)	35/61 (57.4)	24.0 (9.7, 38.2)[1]
Level 2 (>10)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (>20)	0/118 (0)	0/61 (0)	0 (0, 0)
Neutrophils, low (10^3 cells/uL)			
Level 1 (<2)	42/118 (35.6)	7/61 (11.5)	24.1 (12.3, 35.9)[1]
Level 2 (<1)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (<0.5)	0/118 (0)	0/61 (0)	0 (0, 0)
Eosinophils, high (10^3 cells/uL)			
Level 1 (>0.65)	6/118 (5.1)	7/61 (11.5)	-6.4 (-15.3, 2.5)
Level 2 (>1.5)	0/118 (0)	1/61 (1.6)	-1.6 (-4.8, 1.5)
Level 3 (>5)	0/118 (0)	0/61 (0)	0 (0, 0)
Coagulation studies			
PT, high (sec)			
Level 1 (>1.1X ULN)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 2 (>1.3X ULN)	0/118 (0)	0/61 (0)	0 (0, 0)
Level 3 (>1.5X ULN)	0/118 (0)	0/61 (0)	0 (0, 0)
PTT, high (sec)			
Level 1 (>1X ULN)	2/118 (1.7)	2/61 (3.3)	-1.6 (-6.6, 3.5)
Level 2 (>1.21X ULN)	1/118 (0.8)	0/61 (0)	0.8 (-0.8, 2.5)
Level 3 (>1.41X ULN)	0/118 (0)	0/61 (0)	0 (0, 0)

Source: adlb.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; dec., decrease; inc., increase; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; PT, prothrombin time; PTT, partial thromboplastin time; ULN, upper limit of normal; WBC, white blood cells

Assessment of Drug-Induced Liver Injury, Study 48

There does not appear to be a signal for liver injury. Of note, however, is that ALT and AST were significantly elevated at baseline for all subjects, a finding consistent with observations in patients with DMD, and consistent with muscle breakdown as a source, rather than liver injury (McMillan et al. 2011). Therefore, in patients with DMD, GGT and glutamate dehydrogenase (GLDH) are used to evaluate liver injury because they are more specific and do not capture muscle-injury related increases that are seen with ALT and AST.

Four subjects in the givinostat-treated group had bilirubin >2x ULN (2.11- 2.67x ULN), all of whom had elevated transaminases at baseline and throughout the trial. In each of these 4 cases, the elevated bilirubin was transient and peak bilirubin did not coincide with the peak transaminases. None of these 4 cases experienced elevated GGT levels, indicating no concern for liver failure.

There were an additional 3 subjects in the givinostat group that had elevated GGT values (>45 U/L) during the study. Two of these were listed as adverse events ((b) (6) on Days 291-334

that was not serious, did not result in a dosage change, and resolved spontaneously; and (b) (6) noted to have elevated GGT at follow-up on Day 603 that was not considered serious). The third subject ((b) (6) had elevated GGT at baseline. None of these cases were not associated with elevated bilirubin values.

Vital Signs Analyses, Study 48

There were no clinically significant patterns or trends observed in abnormalities in vital signs (including systolic and diastolic blood pressure, pulse rate, and temperature) in Study 48.

7.6.1.7. Subgroup Analyses, Study 48

Table 48 summarizes the frequency of SAEs by demographic groups in Study 48. There were too few subjects of race other than White, ethnicity other than "not Hispanic or Latino," or age greater than 12 years old to identify differences in the rates of SAEs. There were also too few subjects in the US to compare SAEs in the US and non-US subgroups.

Table 48. Overview of Serious Adverse Events by Demographic Subgroup, Safety Population, Study 48

Characteristic	Givinostat N=118 n/N_s (%)	Placebo N=61 n/N_s (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Sex			
Male	8/118 (6.8)	2/61 (3.3)	3.5 (-2.9, 9.9)
Age group, years			
Adolescent (≥12 to <18)	3/18 (16.7)	0/12 (0)	16.7 (-0.5, 33.9)
Children (6 to <12)	5/100 (5.0)	2/49 (4.1)	0.9 (-6.1, 7.9)
Race			
Asian	0/4 (0)	0/2 (0)	0 (0, 0)
Black or African American	1/3 (33.3)	0/0 (NA)	NA
Other	0/5 (0)	0/2 (0)	0 (0, 0)
White	7/106 (6.6)	2/57 (3.5)	3.1 (-3.6, 9.8)
Ethnicity			
Hispanic or Latino	1/9 (11.1)	0/3 (0)	11.1 (-9.4, 31.6)
Not Hispanic or Latino	7/109 (6.4)	2/58 (3.4)	3.0 (-3.6, 9.5)
Is in United States			
United States	1/24 (4.2)	1/19 (5.3)	-1.1 (-13.9, 11.7)
Non-United States	7/94 (7.4)	1/42 (2.4)	5.1 (-2.0, 12.1)

Source: adae.xpt; Software: R
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with adverse event; NA, not applicable; N_s, total number of patients for each specific subgroup and were assigned to that specific arm

Table 49 summarizes the frequency of AEs by demographic groups in Study 48. Similar to above, there were too few subjects in each subgroup to determine differences in frequency of adverse events.

Table 49. Overview of Adverse Events by Demographic Subgroup, Safety Population, Study 48

Characteristic	Givinostat N=118 n/N_s (%)	Placebo N=61 n/N_s (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Sex			
Male	112/118 (94.9)	57/61 (93.4)	1.5 (-5.9, 8.8)
Age group, years			
Adolescent (≥12 to <18)	17/18 (94.4)	12/12 (100)	-5.6 (-16.1, 5.0)
Children (6 to <12)	95/100 (95.0)	45/49 (91.8)	3.2 (-5.6, 11.9)
Race			
Asian	4/4 (100)	2/2 (100)	0 (0, 0)
Black or African American	3/3 (100)	0/0 (NA)	NA
Other	5/5 (100)	2/2 (100)	0 (0, 0)
White	100/106 (94.3)	53/57 (93.0)	1.4 (-6.6, 9.3)
Ethnicity			
Hispanic or Latino	8/9 (88.9)	2/3 (66.7)	22.2 (-34.9, 79.4)
Not Hispanic or Latino	104/109 (95.4)	55/58 (94.8)	0.6 (-6.3, 7.5)
Is in United States			
United States	22/24 (91.7)	18/19 (94.7)	-3.1 (-18.0, 11.9)
Non-United States	90/94 (95.7)	39/42 (92.9)	2.9 (-5.9, 11.7)

Source: adae.xpt; Software: R
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with adverse event; NA, not applicable; N_s, total number of patients for each specific subgroup and were assigned to that specific arm

7.6.1.8. Exposure-Adjusted Analyses, Study 48

Not applicable.

7.7. Key Safety Review Issues

7.7.1. Thrombocytopenia

Issue

Thrombocytopenia was among the most frequently reported adverse events in Study 48, and a reason for dose modification. It was also the key safety finding that led to adjustment in the starting dose for subjects enrolled after protocol version 4.0.

Background

Thrombocytopenia was an adverse event of special interest since this is a previously identified major dose limiting toxicity of givinostat from earlier studies, including Study 43, and a known toxicity of other previously approved HDAC inhibitors. According to (Bishton et al. 2011), administration of this class to knockout mice demonstrated megakaryocytosis and increased thrombopoietin levels with subsequent rebound thrombocytosis with drug cessation. Furthermore, givinostat is being studied in polycythemia vera where platelet count reduction is a desired outcome (Rambaldi et al. 2020).

Non-clinical studies in mouse and rat demonstrating dose-related decreases in platelets, which were also associated with increased prothrombin time and myeloid atrophy in the bone marrow in the rat.

Assessment

Subjects with platelet counts "< Lower Limit of Normal" were excluded from the study. Study 48 included rules of permanent cessation for platelet count ≤50 x 10^9/L, confirmed on 2 consecutive days. The study drug was temporarily stopped if subjects had platelets count <75 x 10^9/L, but >50 x 10^9/L, and repeat laboratory values were obtained after 1 week. Dose was to be reduced by 20% for platelets ≤150 x 10^9/L.

Treatment emergent adverse events (TEAEs) of platelet count decreased and thrombocytopenia were both reported only in the givinostat group (33%) and not the placebo cohort. These AEs were classified as either mild or moderate and did not lead to treatment discontinuation.

Laboratory findings of thrombocytopenia with platelet counts less than 140 x 10^9 cells/L were reported in 34% of givinostat patients and in no placebo patients. The givinostat dose was reduced in 28% of subjects due to thrombocytopenia based on protocol-driven reductions. As indicated above, approximately halfway through the study, the protocol was modified to initiate subjects on a lower dose in an attempt to decrease the number of dose reductions due to thrombocytopenia.

None of the laboratory findings of thrombocytopenia in Study 48 led to serious bleeding events. Three subjects who had adverse events of contusions had concurrent thrombocytopenia (platelets less than 150 X 10^9/L), including subject (b) (6) who had an episode of epistaxis and an episode of contusion, both concurrent with thrombocytopenia. Subject (b) (6) with thrombocytopenia (platelets as low as 88 x 10^9/L) also reported a concurrent adverse event of hematoma. Subject (b) (6) had ear bleeding that was not associated with decreased platelets and had anal bleeding due to constipation with concurrent levels of low platelets as low as 143 x 10^9/L. Three subjects with epistaxis ((b) (6)) had concurrent low levels of platelets less than 150 x 10^9/L, although the clinical significance of the low platelet levels was unclear. Of note, episodes of epistaxis in general were common during the study, occurring in 7 subjects (6%) treated with givinostat and 5 subjects (8%) treated with placebo.

In total, there were 6 subjects who had a platelet value below 100 x 10^9/L during Study 48. All of these findings were classified as mild, and they all resulted in dose reduction. Three of these subjects ((b) (6) recovered normal platelet numbers during the treatment period, while three did not ((b) (6) -- noted above who experienced both epistaxis and contusion). There was a single subject ((b) (6)) who had a platelet count of 60 x 10^9/L but did not have any associated bleeding events.

The median minimum value of platelet counts over time was 162 x 10^9/L for the givinostat group and 266 x10^9/L for the placebo group. Mean change from baseline in the givinostat group was -93 x 10^9/L compared to 1.0 x 10^9/L in the placebo group. There was a dose-related decrease in platelets, and the greatest decrease in platelets in the givinostat group was seen for dose level A (median decrease from baseline of -116 x 10^9/L) indicating that the effect on platelets was greatest with the highest dose. The median decrease in platelets improved with dose reduction. (Source: NDA submission, dsc-14-2357-48-report-body p. 208). The median time to the platelet count nadir was 88.0 days.

The Applicant conducted pharmacometrics modelling and determined that baseline platelet count was negatively correlated to weight and age, with younger patients and those in lower weight categories having higher platelet counts and older patients and those in higher weight brackets having worse thrombocytopenia (Source: NDA submission, Module 2.7.4 p. 64). Also see details in Section 14.5.4.1 for PK/PD analysis regarding thrombocytopenia.

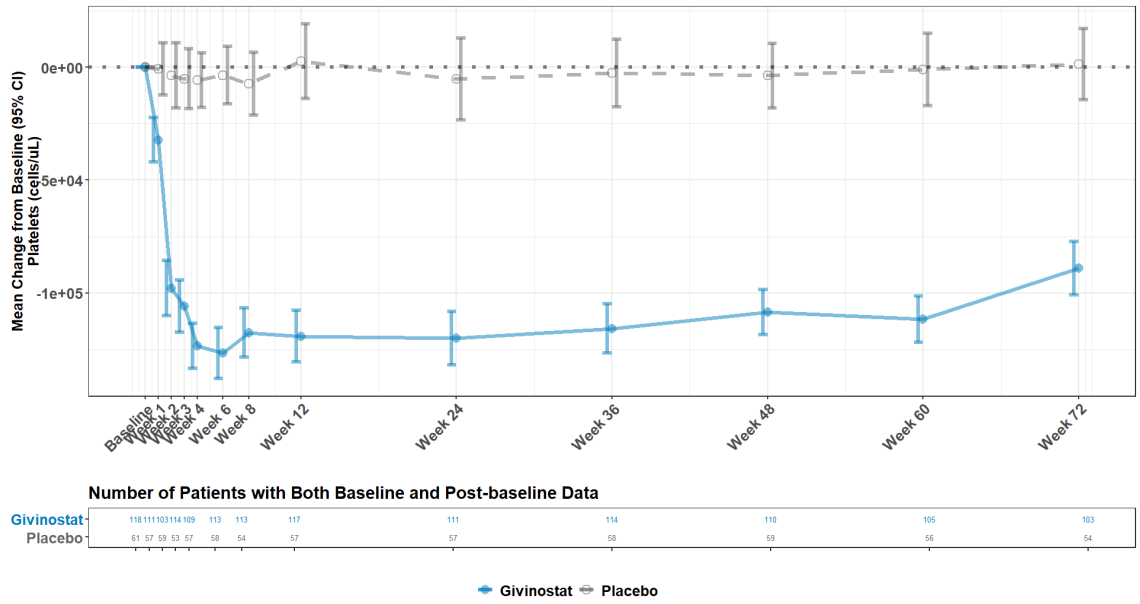
Table 50 assesses platelet reduction in the safety population for Study 48 as analyzed by the review team which confirms the Applicant's findings of 33% of subjects experiencing thrombocytopenia compared to none in the placebo group. As shown in Figure 16 and Figure 17, the effect on platelet counts occurs early in the course of treatment and persists throughout.

Table 50. Adverse Events of Special Interest Assessment, Platelet Reduction, Safety Population, Study 48

Platelet Reduction Assessment	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
AE grouping related to AESI	39 (33.1)	0	33.1 (24.6, 41.5)[1]
Platelet count decreased	22 (18.6)	0	18.6 (11.6, 25.7)[1]
Thrombocytopenia	19 (16.1)	0	16.1 (9.5, 22.7)[1]
Maximum severity			
Death	0	0	0 (0, 0)
Life-threatening	0	0	0 (0, 0)
Severe	0	0	0 (0, 0)
Moderate	5 (4.2)	0	4.2 (0.6, 7.9)[1]
Mild	34 (28.8)	0	28.8 (20.6, 37.0)[1]
Serious	0	0	0 (0, 0)
Deaths	0	0	0 (0, 0)
Resulting in discontinuation	0	0	0 (0, 0)
Relatedness per Investigator	38 (32.2)	0	32.2 (23.8, 40.6)[1]

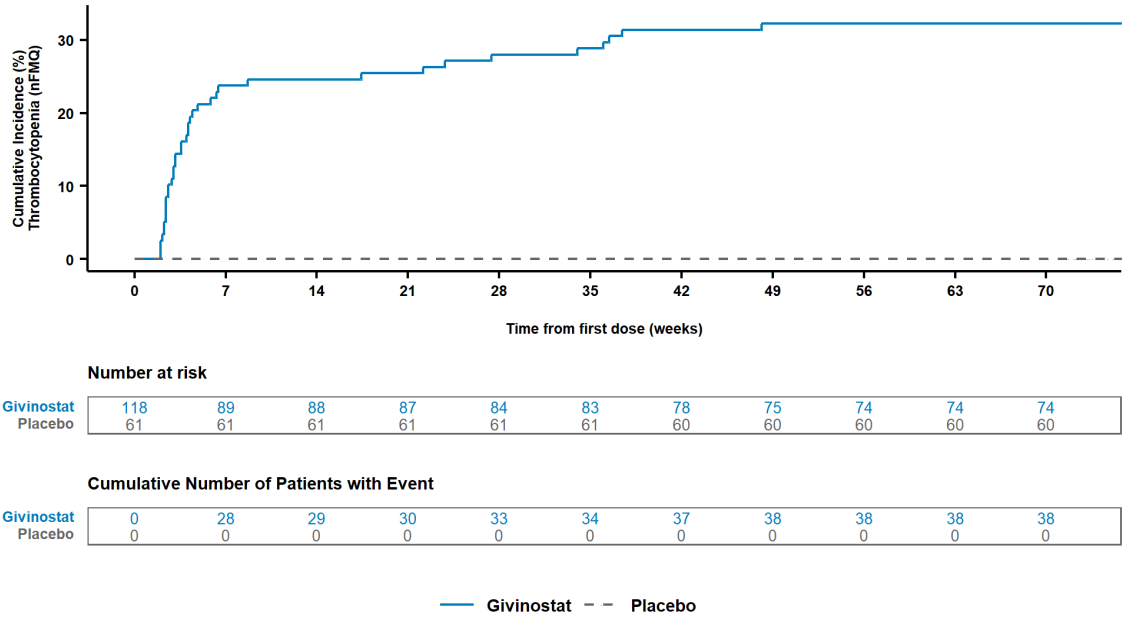
Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; AESI, adverse events of special interest; CI, confidence interval; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event

Figure 16. Mean Laboratory (Hematology) Data Change From Baseline Over Time, Safety Population, Study 48



Source: adae.xpt; Software: R
Abbreviations: CI, confidence interval

Figure 17. Time to Onset of Thrombocytopenia (NFMQ), Safety Population, Study 48



Source: adae.xpt; Software: R
Note: FMQ Thrombocytopenia contains: Platelet count decreased, Thrombocytopenia
Abbreviations: FMQ, FDA medical query; NFMQ, narrow U.S. Food and Drug Administration medical query

Table 51 below shows dose reductions for patient with thrombocytopenia based on initial givinostat dosing. Of 19 total patients who reduced their dose due to low platelets, 12 started the trial on the highest dose (Dose A) and reduced the dose to Dose B. Of these, 2 reduced the dose even further to Dose C. This indicates a dose-response relationship for thrombocytopenia as it affects dose reduction.

Table 51. Dose Reduction for Patients With Thrombocytopenia Based on Givinostat Dose Schedule, Study 48

Dosing Pathway	USUBJID	Symptom Start Day (Day of Study)	Symptom End Day (Day of Study)	Action Taken	AE Outcome
Schedule AB	(b) (6)	44	56	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		14	59	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		21	168	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		21	249	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		18	60	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		41	164	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		20		DOSE REDUCED	NOT RECOVERED/NOT RESOLVED
Schedule AB		22	68	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		45	85	DOSE REDUCED	RECOVERED/RESOLVED
Schedule AB		30	50	DOSE REDUCED	RECOVERED/RESOLVED
Schedule ABC		45	62	DOSE REDUCED	RECOVERED/RESOLVED
Schedule ABC		17	125	DOSE REDUCED	RECOVERED/RESOLVED
Schedule BC		28	90	DOSE REDUCED	RECOVERED/RESOLVED
Schedule BC		34		DOSE REDUCED	RECOVERING/RESOLVING
Schedule BC		337	368	DOSE REDUCED	RECOVERED/RESOLVED
Schedule BC		122		DOSE REDUCED	NOT RECOVERED/NOT RESOLVED
Schedule BC		255	313	DOSE REDUCED	RECOVERED/RESOLVED
Schedule BC		84	135	DOSE REDUCED	RECOVERED/RESOLVED
Schedule BC		258	342	DOSE REDUCED	RECOVERED/RESOLVED

Source: Clinical data scientist review
Note: Schedule AB = patient started on dose 37.5 mg, then reduced to 25 mg
Note: Schedule ABC = patient started on dose 37.5 mg, then reduced to 25 mg and then reduced by an additional 20%
Note: Schedule BC = Patient started on dose 25 mg and then reduced by an additional 20%
Abbreviations: USUBJID, unique subject identifier

Conclusion

There appears to be a clear dose-related effect of givinostat on platelet levels, but the extent of this is not fully characterized given the dosing changes that occurred in Study 48, and the small number of subjects enrolled in Study 48 overall and in each dose group. There were no events of

Reference ID: 5351242

serious bleeding related to thrombocytopenia in the Study 48; however, this remains a true concern in the overall DMD population, especially given the muscle weakness and predisposition to falls. Prescribers must be aware and knowledgeable about this adverse effect and subsequent related dose changes that may be needed.

Further investigation is required to characterize the incidence, frequency, and severity of thrombocytopenia and bleeding events in patients with DMD. Enhanced pharmacovigilance will be requested for thrombocytopenia, and there will be a description of hematologic abnormalities, including thrombocytopenia, in the Warnings and Precautions section of the Prescribing Information, including guidelines for monitoring and criteria for dosage adjustment. Additionally, we will require a Post-Marketing Requirement to require the Applicant to conduct a prospective observational registry to characterize the incidence, frequency, and severity of thrombocytopenia and incidence, frequency, and severity of serious events of bleeding in patients with DMD exposed to givinostat.

Given the rarity of DMD and the severity of disease relative to the observed risks, which are monitorable, as well as the potential benefit of givinostat, a risk evaluation and mitigation strategy is not required. Refer to Dr. Donella Fitzgerald's Division for Risk Management review for additional details.

7.7.2. Hypertriglyceridemia

Issue

Hypertriglyceridemia was a common reported adverse event in Study 48, and a significant reason for dose adjustments and treatment discontinuation.

Background

Hypertriglyceridemia was an adverse event of special interest, identified due to findings in monkey studies, as well signals seen in earlier studies of givinostat in the non-DMD population. The mechanism by which this occurs is unclear.

Assessment

Hypertriglyceridemia was identified as a major safety signal in Study 48. A total of 27 subjects (23%) in the treatment group experienced TEAEs of elevated triglyceride levels (one of whom had familial hypertriglyceridemia) compared to 4 subjects (7%) in the placebo group. Most of the events were mild or moderate in severity, though one was classified as severe. In the Study 48 protocol, the Applicant instructed that the drug should be temporarily stopped if triglyceride levels increased above 300 mg/dL in fasting condition, and the level should then be measured every 2 weeks until levels return. (b) (4). Five subjects had triglyceride levels above 500 mg/dL, and all but one of these started the study with elevated levels. Hypertriglyceridemia resulted in discontinuation in 3% of subjects taking givinostat and dose modification in 8% of subjects.

The results of elevated triglyceride assessments in the safety population are analyzed in Table 52 and Figure 18.

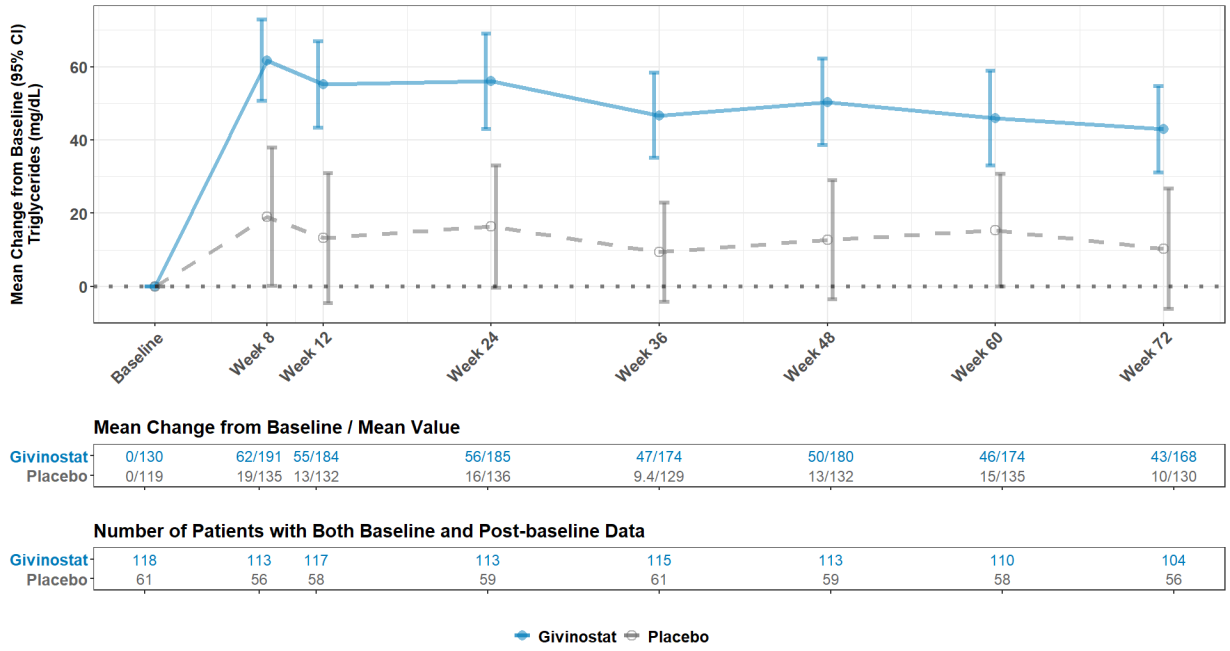
Reference ID: 5351242

Table 52. Adverse Events of Special Interest Assessment, Elevated Triglycerides, Safety Population, Study 48

Elevated Triglycerides Assessment	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
AE grouping related to AESI	27 (22.9)	4 (6.6)	16.3 (6.5, 26.1)[1]
Blood triglycerides increased	14 (11.9)	3 (4.9)	6.9 (-1.0, 14.9)
Familial hypertriglyceridemia	1 (0.8)	0	0.8 (-0.8, 2.5)
Hypertriglyceridemia	14 (11.9)	1 (1.6)	10.2 (3.6, 16.9)[1]
Maximum severity			
Death	0	0	0 (0, 0)
Life-threatening	0	0	0 (0, 0)
Severe	1 (0.8)	0	0.8 (-0.8, 2.5)
Moderate	7 (5.9)	2 (3.3)	2.7 (-3.5, 8.8)
Mild	19 (16.1)	2 (3.3)	12.8 (4.8, 20.8)[1]
Serious	0	0	0 (0, 0)
Deaths	0	0	0 (0, 0)
Resulting in discontinuation	3 (2.5)	0	2.5 (-0.3, 5.4)
Relatedness	26 (22.0)	4 (6.6)	15.5 (5.8, 25.2)[1]

Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; AESI, adverse events of special interest; CI, confidence interval; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event

Figure 18. Mean Laboratory (Triglyceride) Data Change From Baseline Over Time, Safety Population, Study 48



Source: adlb.xpt; Software: R
Note: Figures do not include time points with data from fewer than 10% of randomized/enrolled patients in all treatment groups.
Abbreviations: CI, confidence interval

Conclusion

There is a dose-related increase in triglycerides identified with givinostat. Increased triglycerides should be described in the Warnings and Precautions section of the prescribing information and it will be important to monitor triglycerides closely in all DMD patients who are taking givinostat. Dietary modifications may also be important in management of this adverse reaction.

Dose modifications are recommended for levels >300 mg/dL, which is how it was studied, and discontinuation is recommended for persistent levels >300 mg/dL despite adequate dietary intervention and dose modification. The frequency of the monitoring may be reduced after the first year of treatment, but should occur at least on the 3rd month, 6th month, and then every 6 months.

7.7.3. Gastrointestinal Disturbances

Issue

Gastrointestinal disturbances, including diarrhea, nausea/vomiting, abdominal pain, and constipation, were frequently reported in Study 48. In a few cases, these side effects were serious or were the reason for drug discontinuation.

Background

Gastrointestinal disturbances are known clinical toxicities associated with other approved HDAC inhibitors. Published literature cites nausea, vomiting, and anorexia as the most common higher grade adverse events observed with this class of drugs (Subramanian et al. 2010). Nonclinical studies also described liquid or loose feces in the dog, along with inflammatory changes in the small intestines and reduced gastrointestinal tolerability of givinostat affecting absorption.

Assessment

In Study 48, gastrointestinal issues were more common in the givinostat group, compared to the placebo group. More specifically, diarrhea was seen in 37% of the givinostat group compared to 20% of the placebo cohort, reported as severe in one case and otherwise mild or moderate in nature. This is depicted in Table 53 below. Diarrhea was usually seen in the first few weeks of dosing, shown in Figure 19.

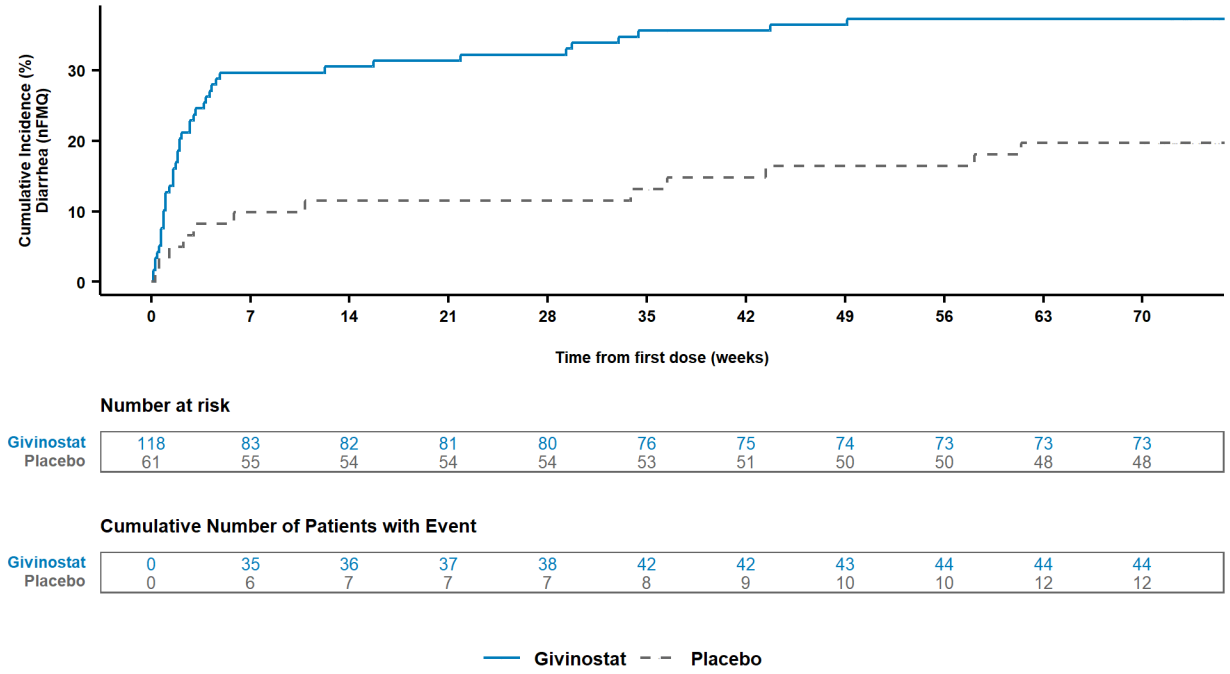
Table 53. Adverse Events of Special Interest Assessment, Diarrhea-Related, Safety Population, Study 48

FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Diarrhea (FMQ)	44 (37.3)	12 (19.7)	17.6 (3.4, 30.1)[1]
Diarrhea	43 (36.4)	11 (18.0)	18.4 (4.4, 30.6)[1]
Diarrhea hemorrhagic	1 (0.8)	0	0.8 (-5.1, 4.7)
Dysentery	0	1 (1.6)	-1.6 (-8.8, 1.6)

FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Maximum severity			
Death	0	0	0.0 (-6.0, 3.2)
Life-threatening	0	0	0.0 (-6.0, 3.2)
Severe	1 (0.8)	0	0.8 (-5.1, 4.7)
Moderate	3 (2.5)	1 (1.6)	0.9 (-6.4, 5.9)
Mild	40 (33.9)	11 (18.0)	15.9 (2.0, 28.0)[1]
Serious	0	0	0.0 (-6.0, 3.2)
Deaths	0	0	0.0 (-6.0, 3.2)
Resulting in discontinuation	2 (1.7)	0	1.7 (-4.3, 6.0)
Relatedness	26 (22.0)	2 (3.3)	18.8 (9.0, 27.7)[1]
Number of patients with adverse events with end dates on or before treatment end dates	37/44 (84.1)	11/12 (91.7)	-7.6 (-23.8, 21.2)
Duration, days (from AE start date to AE end date)			
Mean (SD)	34.4 (103.1)	32.5 (84.5)	NA
Median (Q1, Q3)	6 (2, 15)	2 (2, 6.5)	NA
Min, max	1, 589	1, 284	NA
Number of patients with adverse events (occurred on or before treatment end date) with end dates missing (no end dates reported, assumed that AE continuing)	4/44 (9.1)	1/12 (8.3)	0.8 (-27.4, 15.4)
Number of patients with adverse events (occurred on or before treatment end date) with end dates after treatment end dates	5/44 (11.4)	0/12 (0)	11.4 (-13.9, 24.1)
Duration, days (from treatment end date to AE end date)			
Mean (SD)	37.2 (33.7)	NA	NA
Median (Q1, Q3)	29 (10, 70)	NA	NA
Min, max	2, 75	NA, NA	NA

Source: adae.xpt; Software: R
[1] indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, Food and Drug Administration medical query; IMP, investigational medicinal product; max, maximum; min, minimum; N, number of patients in treatment arm; n, number of patients with adverse event; NA, not applicable; Q, quartile; SD, standard deviation

Figure 19. Time to Onset of Diarrhea (NFMQ), Safety Population, Study 48



Source: adae.xpt; Software: R
Note: FMQ Diarrhea contains: Diarrhoea, Diarrhoea haemorrhagic, Dysentery
Abbreviations: FMQ, Food and Drug Administration medical query; NFMQ, narrow FMQ, Food and Drug Administration medical query

One subject experienced severe diarrhea ([redacted] (b) (6)) and was admitted to the hospital for rehydration in the setting of influenza, which is described above under SAEs. (Section 7.6.1.3). This subject also experienced severe vomiting (see below).

Nausea, which was characterized as mild in all cases in the givinostat cohort, was seen in 8% of subjects taking givinostat compared to 7% in the placebo group, seen in Table 54.

Table 54. Adverse Events of Special Interest Assessment, Nausea-Related, Safety Population, Study 48

FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Nausea (FMQ)	9 (7.6)	4 (6.6)	1.1 (-8.8, 8.6)
Nausea	8 (6.8)	4 (6.6)	0.2 (-9.5, 7.6)
Procedural nausea	1 (0.8)	0	0.8 (-5.1, 4.7)
Maximum severity			
Death	0	0	0.0 (-6.0, 3.2)
Life-threatening	0	0	0.0 (-6.0, 3.2)
Severe	0	0	0.0 (-6.0, 3.2)
Moderate	0	1 (1.6)	-1.6 (-8.8, 1.6)
Mild	9 (7.6)	3 (4.9)	2.7 (-6.6, 10.0)
Serious	0	0	0.0 (-6.0, 3.2)
Deaths	0	0	0.0 (-6.0, 3.2)
Resulting in discontinuation	0	0	0.0 (-6.0, 3.2)
Relatedness	3 (2.5)	1 (1.6)	0.9 (-6.4, 5.9)

FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Number of subjects with adverse events with end dates on or before treatment end dates	9/9 (100)	4/4 (100)	0.0 (-31.6, 51.0)
Duration, days (from AE start date to AE end date)			
Mean (SD)	7.1 (10.5)	23 (43.3)	NA
Median (Q1, Q3)	2 (1, 8)	1.5 (1, 23.5)	NA
Min, max	1, 32	1, 88	NA
Number of subjects with adverse events (occurred on or before treatment end date) with end dates missing (no end dates reported, assumed that AE continuing)	0/9 (0)	1/4 (25.0)	-25.0 (-71.2, 12.3)
Number of subjects with adverse events (occurred on or before treatment end date) with end dates after treatment end dates	0/9 (0)	0/4 (0)	0.0 (-51.0, 31.6)

Source: adae.xpt; Software: R
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, Food and Drug Administration medical query; IMP, investigational medicinal product; max, maximum; min, minimum; N, number of patients in treatment arm; n, number of patients with adverse event; Q, quartile; SD, standard deviation

Vomiting in the safety population in Study 48 occurred in 29% of the givinostat group compared to 13% in the placebo group. Most cases were mild, with a total of 5 cases that were classified as moderate or severe. This is show in Table 55.

Table 55. Adverse Events of Special Interest Assessment, Vomiting, Safety Population, Study 48

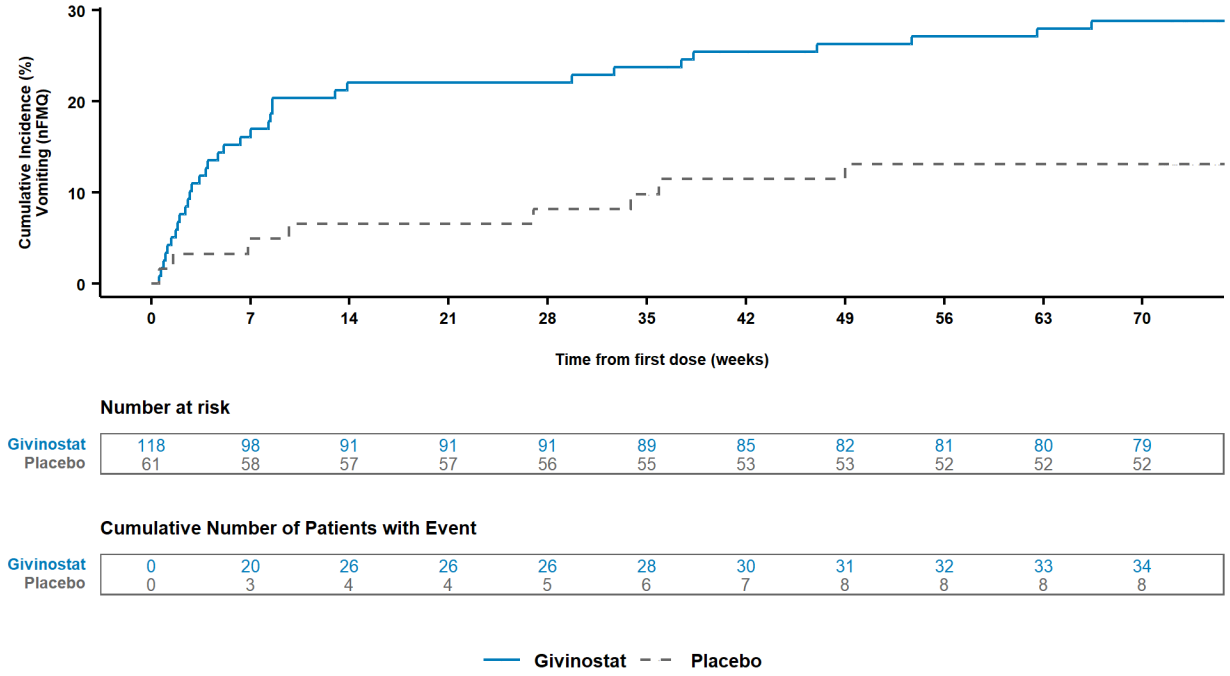
FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Vomiting (FMQ)	34 (28.8)	8 (13.1)	15.7 (2.8, 26.9)[1]
Vomiting	34 (28.8)	8 (13.1)	15.7 (2.8, 26.9)[1]
Maximum severity			
Death	0	0	0.0 (-6.0, 3.2)
Life-threatening	0	0	0.0 (-6.0, 3.2)
Severe	2 (1.7)	0	1.7 (-4.3, 6.0)
Moderate	3 (2.5)	0	2.5 (-3.5, 7.2)
Mild	29 (24.6)	8 (13.1)	11.5 (-1.2, 22.4)
Serious	1 (0.8)	0	0.8 (-5.1, 4.7)
Deaths	0	0	0.0 (-6.0, 3.2)
Resulting in discontinuation	0	0	0.0 (-6.0, 3.2)
Relatedness	7 (5.9)	0	5.9 (-0.1, 11.8)
Number of subjects with adverse events with end dates on or before treatment end dates	34/34 (100)	8/8 (100)	0.0 (-10.4, 33.0)
Duration, days (from AE start date to AE end date)			
Mean (SD)	3.8 (6.1)	2.2 (2.4)	NA
Median (Q1, Q3)	2 (1, 3)	1 (1, 2.2)	NA
Min, max	1, 35	1, 8	NA
Number of subjects with adverse events (occurred on or before treatment end date) with end dates missing (no end dates reported, assumed that AE continuing)	0/34 (0)	1/8 (12.5)	-12.5 (-47.5, -1.1)[1]
Number of subjects with adverse events (occurred on or before treatment end date) with end dates after treatment end dates	0/34 (0)	0/8 (0)	0.0 (-33.0, 10.4)

Source: adae.xpt; Software: R
[1] Indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, Food and Drug Administration medical query; IMP, investigational medicinal product; max, maximum; min, minimum; N, number of patients in treatment arm; n, number of patients with adverse event; Q, quartile; SD, standard deviation

Two subjects had severe vomiting. The first ([redacted](b) (6)) was a 13-year-old boy who presented to the ER and was ultimately hospitalized due to dehydration. There is speculation that his vomiting, which was accompanied by fever, was due to bisphosphonate infusion on the day prior to onset of symptoms. He had no diarrhea. This subject also had temporary treatment interruption a few months later due to hypertriglyceridemia. The second subject ([redacted](b) (6)) with severe vomiting was in the setting of an SAE of influenza, and was described above (Section 7.6.1.3).

The onset of vomiting was usually within the first 7 weeks of treatment, as depicted in Figure 20.

Figure 20. Time to Onset of Vomiting (NFMQ), Safety Population, Study 48



Source: adae.xpt; Software: R
Note: FMQ Vomiting contains: Vomiting
Abbreviations: FMQ, Food and Drug Administration medical query; NFMQ, narrow Food and Drug Administration medical query

Abdominal pain was seen in 34% of the givinostat cohort and 25% of the placebo cohort, all mild or moderate in severity. It was often, but not always, associated with concurrent diarrhea and/or nausea/vomiting. One case of abdominal pain was serious (see narrative in Section 7.6.1.3) but did not result in discontinuation of the study drug, see Table 56. One case of abdominal pain resulted in discontinuation but was not serious as described above (see Section 7.6.1.4).

Table 56. Adverse Events of Special Interest Assessment, Abdominal Pain, Safety Population, Study 48

FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Abdominal pain (FMQ)	40 (33.9)	15 (24.6)	9.3 (-5.2, 22.4)
Abdominal discomfort	1 (0.8)	1 (1.6)	-0.8 (-8.0, 3.2)
Abdominal pain	24 (20.3)	9 (14.8)	5.6 (-7.1, 16.5)
Abdominal pain upper	17 (14.4)	7 (11.5)	2.9 (-8.7, 12.6)
Maximum severity			
Death	0	0	0.0 (-6.0, 3.2)
Life-threatening	0	0	0.0 (-6.0, 3.2)
Severe	0	0	0.0 (-6.0, 3.2)
Moderate	6 (5.1)	2 (3.3)	1.8 (-6.6, 8.0)
Mild	34 (28.8)	13 (21.3)	7.5 (-6.5, 19.9)
Serious	1 (0.8)	0	0.8 (-5.1, 4.7)
Deaths	0	0	0.0 (-6.0, 3.2)
Resulting in discontinuation	1 (0.8)	0	0.8 (-5.1, 4.7)
Relatedness	25 (21.2)	3 (4.9)	16.3 (5.9, 25.5)[1]

FMQ (Narrow) Preferred Term	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Number of subjects with adverse events with end dates on or before treatment end dates	38/40 (95.0)	14/15 (93.3)	1.7 (-11.5, 25.6)
Duration, days (from AE start date to AE end date)			
Mean (SD)	52.9 (120.2)	66.6 (122.6)	NA
Median (Q1, Q3)	7.5 (2.2, 34.8)	5 (1.2, 73.2)	NA
Min, max	1, 574	1, 417	NA
Number of subjects with adverse events (occurred on or before treatment end date) with end dates missing (no end dates reported, assumed that AE continuing)	0/40 (0)	1/15 (6.7)	-6.7 (-30.1, 2.7)
Number of subjects with adverse events (occurred on or before treatment end date) with end dates after treatment end dates	2/40 (5.0)	0/15 (0)	5.0 (-16.0, 16.7)
Duration, days (from treatment end date to AE end date)			
Mean (SD)	72.5 (3.5)	NA	NA
Median (Q1, Q3)	72.5 (71.2, 73.8)	NA	NA
Min, max	70, 75	NA, NA	NA

Source: adae.xpt; Software: R
[1] Indicates that 95% confidence interval excludes zero.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Note: Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Note: Relatedness is determined by investigator.
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, Food and Drug Administration medical query; IMP, investigational medicinal product; max, maximum; min, minimum; N, number of patients in treatment arm; n, number of patients with adverse event; Q, quartile; SD, standard deviation

Constipation was also more common in the givinostat group, seen in 8% compared to 2% in the placebo group. These were mostly mild, as shown in Table 57.

Table 57. Overview of Constipation (Grouped Query), Safety Population, Study 48

Adverse Event Category	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Constipation (GQ)[1]	9 (7.6)	1 (1.6)	6.0 (0.2, 11.7)[1]
Constipation	8 (6.8)	1 (1.6)	5.1 (-0.4, 10.7)
Dyschezia	1 (0.8)	0	0.8 (-0.8, 2.5)
Maximum severity			
Death	0	0	0 (0, 0)
Life-threatening	0	0	0 (0, 0)
Severe	0	0	0 (0, 0)
Moderate	1 (0.8)	0	0.8 (-0.8, 2.5)
Mild	8 (6.8)	1 (1.6)	5.1 (-0.4, 10.7)
Serious	0	0	0 (0, 0)
Deaths	0	0	0 (0, 0)

Adverse Event Category	Givinostat N=118 n (%)	Placebo N=61 n (%)	Givinostat vs. Placebo Risk Difference (%) (95% CI)
Resulting in discontinuation	0	0	0 (0, 0)
Relatedness	1 (0.8)	0	0.8 (-0.8, 2.5)

Source: adae.xpt; Software: R
[1] Group query Constipation contains: Constipation, Dyschezia.
Note: Treatment-emergent adverse events defined as an AE that started or worsened on or after the date of start of the administration of the IMP.
Note: Duration is 18-month double-blind treatment period.
Abbreviations: AE, adverse event; CI, confidence interval; GQ, group query; IMP, investigational medicinal product; N, number of patients in treatment arm; n, number of patients with adverse event

Conclusion

The review team concluded that gastrointestinal concerns need to be monitored in all patients taking givinostat. As noted in the chemistry analysis above, some patients on givinostat had electrolyte disturbances which may have been related to underlying dehydration, potentially from diarrhea and/or vomiting. Gastrointestinal symptoms can be especially challenging to manage in the DMD population given the difficulties with ambulation and muscle weakness that are already present. The benefit-risk assessment for individual patients should consider the potential benefits of givinostat compared to any adverse gastrointestinal effects being experienced at the prescribed dose, or if a reduced dose or drug discontinuation are more appropriate. Gastrointestinal disorders will be included in the Warnings and Precaution section of the prescribing information.

7.7.4. Prolonged QT

Issue

Givinostat is associated with prolonged QTc and therefore poses additional risks for patients with underlying cardiac issues that may be susceptible to ventricular arrhythmias.

Background

HDAC inhibitors such as givinostat have been associated with delayed cardiac repolarization and arrhythmias (Spence et al. 2016). The Applicant conducted a thorough QT (TQT) study (Study ITF/2357/54), titled “A Randomized, Partially Double-Blind, Four-Period, Four Treatment, Crossover Study Investigating the Placebo-Corrected Effects of a Therapeutic Dose (100 mg) and a Supratherapeutic Dose (300 mg) of ITF2357 (givinostat) and Moxifloxacin on QT/QTc Interval in Healthy Male and Female Subjects.”, the protocol for which had been previously reviewed by the Interdisciplinary Review Team for Cardiac Safety (QT-IRT). The study results from Study ITF/2357/54 were submitted in this NDA submission.

Assessment

Refer to the full QT-IRT review dated August 29, 2023, for additional details of the analysis. Per the review, QTcF prolongation was detected in the thorough TQT study (ITF/2357/54). The highest dose administered in Study ITF/2357/54 (300 mg, corresponding to 3-6 mg/kg) provided exposures 6.6-fold the givinostat C_{max} at steady state in subjects with DMD at a therapeutic dose (70 mg daily, approximately 1 mg/kg).

Data analyzed using by-time analysis as the primary analysis did not suggest that a 100 mg dose (corresponding to 1-2 mg/kg) of givinostat is associated with significant QTc prolonging effect. However, the by-time analysis suggests the presence of significant QTc prolonging effect for the 300 mg dose. Integrated nonclinical risk assessment was not performed. The time profile of QTc interval suggests that the metabolites could be causing QTc prolongation because the T_{max} for givinostat is around 2 hours post-dose, but the largest mean QTc effect occurred around 5 hours post-dose, which is consistent with T_{max} of metabolites ITF2374 and/or ITF2375.

The Applicant has not performed organ impairment studies to identify the high-clinical exposure scenario of parent or metabolites, so it is not known whether the exposures achieved in the TQT study cover the high exposure scenario.

Table 58. Clinical QT Study findings, Study ITF/2357/54

ECG parameter	Treatment	Time (h)	ΔΔQTcF (msec)	90% CI (msec)
QTcF	Givinostat 100 mg (corresponds to 1-2 mg/kg)	5.0	5.5	2.0 to 9.0
QTcF	Givinostat 300 mg (corresponds to 3-6 mg/kg)	5.0	13.6	10.1 to 17.1

Source: QT-Interdisciplinary Review Team Review
Abbreviations: CI, confidence interval; QTcF, heart-rate corrected QT interval by Fridericia's cube root formula

Conclusion

The review completed by the QT-IRT concludes that givinostat use should be avoided in any patient with an increased risk of ventricular arrhythmias, including those with congenital long QT syndrome, coronary artery disease, electrolyte disturbance, and concomitant use of other medications known to cause QT prolongation. This risk for QT prolongation will be described as a Warning and Precaution in labeling, and ECGs will be recommended prior to initiating treatment in any patients who are at increased risk for ventricular arrhythmias if use cannot be avoided. Additionally, per discussion with the QT-IRT, the prescribing information will inform prescribers to withhold givinostat if the QTc interval is >500 ms or the change from baseline is >60 ms.

Additional risk factors that could lead to QT prolongation, but are less likely to occur in patients with DMD, include significant cardiac disease, recent myocardial infarction, heart failure, unstable angina, bradyarrhythmias, uncontrolled hypertension, high degree of atrioventricular block, severe aortic stenosis, or uncontrolled hypothyroidism. Cardiologists are an important part of the care team for patients with DMD and will already be aware of additional risk factors for QT prolongation and can make informed decisions regarding benefit-risk in individual patients.

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8. Therapeutic Individualization

8.1. Intrinsic Factors

Age

Dose adjustments are not recommended on the basis of age. Baseline age was evaluated as a covariate in the pop PK analysis for healthy adult subjects. However, DMD patients were mostly pediatric subjects with a mean age of 9.54 years, and age is correlated with body weight. The effect of age was not tested in the formal model-based covariate analysis.

Body Weight

The popPK analyses show that the PK of givinostat can be affected by body weight (see Section 14.5.1). The recommended dose of givinostat is based on body weight with individual fixed doses for four different weight ranges.

Gender

The effect of gender is not relevant for the DMD patient population, which occurs predominantly in males.

Hepatic Impairment

The Applicant did not perform dedicated studies in subjects with hepatic impairment. Subjects with liver disease or impairment (current or in the past) were excluded from Study 48. The Applicant did not include dose adjustment recommendation for patients with hepatic impairment in the label.

Considering givinostat is extensively metabolized, the impact of hepatic impairment on drug elimination cannot be excluded. The Applicant did not conduct mass balance and absolute bioavailability studies. In humans, only urinary recovery data is available, indicating very minimal administered drug was cleared unchanged in the urine. There was no fecal recovery data available from human studies of givinostat. Further, based on available metabolism information from in vitro and in vivo, it is not feasible to reasonably estimate the contribution of hepatic metabolism.

Evaluating the available clinical experience of givinostat in pediatric subjects, the highest dose studied was 50 mg bid in the phase 2 study (Study 43). The exposure of givinostat ($AUC_{0\text{-}24hr}$ at steady state) in this study is close to 2-fold of the highest therapeutic exposure seen in the phase 3 study. It is worth noting that only 12 subjects were dosed with 50 mg bid and this dose level was considered not tolerated due to emerging adverse events, i.e., the decrease in platelets (see Clinical Study Report DSC-11-2357-43 (Italfarmaco 2019)).

Overall, there is not enough information to evaluate the safety when givinostat is dosed in subjects with hepatic impairment. A post-market requirement has been issued to the Applicant for a dedicated study in subjects with hepatic impairment.

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Renal Impairment

The Applicant did not perform dedicated studies in subjects with renal impairment. Subjects with inadequate renal function, as defined by serum cystatin C>2 times the upper limit of normal, were excluded from Study 48. The Applicant did not provide recommendations for dose adjustment in patients with renal impairment.

Only minimal amount of givinostat is excreted intact in the urine, indicating the contribution of renal elimination of the drug is minimal. According to the draft FDA guidance Pharmacokinetics in Patients with Impaired Renal Function – Study Design, Data Analysis, and Impact on Dosing (September 2020), a dedicated study in subjects with renal impairment is not needed. Dose adjustment in patients with renal impairment is not necessary.

8.2. Extrinsic Factors

Food Effect

A dedicated food effect study using the to-be-marketed suspension formulation was not conducted. However, the evaluation of food effect was assessed using a prototype liquid-filled givinostat capsule formulation in a single dose study at a dose of 100 mg both in the fasted state and after completion of a high fat standard meal. The results (see Section 14.2) indicated that food had an effect on the PK parameters of givinostat. Higher concentrations of givinostat and ITF2375 were observed in the fed state compared to under fasting conditions (about 40% and 23% increase in AUC and C_{max} respectively for givinostat), while ITF2374 had slightly higher concentrations observed in the fasted state compared to the fed state.

The Applicant considers that the food effect on the liquid suspension (to-be-marketed formulation) would provide a similar increase of givinostat systemic exposure to that observed with the capsules. This was based on the similarity of suspension formulation and liquid-filled capsule formulation as well as the insignificance of formulation effect on givinostat PK based on popPK analyses. Since the proposed label recommends dosing with food and the pivotal phase 3 study was conducted with drug administration after food, no additional food effect study is recommended in postmarket setting.

The clinical significance of the changes in exposure due to food effect is expected to be minimal but cannot be ruled out. The drug administration section of the Prescribing Information will recommend taking givinostat with food, which is the same as the dosing instruction in the pivotal phase 3 study (Study 48) . This will also be useful to aid in masking the bitter taste of givinostat product.

Drug Interactions

Effect of Other Drugs on Givinostat

In vitro data showed that givinostat's metabolism does not involve CYPs; therefore, the DDI potential for givinostat to be affected by modulators of CYPs is low. Givinostat is a substrate of intestinal P-gp and breast cancer resistance protein (BCRP) transporters. The clinical DDI Study (See Section 14.2) for givinostat with co-administration of clarithromycin (a strong P-gp inhibitor) showed an increase C_{max} of givinostat of about 40% without significant change of AUC (meet bioequivalence criterion 90% CI of AUC ratio within 80-125%). This increase of

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C_{max} is not clinically impactful considering the flexible dosing regimen with opportunity to reduce the dose to mitigate safety and tolerability risks. The effect of BCRP inhibitor on givinostat PK was not studied in a clinical study. However, it is expected the effect of BCRP inhibitor on givinostat PK is smaller than the P-gp inhibitor based on the comparison of the two transporters mediated efflux ratios determined in the in vitro cell models. Overall, dose adjustment is not recommended for givinostat when taking with other concomitant drugs.

Effect of Givinostat on Other Drugs

The in vitro studies indicated the inhibition of the intestinal CYP3A4 by givinostat could not be excluded when doses ≥10 mg were administered. The in vitro induction studies also showed givinostat is a moderate to strong inducer of CYP3A4. In addition, givinostat has the potential to inhibit intestinal P-gp transporter and the renal uptake transporter, OCT2 (See Section 14.1).

The Applicant conducted a study in healthy volunteers (Study No. ITF/2357/55, Module 2.7.2) to determine the potential of givinostat as an inhibitor and inducer and its effect on the PK of midazolam (sensitive index CYP3A substrate) and dabigatran etexilate (P-gp substrate). The results (see Section 14.2) indicated that:

- Givinostat has no significant inhibition or induction on hepatic 3A4 but does have weak inhibition on intestinal 3A4
- Givinostat does not likely have inhibition on P-gp transporter

An increase in creatinine (OCT2 substrate) with givinostat treatment was seen in the phase 3 study, with a mean increase of 4.76 umol/L from baseline (19.24 umol/L) to the end of treatment (24.00 umol/L), observed within the first 2 months of treatment, remaining similar elevation during entire study (see Clinical Study Report DSC-14-2357-48). Overall, the inhibition of givinostat on OCT2 appears mild.

Immunizations

Immunizations with vaccines during givinostat therapy has not been studied.

8.3. Plans for Pediatric Drug Development

Givinostat for the treatment of DMD was granted orphan drug designation on April 12, 2013, and is exempt from PREA requirements. Givinostat has been studied in pediatric patients 6 years of age and older.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

The effects of givinostat on pregnancy and lactation have not been studied. Studies were only performed in males, and they were required to use adequate contraception including true abstinence or condom with a spermicide along with partner contraception. No pregnancies were reported in givinostat studies in DMD patients.

9. Product Quality

Approval With Postmarketing Commitments PMC

The Office of Pharmaceutical Quality (OPQ) review team has assessed NDA 217865 with respect to chemistry, manufacturing, and controls (CMC) and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such, OPQ recommends approval of this NDA from a quality perspective. The chemistry, manufacturing, and controls postmarketing commitments and (any other post-approval quality agreements) between OPQ and the Applicant are listed below should be included in the action letter.

- Perform experimental activities to evaluate if the drug product matrix can be suppressed (e.g., drug product dilutions or interfering matrix precipitation/liquid extraction with recovery evaluation).
  - *Final reports submission: July 2024*
- If matrix interference can be suppressed: Test the three drug product submission batches at their 36 months stability endpoint at 25°C/60% RH.
  - *Final protocol submission: July 2024*
  - *Final report submission: December 2024*
- In addition, to assess any trending, test the first three post-approval batches at multiple time points - from release through the end of proposed shelf-life.
  - *Results - submitted in the NDA annual reports.*
- If matrix interference cannot be suppressed: Update regarding the on-going leachable study with simulant solvent and provide an overall risk assessment to evaluate if there is any significant risk associated to presence of leachables in the drug product.
  - *Interim reports* (b) (4) *submission: December 2024*
  - *Final reports* (b) (4) *submission: December 2025*

9.1. Device or Combination Product Considerations

Not applicable.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

The results of the clinical site inspections support the conclusion that the studies were adequately conducted, and that the data generated support the proposed indication. Review of the financial disclosures did not raise concerns about the validity or reliability of the data.

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11. Advisory Committee Summary

Not applicable.

III. Additional Analyses and Information

12. Summary of Regulatory History

Givinostat was developed by Italfarmaco S.p.A under IND 126598 for the treatment of Duchenne muscular dystrophy (DMD). In the Division's August 20, 2015, Pre-IND written responses, the Division responded to the Sponsor's proposed clinical development plans for givinostat. The Division informed the Sponsor that carcinogenicity studies could be conducted postmarketing if the available nonclinical and clinical data supported such a strategy due to the serious nature of DMD. (b) (4) The Sponsor additionally proposed the use of MRI to evaluate the effects of givinostat versus placebo to preserve muscle tissue. The Division indicated that their proposed histological and MRI endpoints were not adequately described and that the discussions for study design and analysis were premature prior to agreement on clinically meaningful primary and key secondary endpoints. The Division encouraged the use of clinical primary endpoints, such as stair climb or rise time. Additionally, the Division expressed concerns with known safety information regarding bone marrow suppression and reductions in platelets and neutrophils from studies in other indications.

The initial IND was submitted to the Agency on May 25, 2016. Initial reviews described givinostat as an orally active hydroxamic acid derivative possessing a potent histone deacetylase ([HDAC] class I and II) inhibitory activity. The Sponsor stated that there had been 19 completed non-DMD studies with human exposure to 505 subjects, of which 31 were pediatric subjects (ages 2-18). In addition, there was a phase 2 exploratory study being conducted in Italy, in 20 patients with DMD at the time of the IND submission. Therefore, the initial IND proposed a phase 3 clinical study (Study 48) in ambulant males 6 years of age or older with DMD. The study was allowed to proceed on June 22, 2016. Givinostat was granted orphan drug designation on April 12, 2013, fast track designation on August 29, 2016, and rare pediatric disease designation on September 22, 2020, for the treatment of DMD.

A Type C Guidance meeting was requested to ensure that all aspects of the clinical program in the IND May Proceed letter were adequately addressed (written responses dated April 27, 2018). The Division noted that there was a potential for subject unblinding with interruption of treatment or increased laboratory monitoring that could introduce bias on effort-dependent assessments. The Division encouraged the Sponsor to explore options to provide matching placebo to subjects who need to have the study treatment interrupted. The Division additionally recommended that the study have at least two dose arms given the safety profile of givinostat. Type C Guidance meetings were also held in January 2019 and May 2020 to discuss the continued development plan for givinostat. In the written responses sent May 11, 2020, the Division strongly advised against decreasing the sample size following the interim analysis for Study 48.

A Type C meeting was held on August 25, 2022, to discuss a proposed NDA submission based on the results of a complete phase 3 study (Study 48) and an ongoing open label study (Study 51)

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(minutes dated September 23, 2022). The Division expressed concern that the data from Study 48 and Study 51 were not sufficient to form the basis of an NDA submission for givinostat due to concerns regarding the ability to establish an adequate benefit risk profile due to the dose modifications made during the study and difficulty selecting a safe and effective dose, in addition to the substantial concern regarding the potential for unblinding due to adverse events (AEs). The Division also reiterated their concerns with the changes to the statistical analysis plan after the futility interim analysis. In follow-up to the August 25, 2022, meeting, another Type C meeting was requested to further discuss dose rationale, study blinding and potential sources of confirmatory evidence to support a marketing application (preliminary comments dated January 27, 2023). The Division noted that while the results of Study 48 were encouraging, there were still concerns about the adequacy of the safety database, particularly the limited exposure at the highest dose, to adequately characterize the safety of givinostat, as well as overall robustness of the single study to inform the risk-benefit-risk assessment of givinostat in patients with DMD. No pre-NDA meeting was held with the Applicant.

Because givinostat for the treatment of DMD was granted orphan designation, the Applicant is exempted from the Pediatric Research Equity Act requirements. No written requests have been issued under Best Pharmaceuticals for Children Act.

NDA 217865 was submitted on April 21, 2023, and was filed on June 20, 2023, with filing issues identified. The Division stated that the ability of Study 48 to serve as an adequate and well-controlled study to contribute to the substantial evidence of givinostat in the treatment in patients with DMD would be a matter of review. The Division also reiterated their concerns regarding the adequacy of the safety database. This original NDA received priority review designation and was reviewed under “The Program.” The proprietary name review request was submitted on April 21, 2023, and Duvyzat was found to be conditionally acceptable on July 21, 2023.

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

13.1.1. Pharmacology

Givinostat (ITF2357) is a small molecule pan-HDAC inhibitor. According to the Applicant, inhibition of HDAC hyperactivity in DMD patients is thought to promote muscle regeneration by reducing inflammation, improving muscle regeneration, and delaying fibrosis.

In vitro studies in human peripheral blood mononuclear cells (hPBMC) indicated half maximal inhibitory concentration values of 52, 120, 11, 49, 9, 560, 336, 343, 332, and 59 nM for HDAC 1, 2, 3, 8, 6, 4, 5, 7, 9, and 10, respectively. Potential effects of givinostat on inflammation were demonstrated in lipopolysaccharide-stimulated hPBMC, in which the proinflammatory cytokines TNFα, IL1β, and IFNγ were suppressed by concentrations of givinostat up to 1 µM.

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Additionally, profibrotic signaling pathways and gene expression that are mediated by TGF-β were suppressed by 250 nM givinostat in human DMD patient-derived fibroblasts.

In vivo proof-of-concept studies intended to support the development of givinostat for DMD were conducted in *mdx* mice, which harbor a nonsense point mutation in exon 23 that results in production of a truncated and nonfunctional dystrophin protein. Studies were conducted in the C57BL/10ScSn-Dmd^{mdx}/J (i.e., B10.*mdx*) and D2.B10-Dmd^{mdx}/J (i.e., D2.*mdx*) mice which were intended to model the IAAM (mild) and VTTT (severe) haplotypes, respectively, in DMD. The Applicant's use of the D2 mouse to model the severe phenotype is supported by literature reports that indicate the D2.*mdx* mouse exhibits more rapid clinical decline and greater severity of muscle damage, impaired muscle regeneration, muscle wasting, and intramuscular fibrosis relative to age-matched B10.*mdx* mice (Hammers et al. 2020). In both models, daily oral administration of givinostat delayed decreases in treadmill exhaustion, grip strength, and muscle fibrosis and adipocyte infiltration.

Secondary and safety pharmacology studies did not indicate the potential for Off-Target toxicity, or adverse CNS, cardiovascular, or respiratory effects.

13.1.2. PK/ADME

Standard pharmacokinetic (PK) studies were performed in male SD rats, beagle dogs, and cynomolgus monkeys administered givinostat by oral or IV administration. Oral bioavailability ranged from 21% in rats to approximately 12% in dogs and monkeys; plasma $t_{1/2}$ was generally between 1 and 2 h in rats, dogs, and monkeys. Administration of radiolabeled givinostat to male rats resulted in accumulation of radioactivity in the stomach, intestines, kidneys, ureter, and urinary bladder. The major circulating human metabolites (i.e., ITF2374 and ITF2375) were formed in all species tested.

13.1.3. Single Dose Toxicology

Single dose toxicology studies following IV or oral dosing were conducted in CD1 mice and SD rats. IV doses of 0, 100, 150, 155, 160, or 200 mg/kg resulted in respiratory arrest at doses greater than 100 mg/kg; clinical signs at all doses included respiratory difficulty, tremors or clonic convulsions, and slight hypokinesia. In male and female rats, single IV doses of 0, 96, 120, or 150 mg/kg resulted in death at doses greater than 96 mg/kg, and clinical signs such as shallow breathing, hypoactivity, and piloerection at all doses.

In oral studies, administration of 0 or 1000 mg/kg givinostat in male and female mice resulted in hypokinesia and dyspnea. In rats, oral administration of 0, 1560, 2434, or 5000 mg/kg givinostat resulted in death at doses above 2434 mg/kg, which was accompanied by histologic signs of kidney medulla congestion. Clinical signs in rats included transient salivation, hunched posture, diarrhea, and hypoactivity at all doses, shallow breathing at doses above 1560 mg/kg, and dilation and urine-stained perineum at the high dose.

Single IV administration of the metabolite ITF2374 (20, 32, 50 mg/kg) resulted in mortality at all doses (1/5 LDM, 4/5 MDM, 2/5 MDF, 5/5 HDM, 3/5 HDF). The Applicant calculated an LD_{50} for males (25.2 mg/kg) and females (41.2 mg/kg), suggesting that males were more sensitive than females. Clinical signs following IV administration of ITF2374 included sedation, clonic

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convulsions, and shallow breathing. IV administration of the metabolite ITF2375 (100 mg/kg, 5M and 5F; 80 mg/kg, 5M) resulted in sedation and shallow breathing at both doses and death in 2 HDM.

13.1.4. Repeat-Dose Toxicology

R epeat dose studies were conducted in SD rats, cynomolgus monkeys, and beagle dogs. Primary toxicities included transient CNS signs in rat and monkey, decreases in WBC counts and bone myeloid series in rats, and bile duct hyperplasia and thymic atrophy in monkeys. Repeat oral dosing was also assessed over a 4-week period in male and female beagle dogs, but further testing in dog was not conducted due to GI toxicity consisting of vomiting, loose or liquid stools, and histological findings of inflammation in the esophagus and small intestine.

Rats

The toxicity of repeat IV and oral dosing was assessed in male and female SD rats. Daily IV doses of 0, 5, 25, or 75 mg/kg givinostat were to be administered for 4 weeks; however, dosing was terminated on Day 21 due to injection site toxicity. Transient clinical signs were observed at the high dose and included sedation, hypomotility, and piloerection.

Repeat oral dosing in male and female SD rats was conducted in studies of 4, 13, and 26-weeks' duration, with doses up to 250, 160, and 90 mg/kg, respectively. Primary toxicities included transient clinical signs (salivation, piloerection, ungroomed coat), reductions in WBC counts and bone marrow cellularity, decreases in body weight gain, and adrenal gland atrophy; adverse effects were not observed over the recovery periods. Based on the 26-week study, the NOAEL for oral dosing in rats was 10 mg/kg (givinostat Cmax = 16.6 ng/mL, AUC0-t = 45.1 ng*h/mL; ITF2374 Cmax = 5.2 ng/mL, AUC0-t = 34.1 ng*h/mL; ITF2375 Cmax = 173.9 ng/mL, AUC0-t = 528.3 ng*h/mL).

Monkeys

Repeat oral doses were evaluated in studies of 4, 13, and 39 weeks' duration. In the 4-week study, doses of 0, 10, 30, or 90 mg/kg were planned; however, dosing in the HD groups was discontinued between Days 8 and 9 due to clinical signs including low food intake, vomiting, hunched posture, piloerection, salivation, and underactivity. One HDM and 2 HDF were euthanized on Day 12 due to declining clinical condition. After a 20-day drug-free period, the HD was reduced to 60 mg/kg and dosing was reinitiated; however, all HDM and HDF were terminated 9 days later due to emergence of similar clinical signs. Based on toxicity in the 4-week study, the high dose for subsequent repeat-dose studies was reduced to 30 mg/kg. Primary toxicities in the 13 and 39-week studies included reductions in WBC and elevations in ALT and bilirubin, which were accompanied by histologic signs of diffuse bile duct hyperplasia. Additional histopathology findings included thymus atrophy and decreased bone marrow cellularity. Bile duct, thymus, and bone marrow histopathology remained present after recovery periods of 4 or 8 weeks in the 13 and 39-week studies, respectively, but appeared to be resolving. The NOAEL in the 39-week oral study was 10 mg/kg (givinostat Cmax = 11 ng/mL, AUC0-t =

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18 ng*h/mL; ITF2374 Cmax = 8 ng/mL, AUC0-t = 19 ng*h/mL; ITF2375 Cmax = 548 ng/mL, AUC0-t = 999 ng*h/mL).

13.1.5. Genetic Toxicology

Genetic toxicology was initially assessed in in vitro (Ames, in vitro mammalian chromosomal aberration in human lymphocytes) and in vivo (rat micronucleus, mammalian cell mutation [i.e., thymidine kinase assay], and rat liver DNA repair) assays. Givinostat was negative in the clastogenicity assays; however, significant increases in reversions in the Ames assay indicated the potential for mutagenicity. To further assess the mutagenic potential of givinostat, the Applicant conducted a transgenic rat mutagenesis assay (Big Blue) that included assessment of the Pig-a endpoint. Based on a review of these data by the PTCC Genetic Toxicology Subcommittee, it was determined that the risk of in vivo mutation was negligible for givinostat. The Applicant was therefore informed in a Type C meeting (May 11, 2020) that, due to the nature of the proposed indication (i.e., DMD), carcinogenicity studies for givinostat may be deferred until post approval.

13.1.6. Reproductive Toxicology

A complete battery of reproductive and developmental toxicology studies was conducted with orally administered givinostat. To assess fertility, doses of 0, 40, 80, and 160 mg/kg were administered in male and female rats prior to pairing and continuing to gestation day (GD) 7 in females. No adverse effects were observed on fertility parameters. Uterine findings consisted of an increased number of corpora lutea at doses greater than 40 mg/kg and increased pre- and postimplantation loss at all doses.

Embryofetal development was assessed in pregnant rats and NZW rabbits. In rats, administration of givinostat (0, 40, 80, and 160 mg/kg) during GDs 6-17 resulted in reductions in maternal body weight gain and food consumption at the high dose. Following cesarean section, drug-related effects consisted of reductions in mean fetal and placenta weights of up to 15 and 13%. Examination of the fetuses indicated an increased incidence of skeletal (thoracic vertebral abnormalities, 13/14 ribs, and incomplete vertebral ossification) and visceral (small, absent, or rudimentary thyroid) variations at the high dose, and visceral (partially undescended thymus and rudimentary or absent kidney papilla) variations at the mid and high doses. At the same doses administered to rabbits during GDs 6-19, severe GI toxicity resulted in premature sacrifice at the high dose due to declining clinical condition. As a result, there were too few fetuses to evaluate at that dose. No adverse effects were observed in rabbit fetuses at the low and mid doses.

In a pre- and postnatal development study in rat, administration of givinostat (0, 40, 80, and 160 mg/kg) from GD 6 to postnatal day (PND) 20 resulted in reduced number of litters with liveborn pups, increases in stillborn pups, total litter loss, and decreases in pups surviving to PND 21. Delayed growth was seen in offspring at the high dose, but there were no differences in time to sexual maturity. Neurobehavioral assessments conducted postweaning indicated increases in

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inactivity and decreases in total distance traveled at all doses in males. In females, there were decreases in total distance travelled in MDF and HDF offspring. When offspring were mated, no adverse effects were observed on reproductive performance or fertility; upon caesarean section, there were no drug-effects on numbers of corpora lutea, implantations, viable embryos, % implantations, or pre or postimplantation loss.

13.1.7. Juvenile Animal Toxicity

A 4-week study, with an 11-week recovery period was conducted in juvenile rats administered 0, 20, 60, and 180 mg/kg givinostat by daily oral gavage. However, upon review, the study was determined to be inadequate due to the age at which dosing was initiated in the animals (i.e., postnatal day 28) and inadequate group sizes.

13.1.8. Other Toxicology Studies

Exposure to light did not result in degradation of givinostat when evaluated by high performance liquid chromatography. Givinostat and the metabolites ITF2375 and ITF2375 did not absorb light within the 290 to 700 nm spectrum.

13.2. Individual Reviews of Studies Submitted With the New Drug Application

13.2.1. Introduction

The pivotal juvenile animal toxicity study was submitted only to the NDA.

13.2.2. Juvenile Animal Toxicity

Table 59. Study Design

Study Information	Study Details
Study title	13-Week Juvenile Toxicity Study of ITF2357 by the Oral (Gavage) Route in the Rat with a 8-Week Recovery Period
Study no.	20313876
Study report location	docuBridge, SDN 3, 4.2.3.5.4
Conducting laboratory and location	(b) (4)
Duration	13 weeks, with 8-week recovery
GLP compliance	Yes
Drug, lot #, and % purity	ITF2357, Lot No. 33, 99.8% pure
Doses	Days 1 to 21: 0, 10, 20, 40 mg/kg Days 22 to 42: 0, 15, 30, 60 mg/kg Days 43 to 92: 0, 15, 45, 90 mg/kg

Study Information	Study Details
Frequency of dosing	Daily
Number/sex/group	Main study: 16/sex/group Recovery Study: 20/sex/group TK Study (Control): 3/sex/group TK Study (Drug): 15/sex/group
Dose volume	5 mL/kg
Formulation/vehicle	Purified water
Route of administration	ORAL GAVAGE
Species	Rat
Strain	Sprague Dawley
Age at start of experiment	7 days
Period of development studied	Juvenile (1-14 weeks of age)
Comment on study design and conduct	Doses were increased on Days 22 (LD, MD, HD) and 43 (MD and HD) to maintain consistent systemic exposure. Dosing was initiated on postnatal Day 7.
Parameters and key endpoints evaluated	Mortality, clinical observations, body weights, food consumption, ophthalmology, sexual maturation, behavioral tests (learning and memory, locomotor activity, and auditory startle reflex), left tibia length, clinical pathology parameters (hematology, clinical chemistry and urinalysis), splenic lymphocyte subpopulations, toxicokinetic parameters, bone densitometry, organ weights and macroscopic and microscopic examinations
Dosing solution analysis	All dosing solutions were within the acceptance criteria (± 15%).

Source: Reviewer-created table derived from Study Report 20313876
Abbreviations: GLP, good laboratory practice; HD, high dose; LD, low dose, MD, medium dose; TK, toxicokinetic

Observations and Results

Mortality and Clinical Signs

Animals were monitored twice daily (BID) for mortality or signs of morbidity. Detailed examinations were conducted weekly.

There were no drug-related deaths. Liquid feces were observed at MD and HD during the first week of dosing. CNS signs in 1 HDF, consisting of piloerection, hunched posture, pale appearance, swollen forelimb/forepaw, lameness, abnormal gait, decreased activity and cold to touch condition, were observed between PNDs 22 and 26; however, based on absence of these clinical signs after PND 26 and the absence of similar findings in other HDM or HDF, it was unclear if these findings were drug related.

Body Weight and Food Consumption

Body weight and food consumption were assessed twice weekly during the dosing period, and weekly over the recovery period. There were no drug effects on body weight or food consumption.

Ophthalmoscopy

Ophthalmoscopy parameters were assessed by indirect ophthalmoscopy during the first week after weaning and at the end of the dosing period. There were no drug-related findings.

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Hematology, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected from fasted animals during Weeks 14 and 22. There were no drug effects on hematology, clinical chemistry, or urinalysis parameters.

Immunophenotyping

Splenic lymphocyte populations were assessed at necropsy. There were no drug effects on leucocytes, viable cells, T lymphocytes, T-Helper cells (Th), Cytotoxic T lymphocytes, B lymphocytes, Natural Killer or Natural Killer T lymphocytes.

Sexual Maturation

Vaginal opening and balanopreputial cleavage were assessed from PND 28 and 38, respectively; there were no drug-effects on sexual maturation.

Reproductive Capacity

Not assessed.

CNS/ Neurobehavioral Assessment

L earning and memory was assessed in all main study (Weeks 11-12) and recovery (Weeks18-19) animals using the Cincinnati water maze. There were no drug-related effects on learning and memory.

Locomotor activity (ambulatory and fine movement) in a novel environment was assessed by open field in all main and recovery group animals during Weeks 12 and 20, respectively. Auditory startle reflex was assessed using a pressure sensor platform to assess reflex in response to acoustic signals in all main and recovery group animals during Weeks 13 and 20, respectively.

Increases in locomotor activity were observed in drug-treated groups, with effects on fine movements and ambulation most evident in MD and HD recovery females but also seen at the HD in females at the end of the dosing period and at the high dose in males at the end of the recovery period (Tables 1-4).

Reference ID: 5351242

Table 60. Open Field, Dosing Period, Males

		Interval (Count, % Habitation)										
	Statistic	1	2		3		4		5		6		
Dose		Count	Count	%	Count	%	Count	%	Count	%	Count	%	Total
Fine movement													
0/0/0	Mean	1195	636	-46	359	-70	247	-80	255	-77	256	78	2948
	SD	307	263	22	230	21	223	18	277	26	260	20	945
10/15/15	Mean	1090	640	-42	509	-55	325	-67	290	-73	377	-66	3231
	SD	310	294	26	310	27	290	28	302	27	301	25	1216
20/30/45	Mean	1074	589	-43	525	-48	369	-63	408	-59	434	-57	3373
	SD	264	250	22	232	25	274	31	266	33	210	22	806
40/60/90	Mean	1290	662	-48	563	-55	368	-69	421	-65	399	-69	3702
	SD	181	240	19	239	19	276	28	289	25	261	19	914
Ambulation													
0/0/0	Mean	243	95	-61	46	-82	25	-89	39	-83	38	-83	486
	SD	113	55	21	39	18	27	12	49	21	56	22	226
10/15/15	Mean	219	93	-54	73	-67	39	-78	41	-84	57	-75	521
	SD	97	58	35	54	22	45	25	51	18	58	25	253
20/30/45	Mean	213	84	-58	82	***-59****	62	-70	57	-70	54	-73	551
	SD	92	57	29	56	27	57	30	49	30	39	20	225
40/60/90	Mean	235	92	-61	90	***-60****	42	-79	48	-76	55	-78	562
	SD	60	65	25	49	21	46	26	40	22	51	20	190

Source: Reviewer-created table derived from Study Report 20313867
* Statistically significant different vs control

Reference ID: 5351242

Table 61. Open Field, Dosing Period, Females

Dose	Statistic	Interval (Count, % Habitation) 1 Count	2 Count	2 %	3 Count	3 %	4 Count	4 %	5 Count	5 %	6 Count	6 %	Total
Fine movement													
0/0/0	Mean	1045	749	-28	527	-50	363	-63	407	-58	378	-63	3469
	SD	306	297	18	314	28	281	29	255	27	330	37	1085
10/15/15	Mean	1218	715	-38	652	-44	429	-62	408	-63	490	-59	3912
	SD	367	216	21	261	22	289	28	304	28	365	27	1212
20/30/45	Mean	1021	***504****	***-52****	373	-60	343	-57	257	-70	283	-74	2782
	SD	310	294	23	187	25	291	49	279	33	253	22	997
40/60/90	Mean	***1322****	874	-34	596	-55	575	-55	522	-60	344	-73	***4233****
	SD	244	419	28	371	26	188	16	271	22	308	25	921
Ambulation													
0/0/0	Mean	292	163	-34	126	-55	76	-60	114	-47	89	-37	860
	SD	157	106	43	127	49	77	52	107	58	101	137	450
10/15/15	Mean	362	160	-54	131	-62	85	-76	83	-71	85	-75	906
	SD	150	96	23	73	21	74	19	90	32	79	22	332
20/30/45	Mean	299	99	***-70****	72	-58	69	-35	60	-63	48	-84	648
	SD	142	88	26	61	87	80	172	77	62	47	16	299
40/60/90	Mean	393	195	-53	134	-68	107	-69	105	-73	44	-78	977
	SD	114	111	21	107	24	65	24	73	22	55	17	310

Source: Reviewer-created table derived from Study Report 20313867
* Statistically significant different vs control

Reference ID: 5351242

Table 62. Open Field, Recovery Period, Males

Dose	Statistic	Interval (Count, % Habitation) 1 Count	2 Count	2 %	3 Count	3 %	4 Count	4 %	5 Count	5 %	6 Count	6 %	Total
Fine movement													
0/0/0	Mean	663	430	-28	284	-50	330	-45	269	-55	243	-54	2220
	SD	250	249	51	154	40	162	40	192	32	201	55	821
10/15/15	Mean	599	379	-13	261	-49	212	-48	221	-24	293	4	1965
	SD	363	274	84	220	43	214	68	206	119	229	185	1012
20/30/45	Mean	812	440	-18	332	-32	261	-65	247	-56	268	-52	2360
	SD	407	292	107	246	118	243	26	232	56	240	64	1054
40/60/90	Mean	887	525	-37	398	-54	235	-72	322	-61	270	-66	2637
	SD	242	235	28	278	28	152	20	193	25	186	26	830
Ambulation													
0/0/0	Mean	79	39	31	27	35	38	-21	25	-38	24	-45	232
	SD	52	33	372	24	389	30	108	31	105	30	93	124
10/15/15	Mean	74	38	90	30	-41	21	*-77**	25	211	40	239	228
	SD	61	38	446	36	65	40	39	43	1155	51	1031	184
20/30/45	Mean	119	47	-37	38	*-53*	25	*-77**	28	-68	29	-67	287
	SD	68	42	85	42	71	31	26	35	50	29	38	143
40/60/90	Mean	*125**	63	-36	47	*-59*	23	*-76**	33	-69	31	-71	321
	SD	57	36	51	46	39	24	31	26	31	31	31	134

Source: Reviewer-created table derived from Study Report 20313867
* Statistically significant different vs control

Table 63. Open Field, Recovery Period, Females

Dose	Statistic	Interval (Count, % Habitation)												Total
		1	2		3		4		5		6			
		Count	Count	%	Count	%	Count	%	Count	%	Count	%		
Fine movement														
0/0/0	Mean	873	464	-46	309	-60	380	-55	257	-65	245	-69		2526
	SD	196	218	25	229	35	249	29	256	40	191	25		665
10/15/15	Mean	1090	678	-37	398	-66	393	-61	332	-65	307	-70		***3198****
	SD	357	304	24	303	20	256	27	238	31	271	27		984
20/30/45	Mean	***1247****	***775****	***-39***	501	-61	422	-66	249	-78	366	-68		***3580****
	SD	394	370	19	297	19	356	27	221	20	260	25		1296
40/60/90	Mean	***1122****	***786****	-29	***595****	-45	448	-57	295	-74	260	-75		***3506****
	SD	316	282	25	286	29	288	30	250	23	315	32		1338
Ambulation														
0/0/0	Mean	230	98	-56	54	-71	96	-55	43	-74	54	-69		575
	SD	92	64	27	68	39	75	31	58	38	49	31		203
10/15/15	Mean	269	118	-56	46	-85	76	-67	56	-77	48	-80		612
	SD	103	67	21	56	18	62	30	58	26	71	33		203
20/30/45	Mean	318	***174****	***-50***	***105****	-68	74	-76	39	-85	58	-75		767
	SD	176	143	21	95	30	78	23	47	19	57	26		436
40/60/90	Mean	***336****	***197****	-42	***147****	***-57****	83	-75	61	-84	78	-79		901
	SD	102	90	21	100	27	82	24	66	18	106	29		443

Source: Reviewer-created table derived from Study Report 20313867
* Statistically significant different vs control

There were no clear effects on auditory startle amplitude or habituation, but a decrease in percent prepulse inhibition was observed in HD males at the end of the dosing period (statistically significant for 68 db) but not during the recovery period (see sponsor's table below).

Table 64. Group Mean Auditory Startle Reflex

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Group Mean Auditory Startle Reflex - Dosing Period
Prepulse Inhibition - Group Mean values (Newton)

Females

	Prepulse + Pulse and Pulse Trials (N)				% of Prepulse Inhibition		
Group 1, 0/0/0 mg/kg/day	No PP	PP 68db	PP 71db	PP 77db	PP 68db	PP 71db	PP 77db
Mean	2.658	2.018	1.502	0.997	-21.5	-41.2	-60.4
SD	1.021	0.643	0.492	0.327	11.8	12.0	9.5
N	16	16	16	16	16	16	16
Group 2, 10/15/15 mg/kg/day							
Mean	3.000	2.432	1.890	1.307	-16.7	-36.2	-55.5
SD	1.440	1.121	0.973	0.634	16.6	17.7	13.7
N	16	16	16	16	16	16	16
Group 3, 20/30/45 mg/kg/day							
Mean	2.383	1.733	1.389	1.005	-25.1	-40.4	-56.2
SD	1.199	0.902	0.817	0.553	19.0	19.8	14.7
N	16	16	16	16	16	16	16
Group 4, 40/60/90 mg/kg/day							
Mean	2.550	2.163	1.778	1.076	-14.2	-30.1	-58.4
SD	1.179	1.051	0.898	0.667	23.1	14.0	11.9
N	16	16	16	16	16	16	16

Source: Sponsor's table from Study Report 20313867

Bone Evaluation

Bone length and density were assessed in 10/sex/group at the end of the main and recovery periods. Bone length was assessed by measuring femur length, and bone density was assessed by dual energy X-ray on femur and L4 lumbar vertebra. There were no drug effects on tibia length. However, decreases in tibia and vertebra bone density were seen at all doses in males and in MDF and HDF after the dosing period. After the recovery period, tibia and vertebra bone density remained lower in MDM and HDM. In females, there were dose-dependent decreases in tibia density after the recovery period.

Reference ID: 5351242

Table 65. Bone Mineral Density (g/cm2)

	Males (mg/kg)				Females (mg/kg)			
Measurement	**0/0/0**	**10/15/15**	**20/30/45**	**40/60/90**	**0/0/0**	**10/15/15**	**20/30/45**	**40/60/90**
Dosing Period								
Tibia	0.257	0.257	0.253	0.246	0.246	0.247	0.237	0.233
epiphysis	0.244	0.234	0.233	0.223	0.244	0.246	0.232	0.231
metaphysis	0.246	0.247	0.242	0.225	0.265	0.267	0.244	0.231
diaphysis	0.188	0.185	0.182	0.173	0.184	0.185	0.176	0.170
Vertebra	0.262	0.257	0.251	0.252	0.244	0.250	0.233	0.231
Recovery Period								
Tibia	0.295	0.293	0.288	0.286	0.267	0.274	0.27	0.261
epiphysis	0.283	0.279	0.279	0.276	0.271	0.275	0.272	0.258
metaphysis	0.256	0.255	0.252	0.244	0.256	0.274	0.258	0.245
diaphysis	0.226	0.228	0.218	0.214	0.209	0.206	0.203	0.198
Vertebra	0.279	0.281	0.276	0.273	0.257	0.264	0.261	0.254

Source: Reviewer-created table derived from study report 2031876

Gross Pathology and Organ Weights

Small adrenal gland was observed in 5/15 HDM, correlated with a dose-dependent decrease in adrenal gland weight. Prostate weights were decreased in HDM. There were no drug-related gross findings or effects on organ weights in the recovery groups.

Table 66. Adrenal Gland Weight (Dosing Period)

Measurement	0/0/0 mg/kg	10/15/15 mg/kg	20/30/45 mg/kg	40/60/90 mg/kg
Males				
Adrenal gland				
Absolute wt. (g)	0.06617	0.06197	0.05353	0.04424
Relative (% brain)	2.96239	2.81703	2.40134	2.01823
Relative (% body)	0.01379	0.01258	0.01044	0.00874
Prostate				
Absolute wt. (g)	1.07156	1.09386	1.00178	0.77374
Relative (% brain)	48.02406	49.71428	44.83579	35.51321
Relative (% body)	0.22427	0.22236	0.19396	0.15605
Females				
Adrenal gland				
Absolute wt. (g)	0.07427	0.06966	0.06281	0.05478
Relative (% brain)	3.70059	3.45979	3.09299	2.73735
Relative (% body)	0.02738	0.02516	0.02301	0.01936

Source: Reviewer-created table derived from Study Report 20313876
Abbreviations: wt, weight

Histopathology

- Adequate Battery: Yes
- Signed Pathology Report: Yes
- Peer Review: Yes

Findings: There were no drug-related histopathology findings in main-study or recovery groups.

Toxicokinetics

Standard toxicokinetic parameters were assessed on Days 1, 22, 43, and 92 for givinostat and the metabolites, ITF2374 and ITF2375. There were no sex differences in time to maximum plasma concentration, C_{max}, or AUC.

Table 67. TK Parameters for Givinostat

		Males			Females		
Study Day	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)
1	10	2	57.7	255	1	58.9	289
	20	1	113	513	1	129	567
	40	2	164	1030	2	258	1190
22	15	1	6.47	31.5	1	5.07	23.1
	30	2	24.7	89.8	2	45.6	121
	60	2	62.4	192	2	108	285
43	15	1	3.62	13.7	2	5.89	18.6
	45	2	32.6	98	2	58.7	227
	90	2	142	531	2	179	501

		Males			Females		
Study Day	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)
92	15	2	7.64	39.7	1	14	35.1
	45	2	104	502	1	103	231
	90	2	290	1390	2	186	790

Source: Reviewer-created table derived from Study Report 20313876
Abbreviations: AUC_{0-24}, area under the curve from 0 to 24 hours; C_{max}, maximum plasma concentration; TK, toxicokinetic; T_{max}, time to maximum concentration

Table 68. TK Parameters for ITF2374 (Metabolite)

		Males			Females		
Study Day	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)
1	10	4	28.3	418	4	26.8	337
	20	4	46.1	609	4	51.7	712
	40	4	68	1020	2	69.6	949
22	15	4	15.1	119	4	10.5	139
	30	8	15	200	2	18.5	205
	60	8	36.8	503	4	51.2	417
43	15	4	9.8	107	4	17.6	244
	45	8	31.7	390	4	38.5	527
	90	8	45.2	588	8	76.9	1090
92	15	4	19.2	167	8	21.9	282
	45	8	60	742	8	80.5	1160
	90	8	136	1950	8	195	2730

Source: Reviewer-created table derived from Study Report 20313876
Abbreviations: AUC_{0-24}, area under the curve from 0 to 24 hours; C_{max}, maximum plasma concentration; TK, toxicokinetic; T_{max}, time to maximum concentration

Table 69. TK Parameters for ITF2375 (Metabolite)

		Males			Females		
Study Day	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)
1	10	2	110	1180	4	109	1260
	20	2	219	2100	2	236	2120
	40	4	448	4350	2	473	4600
22	15	1	47.8	216	1	40.3	298
	30	2	113	631	2	207	769
	60	2	221	1300	2	295	1580
43	15	1	28.2	158	2	37.8	231
	45	2	137	886	2	226	1200
	90	2	376	2700	2	651	3110
92	15	4	50.5	439	1	95.9	501
	45	2	452	3160	1	311	1910
	90	2	1100	9590	2	799	6190

Source: Reviewer-created table derived from Study Report 20313876
Abbreviations: AUC_{0-24}, area under the curve from 0 to 24 hours; C_{max}, maximum plasma concentration; TK, toxicokinetic; T_{max}, time to maximum concentration

Conclusion

Dosing in the pivotal juvenile animal toxicity study was informed by a 4-week dose range-finding study in which givinostat was administered orally at doses of 0, 20, 60, and 180 mg/kg male and female rats beginning on PND 25. In the dose-range finding study, toxicity was

observed at the high dose (i.e., decreases in tibia length, body weight, reticulocytes, platelets, WBC count, and bone-marrow erythroid series). In the pivotal juvenile animal toxicity study, no adverse effects on developmental parameters were observed; drug-related findings consisted of decreased adrenal gland size and weight, and decreased prostate gland weight at the high dose at the end of the dosing period only.

14. Clinical Pharmacology

14.1. In Vitro Studies

In vitro studies conducted to characterize the absorption, distribution, metabolism and excretion and drug-drug interaction (DDI) potentials are summarized in Table 70. Apparent permeability (Papp) of givinostat was found to be moderate based on two different in vitro models (Studies TR1608/E and TR1876/E), Caucasian colon adenocarcinoma (Caco-2) and Madin-Darby canine kidney (MDCK)II multidrug resistance protein (MDR)1.

Givinostat is highly bound to plasma proteins (Study TR1683/E). The binding in human plasma was 95.9 ± 1.3%, independent of plasma concentrations in a range that largely covers the therapeutic plasma levels. Blood to plasma ratio was slightly above 1 in all species and in humans (human blood to plasma ratio; RB/P = 1.3), suggesting a moderate accumulation in the red blood cells (Study TR2411/I).

Givinostat is extensively metabolized and up to 15 metabolites have been identified in in vitro and in vivo experiments. To determine the enzymes involved in the main metabolic reactions of givinostat, metabolism was studied in several hepatic and extrahepatic fractions and recombinant enzymes. The results showed that the metabolism in humans does not involve the cytochrome P450s (CYPs) and UGTs. ITF2440 is a very important metabolite resulted in human hepatocytes (highest metabolite to parent ratio in human plasma), but not a specific enzyme was identified for this metabolite. Human mitochondrial amidoxime reducing components (mARCs) 1 and 2 are both involved in the reduction of the hydroxamic acid of givinostat to the amide ITF2374 (Study TR2305/E). The hydrolysis of the hydroxamic acid of givinostat to form the carboxylic acid ITF2375 occurs mainly in the blood (Study TR2411/I); liver and intestine carboxylesterase are not involved in this reaction, as confirmed by experiments with the human recombinant enzymes hCES1 and hCES2 (Study TR2471/I).

The potential DDI risk associated with givinostat as a victim and perpetrator was also assessed in the in vitro studies. Givinostat is not a substrate of CYPs or UGTs. In vitro studies indicate that givinostat is a substrate of the intestinal transporters: P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), not a substrate of OCTs, organic anion transporters (OATs), and OATPs.

Givinostat and its metabolites ITF2374, ITF2375, ITF2440 and ITF2563 were investigated as inhibitors of the main CYP subfamilies, both as direct inhibitors, determining the inhibition constant (Ki), and as time dependent inhibitors (inhibition enhanced by preincubation) performing half maximal inhibitory concentration shift experiments. Based on the in vitro results, no relevant DDI potential of givinostat for CYP 1A2, 2C9, 2C19, 2D6, 2B6, 2C8 and 3A4 inhibition is expected. However, the inhibition of the intestinal CYP3A4 could not be

excluded when doses ≥10 mg were administered. In vitro induction studies showed that Givinostat might be a potential inducer of CYP1A2, 2B6 at concentrations up to 3 μM and of CYP3A4 at concentration up to 0.3μM. Givinostat is a moderate inducer for CYP1A2 and 2B6 and a moderate to strong inducer of CYP3A4. The inhibitory and inductive potential of Givinostat towards CYP3A4 was investigated in a clinical DDI Study (Study ITF/2357/55).

The in vitro inhibition activity of givinostat on P-gp(MDR1) and BCRP (intestinal transporters), OAT1, OAT3, OCT2, MATE1 and MATE2-K (renal transporters) and OATP1B1, OATP1B3 and OCT1 (hepatic uptake transporters) was investigated. Givinostat has the potential to inhibit the intestinal transporter P-gp and BCRP. Givinostat showed the potential to inhibit the renal uptake transporter OCT2, but inhibition of the other two intestinal transporters MATE1 and MATE2-K can be excluded. Givinostat's effect on OCT2 was evaluated in the clinical trials by investigating the disposition of the endogenous substrate, creatinine.

Investigation on transporter proteins inhibition by the metabolites ITF2375, ITF2440 and

ITF2563 have been conducted showing no relevant DDI potential for these metabolites on P-gp, BCRP, OAT1, OAT3, OCT2, MATE1, MATE2-K, OATP1B1, OATP1B3 and OCT1.

Table 70. Summary of In Vitro Studies to Characterize ADME and DDI Risk of Givinostat

Study Type	Study Number	Biomaterial
Absorption studies		
Permeability	TR1608/E	Caco-2
Permeability	TR1876/E	Caco-2 and MDCK-MDR1
Distribution studies		
Protein binding	TR1683/E	Human plasma
Blood:plasma partitioning	TR2411/I	Human blood and plasma
Metabolism studies		
In vitro stability & metabolism	TR2411/I	Human blood and plasma
In vitro metabolic stability	SR44/13	Human hepatocytes
In vitro metabolism	TR2262/I	Human hepatocytes
In vitro metabolic stability	SR42/13	Human liver S9 fraction
In vitro stability & metabolism	TR2488/I	Human cytosol
In vitro stability & metabolism	TR2412/I	Human liver, kidney & intestinal microsomes
In vitro stability	TR2489/I	Human mitochondria
In vitro metabolism	TR2305/E	Human recombinant mARC
In vitro metabolism	TR2263/I	Human recombinant cytochrome P450
In vitro metabolism	TR2471/I	Human recombinant carboxylesterases

Study Type	Study Number	Biomaterial
PK drug interaction studies		
In vitro inhibition (CYP450)	TR2126/I, TR2149/I, TR2205/I, TR2216/I, TR2225/I, TR2271/I, TR2275/I, TR2273/I, TR2296/I, TR2297/I, TR2298/I, TR2299/I	Human liver microsomes with probe substrates for CYP450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP3A4, CYP2D6, CYP2B6, CYP2C8)
In vitro induction (CYP450)	TR2251/E, TR2252/E	Human hepatocytes (mRNA of CYP1A2, CYP2B6, CYP3A4)
In vitro substrate (drug transporter)	TR2329/E, TR1876/E ADM-22-4264a	MDCK-BCRP, Caco-2 and MDCK-MDR1, HEK-293-OATP1B1, HEK-293-OATP1B3
In vitro inhibition (drug transporter)	TR2178/E, TR2328/E, TR2430/E, TR2431/E, TR2470/I, TR2404/E, TR2405/E, ADM-22-4203a, ADM-22-4203b	Cell lines overexpressing human transporters

Source: 2.7.2 Summary of Clinical Pharmacology Studies Page 14
Abbreviations: ADME, absorption, distribution, metabolism and excretion; BCRP, breast cancer resistance protein; CYP450, cytochrome P450; DDI, drug-drug interaction; HEK, human embryonic kidney cell; MARC, Murine CCL7; MDCK, Madin-Darby canine kidney cell; MDR1, multidrug resistance 1; MRNA, messenger ribonucleic acid; OAT, organic anion transporter; PK, pharmacokinetic

14.2. In Vivo Studies

The pharmacokinetics of givinostat and major metabolites were characterized in both healthy volunteers and DMD patients. The clinical studies conducted are summarized in Table 71. The key studies (food effect and DDI studies) are described in subsections below.

Table 71. Summary of Clinical Pharmacology Studies

Study No.	Studied Population	Brief Description	Dose & Formulation	No. of Subjects
Healthy volunteers				
DM/00/2357/01 (Study 01)	Healthy volunteers	Single ascending dose	50 mg[1] 100mg[1] 200 mg[1] 400 mg[1] 600 mg[1]	40
DM/00/2357/03 (Study 03)	Healthy volunteers	Multiple ascending dose	50 mg QD[1] 100 mg QD[1] 200 mg QD[1] 100 mg BID[1]	41
DM/00/2357/04 (Study 04)	Healthy volunteers	Food effect	100 mg QD[1]	12
ITF/2357/54 (Study 54)	Healthy volunteers	Thorough QT/QTc	100 mg QD[2] 300 mg QD[2]	34
ITF/2357/55 (Study 55)	Healthy volunteers	Drug-drug Interaction	50 mg BID[2] 50 mg[2]	54

Study No.	Studied Population	Brief Description	Dose & Formulation	No. of Subjects
Neuromuscular disease				
DSC/11/2357/43 (Study 43)	Duchenne muscular dystrophy	Phase 2	25 mg BID[1,2] 37.5 mg BID[1,2] 50 mg BID[1,2] 25 - 60 mg BID[2] in extension phase	20
DSC/14/2357/48 (Study 48)	Duchenne muscular dystrophy	Phase 3	10.63-70 mg BID according to body weight[2]	179
Other patient populations				
DSC/03/2357/06	Crohn's disease	Phase 2	50 mg QD 50 mg BID 100 mg QD 100 mg BID	106
DSC/03/2357/07	Plaque psoriasis	Dose finding Phase 2a	50 mg QD 50 mg BID 100 mg QD 100 mg BID	55
DSC/05/2357/19	Systemic onset juvenile idiopathic arthritis	Phase 2	0.75 mg/kg BID	17
DSC/06/2357/23	Crohn's disease	Phase 2	50 mg BID	51
DSC/08/2357/36	Polyarticular course	Phase 2	0.5 mg/kg BID 0.75 mg/kg BID	16
DSC/12/2357/45	JAK2 positive polycythemia vera	Phase 1b/2	Part A 50 mg BID 150 mg QD 100 mg BID Part B 100 mg BID	48

Source: 2.7.2 Summary of Clinical Pharmacology Studies Page 18
[1] Capsule
[2] Oral suspension
Abbreviations: BID, twice daily; JAK2, non-receptor tyrosine kinase; QD, once daily; TQT, thorough QT

Food Effect Study (Study DM/00/2357/04)

Study Design

An open-label, randomized, crossover, single dose study, with 12 healthy males. Subjects received a single oral 100 mg dose of givinostat (two 50 mg capsules) in the fasting state and after a high-fat diet standard meal. The fasting and fed periods were separated by a washout period of at least one week. In each period, blood samples were collected up to 24 hours post-dose.

PK Results

The summary of the statistical comparison of the C_{max} and AUC parameters for givinostat between the fasting and fed conditions is presented in Table 72. A high fat meal slightly increased givinostat's exposure compared to the fasted state. Similar increase of exposure of the metabolite, ITF2375 was also observed under fed conditions compared with fasting. For both compounds, the median time to maximum plasma concentration was slightly later (delayed from

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2 to 3 hours). The exposure of the metabolite ITF2374 were very slightly decreased (about 15%) under fed condition than fasted (data not shown).

Table 72. Summary of PK Parameters of Givinostat and Statistical Comparisons Between Fed and Fasted Condition

Parameter	Fed Geom. Mean (Range)	Fasted Mean (Range)	Estimated Ratio (Fed:Fasted) With 90%CI
$AUC_{0\text{-}inf}$ [ng.h/ml]	749(496-1197)	533(69-853)	1.40 (1.04-1.90)
$AUC_{0\text{-}t}$ [ng.h/ml]	703(447-1142)	498(54-819)	1.41 (1.02-1.95)
C_{max} [ng/ml]	105 (66.3-150)	84.7(8.2-187)	1.23 (0.79-1.93)

Source: Clinical Study Report DM/00/2357/04 Tables 3 and 6, page 37, 40.
Abbreviations: $AUC_{0\text{-}t}$, area under the curve up top the last quantifiable time-point; $AUC_{0\text{-}inf}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; Geom., geometric; PK, pharmacokinetic

Food effect evaluation was conducted using a prototype formulation (liquid-filled capsules), and not the to-be-marketed suspension formulation. The results indicated that high fat meals increased givinostat exposure in both AUC (about 40%) and C_{max} (about 23%) compared to those observed under fasted state.

The Applicant considered that the food effect for the liquid suspension would provide a similar increase of givinostat systemic exposure to that observed with the capsules. The justifications included: 1) the similarity of the formulation between capsule and suspension; 2) the insignificant effect of formulation (capsule versus suspension) on givinostat PK based on population pharmacokinetic (popPK) analysis. It is worth noting that the current proposed label recommends taking givinostat with food. In the phase 3 Study, givinostat (in the to-be-marketed suspension formulation) was administered with food, which was mainly intended to aid in masking the bitter taste of givinostat product. Overall, it was determined that a new food effect study in the to-be-marketed formulation is not necessary.

Clinical DDI Study (ITF/2357/55) to Investigate the DDI Potential of Givinostat as a Perpetrator and Victim and PK of Givinostat as Well as Major Metabolites

This study was conducted in healthy subjects to investigate the DDI potential of givinostat on CYP 3A4 and P-gp, the effect of P-gp inhibitor on givinostat PK, as well as the plasma and urine PK of givinostat and its metabolites following single and multiple oral doses of givinostat.

Study Design

Population: Healthy male and nonpregnant, non-lactating female with age ≥18 and ≤55 years; a total of 54 subjects was enrolled.

Objectives

The Study consisted of 3 parts with different objectives:

Part 1: to investigate the potential inhibitory and inducing effect of oral givinostat on CYP 3A4 enzyme (midazolam as the substate) and P-gp transporter (dabigatran as the substrate)

Part 2: to assess the effect of P-gp inhibitor (oral clarithromycin) on the PK of givinostat

Part 3: to evaluate the plasma and urine PK of givinostat and its metabolites following single and multiple oral doses of givinostat

Treatment

Part 1: On Days 1, 6 and 17, single doses of midazolam 1 mg IV and dabigatran etexilate 75 mg were administered 1 hour after the planned morning time of givinostat administration. On Days 2, 7 and 18, a single oral dose of midazolam 2.5 mg oral solution was administered 1 hour after the planned morning time of givinostat administration.

From Day 4 to Day 18, givinostat 50 mg as oral suspension was administered twice daily in the morning and in the evening. On Day 19, only the morning dose was administered.

Midazolam and dabigatran etexilate were administered following an overnight fasting of at least 8 hours and subjects remained fasted until at least 3 hours post-dose. Oral midazolam and dabigatran etexilate were administered with 150 mL of water.

Part 2: On Days 1 and 8, givinostat 50 mg as oral suspension was administered as a single dose, 1 hour after the planned morning time of clarithromycin administration. Givinostat was administered following an overnight fasting of at least 8 hours and subjects remained fasted until at least 3 hours post-dose. Except for water given with clarithromycin (150 mL), no fluids were allowed from 1 hour before the morning dosing until 2 hours post-dose. From Day 4 to Day 10, clarithromycin 500 mg film-coated tablet was administered twice daily, in the morning and in the evening.

Part 3: On Day 1 and Day 13, givinostat 50 mg as oral suspension was administered as a single dose, in the morning, following an overnight fasting of at least 8 hours and subjects remained fasted until at least 4 hours post-dose. From Day 5 to Day 12, givinostat 50 mg as oral suspension was administered twice daily, in the morning and in the evening.

PK Sampling

Part 1

Midazolam IV: at pre-dose and at 2 minutes, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12 and 24 hours after the administration of intravenous midazolam, on Days 1, 6 and 17, for the determination of midazolam and 1-hydroxymidazolam plasma concentrations.

Midazolam Oral: at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after dosing on Days 2, 7 and 18 for the determination of midazolam and 1-hydroxymidazolam plasma concentrations. The 24-hour blood collection following intravenous midazolam was assumed as the pre-dose sampling for oral midazolam.

Dabigatran etexilate: at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours after the administration of dabigatran etexilate, on Days 1, 6 and 17, for the determination of total and free dabigatran plasma concentrations.

Part 2

Givinostat: at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 15, 24, 36, 48, 60 and 72 hours after the administration of givinostat, on Days 1 and 8, for the determination of givinostat.

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Part 3

Plasma: at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 15, 24, 36, 48, 60, 72, 84 and 96 hours after the administration of givinostat, on Days 1 and 13, for the determination of givinostat and its metabolites (ITF2374, ITF2375, ITF2440 and ITF2563) plasma concentrations. Additionally, samples were collected before the morning dose of givinostat on Days 9, 10, 11 and 12, for the determination of pre-dose (Ctrough) plasma concentration of givinostat and metabolites.

Urine: on Day -1 (Baseline) and in the following intervals, on Days 1 and 13, for the determination of the amounts of givinostat and its metabolites (ITF2374, ITF2375, ITF2440 and ITF2563) excreted in urine: 0-12, 12-24, 24-36, 36-48, 48-72 and 72-96 post-dose.

Results

Part 1: Effect of Givinostat on the PK of Midazolam

A total of 26 healthy subjects were enrolled to Part 1 Study.

IV midazolam: The statistics of midazolam pharmacokinetic parameters following IV administration of midazolam alone (Day 1) and co-administration of midazolam IV and givinostat on Day 6 (after 2 days of administration of givinostat) showed minimal impact of midazolam PK, indicating givinostat has minimal inhibitory effect on hepatic CYP3A4 enzyme (see Clinical Study Report ITF/2357/55 Page 13-14). After 13 days of givinostat (on Day 17), it observed very slight increase (20%-30%) of AUC of midazolam and 1-hydroxymidazolam (see Clinical Study Report ITF/2357/55 Page 14). These results indicated that givinostat does not have significant inhibition and induction on hepatic 3A4.

Oral midazolam: Summary statistics of midazolam pharmacokinetic parameters following oral administration of midazolam alone (Day 2) and co-administration of oral midazolam and givinostat on Day 7 (after 3 days of administration of givinostat) was presented in Table 73 and Table 74. The results showed midazolam exposure after oral administration increased 24 % in C_{max} and 39% in AUC, and the exposure of metabolite,1-hydroxymidazolam increased 17% in C_{max} and 24% in AUC. The increase of givinostat exposure indicates givinostat has weak inhibitory effect on hepatic CYP3A4 enzyme. After 14 days of givinostat coadministration, it observed about 39% increase C_{max} and 67% increase of AUC of midazolam (Table 75). Similar exposure increase of 1-hydroxymidazolam as to midazolam (Table 76) was also observed (on Day 18). These results indicated that givinostat did not exhibit induction effect on intestinal CYP3A4 while larger inhibition of intestinal CYP3A4 was found with longer treatment of givinostat.

Table 73. Statistical Summary for Midazolam PK of Oral Administration With the Coadministration of Givinostat (From Day 4-7)

Parameter	Total ISCV%	Geometric LSmeans: Oral Midazolam Alone (Day 2)	Geometric LSmeans: Oral Midazolam Co-Administered with Givinostat (Day 7)	GMR (%)	90% CI
C_{max}	17.2	12695.07	15744.30	124.02	114.38 – 134.47
AUC_{0-t}	14.9	36483.20	50134.44	137.42	128.09 – 147.43
$AUC_{0-\infty}$	15.1	38471.79	53379.48	138.75	129.05 – 149.18

Source: Clinical Study Report ITF/2357/55, page 18
Note: Geometric LS means is given in pg/mL for C_{max} and pg.h/mL for AUC GMR.
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; GMR, geometric mean ratio; ISCV, intra-subject coefficient of variation; LS, least squares; PK, pharmacokinetic

Table 74. Statistical Summary for 1-Hydroxymidazolam PK After Midazolam Oral Administration With the Coadministration of Givinostat (From Day 4-7)

Parameter	Total ISCV%	Geometric LSmeans: Oral Midazolam Alone (Day 2)	Geometric LSmeans: Oral Midazolam Co-Administered with Givinostat (Day 7)	GMR (%)	90% CI
C_{max}	24.5	4451.49	5229.06	117.47	104.78 – 131.69
AUC_{0-t}	14.6	10871.01	14264.35	131.21	122.47 – 140.59
$AUC_{0-\infty}$	15.8	11914.16	15986.40	134.18	124.19 – 144.97

Source: Clinical Study Report ITF/2357/55, page 18
Note: Geometric LSmeans is given in pg/mL for Cmax and pg.h/mL for AUC GMR.
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; GMR, geometric mean ratio; ISCV, intra-subject coefficient of variation; LS, least squares; PK, pharmacokinetic

Table 75. Statistical Summary for Midazolam PK of Oral Administration With the Coadministration of Givinostat (From Day 4-18)

Parameter	Total ISCV%	Geometric LSmeans		GMR (%)	90% CI
		Oral Midazolam Alone (Day 2)	Oral Midazolam Co-Administered with Givinostat (Day 18)		
C_{max}	16.7	12695.07	17678.67	139.26	128.71 – 150.67
AUC_{0-t}	14.3	36483.20	57559.53	157.77	147.49 – 168.76
$AUC_{0-\infty}$	15.1	38436.77	62558.70	162.76	151.13 – 175.28

Source: Clinical Study Report ITF/2357/55, page 18
Note: Geometric LS means is given in pg/mL for C_{max} and pg.h/mL for AUC GMR.
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; GMR, geometric mean ratio; ISCV, intra-subject coefficient of variation; LS, least squares; PK, pharmacokinetic

Table 76. Statistical Summary for 1-Hydroxymidazolam PK of Oral Administration With the Coadministration of Givinostat (From Day 4-18)

Parameter	Total ISCV%	Geometric LSmeans		GMR (%)	90% CI
		Oral Midazolam Alone (Day 2)	Oral Midazolam Co-Administered with Givinostat (Day 18)		
C_{max}	31.6	4451.49	6303.67	141.61	122.36 – 163.88
AUC_{0-t}	18.3	10871.01	17208.29	158.30	145.23 – 172.54
$AUC_{0-\infty}$	18.1	11914.16	19205.92	161.20	147.80 – 175.82

Source: Clinical Study Report ITF/2357/55 Page 18-19
Note: Geometric LS means is given in pg/mL for Cmax and pg.h/mL for AUC GMR.
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; GMR, geometric mean ratio; ISCV, intra-subject coefficient of variation; LS, least squares; PK, pharmacokinetic

These study results indicated that givinostat does not have significant inhibition or induction on hepatic 3A4 but has weak inhibition on intestinal 3A4.

Part 2

A total of 20 subjects were enrolled in Part 2 Study.

Effect of Givinostat on the PK of Dabigatran (a P-gp Substrate)

The statistics of dabigatran pharmacokinetic parameters following oral administration alone (Day 1) and co-administration of oral dabigatran with givinostat on Day 6 (after 2 days of administration of givinostat) and Day 17 (after 13 days of administration of givinostat) showed smaller exposure of dabigatran with co-administration of givinostat (Table 77). The exposure of

total dabigatran (total of dabigatran and dabigatran-acyl-β-D-glucuronide) and free dabigatran on Day 6 and Day 17 are similar.

The reduction of total dabigatran's exposure with co-administration of givinostat for two days is not expected since inhibition of P-gp would result in higher exposure of dabigatran. Meanwhile, the reduced dabigatran exposure is unlikely caused by P-gp induction, as the induction effect often appears after long-term use of inducer. It is also expected that the induction effect should be more obvious after 13 days than 2 days of administration of givinostat but the exposure of dabigatran is similar on Day 6 and Day 17. In addition, P-gp is co-regulated along with CYP3A and is less inducible than CYP3A. If there is an induction of P-gp, then it should come with CYP 3A4 induction as well, which was not observed in the midazolam DDI Study.

Overall, based on these data, the effect of givinostat on dabigatran PK is not strong and it is unlikely that givinostat has an inhibitory effect on P-gp transporter.

The reduction of dabigatran exposure with co-administration of givinostat might be caused by other mechanism. One possibility might be that givinostat inhibits carboxylesterase which catalyzed the conversion of the prodrug dabigatran etexilate to the intermediate BIBR 1087 and to dabigatran.

Table 77. Statistical Summary for Dabigatran PK (Total of Dabigatran and Dabigatran-Acyl-β-D-Glucuronide) of Oral Administration With the Coadministration of Givinostat

Parameter	Total ISCV%	Geometric LSmeans		GMR (%)	90% CI
		Dabigatran Etexilate Alone (Day 1)	Dabigatran Etexilate Co-Administered with Givinostat (Day 6)		
C_{max}	42.4	69.44	51.17	73.68	60.77 – 89.33
AUC_{0-t}	39.3	552.26	386.05	69.90	58.43 – 83.64
$AUC_{0-\infty}$	37.8	563.09	393.93	69.96	58.83 – 83.20

Parameter	Total ISCV%	Geometric LSmeans		GMR (%)	90% CI
		Dabigatran Etexilate Alone (Day 1)	Dabigatran Etexilate Co-Administered with Givinostat (Day 17)		
C_{max}	44.8	69.44	45.35	65.31	53.33 – 79.98
AUC_{0-t}	37.8	552.26	386.23	69.94	58.82 – 83.15
$AUC_{0-\infty}$	36.0	563.09	396.25	70.37	59.64 – 83.03

Source: Clinical Study Report ITF/2357/55 Page 23-24
Note: Geometric LS means is given in ng/mL for C_{max} and ng.h/mL for AUC
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; GMR, geometric mean ratio; ISCV, intra-subject coefficient of variation; LS, least squares; PK, pharmacokinetic

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Effect of Clarithromycin (P-gp Inhibitor) on the PK of Givinostat

Co-administration with clarithromycin leads to a slight increase of givinostat C_{max} about 39%, while for givinostat AUC_{0-t} and $AUC_{0-\infty}$ the geometric mean ratio and corresponding 90% CI are within the [80 –125%] acceptance limits for the comparable bioavailability (see Table 78).

Table 78. Statistical Summary for Givinostat PK After Co-Administration With Clarithromycin

Parameter	Total ISCV%	Geometric LSmeans		GMR (%)	90% CI
		Givinostat Alone (Day 1)	Givinostat Co-Administered with Clarithromycin (Day 8)		
C_{max}	12.9	54.04	75.35	139.43	129.73 – 149.86
AUC_{0-t}	8.9	344.67	406.61	117.97	112.24 – 124.00
$AUC_{0-\infty}$	8.9	349.25	411.69	117.88	112.13 – 123.92

Geometric LSmeans values are given in ng/mL for C_{max} and ng.h/mL for AUC
GMR corresponds to the ratio of the Geometric LSmeans of Givinostat Co-Administered with Clarithromycin (Day 8) and the Geometric LSmeans of Givinostat Alone (Day 1)
Source: Clinical Study Report ITF/2357/55 Page 28
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; GMR, geometric mean ratio; ISCV, intra-subject coefficient of variation; LS, least squares; PK, pharmacokinetic

Based on the results, givinostat is a substrate for intestinal P-gp transporter and co-administration with the strong P-gp inhibitor clarithromycin caused an increase of about 40% in givinostat C_{max} but had a minimal effect on givinostat AUC. This increase of C_{max} is not considered clinically meaningful, considering the flexible dosing regimen with opportunity to reduce the dose to mitigate safety and tolerability risk.

Part 3

A total of 8 subjects enrolled in Part 3 study.

Plasma PK

The plasma PK profiles of givinostat and metabolites following single dose on Day 1 and multi-dose administration (on Day 13) of givinostat are shown in Figure 21.

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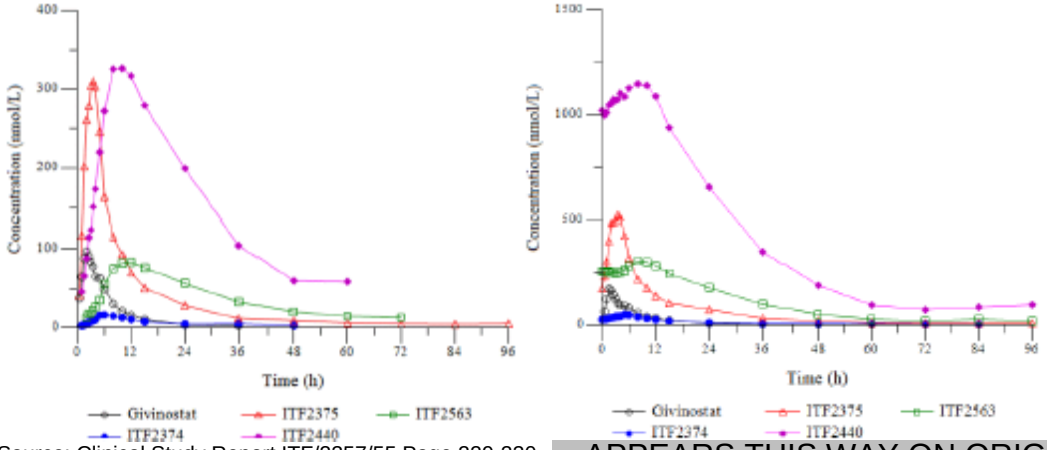
Figure 21. Geometric Mean Plasma Concentration Versus Time Profiles for Givinostat and Metabolites Following Day 1 and Day 13 Dose

A: Day 1 B: Day 13



Source: Clinical Study Report ITF/2357/55 Page 229-230

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Summary statistics of the plasma pharmacokinetic parameters for givinostat and metabolites (ITF2374, ITF2375, ITF2440 and ITF2563) following single (Day 1) are presented in Table 79, and for multiple-dose administration (Day 13) of givinostat are presented in Table 80.

Table 79. Summary of Plasma PK Parameters of Givinostat and Metabolites After Single Dose 50 mg

Parameter (unit)	Givinostat (n = 8)	ITF2374 (n = 8)	ITF2375 (n = 8)	ITF2440 (n = 8)	ITF2563 (n = 8)
C_{max} (ng/mL)	48.06 (25.8%) [38.86 – 59.45]	7.34 (45.7%) [5.10 – 10.57]	132.20 (40.2%) [95.66 – 182.70]	88.77 (16.4%) [77.47 – 101.74]	15.38 (13.8%) [13.71 – 17.25]
T_{max} (h)	1.50 (1.00 – 3.50)	5.50 (5.00 – 12.00)	3.50 (2.00 – 4.00)	9.00 (8.00 – 12.00)	10.00 (8.00 – 12.00)
C_{trough} (ng/mL)	6.75 (22.3%) [5.61 – 8.11]	4.14 (42.6%) [2.94 – 5.83]	28.47 (53.3%) [18.74 – 43.26]	81.58 (14.9%) [72.09 – 92.32]	14.75 (11.1%) [13.44 – 16.18]
$AUC_{0-\tau}$ (ng.h/mL)	245.73 (23.2%) [202.92 – 297.57]	52.48 (42.2%) [37.40 – 73.64]	806.71 (40.6%) [581.96 – 1118.27]	681.56 (25.8%) [551.33 – 842.55]	99.71 (23.1%) [82.39 – 120.67]
AUC_{0-t} (ng.h/mL)	300.45 (21.3%) [252.01 – 358.20]	93.30 (51.0%) [62.41 – 139.48]	1223.60 (47.5%) [839.34 – 1783.77]	2345.60 (12.7%) [2110.56 – 2606.81]	439.59 (13.0%) [394.66 – 489.65]
$AUC_{0-\infty}$ (ng.h/mL)	305.72 (21.0%) [257.04 – 363.63]	99.01 (49.1%) [67.16 – 145.98]	1239.34 (47.8%) [847.95 – 1811.38]	2431.31 (12.1%) [2198.19 – 2689.16]	457.95 (13.4%) [409.64 – 511.95]
$\%AUC_{extrap}$ (%)	1.69 (21.7%) [1.41 – 2.02]	5.39 (39.8%) [3.91 – 7.42]	0.93 (97.7%) [0.47 – 1.84]	3.46 (20.9%) [2.91 – 4.11]	3.96 (16.3%) [3.46 – 4.54]
λ_z (1/h)	0.097 (15.6%) [0.085 – 0.110]	0.094 (33.0%) [0.072 – 0.123]	0.062 (34.2%) [0.047 – 0.082]	0.060 (15.2%) [0.052 – 0.068]	0.055 (27.8%) [0.044 – 0.069]
$t_{1/2}$ (h)	7.16 (15.7%) [6.29 – 8.16]	7.40 (32.8%) [5.66 – 9.67]	11.21 (34.4%) [8.48 – 14.83]	11.65 (15.4%) [10.25 – 13.24]	12.57 (27.8%) [10.01 – 15.79]

Source: Clinical Study Report ITF/2357/55 Page 232 Table 3.E.1 and 3.E.2
Note: Values are Gmean with geometric coefficient of variation (GCV%) within parenthesis and 95% confidence interval of the Gmean within square brackets
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; AUC_{extrap}, area under the curve extrapolated; C_{max}, maximum plasma concentration; GCV%, geometric coefficient of variation; G_{mean}, geometric mean; n, number; PK, pharmacokinetic; ss, steady state; T_{max}, time to maximum concentration; $t_{1/2}$, half-life

Table 80. Summary of PK Parameters of Givinostat and Metabolites for 50 mg BID at SS

Parameter (unit)	Givinostat (n = 8)	ITF2374 (n = 8)	ITF2375 (n = 8)	ITF2440 (n = 8)	ITF2563 (n = 8)
$C_{max,ss}$ (ng/mL)	75.50 (27.0%) [60.47 – 94.28]	20.52 (38.6%) [15.02 – 28.02]	217.29 (38.1%) [159.76 – 295.52]	306.60 (15.2%) [270.25 – 347.84]	55.37 (20.3%) [46.82 – 65.48]
$T_{max,ss}$ (h)	1.50 (1.50 – 3.00)	5.50 (5.00 – 6.00)	3.50 (2.05 – 4.00)	10.00 (4.00 – 12.00)	9.00 (6.00 – 10.00)
C_{trough} (ng/mL)	13.16 (28.7%) [10.40 – 16.64]	10.18 (50.8%) [6.82 – 15.20]	56.12 (60.6%) [35.15 – 89.59]	279.96 (15.6%) [245.91 – 318.73]	50.11 (18.8%) [42.90 – 58.54]
$AUC_{0-\tau,ss}$ (ng.h/mL)	408.55 (16.2%) [357.02 – 467.52]	179.63 (41.9%) [128.38 – 251.35]	1518.21 (50.5%) [1019.48 – 2260.93]	3395.41 (15.8%) [2978.26 – 3870.99]	586.72 (19.3%) [499.88 – 688.63]
AUC_{0-t} (ng.h/mL)	554.78 (17.5%) [479.71 – 641.59]	328.36 (53.9%) [215.25 – 500.90]	2609.03 (68.2%) [1556.35 – 4373.73]	9262.28 (27.4%) [7393.79 – 11602.95]	1681.26 (21.8%) [1403.67 – 2013.74]
$AUC_{0-\infty}$ (ng.h/mL)	562.42 (17.5%) [486.53 – 650.15]	337.71 (53.2%) [222.41 – 512.79]	2754.89 (77.8%) [1549.89 – 4896.75]	9526.56 (30.2%) [7440.53 – 12197.43]	1715.62 (23.0%) [1418.73 – 2074.63]
$\%AUC_{extrap}$ (%)	1.33 (20.6%) [1.13 – 1.58]	2.56 (45.2%) [1.79 – 3.68]	2.13 (220.0%) [0.70 – 6.46]	1.84 (112.1%) [0.87 – 3.92]	1.53 (82.8%) [0.83 – 2.79]
λ_z (1/h)	0.067 (24.8%) [0.054 – 0.082]	0.064 (22.5%) [0.053 – 0.077]	0.037 (71.6%) [0.022 – 0.063]	0.047 (49.8%) [0.032 – 0.070]	0.052 (31.5%) [0.040 – 0.068]
$t_{1/2}$ (h)	10.40 (24.7%) [8.48 – 12.75]	10.82 (22.8%) [8.97 – 13.06]	18.88 (73.1%) [10.93 – 32.62]	14.60 (49.8%) [9.85 – 21.65]	13.23 (31.0%) [10.27 – 17.04]

Source: Clinical Study Report ITF/2357/55 Page 232 Table 3.F.1 and 3.F.2
Note: Values are Gmean with geometric coefficient of variation (GCV%) within parenthesis and 95% confidence interval of the Gmean within square brackets
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; AUC_{extrap}, area under the curve extrapolated; BID, twice daily; C_{max}, maximum plasma concentration; GCV%, geometric coefficient of variation; G_{mean}, geometric mean; n, number; PK, pharmacokinetic; ss, steady state; T_{max}, time to maximum concentration; $t_{1/2}$, half-life

The ratios of individual metabolites to the parent PK parameters are summarized in Table 81 for both single dose (Day 1) and steady state (Day 13). The accumulation index for givinostat and metabolites are summarized in Table 82.

Table 81. Summary of Metabolic Ratios for the Plasma PK Parameters

Metabolite	Givinostat (Day 1) (n = 8)	Givinostat (Day 13) (n = 8)
ITF2374	33.7 (46.9%) [23.2 – 48.9]	62.4 (42.5%) [44.4 – 87.7]
ITF2375	420.4 (42.3%) [299.5 – 590.0]	507.9 (62.0%) [315.3 – 818.2]
ITF2440	1302.6 (19.6%) [1107.7 – 1531.7]	2774.4 (29.2%) [2184.1 – 3524.3]
ITF2563	354.3 (24.5%) [289.5 – 433.7]	721.5 (25.1%) [586.8 – 887.3]

n – Number of Subjects
Values are geometric mean (G_{mean}) of percentage of AUC metabolite/parent ratio, with geometric coefficient of variation (GCV%) within parenthesis and 95% confidence interval (CI) of the G_{mean} within square brackets
Source: Clinical Study Report ITF/2357/55 Page 235 Table 3.G
Abbreviations: CI, confidence interval; GCV%, geometric coefficient of variation; G_{mean}, geometric mean; PK, pharmacokinetic

Table 82. Summary of Accumulation Index

Assessment	Parameter (unit)	Givinostat (n = 8)	ITF2374 (n = 8)	ITF2375 (n = 8)	ITF2440 (n = 8)	ITF2563 (n = 8)
Accumulation Index	C_{max}	1.57 (24.5%) [1.28 – 1.92]	2.80 (44.6%) [1.96 – 3.99]	1.64 (34.7%) [1.24 – 2.18]	3.45 (19.9%) [2.93 – 4.07]	3.60 (19.2%) [3.07 – 4.22]
	C_{trough}	1.95 (17.7%) [1.68 – 2.26]	2.46 (16.0%) [2.15 – 2.81]	1.97 (22.1%) [1.64 – 2.36]	3.43 (21.0%) [2.89 – 4.08]	3.40 (18.6%) [2.91 – 3.96]
	$AUC_{0-\tau}$	1.66 (15.0%) [1.47 – 1.88]	3.42 (35.2%) [2.57 – 4.56]	1.88 (33.5%) [1.43 – 2.47]	4.98 (30.0%) [3.90 – 6.37]	5.89 (24.6%) [4.80 – 7.21]
Time Dependency	$AUC_{0-\tau,Day\ 13}/AU_{0-\infty,Day\ 1}$	1.34 (11.4%) [1.22 – 1.47]	1.81 (23.1%) [1.50 – 2.20]	1.22 (19.5%) [1.04 – 1.44]	1.40 (14.1%) [1.24 – 1.57]	1.28 (21.3%) [1.07 – 1.53]

n - Number of Subjects
Values are geometric mean (G_{mean}) of Day 13/Day 1 ratio of each parameter with geometric coefficient of variation (GCV%) within parenthesis and 95% confidence interval (CI) of the Gmean within square brackets
Source: Clinical Study Report ITF/2357/55 Page 236 Table 3.H.1 and 3.H.2
Abbreviations: AUC_{0-t}, area under the curve up top the last quantifiable time-point; $AUC_{0-\infty}$, area under the curve from 0 to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; $C_{trough,}$ trough concentration; GCV%, geometric coefficient of variation; G_{mean}, geometric mean

Urine PK

Summary statistics of givinostat and metabolites (ITF2374, ITF2375, ITF2440 and ITF2563) urinary pharmacokinetic parameters following single dose (Day 1) is presented in Table 83.

Table 83. Summary Statistics of Urinary PK Parameters for Givinostat and Metabolites (ITF2374, ITF2375, ITF2440, and ITF2563) Following Single Dose

Parameter (unit)	Givinostat (n = 8)	ITF2374 (n = 8)	ITF2375 (n = 8)	ITF2440 (n = 8)	ITF2563 (n = 8)
R_{max} (mg/h)	0.051 (50.8%) [0.034 – 0.077]	0.015 (25.9%) [0.012 – 0.019]	0.006 (44.6%) [0.004 – 0.009]	0.276 (25.9%) [0.223 – 0.342]	0.107 (23.6%) [0.088 – 0.130]
tu_{max} (h)	6.00 (6.00 – 6.00)	6.00 (6.00 – 18.00)	6.00 (6.00 – 6.00)	6.00 (6.00 – 18.00)	12.00 (6.00 – 18.00)
Amt_{CUM} (mg)	0.782 (39.2%) [0.570 – 1.073]	0.417 (24.9%) [0.340 – 0.512]	0.093 (49.6%) [0.063 – 0.137]	9.179 (15.6%) [8.067 – 10.446]	3.985 (18.6%) [3.415 – 4.650]
$AURC_{0-t}$ (mg)	0.532 (31.1%) [0.413 – 0.686]	0.354 (27.4%) [0.283 – 0.444]	0.063 (55.3%) [0.041 – 0.097]	8.019 (16.6%) [6.985 – 9.205]	3.571 (19.9%) [3.028 – 4.212]
REC% (%)	1.565 (39.1%) [1.142 – 2.146]	0.868 (24.9%) [0.707 – 1.065]	0.193 (49.8%) [0.130 – 0.285]	30.072 (15.6%) [26.426 – 34.220]	18.852 (18.6%) [16.156 – 21.998]
CL_R (L/h)	2.604 (28.6%) [2.061 – 3.291]	4.473 (44.1%) [3.145 – 6.361]	0.076 (26.8%) [0.061 – 0.094]	3.913 (23.7%) [3.219 – 4.758]	9.065 (25.8%) [7.331 – 11.209]

Source: Clinical Study Report ITF/2357/55 Page 247 Table 3.I.1 and 3.I.2
Abbreviations: Amt_{cum}, cumulative amount of drug excreted in urine; $AURC_{0-t}$, area under the urine excretion rate curve; CL_R, renal clearance; n, number; PK, pharmacokinetic; REC%, percentage of drug recovered in urine (over dose);.R_{max}, maximum observed urinary excretion rate; tu_{max}, time of occurrence of maximum urinary excretion rate

The urine data on Day 13 were also collected. However, it is difficult to interpretate the results due to accumulation.

For plasma PK, the accumulation index was 1.57 to 1.95 for givinostat, 2.80 to 2.46 for ITF2374, 1.64 to 1.97 for ITF2375, 3.45 to 3.43 for ITF2440, and 3.60 to 3.40 for ITF2563. On Day 9, after 5 days of twice daily givinostat administration, all subjects have reached steady-state plasma concentrations for givinostat and its metabolites. Based on metabolic ratios, on Day 1, after a single oral administration, the higher exposure in plasma was observed for the metabolite ITF2440, with an AUC about 13-fold higher than the parent compound. Exposures higher than

Reference ID: 5351242

givinostat were observed also for the metabolites ITF2563 (about 3.5-fold) and ITF2375 (about 4-fold), while the metabolite ITF2374 showed the lower exposure (about 0.3-fold). On Day 13, at steady state, metabolic ratios increased for all metabolites, especially for those with the higher accumulation index such as ITF2440, ITF2563 and ITF2374, that almost doubled respect to day 1 (AUC about 28-, 7- and 0.6-fold, respectively). Exposure to ITF2375 instead was only slightly increased (5-fold).

Givinostat was mainly excreted in urine in the interval of 0-12 hours, and the percentage of drug recovered in urine was low (<3%). Givinostat metabolite ITF2440 was the compound with the highest amount recovered in urine (30% following single dose).

14.3. Bioanalytical Method Validation and Performance

The bioanalytical method validation reports and sample analysis reports for the quantitation of givinostat (ITF 2357), and metabolites in human plasma and urine was reviewed and found adequate. Liquid Chromatography with tandem Mass Spectrometry methods were used for assays of givinostat and metabolites (including ITF 2374, ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 in Human plasma and urine, and these methods are acceptable. The bioanalytical method information and validation results are summarized in Table 84 to Table 87.

Table 84. Summary of Givinostat and ITF 2374 Assay Analytical Method Validation in Heparinized Human Plasma for Study 48

Parameter	Givinostat	ITF2374
Method #	TM.3251	TM.3251
Validation report #	177049AQSM	207118AXWR
IS	ITF2400	ITF2400
Lower limit of quantitation (ng/mL)	1 ng/mL	10 to 5000ng/mL
Average recovery of drug (%)	98.79%	64.44%
Average recovery of IS (%)	98.37%	67.51%
Calibration curve concentration range and linearity r^2	1-100 ng/mL $r^2 \geq 0.9938$	10 to 5000ng/mL $r^2 \geq 0.9962$
Qc intra-run precision (%)	1.24 to 4.75%	1.63 - 8.87%
Qc intra-run accuracy (%)	-4.11 to 19.83%	-4.87 to 6.95%
Qc inter-run precision (%)	3.04 to 8.76%	3.73 - 6.98%
Qc inter-run accuracy (%)	-2.04 to 8.56%	-0.79 to 2.82%
Sensitivity	Intra-run LLQC accuracy 0.54% Intra- run LLQC precision 10.08%	Intra-run LLQC accuracy - 0.51% Intra- run LLQC precision 4.72%
Matrix effect (mean IS-normalized matrix factor)	No significant interference observed	No significant interference observed
Selectivity	No interferences by metabolites and commonly used drugs	No interferences by metabolites and commonly used drugs
Dilution integrity	Bias: 8.80% CV: 3.87%	Bias: -1.72% CV: 2.11%
Freeze and thaw stability in matrix	6 cycles at -20°C and 4 cycles at -80°C	4 cycles at -20°C and -80°C

Parameter	Givinostat	ITF2374
Short- and long-term stability in matrix	23h50min at room temperature 22h35min at 4°C 707 days at -20ºC 6 and 203 days at -80°C	22h20min at room temperature 47h08min at 4°C 64 days at -20ºC 170 days at -80°C
Extract stability	95h25min at room temperature	95h15min at 4°C
Incurred sample reproducibility	98.57% of samples meet criteria	98.57% of samples meet criteria

Source: Validation report 177049AQSM and 207118AXWR
Abbreviations: CV, coefficient of variation; IS, internal standard; LLQC, lower limit of quantification; Qc, quality control

Table 85. Summary of Analytical Method Validation for ITF2374, ITF2375, ITF2440, ITF2563, and ITF2955 in Human Plasma

Parameter	ITF 2374	ITF2375	ITF2440	ITF2563	ITF2955
Method #	TM.3215	TM.3216	TM.3154		
Validation report #	217050AZOM	217051AZON	207116AXWJ		
IS	ITF2400	ITF2361	ITF2440 -d10	ITF2563 -d4	ITF2955 -d10
Lower limit of quantitation (ng/mL)	1 ng/mL	2 ng/mL	10 ng/mL	2 ng/mL	1 ng/mL
Average recovery of drug (%)	86.22%	56.24%	93.77%	93.90%	95.01%
Average recovery of IS (%)	89.84%	85.64%	92.43%	92.54%	95.91%
Calibration curve concentration range and linearity r^2	1-100ng/mL r^2≥0.9907	2-800ng/mL r^2≥0.9930	10-1000ng/mL r^2≥0.9991	2-200ng/mL r^2≥0.9972	1-100ng/mL r^2≥0.9973
Qc intra-run precision (%)	2.11 to 13.12%	1.67 to 12.51%	1.04 to 4.25%	1.08 to 6.42%	-6.67 to 8.83%
Qc intra-run accuracy (%)	-7.84 to 10.22%	-7.33 to 8.92%	-3.28 to 4.89%	-3.31 to 6.58%	0.88 to 11.27%
Qc inter-run precision (%)	7.51 to 9.07%	3.06 to 10.79%	2.37 to 4.08%	2.53 to 5.93%	2.78 to 7.97%
Qc inter-run accuracy (%)	-1.42 to 2.65%	-2.43 to 4.23%	-1.41 to 1.52%	-0.56 to 2.25%	-1.67 to 4.22%
Sensitivity	Inter-run LLQC accuracy -1.00% Inter- run LLQC precision 8.62%	Inter-run LLQC accuracy 1.36% Inter- run LLQC precision 10.79%	Inter-run LLQC accuracy - 1.41% Inter- run LLQC precision 3.40%	Inter-run LLQC accuracy - 0.56% Inter- run LLQC precision 5.93%	Inter-run LLQC accuracy - 1.67% Inter- run LLQC precision 7.79%
Matrix effect (mean IS-normalized matrix factor)	No significant interference observed				
Selectivity	No interferences from matrix and commonly used drugs (8 batches)				
Dilution integrity	Bias: -0.80% CV: 5.16%	Bias: 4.90% CV: 0.99%	Bias: 4.36% CV: 1.86%	Bias: 2.62% CV: 1.08%	Bias: 5.20% CV: 1.69%
Freeze and thaw stability in matrix	6 cycles at -20°C and 4 cycles at -80°C	4 cycles at -20°C and -80°C	4 cycles at -20°C and -80°C		

Parameter	ITF 2374	ITF2375	ITF2440	ITF2563	ITF2955
Short- and long-term stability in matrix	23h50min at room temperature; 23h57min at 4°C; 565 days at -20ºC; 565 days at -80°C	24h at room temperature; 23h45min at 4°C; 9 days at -20ºC; 169 days at -80°C	30h30min at room temperature; 30h10min at 4°C; 155 days at -20ºC; 155 days at -80°C		
Extract stability	95h10min at room temperature	72h05min at room temperature	24h24min at 4°C		
Incurred sample reproducibility	100% of samples meet criteria	94% of samples meet criteria	98% of samples meet criteria	94% of samples meet criteria	59% of samples meet criteria[1]

Source: Validation report# 217050AZOM, # 217051AZON, #207116AXWJ (method was used in Study 54)
[1] The impact was justified as minimal as the concentration of IFT2955 is extremely low in the plasma.
Abbreviations: CV, coefficient of variation; IS, internal standard; LLQC, lower limit of quantification; Qc, quality control

Table 86. Summary of Analytical Method Validation for Givinostat, ITF2374, and ITF2375 in Human Urine

Parameter	Givinostat (ITF 2357)	ITF2374	ITF2375
Method #	TM.3232		
Validation report #	207118AXWR		
IS	ITF2400	ITF2400	ITF2361
Lower limit of quantitation (ng/mL)	10 ng/mL	5 ng/mL	4 ng/mL
Average recovery of drug (%)	64.44%	70.4%	56.35%
Average recovery of IS (%)	67.51%	67.51%	68.57%
Calibration curve concentration range and linearity r^2	10 to 5000ng/mL $r^2 \geq 0.9962$	5-2500 ng/mL $r^2 \geq 0.9950$	4-800 ng/mL $r^2 \geq 0.9936$
Qc intra-run precision (%)	1.63 to 8.87%	2.06 to 10.14%	2.71 to 13.53%
Qc intra-run accuracy (%)	-4.87 to 6.95%	-12.13 to 5.69%	-11.25 to 10.32%
Qc inter-run precision (%)	3.73 to 6.98%	5.35 to 8.63%	3.69 to 10.01%
Qc inter-run accuracy (%)	-0.79 to 2.82%	-7.21 to 0.44%	-1.67 to 2.38%
Sensitivity	Inter-run LLQC accuracy -0.51% Inter- run LLQC precision 4.72%	Inter-run LLQC accuracy -7.21% Inter- run LLQC precision 8.63%	Inter-run LLQC accuracy -1.67% Inter- run LLQC precision 10.01%
Matrix effect (mean IS-normalized matrix factor)	No significant interference observed	No significant interference observed	No significant interference observed
Selectivity	No interferences from matrix and commonly used drugs		
Dilution integrity	Bias: -1.72% CV: 2.11%	Bias: -0.54% CV: 2.59%	Bias: -1.20% CV: 2.15%
Freeze and thaw stability in matrix	4 cycles at -20°C and -80°C		
Short- and long-term stability in matrix	22h20min at room temperature; 47h08min at 4°C; 64 days at -20ºC; 170 days at -80°C		
Extract stability	95h15min at 4°C		
Incurred sample reproducibility	99% of samples meet criteria	75% of samples meet criteria	78% of samples meet criteria

Source: Validation report: 207118AXWR
Abbreviations: CV, coefficient of variation; IS, internal standard; LLQC, lower limit of quantification; Qc, quality control

Table 87. Summary of Analytical Method Validation for ITF2440, ITF2563, and ITF2955 in Human Urine

Parameter	ITF2440	ITF2563	ITF2955
Method #	TM.3155		
Validation report #	207117AXWP		
IS	ITF2400-d10	ITF2563-d4	ITF2955-d10
Lower limit of quantitation (ng/mL)	150 ng/mL	10 ng/mL	10 ng/mL
Average recovery of drug (%)	96.89%	98.27%	93.31%
Average recovery of IS (%)	89.24%	89.76%	88.82%
Calibration curve concentration range and linearity r^2	150 to 75000ng/mL $r^2 \geq 0.9983$	10-5000 ng/mL $r^2 \geq 0.9978$	10-5000 ng/mL $r^2 \geq 0.9972$
Qc intra-run precision (%)	0.70 to 5.35%	0.75 to 5.98%	0.72 to 18.27%
Qc intra-run accuracy (%)	-3.21 to 2.45%	-4.12 to 4.43%	-13.44 to 3.92%
Qc inter-run precision (%)	1.83 to 3.53%	1.85 to 4.03%	5.00 to 11.00%
Qc inter-run accuracy (%)	-1.68 to 1.51%	-2.23 to 2.12%	-5.98 to -1.70%
Sensitivity	Inter-run LLQC accuracy- 1.51% Inter- run LLQC precision 2.07%	Inter-run LLQC accuracy-2.12% Inter- run LLQC precision 3.04%	Inter-run LLQC accuracy-2.29% Inter- run LLQC precision 11.00%
Matrix effect (mean IS-normalized matrix factor)	No significant interference observed	No significant interference observed	No significant interference observed
Selectivity	No interferences from matrix and commonly used drugs		
Dilution integrity	Bias: 6.38%CV: 1.46%	Bias: 3.02%CV: 1.79%	Bias: 4.01%CV: 2.00%
Freeze and thaw stability in matrix	4 cycles at -20°C and -80°C		
Short- and long-term stability in matrix	24h at room temperature; 47h58min at 4°C; 44 days at -20ºC; 190 days at -80°C		
Extract stability	102h25min at room temperature		
Incurred sample reproducibility	100% of samples meet criteria	100% of samples meet criteria	100% of samples meet criteria

Source: Validation report: 207117AXWP/AZCE/AZCF
Abbreviations: CV, coefficient of variation; IS, internal standard; LLQC, lower limit of quantification; Qc, quality control

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

Not applicable.

14.5. Pharmacometrics Assessment

The document givinostat-report-final-12dec22-signed.pdf contains a report titled “Modeling and Simulation Report: Assessment of Pharmacokinetics (PK) and Pharmacokinetic-Pharmacodynamic (PK-PD) and Exposure-Response (E-R) Relationships following the Administration of Oral Givinostat in Subjects with Duchene Muscular Dystrophy (DMD)” and was submitted to module 5342 of sequence 0003. This report describes popPK analyses, E-R analyses, and PK/PD simulations. The PK/PD simulations use the model developed in report 0428-2012-r1.pdf.

The document *0428-2012-r1.pdf* contains a report titled "*Technical report: Population PK/PD analysis to assess Thrombocytopenia of Givinostat (ITF2357) in Clinical studies*" and was submitted to module 5342 of sequence 0003. This report describes the development of a PK/PD model for blood platelet count in the context of thrombocytopenia.

14.5.1. Population PK Modeling

The Applicant's popPK analyses are described in report *givinostat-report-final-12dec22-signed.pdf* submitted to module 5335 in sequence 0003. The objectives of the popPK analyses are to utilize (and update as needed) the previously-published givinostat popPK model to:

- Explore clinically relevant covariates as sources of variability
- Use individual model-based PK parameters of givinostat to estimate systemic exposure

The PK dataset includes 3325 observations above the lower limit of quantification from a total of n = 242 individuals. Data used in the popPK analyses came from five clinical studies. Three studies enrolled healthy adult volunteers (n = 105 in the final dataset). Two studies enrolled subjects with DMD (n = 137 in the final dataset). A summary of these studies from which PK data was measured is found in Table 88 and Table 89.

Table 88. Summary of PK Data Available for PopPK Analyses by Study

Studies	Population	Number of Subjects	Number of PK Observations	BLQ
DM/00/2357/01 Study 01 (SAD)	Healthy Adults	30	330	13
DM/00/2357/03 Study 03 (MAD)	Healthy Adults	32	943	10
DM/00/2357/04 Study 04 (Food effect)	Healthy Adults	12	324	18
ITF/2357/54 Study 54 (TQT)	Healthy Adults	31	1109	156
DSC/11/2357/43 Study 43 (Phase 2)	Subjects with DMD	20	189	0
DSC/14/2357/48 Study 48 (Phase 3)	Subjects with DMD	117	674	47

Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, pages 40
Abbreviations: BLQ, below the lower limit of quantitation; DMD, Duchenne muscular dystrophy; MAD, multiple ascending dose; PK, pharmacokinetic; popPK, population pharmacokinetic; SAD, single ascending dose; TQT, thorough QT study

Reference ID: 5351242

Table 89. Study Design and Sampling Schedule for Analyses Used in PopPK, PK/PD, and E-R

Study	Population	Dose	Sampling
Study 01 – SAD	N=40 (30 GIV, 10 PBO) healthy male subjects	50, 100, 200, 400, 600 mg single dose, capsule	• PK: pre-dose, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 h post-dose • PD (platelet): 12,24, 48 h post-dose
Study 03 – MAD	N=41 (3 GIV, 8 PBO) healthy male subjects	50, 100, 200 mg QD. 100 mg BID. capsule	• QD dosing- PK: pre-dose 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24 h post-dosing Day 1 and 7; 48 h after Day 7 dose; at pre-dose on Day 2 to 7 and 2 h post-dose on Day 3 and 5. • BID dosing- PK: pre-dose, 0.5, 1, 2, 4, 6, 8, 10: in addition, at 12, 13, 14, 16, 20, 24 h post-dose on Day 1 and 7; 48 h after Day 7 dosing; at pre-dose of morning doses on Day 2 to 7 and 2 h after morning dosing on Day 3 and 5. • PD (platelet)- pre-dose, and 2, 4, 8, 12 h post dose on Day 1, on Day 2, 3, 5, 6, and on Day 7 at pre-dose, and 2, 4, 8, 12, 24 and 48 h after the last administration.
Study 04 – Food Effect	N=12 healthy male subjects	100 mg single dose, capsule	• PK: pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 24 h post dose • PD (platelet): pre-dose and 24 and 72 h post-dose, and at study exit.
Study 54 – TQT	N=31 health male subjects	100 mg single dose, 300 mg single dose, suspension	• PK: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60 and 72 h post-dose • PD (platelet): pre-dose and 24 and 72 h post-dose, and at study exit.
Study 43 Phase 2	N=20 subjects with DMD	25, 37.5, 50 mg BID, capsule, suspension	• Part 1- PK: pre-dose, 1, 2, 4, and 8 h post-dose after 1 week of treatment • Part 2- PK: additional pre-dose and post-dose samples collected on Visit 2, 3, 4, 6 (0-2 h, 2-4 h, 4-6 h, 6-8 h). At Visit 10, PK samples collected pre-dose and at 1, 2, 4, and 8 h post-dose. • PD (platelet): pre-dose and weekly post-dose in Part 1; at Month 0, 1, 2, 3, 4.5, 6, 7.5, 9, 10.5, 12 and end of study in Part 2; additional measurements collected every two months in the extension phases.

Reference ID: 5351242

Study	Population	Dose	Sampling
Study 48 Phase 3	N=179 (2:1 vs. PBO), 120 subjects “Target” (VL MFF ≥ 5% to ≤ 30% MRS)	PBO; Dose A, Dose B, Dose C[1].	• PK: pre-dose, 1, 2, 4, and 8 h post-dose after 1 week of treatment • PD (platelet): pre- dose and on Week 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, and 72. • Molecular markers: Baseline, Month 12, Month 18 • Efficacy: Month 18

Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, pages 22 to 24
[1] Dose adopted in Study 48: i) Dose A: 20, 25, 30, 35, 40, 50, 55, 60, 70 mg BID; ii) Dose B: 13.3, 16.7, 20, 23.3, 26.7, 33.3, 36.7, 40, 46.7 mg BID; iii) Dose C: 10.6, 13.4, 16, 18.6, 21.4, 26.6, 29.4, 32, 37.4 BID. Each dosing regimen is referred to the following body weight ranges: ≥10-< 12.5 kg, ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, ≥70 kg, respectively.
Abbreviations: BID, twice daily; DMD, Duchenne muscular dystrophy; E-R, exposure-response; GIV, givinostat; MAD, multiple ascending dose; MRS, magnetic resonance spectroscopy; N, number; PBO, placebo; PD, pharmacodynamic; PK, pharmacokinetic; popPK, population pharmacokinetic; TQT; thorough QT study; QD, once daily; SAD, single ascending dose; VL MFF, vastus lateralis muscle fat fraction

The popPK analyses were performed using the non-linear mixed effects modeling approach with the NONMEM version 7.4.3 software package. The final model includes two compartments, first order absorption following oral administration, and lag time for oral absorption. The model is parameterized in terms of apparent oral clearance (CL/F), apparent volume of distribution of the central compartment (V2/F), apparent intercompartmental clearance (Q/F), and apparent volume of distribution of the peripheral compartment (V3/F). The final includes meal status as a covariate on bioavailability (F1). Allometric scaling was applied to CL/F using a power model with weight normalized to the median body weight in the population (40.5 kg). Between subject variability was estimated for Cl/F, V2/F, and F1. Residual variability wad modeled using a combined proportional and additive model. Parameter estimates for the final givinostat popPK model (run2cmtwt8ffed) are in Table 90.

Table *90*. Parameter Estimates for the Final Givinostat PopPK Model (Run2cmtwt8ffed)

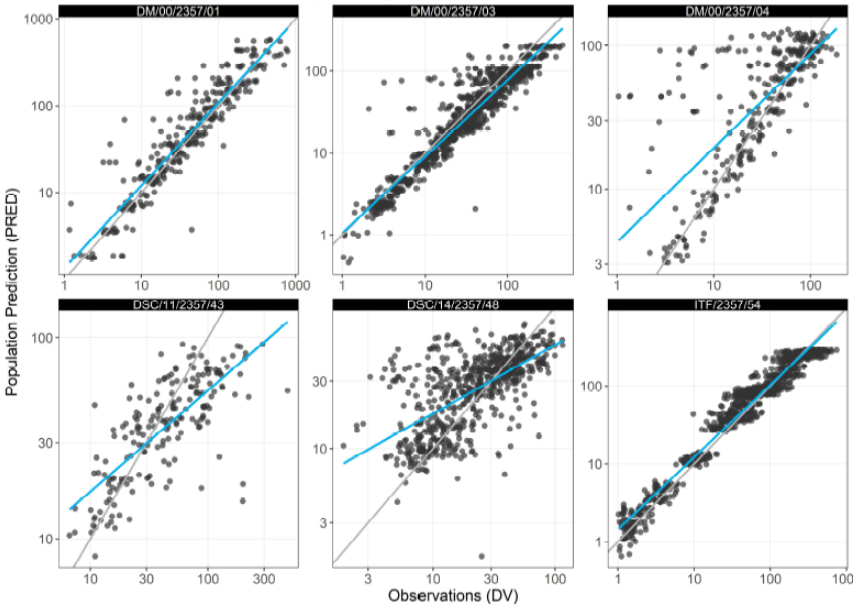
Parameter	Description	Estimate (Shrinkage %)	RSE(%)	Estimate (Natural Scale)
CL/F (L/h)	Estimated on log scale	4.76	1.0	117
V_2/F (L)	Estimated on log scale	5.33	1.4	206
Q/F (L/h)	Estimated on log scale	3.80	1.4	44.7
V_3/F (L)	Estimated on log scale	6.33	0.7	561
F1 (unitless)	Natural scale	1 (FIX)	NA	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: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 51 APPEARS THIS WAY ON ORIGINAL
Note: From file run2cmtwt8ffed.mod in 0003\m5\datasets\ital-pmxgivinostat-3392\analysis\legacy\programs. The central column shows the parameter estimate with a % shrinkage estimate (if applicable) presented in parentheses. Median weight is 40.5 kg.
Abbreviations: CL, clearance; 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; popPK, population 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.

Reference ID: 5351242

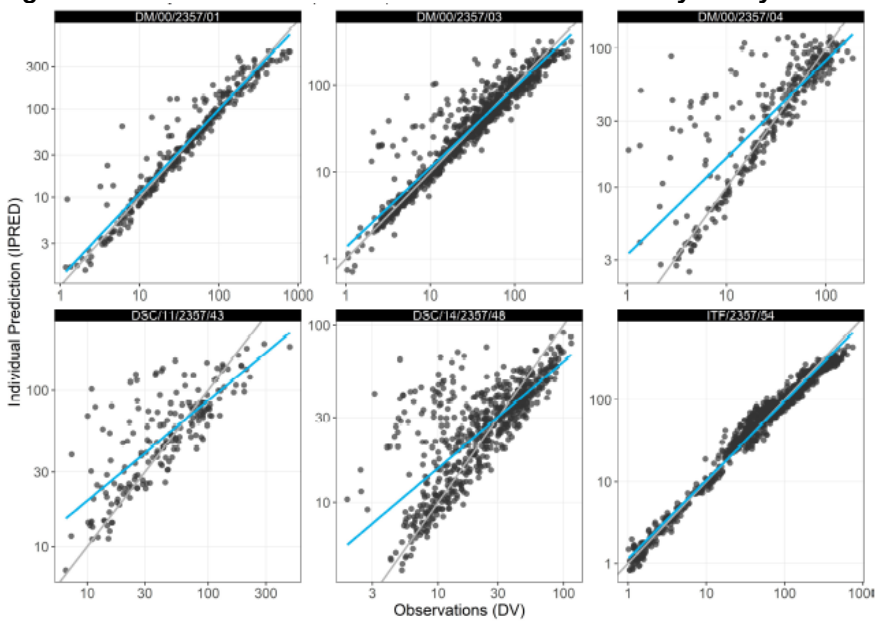
Key diagnostic plots are shown below.

Figure 22. Population Prediction Versus Observation by Study for Final PopPK Model



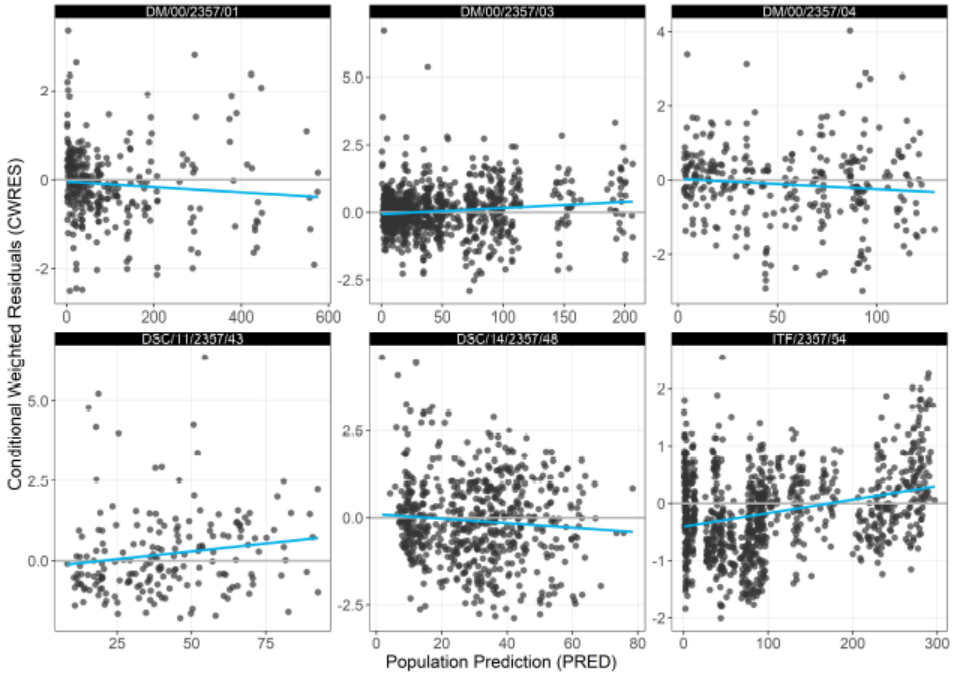
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 51
Note: Dots- observed values; blue solid lines- linear regression fits; gray solid lines: identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable (observations); popPK, population pharmacokinetic; PRED, population predictions

Figure 23. Individual Prediction Versus Observation by Study for Final PopPK Model



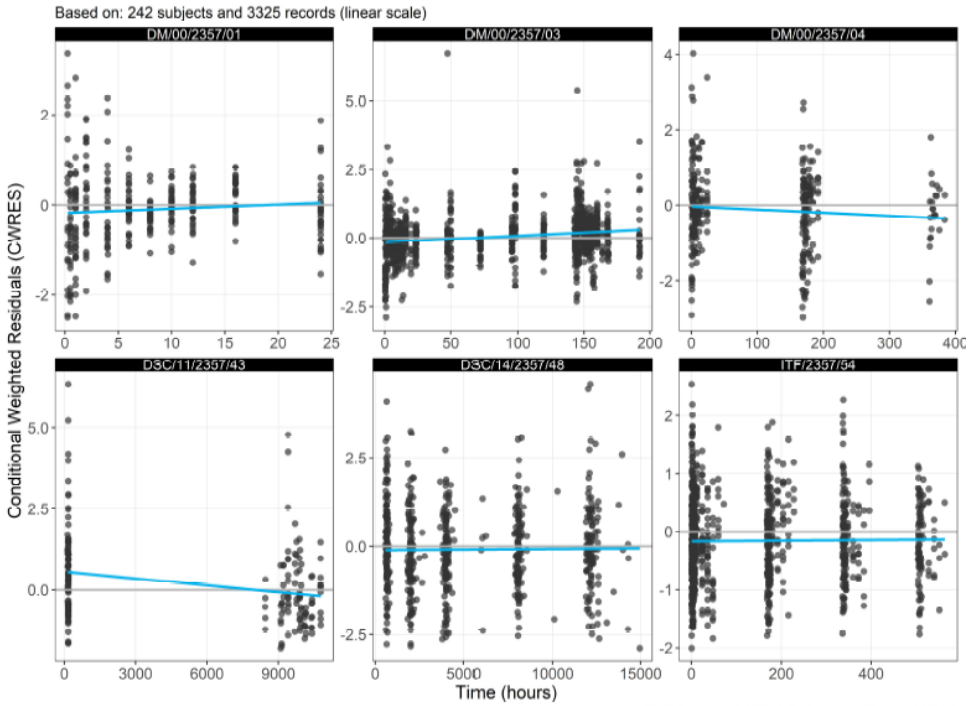
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 53
Note: Dots- observed values; blue solid lines- linear regression fits; gray solid lines- identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable (observations); IPRED, individual predictions; popPK, population pharmacokinetic

Figure 24. Conditional Weighted Residual Versus Population Prediction by Study for Final PopPK Model



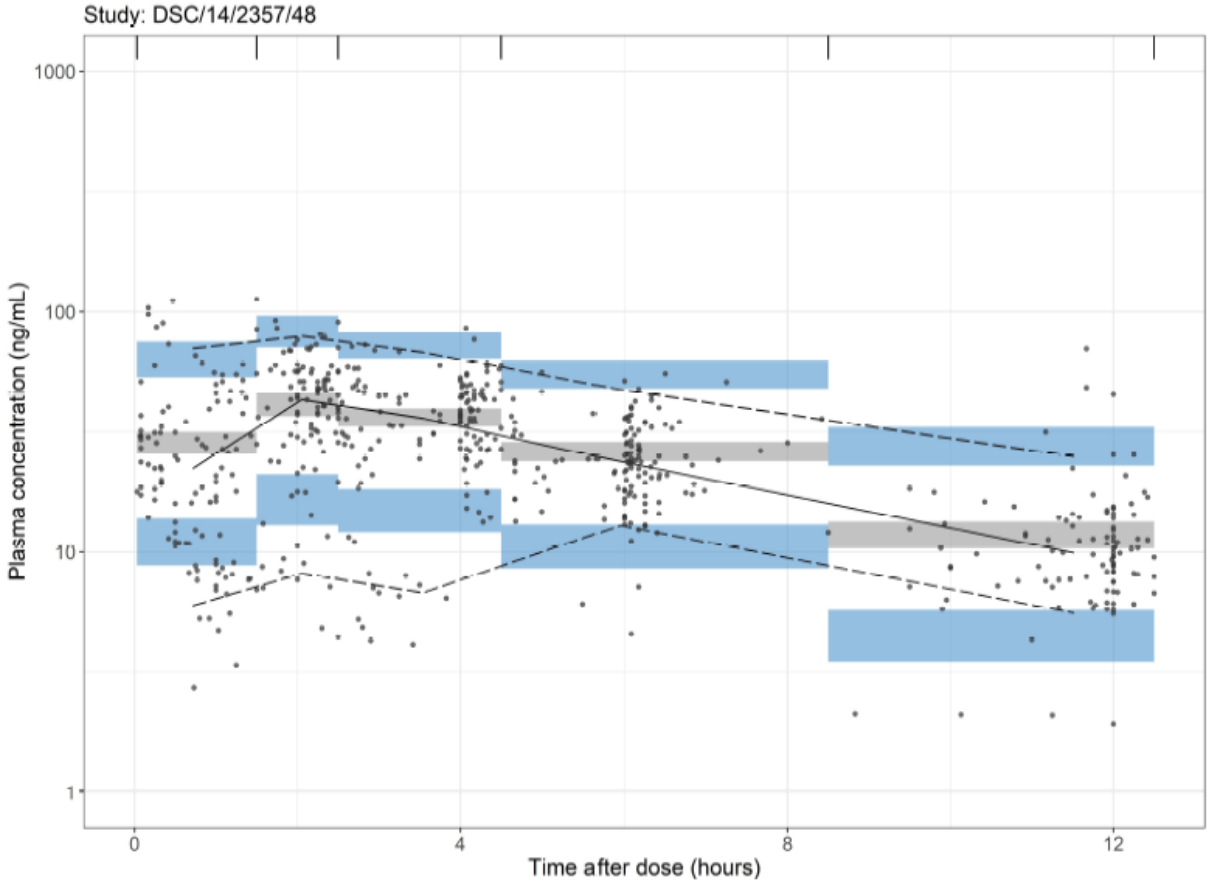
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 54
Note: Dots- observed values; blue solid lines- linear regression fits; gray solid lines- identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; popPK, population pharmacokinetic; PRED, population predictions

Figure 25. Conditional Weighted Residual Versus Time by Study for Final PopPK Model



Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 55
Note: Dots- observed values; blue solid lines- linear regression fits; gray solid lines- identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; popPk, population pharmacokinetic

Figure 26. Visual Predictive Check for Final PopPK Model for Study 48 Subjects



Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 61
Note: 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.
Abbreviations: CI, confidence interval; popPK, population pharmacokinetic

The eta shrinkage values (17.6% to 30.4%) do not raise concerns about the interpretability of the diagnostic plots.

The diagnostic plots in Figure 22, Figure 23, Figure 24 indicate that the model performs best in studies 01, 03, and 54. The majority of conditional weighted residuals are within ± 2 standard deviations in Figure 24. The visual predictive check for phase 3 Study 48 (Figure 26) indicates that model captures the central tendency well in DMD patients. The correlation matrix estimates indicate low correlation among parameters. The relative standard error for fixed effects as well as random effects are acceptable. The condition number is 82.57 and does not suggest overparameterization.

Overall, the popPK model for givinostat is acceptable for exposure-response analysis.

14.5.2. Population PK Simulation

The Applicant conducted simulations to predict steady-state PK based on the individual PK parameter estimates and dosing pathway for subjects enrolled in Study 48. The simulated PK values, summarized by treatment regimen in Study 48, are presented in Table 91.

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Table 91. Summary Statistics of Steady State Metrics of Systemic Exposure (C_{max}, C_{min}, and AUC) for Subjects Consistently Receiving Givinostat at the Dose A or B, or Switching From Those to Lower Doses

Treatment Regimen	N	5th	25th	Median	Mean	Geometric Mean	75th	95th
Weighted Average Cumulative AUC_{ss} within the Dosing Interval (ng.h/mL)								
Always A	22*	286	332	396	411	400	470	611
A to B/C	32	200	293	329	341	328	390	505
Always B	46	167	221	276	283	270	326	446
B to C	17	177	205	251	251	245	293	332
AUC_{ss} within the Dosing Interval at the End of Study (ng.h/mL)								
Always A	22*	285	332	393	411	399	467	611
A to B/C	32	187	250	298	315	302	358	489
Always B	46	160	226	271	284	271	328	450
B to C	17	180	208	238	241	237	282	313
$C_{max,ss}$ within the Dosing Interval at the End of Study (ng/mL)								
Always A*	22	41.2	50	57.7	61.7	59.7	72.4	90
A to B/C	32	28	34.8	43.2	43.4	41.8	50.1	64.2
Always B	46	22.2	29.8	37.9	38.8	37.1	43.7	60.6
B to C	17	25.6	30.7	36.7	37.5	36.4	46.4	51.4
$C_{min,ss}$ within the Dosing Interval at the End of Study (ng/mL)								
Always A*	22	9.28	10.4	12.8	13.6	13.1	14.8	22.1
A to B/C	32	5.48	9.15	10.6	11.9	10.8	12.8	20.9
Always B	46	5.18	7.52	8.98	11.1	9.87	11.3	26.3
B to C	17	5.7	6.92	7.31	7.68	7.56	8.33	9.97

Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 62 APPEARS THIS WAY ON ORIGINAL
Note: Always A N=23, 1 PK missing; Dose adopted in Study 48: i) Dose A: 20, 25, 30, 35, 40, 50, 55, 60, 70 mg BID; ii) Dose B: 13.3, 16.7, 20, 23.3, 26.7, 33.3, 36.7, 40, 46.7 mg BID; iii) Dose C: 10.6, 13.4, 16, 18.6, 21.4, 26.6, 29.4, 32, 37.4 BID. Each dosing regimen is referred to the following body weight ranges: ≥10-< 12.5 kg, ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, ≥70 kg, respectively.
Abbreviations: AUC, area under the plasma concentration-time curve, BID, twice daily; C_{max}, maximal plasma concentration; C_{min}, minimal (through) plasma concentration; N, number; PK, pharmacokinetic; ss, steady state

The Applicant provided simulations of givinostat steady-state PK based on the doses applied in Study 48. The simulated AUC within one dosing interval at steady-state ($AUC_{tau,ss}$) for Dose A, Dose B, and Dose C (assuming no missed or skipped doses) can be found in Module 3, Section 6.1.2.

The reviewer conducted PK simulations using the individual PK parameter estimates for subjects in the popPK dataset that were enrolled in Study 48. The reviewer utilized the actual dosing history for each subject in Study 48, including instances of missing one or both daily doses, temporary dose cessation, and dose adjustments. The dosing history for the subjects was obtained from the file adecdb.xpt, submitted to sequence 0019 in response to an information request sent on 2023-07-25 where the Office of Clinical Pharmacology (OCP) requested clarification on the dosing history. The figures present the dose, expressed as mg/kg/day, for each subject over time stratified by dosing pathway. The term “dosing pathway” refers to the

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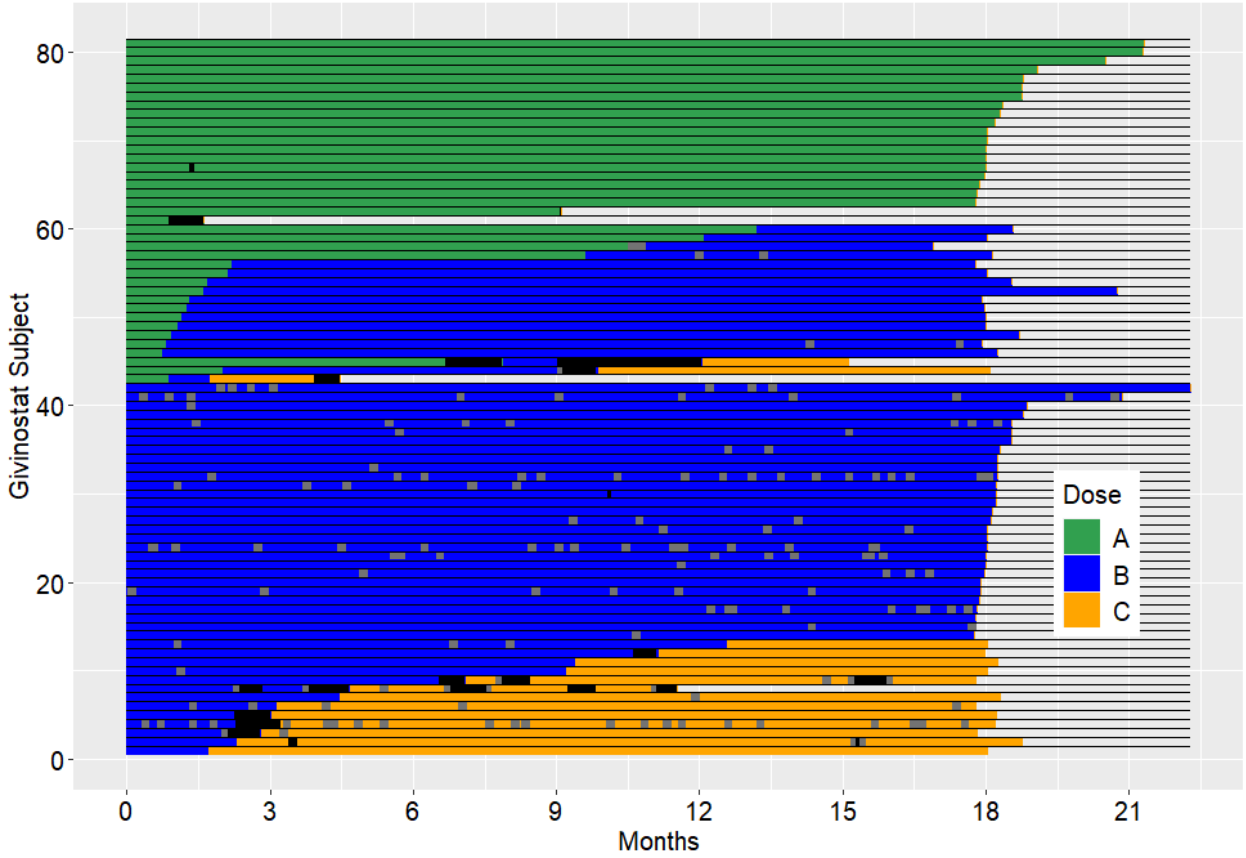
dose level or dose levels that a subject received throughout the course of their treatment in Study 48. The reviewer defines the dosing pathways for subjects in Study 48 as follows:

- **A**: subject remained on Dose A for the entire duration
- **AB**: subject started on Dose A followed by a reduction to Dose B
- **ABC**: subject started on Dose A followed by a reduction to Dose B followed by a reduction to Dose C
- **B**: subject remained on Dose B for the entire duration
- **BC**: subject started on Dose B followed by a reduction to Dose C

Figure 27 shows the dosing pathway of all n = 81 subjects in the intent-to-treat (ITT) population, Target group, randomized to givinostat.

Figure 27. Dose History Overview for Subjects Who Received Givinostat in Phase 3 Study 48



Source: Reviewer's analyses
Note: Each row represents a subject assigned to givinostat treatment in Study 48. Green, blue, and orange squares represent administration of Dose A, Dose B, and Dose C, respectively.
Note: Grey squares represent instances where the subject skipped one of the two daily doses. Black rectangles represent durations where subjects missed at least two administrations connectively (i.e., skipped both daily doses or dosing was temporarily paused).
Note: The end of a horizontal line segment indicates the time after which the subject permanently stopped receiving treatment in Study 48.

The dosing pathway for each subject in phase 3 Study 48 as expressed in mg/kg over time are presented in Figure 28, Figure 29, Figure 30, Figure 31, and Figure 32.

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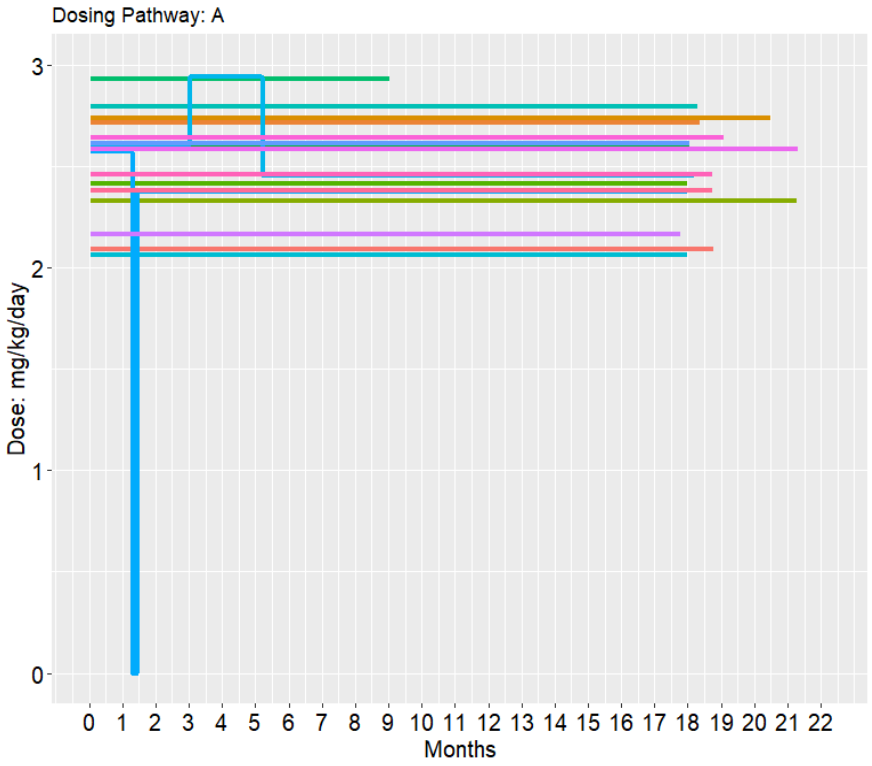
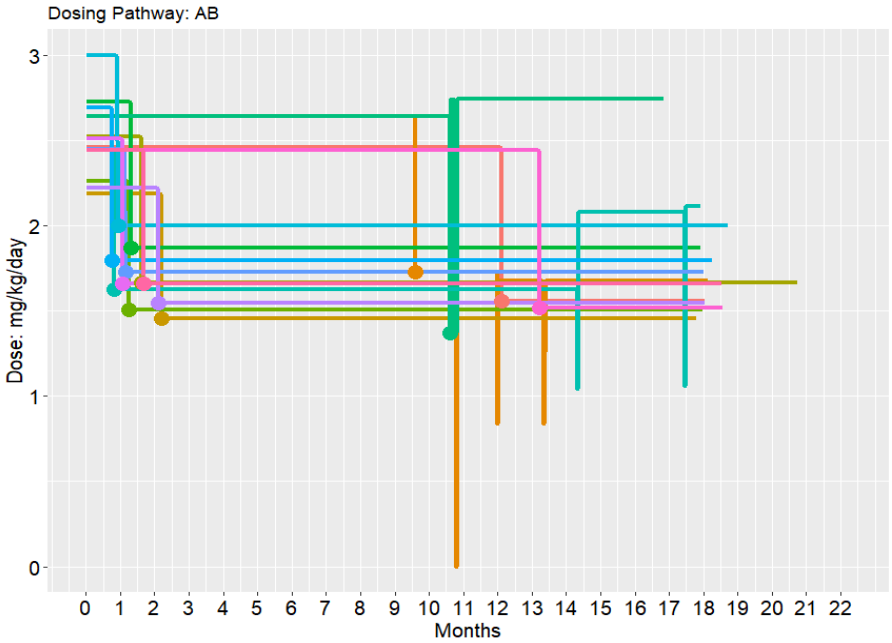
Figure 28. Dose vs. Time for Subjects in Phase 3 Study 48 in Dose Pathway: A



Source: Reviewer's analyses
Note: The horizontal portions of the line segments depict instances where the dose is constant over time. The vertical portions of the line segments depict changes in the dose at the given time. Each series represents an individual subject.

Figure 29. Dose vs. Time for Subjects in Phase 3 Study 48 in Dose Pathway: AB



Source: Reviewer's analyses
Note: The horizontal portions of the line segments depict instances where the dose is constant over time. The vertical portions of the line segments depict changes in the dose at the given time. The dots indicate the time where subjects reduced from Dose A to Dose B. Each series represents an individual subject.

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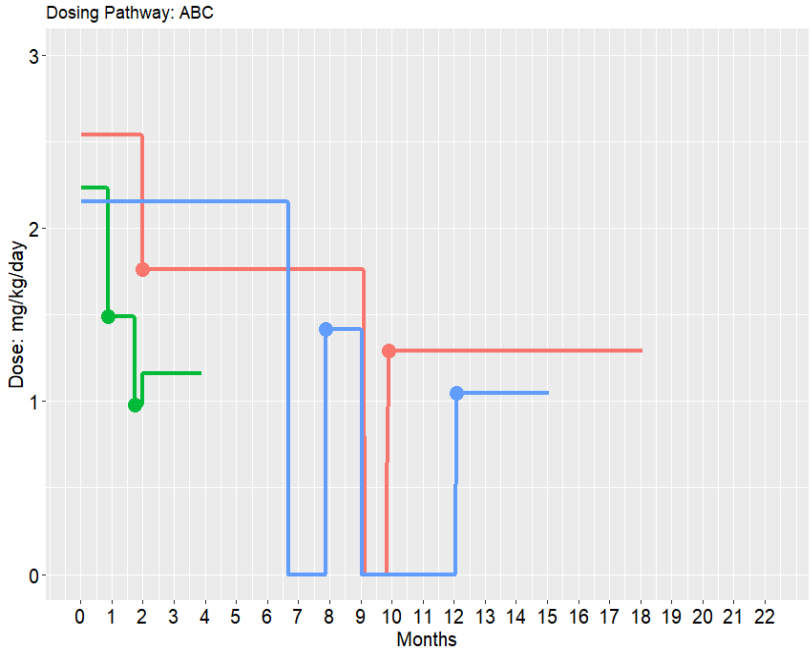
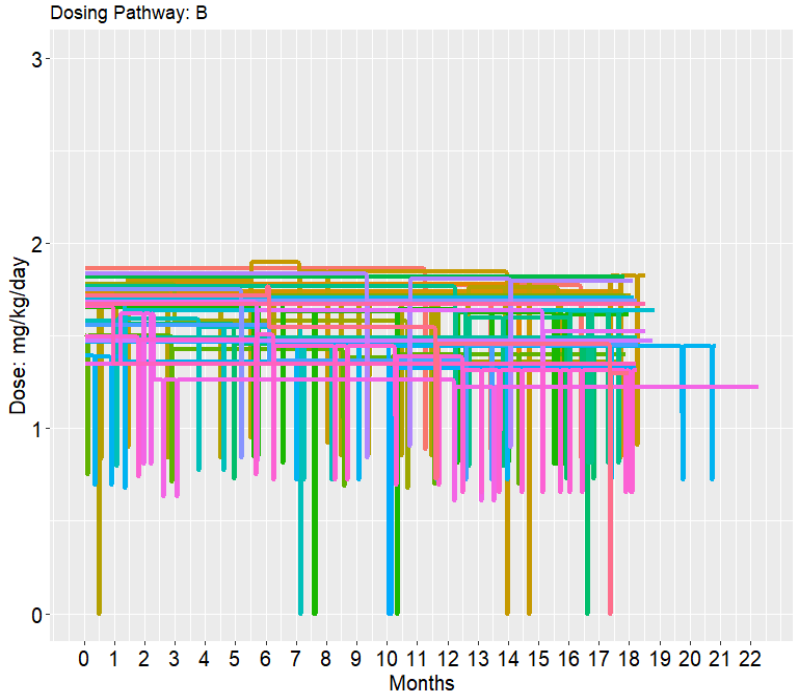
Figure 30. Dose vs. Time for Subjects in Phase 3 Study 48 in Dose Pathway: ABC



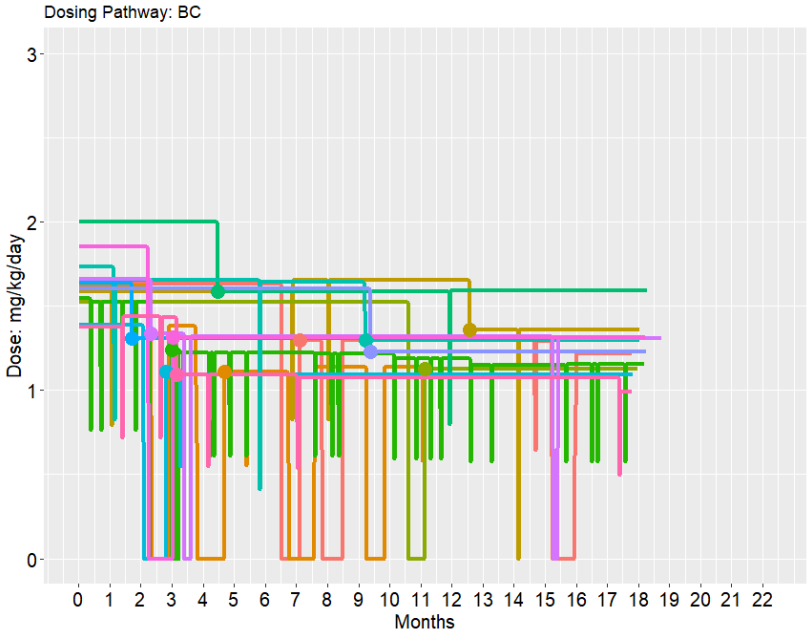
Source: Reviewer's analyses
Note: The horizontal portions of the line segments depict instances where the dose is constant over time. The vertical portions of the line segments depict changes in the dose at the given time. For each subject, the first dot indicates the time where subjects reduced from Dose A to Dose B and the second dot indicates that time where subjects reduced from Dose B to Dose C. Each series represents an individual subject.

Figure 31. Dose vs. Time for Subjects in Phase 3 Study 48 in Dose Pathway: B



Source: Reviewer's analyses
Note: The horizontal portions of the line segments depict instances where the dose is constant over time. The vertical portions of the line segments depict changes in the dose at the given time. Each series represents an individual subject.

Figure 32. Dose vs. Time for Subjects in Phase 3 Study 48 in Dose Pathway: BC

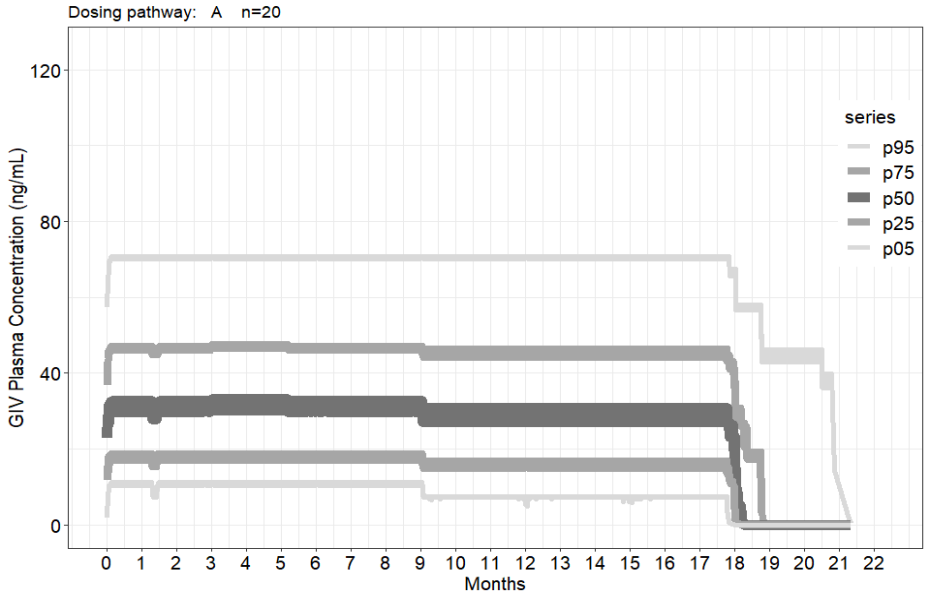


Source: Reviewer's analyses
Note: The horizontal portions of the line segments depict instances where the dose is constant over time. The vertical portions of the line segments depict changes in the dose at the given time The dots indicate the time where subjects reduced from Dose B to Dose C. Each series represents an individual subject.

Multiple subjects in dosing pathway B and BC missed one or both daily doses (see Figure 31 and Figure 32). Multiple subjects in dosing pathway ABC as well as dosing pathway BC underwent periods of temporary dose cessation (for examples see Figure 30 and Figure 32). Due to multiple instances of missed doses and temporary dose cessation, the reviewer computed cumulative AUC from the simulated PK using the history of dose received for each subject. The cumulative AUC was used in the reviewer's independent E-R analyses (see Section 14.5.5.2 in Section 14.5.5.2.1 for details).

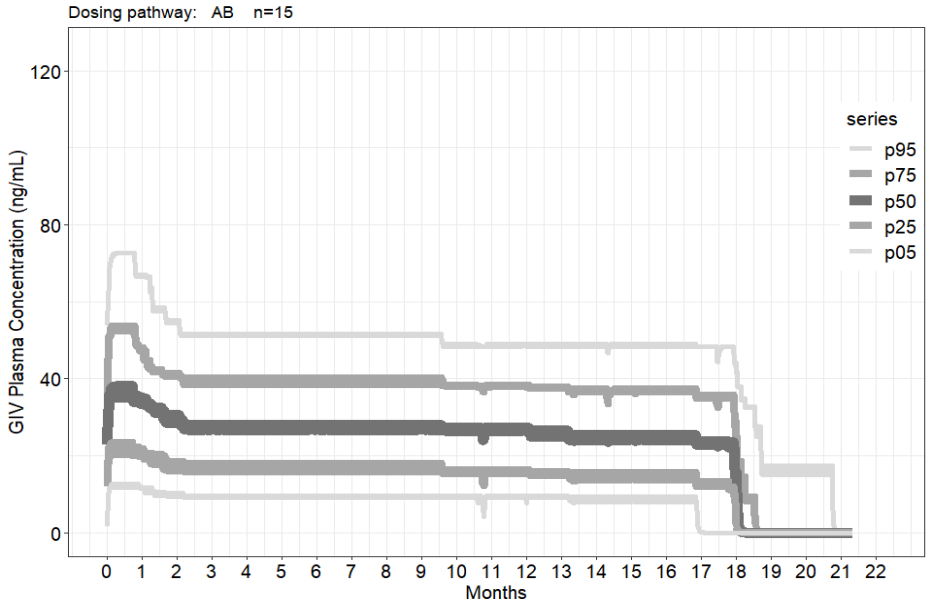
Due to complex dosing in subjects across the population, the reviewer assembled a view of the givinostat PK profile across the population over time, stratified by dosing pathway. Considering the fluctuation of the PK within a dosing interval compared to the timeframe of Study 48, for ease of visualization, the reviewer pooled all simulated PK values within each 12-hour dosing interval and computed percentiles across all subjects at each dosing interval. The resulting summary view the simulated PK data based on the dose received in Study 48 is presented in Figure 33, Figure 34, Figure 35, Figure 36, and Figure 37.

Figure 33. PK Distribution Within Dosing Interval Across Population of Subjects in Phase 3 Study 48 in Dose Pathway: A



Source: Reviewer's analyses
Note: The five series represent the 5th, 25th, 50th, 75th, and 95th percentiles of PK profile within each dosing interval at the given time for all subjects assigned to this dosing pathway.
Abbreviations: GIV, givinostat; n, number; PK, pharmacokinetic

Figure 34. PK Distribution Within Dosing Interval Across Population of Subjects in Phase 3 Study 48 in Dose Pathway: AB



Source: Reviewer's analyses
Note: The five series represent the 5th, 25th, 50th, 75th, and 95th percentiles of PK profile within each dosing interval at the given time for all subjects assigned to this dosing pathway.
Abbreviations: GIV, givinostat; n, number; PK, pharmacokinetic

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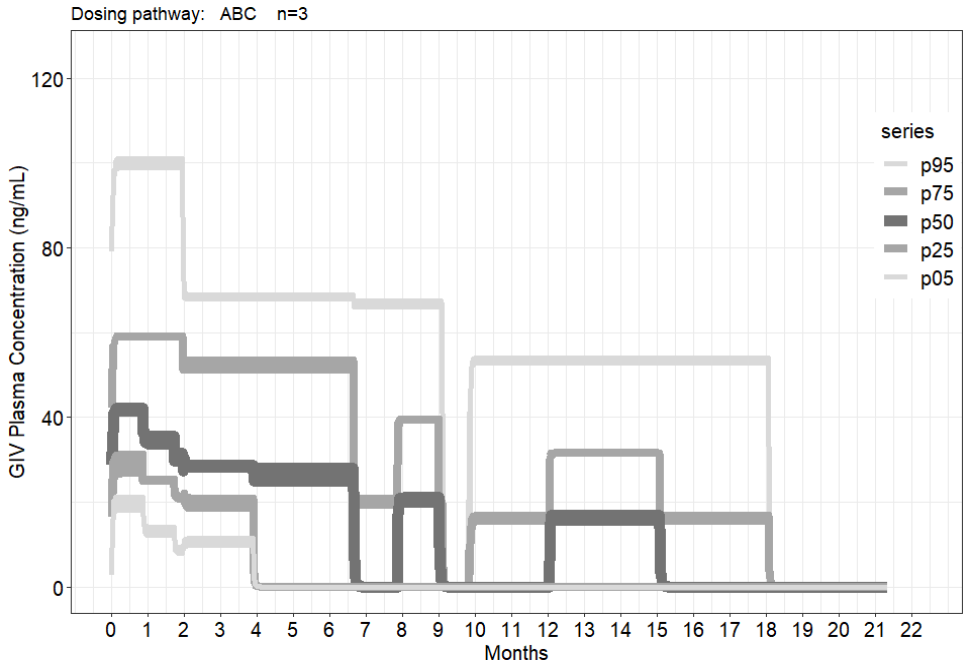
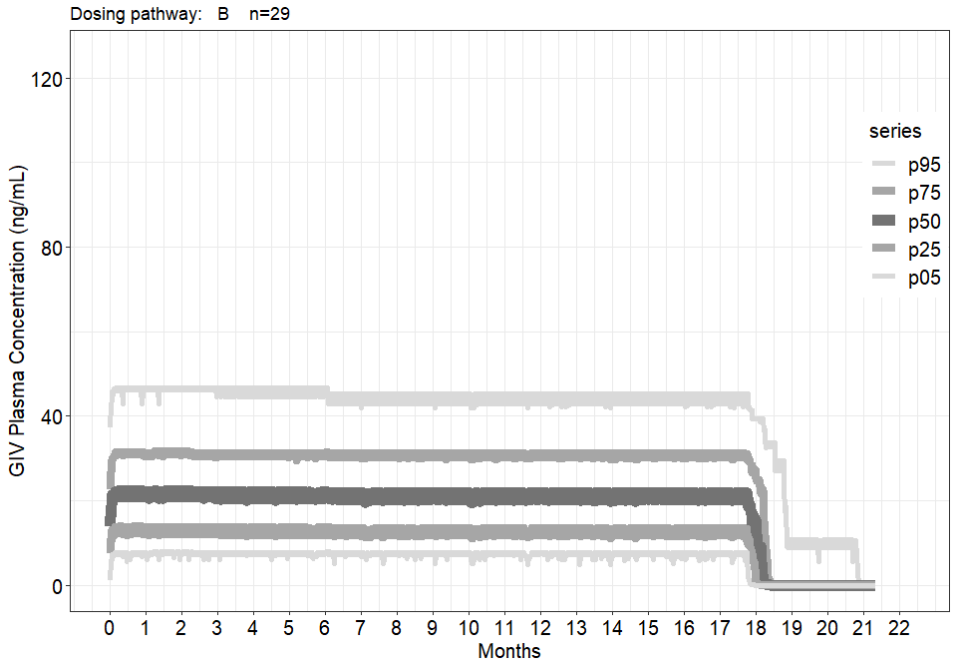
Figure 35. PK Distribution Within Dosing Interval Across Population of Subjects in Phase 3 Study 48 in Dose Pathway: ABC



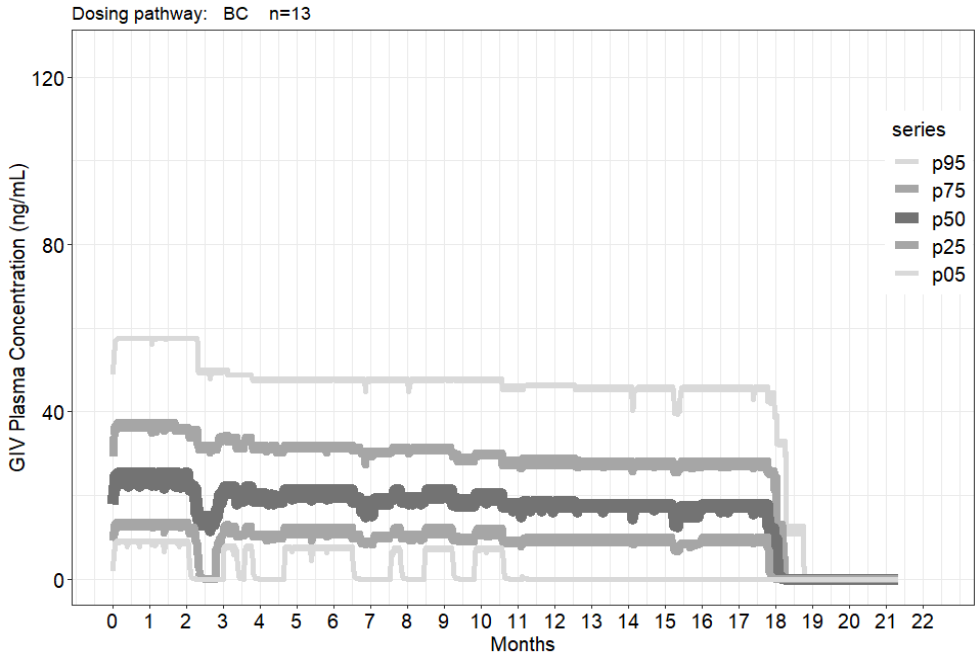
Source: Reviewer's analyses
Note: The five series represent the 5th, 25th, 50th, 75th, and 95th percentiles of PK profile within each dosing interval at the given time for all subjects assigned to this dosing pathway.
Abbreviations: GIV, givinostat; n, number; PK, pharmacokinetic

Figure 36. PK Distribution Within Dosing Interval Across Population Of Subjects in Phase 3 Study 48 in Dose Pathway: B



Source: Reviewer's analyses
Note: The five series represent the 5th, 25th, 50th, 75th, and 95th percentiles of PK profile within each dosing interval at the given time for all subjects assigned to this dosing pathway.
Abbreviations: GIV, givinostat; n, number; PK, pharmacokinetic

Figure 37. PK Distribution Within Dosing Interval Across Population Of Subjects in Phase 3 Study 48 in Dose Pathway: BC



Source: Reviewer's analyses
Note: The five series represent the 5th, 25th, 50th, 75th, and 95th percentiles of PK profile within each dosing interval at the given time for all subjects assigned to this dosing pathway.
Abbreviations: GIV, givinostat; n, number; PK, pharmacokinetic

The simulated PK were applied by the reviewer in independent exposure-response analyses. See Section 14.5.5.2.1 for details.

14.5.3. Population PK/PD Modeling

The objectives of the popPK/PD analyses are: To utilize (and update as needed) the previously developed popPK/PD model to describe the effect of givinostat treatment on the platelet count time course and to explore clinically relevant covariates as sources of variability. Model-based simulations were conducted to describe the PK and PD expected at different givinostat dose levels.

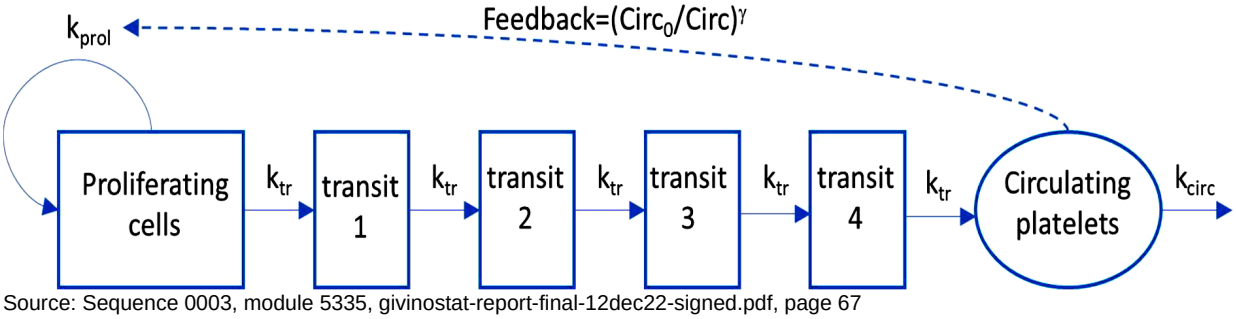
The data used for PK/PD analyses came from three studies (Studies 03, 43, and 48) as presented in Table 89. The Applicant did not include the data from the single dose studies as these studies would only provide information related to baseline platelet count. The final PK/PD dataset included 3241 platelet concentration measurements from n = 169 subjects receiving givinostat. The Applicant report included 2744 observations from n = 137 subjects with DMD. There were no placebo subjects in these PK/PD analyses. The PK exposures were predicted using the PK model described in Section 14.5.1.

The Applicant utilized a model based on a published model of chemotherapy-induced myelosuppression (Friberg et al. 2002). The model is parameterized in terms a compartment representing proliferating cells, 4 transit compartments representing the process of platelet maturation, with the circulating platelets affecting the proliferation rate of the proliferating cells.

Reference ID: 5351242

A schematic presentation of the model with a description of the parameters is shown in Figure 38.

Figure 38. Schematic Representation of the Myelosuppression Model



Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 67
Note: Based on steady state considerations, $k_{prol}=k_{circ}=k_{tr}$; a linear drug effect term (1-slope*concentration) is applied to the proliferative cell generation rate via multiplication.
Note: T_{1-4}, transit compartments represent different states of "maturation" of circulating platelets.
Abbreviations: γ, feedback parameter; $Circ_0$, baseline circulating platelets; Circ, circulating platelets; k_{circ}, rate of clearance of platelets from blood; k_{prol}, proliferation rate; k_{tr}, transit micro constant rate

The final model is parameterized in terms of mean transit time (MTT) to describe the progression through the transit compartments, baseline platelet level, and slope of the presumed inhibitory effect of givinostat on proliferative cell platelet production. Proportional and additive terms are used to describe error. Interindividual variability is estimated for baseline platelet count, MTT, and slope of the drug effect. The only covariate in the final model was weight on baseline platelet level. The parameter estimates for the final model (run5b) are presented in Table 92.

Table 92. Parameter Estimates for the Final PK/PD Model for Givinostat Effect on Platelets (run5b)

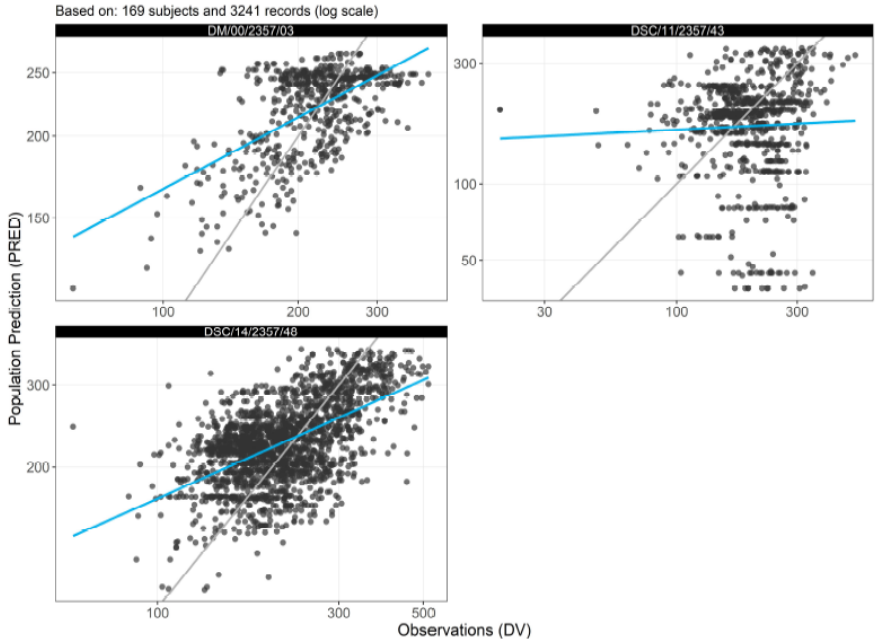
Parameter	Description	Estimate (Shrinkage %)	RSE(%)	Estimate (Natural Scale)
Baseline platelets (10^9/L)	Estimated on log scale	5.76	0.3	316
MTT	Estimated on log scale	4.36	1.0	78.1
Slope mL/ng	Estimated on log scale	-7.0	1.09	0.00091
Proportional RUV	Variance	-1.89	2.1	0.150
Gamma	Estimated on logit	-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: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 73
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

The Applicant encountered minimization problems when using 3 transit compartments and reports that a 4-transit compartment model was more stable. The Applicant reports that for resolving instability problems, residual unexplained variability, initially modeled using both additive and proportional models, was changed to include only a proportional term, whilst the additive term was fixed.

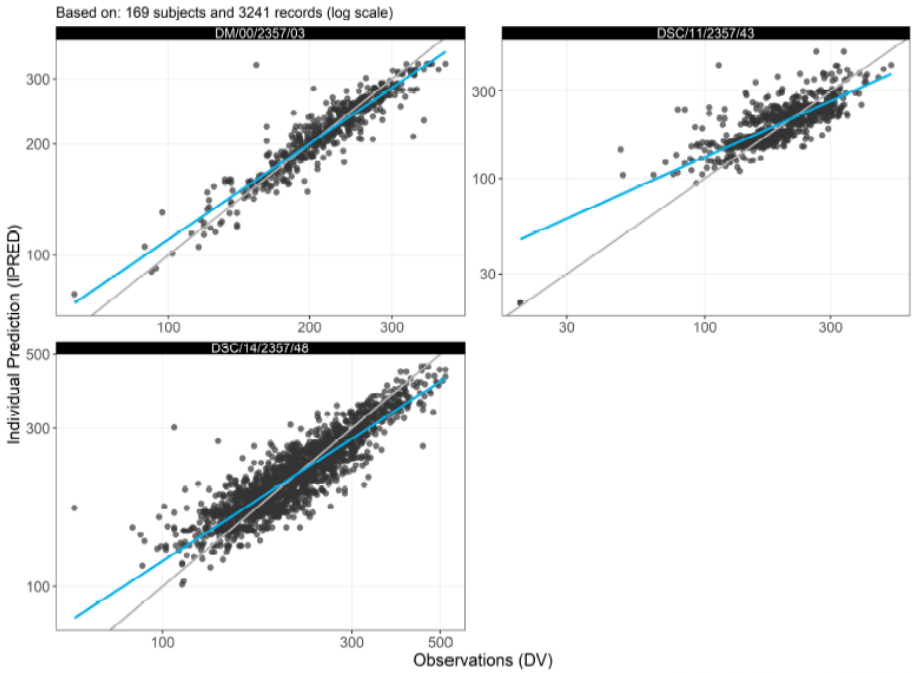
Key diagnostic plots are presented below.

Figure 39. Population Prediction vs. Observation by Study for Final PK/PD Model



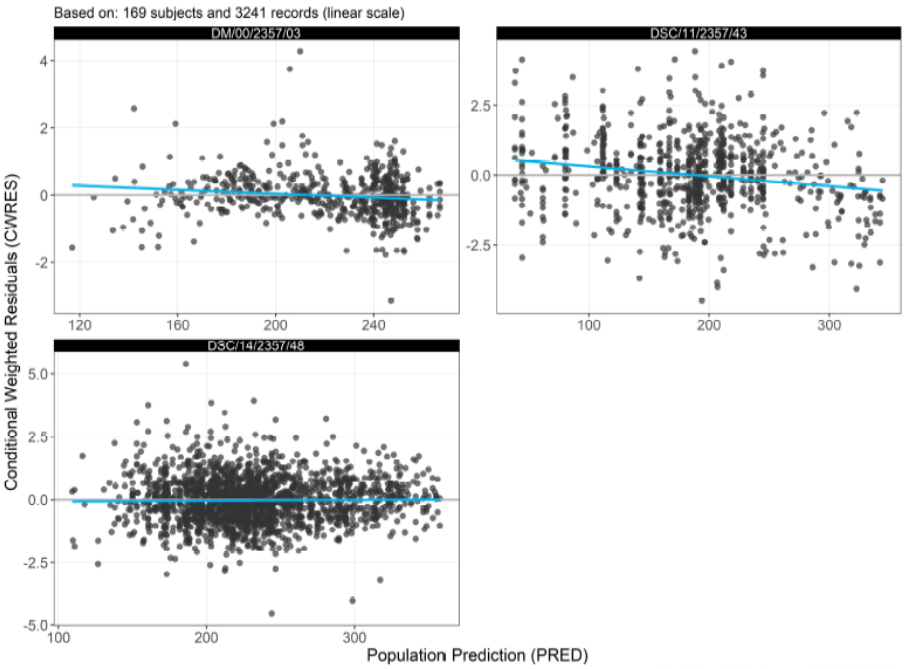
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 74
Note: Symbols: observations; Blue solid lines: linear fits; gray solid lines: identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable (observations); PD, pharmacodynamic; PK, pharmacokinetic; PRED, population predictions

Figure 40. Individual Prediction vs. Observation by Study for Final PK/PD Model



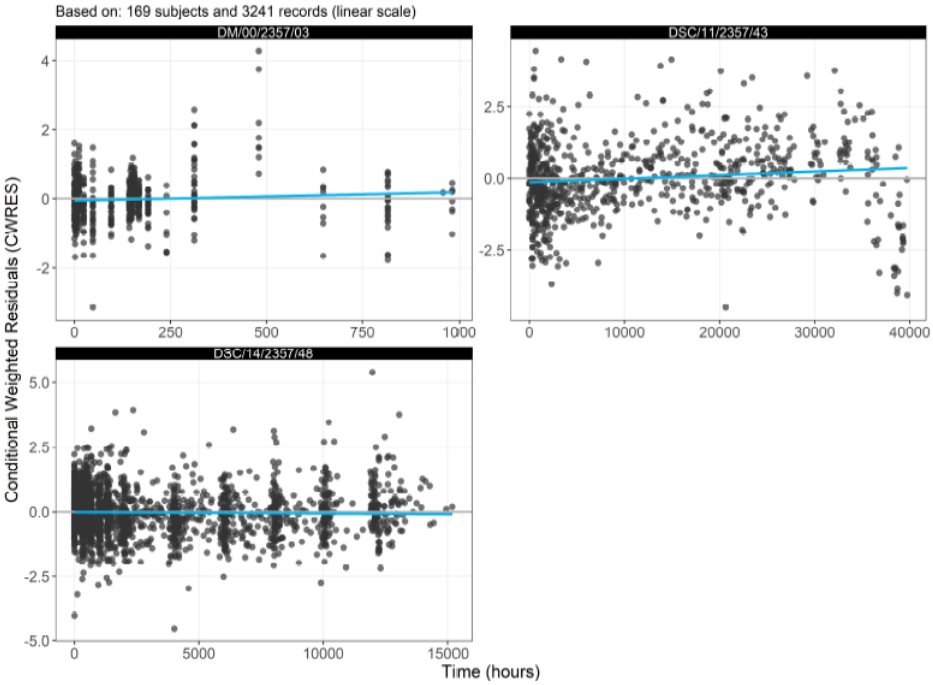
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 75
Note: Symbols- observations; Blue solid lines: linear fits; gray solid lines: identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable (observations); IPRED, individual predictions; PD, pharmacodynamic; PK, pharmacokinetic

Figure 41. Conditional Weighted Residual Versus Population Prediction by Study for Final PK/PD Model



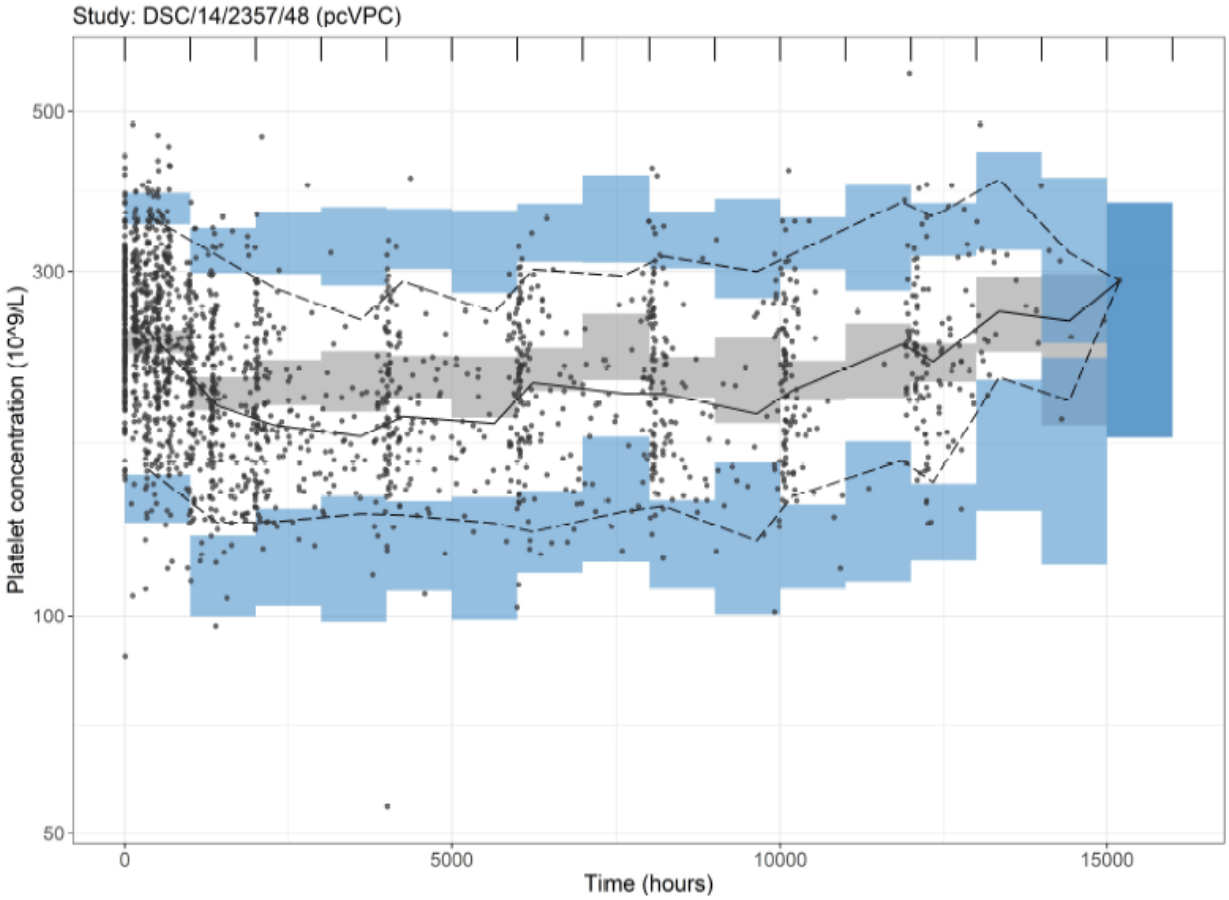
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 76
Note: Dots- observed values; Blue solid lines: linear regression fits; gray solid lines: identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; PD, pharmacodynamic; PK, pharmacokinetic; PRED, population predictions

Figure 42. Conditional Weighted Residual Versus Time by Study for Final PK/PD Model



Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 77
Note: Symbols- observations; Blue solid lines: linear fits; gray solid lines: identity line (observations vs. predictions) or y=0 line (CWRES)
Abbreviations: CWRES, conditional weighted residuals; PD, pharmacodynamic; PK, pharmacokinetic

Figure 43. Visual Predictive Check for Final PK/PD Model for Study 48 Subjects



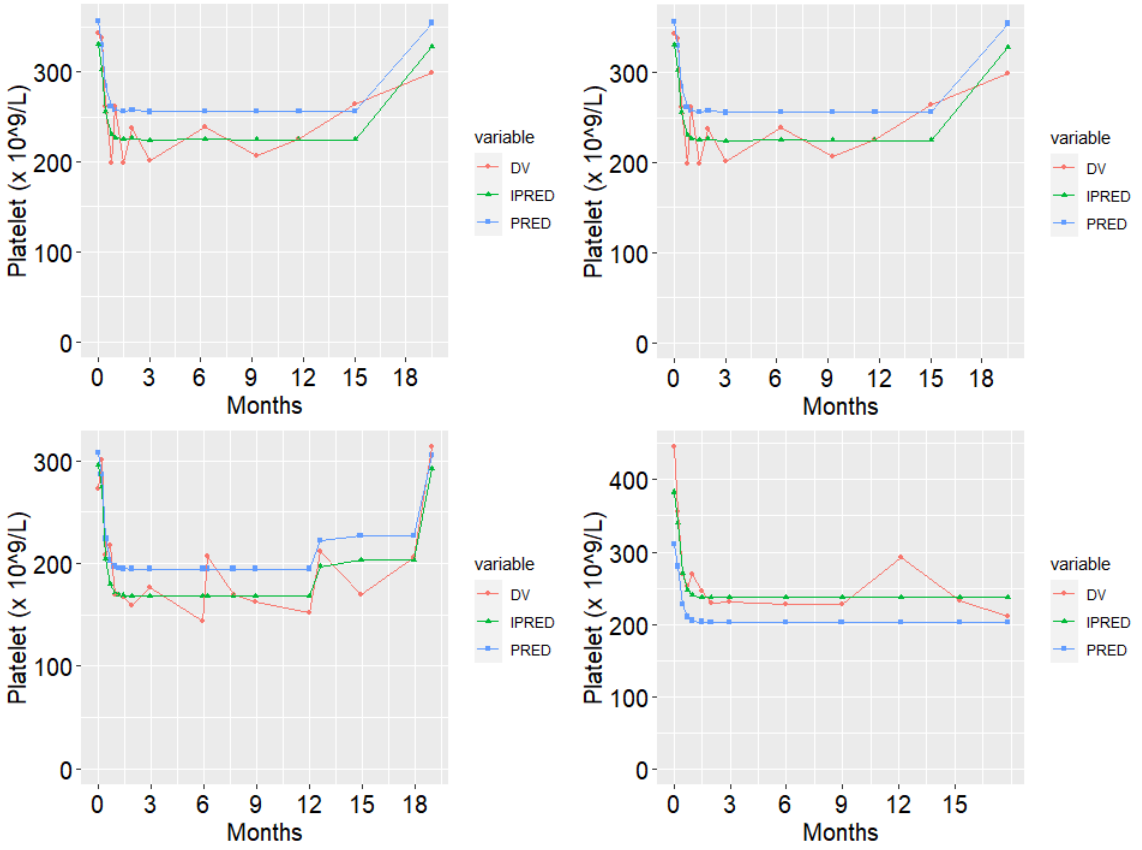
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 80
Note: Symbols represent the observations; solid thick line represents the median of the observations; dashed thick lines represent the 5th and 95th percentiles of the observations; dashed line represents the median 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: CI, confidence interval; pc-VPC, prediction-corrected visual predictive checks; PD, pharmacodynamic; PK, pharmacokinetic

The eta shrinkage for Baseline, MTT, and Slope are 7.7%, 50.1%, and 22.9%.

The pharmacometrics reviewer generated plots of the observed platelet value overlayed with the individual predicted and population predicted platelet values for individual subjects (see Figure 44).

Reference ID: 5351242

Figure 44. Individual Observed and Predicted Platelet Values for Four Representative Subjects



Source: Reviewer's analyses.
Note: Top left subject weight 18.1 kg, top right subject weight 18.1 kg, bottom left subject weighs 32.5 kg, bottom right subject weighs 31.1 kg.
Abbreviations: DV, dependent variable (observations); IPRED, individual predictions; PK, pharmacokinetic; PRED, population predictions

The residual squared error estimates are acceptable for both fixed effects and random effects. The diagnostic plots stratified by study indicate that the final PK/PD model performs best in Study 48. For Study 48, the conditional weighted residuals are generally within ± 2 standard deviations and do not suggest presence of bias with respect to time nor population prediction value. The visual predictive check suggests that the model describes the platelet distribution well over time in Study 48. The condition number, 29.59, does not suggest overparameterization for the final model. Overall, the PK/PD model is acceptable.

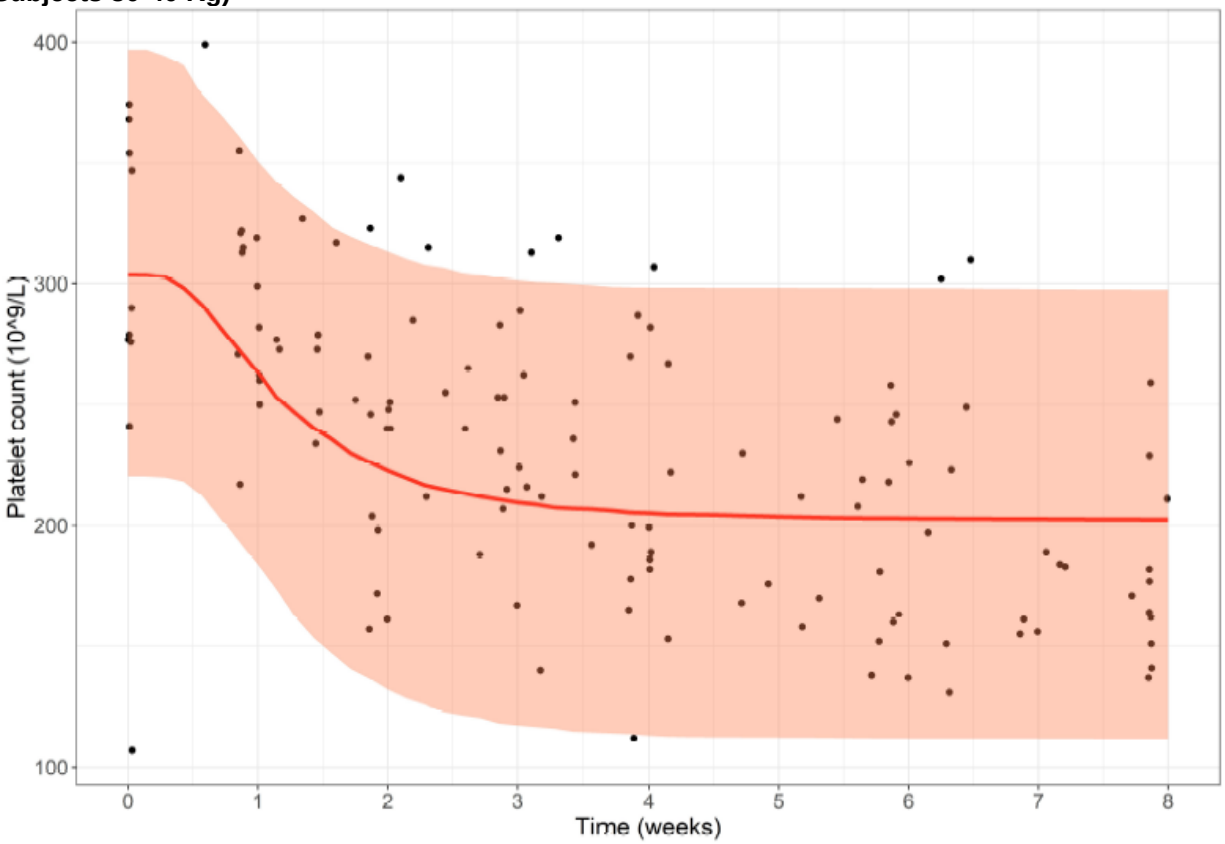
14.5.4. Population PK/PD Simulation

14.5.4.1. Effect of 8 Weeks Treatment With Study 48 Dosing Regimen on Platelet Count

The Applicant utilized the final PK/PD model (see the Section 14.5.3 for details) to simulate the effect of various givinostat dosing regimens on platelet levels over time. In subjects 30 to 40 kg, the PK/PD-simulated effect of dosing regimen B (26.7 mg twice-daily) on the platelet levels across the population is presented in Figure 45.

Reference ID: 5351242

Figure 45. Simulation of the Platelet Count During an 8-Week Dosing Period (26.7 mg BID, in Subjects 30-40 Kg)



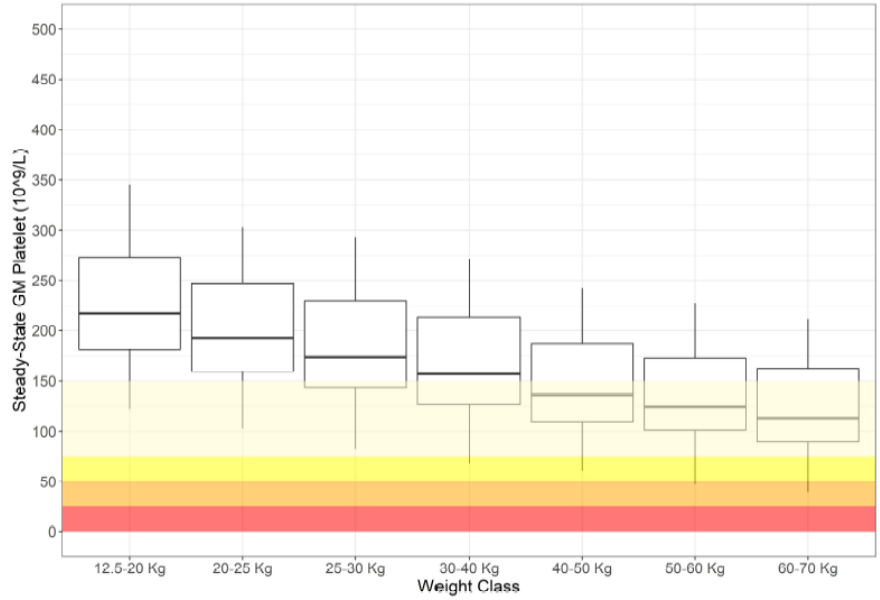
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 86
Note: Solid line is median, ribbon is 5th and 95th PIs of platelet counts simulated from 1000 virtual subjects receiving Dose B with weight in the 30 to 40 kg range. The points are the observed platelet counts for subjects in Study 48 in that started with Dose B weighing 30 to 40 kg.
Abbreviations: BID, twice daily; PI, prediction interval

The Applicant states that platelet counts predicted at 4 weeks were essentially similar to platelet counts predicted at 8 weeks.

The Applicant provides additional PK/PD simulations to assess effects of weight and dose (Dose A, Dose B, or Dose C) on the predicted platelet count at Week 8 (see Figure 46, Figure 47, and Figure 48).

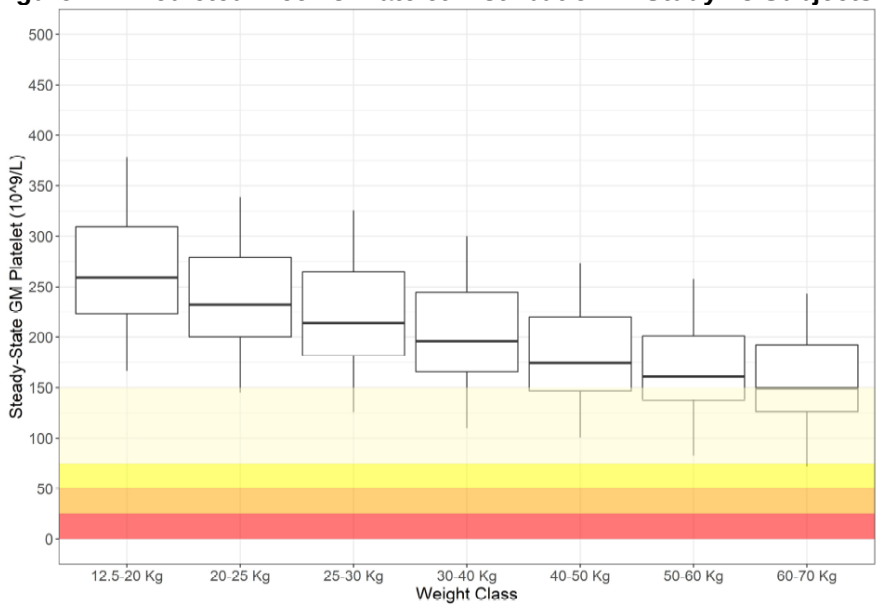
Reference ID: 5351242

Figure 46. Predicted Week 8 Platelet Distribution in Study 48 Subjects: Dose A



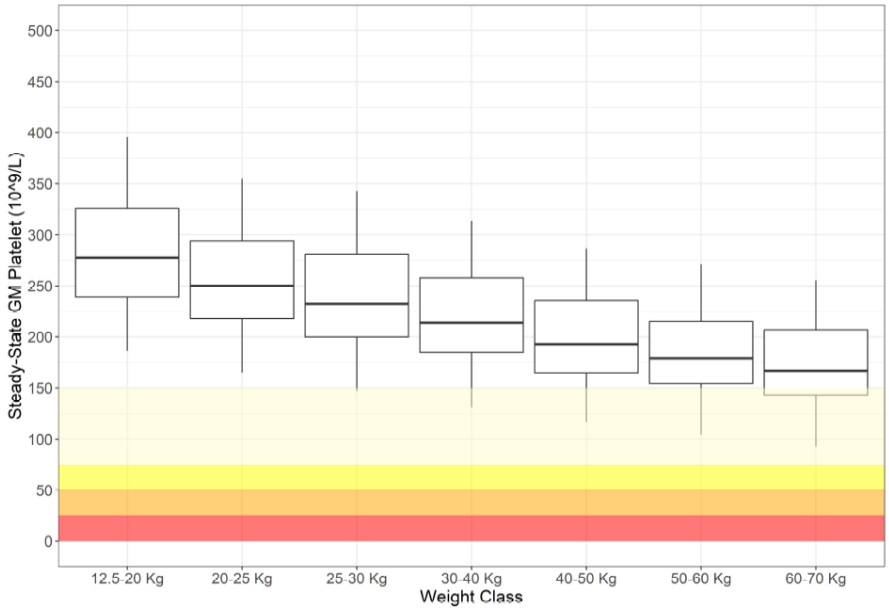
Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 87
Note: Boxes are the 25th and 75th PIs, the line is the geometric mean, and whiskers are the 5th and 95th PIs. Light yellow, yellow, orange and red areas define Grade 1, 2, 3, 4 thrombocytopenia, respectively. Dose A: 25, 30, 35, 40, 50, 55, 60 mg BID for subjects of ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively.
Abbreviations: BID, twice daily; GM, gel matrix; PI, prediction interval

Figure 47. Predicted Week 8 Platelet Distribution in Study 48 Subjects: Dose B



Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 88
Note: Boxes are the 25th and 75th PIs, the line is the geometric mean, and whiskers are the 5th and 95th PIs. Light yellow, yellow, orange and red areas define Grade 1, 2, 3, 4 thrombocytopenia, respectively. Dose B: 16.7, 20, 23.3, 26.7, 33.3, 36.7, 40 mg BID for subjects of body weight, ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively.
Abbreviations: BID, twice daily; GM, gel matrix; PI, prediction interval

Figure 48. Predicted Week 8 Platelet Distribution in Study 48 Subjects: Dose C



Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 89
Note: Boxes are the 25th and 75th PIs, the line is the geometric mean, and whiskers are the 5th and 95th PIs. Light yellow, yellow, orange and red areas define Grade 1, 2, 3, 4 thrombocytopenia, respectively. Dose C: 13.4, 16, 18.6, 21.4, 26.6, 29.4, 32 BID for subjects of body weight ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60- < 70 kg, respectively.
Abbreviations: BID, twice daily; GM, gel matrix; PI, prediction interval

The Week 8 platelet count is summarized by dose and treatment group in Table 93.

Table 93. Summary Statistics of Platelet Count at Steady State at the Different Doses Adopted in Study 48

Body weight (kg)	Platelet Count Geometric Mean, (95%PI)		
	Dose A	Dose B	Dose C
12.5-20 Kg	217 (122; 345)	259 (167; 378)	278 (187; 395)
20-25 Kg	193 (103; 303)	232 (145; 339)	250 (165; 355)
25-30 Kg	174 (82.4; 293)	214 (126; 326)	233 (147; 343)
30-40 Kg	157 (67.7; 271)	196 (110; 300)	214 (131; 314)
40-50 Kg	136 (60.6; 242)	175 (101; 274)	193 (117; 286)
50-60 Kg	124 (47.4; 228)	161 (82.7; 258)	179 (104; 271)
60-70 Kg	113 (39.3; 212)	149 (72.3; 243)	167 (92.3; 255)

Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, page 90
Note: Dose A: 25, 30, 35, 40, 50, 55, 60 mg BID for subjects of body weight ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively;
Dose B: 16.7, 20, 23.3, 26.7, 33.3, 36.7, 40 mg BID for subjects of body weight, ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively;
Dose C: 13.4, 16, 18.6, 21.4, 26.6, 29.4, 32 BID for subjects of body weight ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively.
Abbreviations: BID, twice daily; PI, prediction interval.

The plots depicted in Figure 46, Figure 47, and Figure 48 show shaded bands to depict platelet counts classified as various grades of thrombocytopenia (grade 1 as 75 to <150, grade 2 as 50 to <75, grade 3 as 25 to <50, grade 4 as <25 x 10^9 platelets per liter). The Applicant expressed the simulated platelet counts in terms of the risk of thrombocytopenia by weight and by dose group (see Table 94).

Table 94. Predicted Thrombocytopenia Risk at Steady State by Study 48 Dose Group and Weight

Body Weight	Predicted Incidence of Thrombocytopenia (fraction)											
	Dose A				Dose B				Dose C			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
12.5-20 Kg	0.1	0.01	<0.01	None	0.03	<0.01	None	None	0.01	None	None	None
20-25 Kg	0.18	0.01	<0.01	None	0.06	<0.01	None	None	0.02	None	None	None
25-30 Kg	0.25	0.03	<0.01	<0.01	0.1	<0.01	<0.01	None	0.05	<0.01	None	None
30-40 Kg	0.34	0.05	0.01	<0.01	0.15	<0.01	<0.01	None	0.09	<0.01	<0.01	None
40-50 Kg	0.41	0.06	0.02	0.01	0.24	0.01	<0.01	<0.01	0.15	<0.01	<0.01	None
50-60 Kg	0.49	0.06	0.04	0.01	0.3	0.02	0.01	<0.01	0.2	0.01	<0.01	None
60-70 Kg	0.5	0.09	0.06	0.02	0.37	0.04	0.02	<0.01	0.28	0.02	<0.01	<0.01

Source: Sequence 0003, module 5335, givinostat-report-final-12dec22-signed.pdf, pages 90 and 91
Note: Dose A: 25, 30, 35, 40, 50, 55, 60 mg BID for subjects of body weight ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively;
Dose B: 16.7, 20, 23.3, 26.7, 33.3, 36.7, 40 mg BID for subjects of body weight, ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60-< 70 kg, respectively;
Dose C: 13.4, 16, 18.6, 21.4, 26.6, 29.4, 32 BID for subjects of body weight ≥12.5-< 20 kg, ≥20-< 25 kg, ≥25-< 30 kg, ≥30-< 40 kg, ≥40-< 50 kg, ≥ 50-< 60 kg, ≥60- < 70 kg, respectively.
Abbreviations: BID, twice daily

Applicant concludes:

- The incidence of Week 8 thrombocytopenia was predicted to be lower in subjects with lower body weight (receiving lower dose levels)
- The predicted incidence of thrombocytopenia grade ≥2 is 17%, 6%, and 2% for DMD subjects weighing ≥60-<70 kg, receiving Dose A, B, and C, respectively
- The predicted incidence of thrombocytopenia grade ≥ 2 is 1%, <1% and <1 for DMD subjects weighing 12.5- 20 kg receiving Dose A, B, and C, respectively

The PK/PD simulations indicate that platelet counts are expected to achieve steady-state after approximately 4 weeks of continuous treatment at a fixed dose level. For the proposed dosing (dosing applied in Study 48), thrombocytopenia risk is generally highest in Dose A, lower in Dose B, and lowest in Dose C. Also, within each of the three dose groups (Dose A, Dose B, or Dose C), the thrombocytopenia risk generally increases with increasing weight.

14.5.4.2. Effect of Three Dosing Regimens on PK and Platelet Count

The review team identified a need for dosage optimization with an intent to 1) increase dosing convenience by allowing all dose levels to be administered with a single 5 mL syringe with half mL and whole mL marks; 2) reduce unnecessary complexity in dosing for healthcare by reducing the number of body-weight tiers from nine weight bins (as applied in Study 48) to four weight bins.

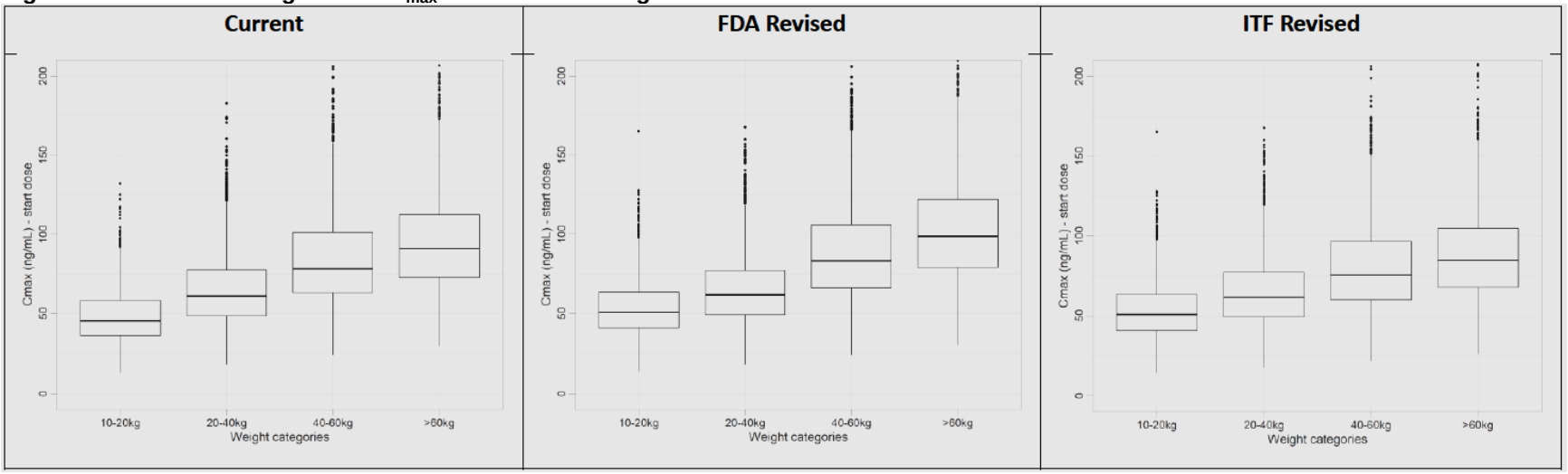
The review team created an alternate regimen with fewer body-weight tiers and requiring a single syringe (referred to as the "FDA Revised Regimen"). This regimen was communicated to the Applicant (via information request sent on 2023-11-28) to be assessed via PK simulations and PK/PD simulations as a potential alternate givinostat dosing regimen.

The Applicant's simulation results assessing the PK and PK/PD (platelet values) for these regimens were submitted to sequence 0038. The PK/PD simulations utilized an adaptive dose adjustment procedure analogous to what was applied in Study 48. The simulated platelet count was assessed for each virtual subject every 2 weeks. Dose reduction was implemented for the subsequent givinostat administration after the first platelet count found below the threshold of 150 x 10^9 / L. Age and body weight data for virtual subjects 2 to 18 years of age were obtained from the NHANES database. One thousand subjects were selected per weight class from the NHANES database.

Their response included analyses of the "FDA Revised Regimen" as well as a new regimen, [redacted] (b) (4) named the "ITF Revised" regimen. The "ITF Revised" regimen has a lower Dose A level for subjects ≥40 kg (compared to the "FDA Revised" regimen) to mitigate thrombocytopenia risk in the higher weight patients. The "ITF Revised" revision is to reduce Dose A from 55 to 50 mg twice-daily for patients 40 kg to <60 kg and reduce the Dose A from 70 to 60 mg twice-daily for patients ≥60 kg.

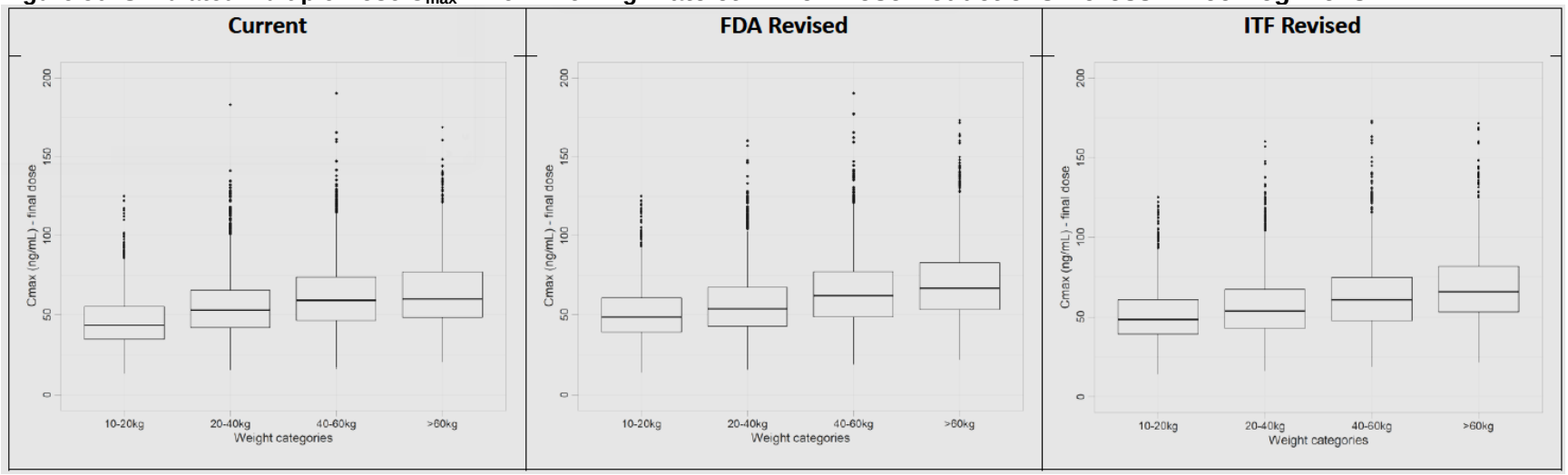
In the Applicant's simulations, the "Current" regimen refers to the dosing regimen applied in Study 48. The single dose PK simulations represent the first administration of Dose A for each dosing regimen. The multiple dose simulations represent the steady-state scenario at the final dose level that each virtual subject ends up on after applying dose reductions based on virtual platelet counts as assessed every two weeks as performed in Study 48. Key results for these simulations are presented in the figures below and in Module 3 Section 6.1.

Figure 49. Simulated Single Dose C_{max} Across Three Regimens



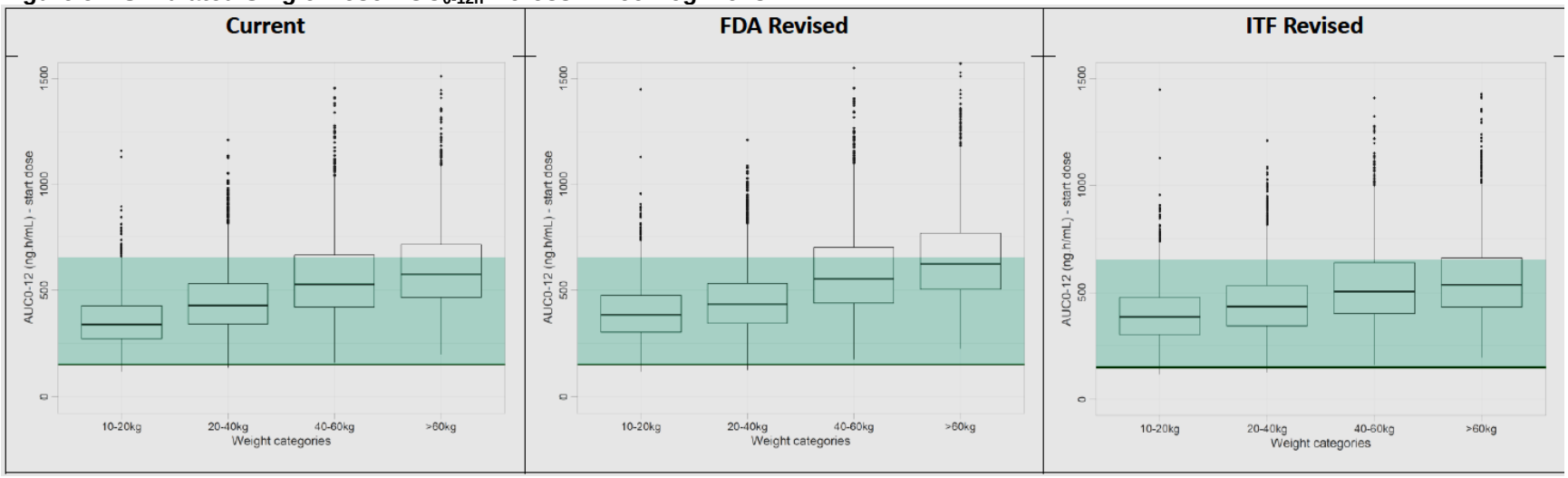
Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 10.
Note: The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). From left to right, the weight categories for each regimen are 10-20 kg, 20-40 kg, 40-60 kg, and >60 kg.
Abbreviations: C_{max}, maximum plasma concentration; FDA, Food and Drug Administration; ITF, givinostat

Figure 50. Simulated Multiple Dose C_{max} When Allowing Platelet-Driven Dose Reductions Across Three Regimens



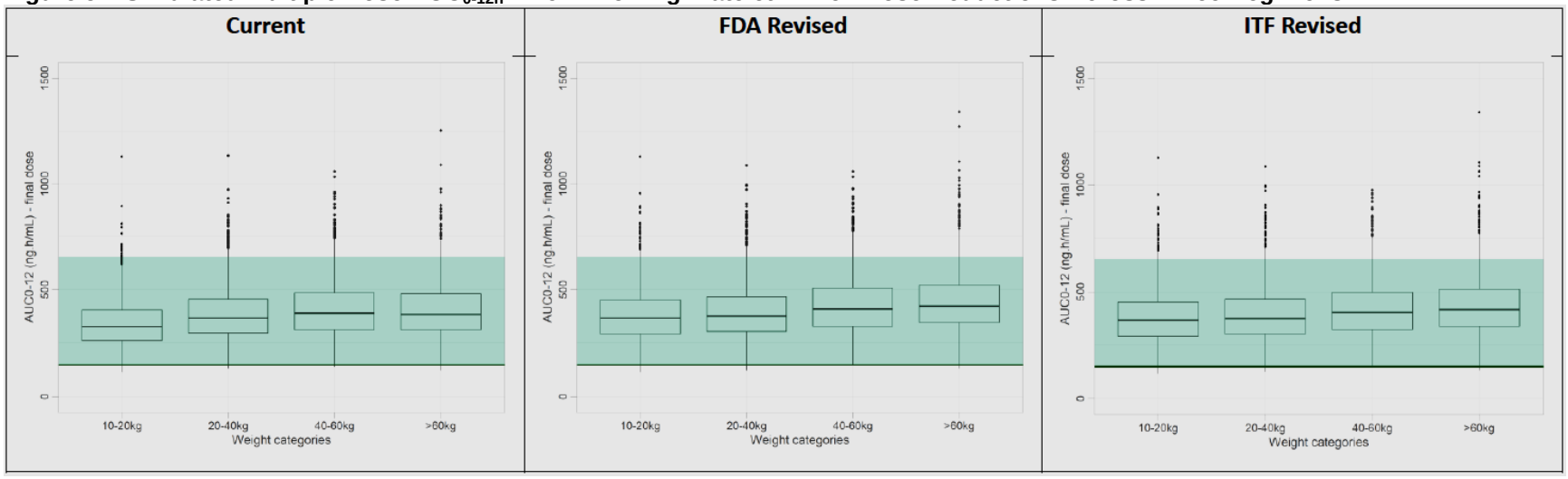
Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 11.
Note: The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). From left to right, the weight categories for each regimen are 10-20 kg, 20-40 kg, 40-60 kg, and >60 kg.
Abbreviations: C_{max}, maximum plasma concentration; FDA, Food and Drug Administration; ITF, givinostat

Figure 51. Simulated Single-Dose AUC_{0-12h} Across Three Regimens



Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 8.
Note: The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). From left to right, the weight categories for each regimen are 10-20 kg, 20-40 kg, 40-60 kg, and >60 kg.
Abbreviations: AUC_{0-12}, area under the curve from 0 to 12 hours; FDA, Food and Drug Administration; ITF, givinostat

Figure 52. Simulated Multiple Dose AUC_{0-12h} When Allowing Platelet-Driven Dose Reductions Across Three Regimens



Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 9.
Note: The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). From left to right, the weight categories for each regimen are 10-20 kg, 20-40 kg, 40-60 kg, and >60 kg.
Abbreviations: AUC_{0-12}, area under the curve from 0 to 12 hours; FDA, Food and Drug Administration; ITF, givinostat

The Applicant summarized the proportion of subjects in the simulation that required a dose reduction based on predicted platelet count by weight group and by dose regimen (see Table 95 and Table 96 below).

Table 95. Proportion of Virtual Subjects Requiring One Dose Reduction Due to Elevated Platelet Counts by Dosing Regimen

Weight	Number of Dose Reductions	% of Subjects (1: Current)	% of Subjects (2: FDA Revised)	% of Subjects (3: ITF Revised)
10 – 20 kg	1	5.6	4.3	4.3
20 – 40 kg	1	15.2	13.5	13.5
40 – 60 kg	1	20.3	17.1	11.4
>60 kg	1	18.2	14.5	7.9

Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 7.
Note: The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg).
Abbreviations: FDA, Food and Drug Administration; ITF, givinostat

Table 96. Proportion of Virtual Subjects Requiring Two Dose Reductions Due to Elevated Platelet Counts by Dosing Regimen

Weight	Number of Dose Reductions	% of Subjects (1: Current)	% of Subjects (2: FDA Revised)	% of Subjects (3: ITF Revised)
10 – 20 kg	2	3.3	7.0	7.0
20 – 40 kg	2	14.5	16.1	16.1
40 – 60 kg	2	35.9	42.0	40.7
>60 kg	2	52.9	61.1	58.8

Source: Sequence 0038, module 1, ir-dose-response-document.pdf, page 7.
Note: The "Current" regimen is the dosing regimen used in Study 48. The "FDA Revised" regimen is similar to Table 8, but with a higher Dose A for subjects ≥ 40 kg (55 mg twice daily for subjects 40 kg to < 60 kg, and 70 mg twice daily for subjects ≥ 60 kg) and a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg). The "ITF Revised" regimen is similar to Table 8, but with a higher Dose B for subjects ≥ 40 kg (40 mg twice daily for subjects 40 kg to < 60 kg, and 50 mg twice daily for subjects ≥ 60 kg).
Abbreviations: FDA, Food and Drug Administration; ITF, givinostat

According to the PK simulations presented in Table 9 in Module 3 Section 6.1, compared to the "Current" regimen, the "FDA Revised" and "ITF Revised" regimens produce exposures within -14% to +7% for AUC and within -13% to +7% for C_{max} across the four weight groups.

The review team determined that the "ITF Revised" dosing regimen is expected to provide comparable efficacy to that observed in Study 48 (due to comparable PK after all expected dose reductions due to thrombocytopenia). In addition, the "ITF Revised" dosing regimen is expected to provide a minor improvement in thrombocytopenia risk over the "FDA Revised" regimen. For subjects ≥40 kg, the "ITF Revised" dosing regimen reduces the risk of dose reduction compared to "FDA Revised Regimen" by ~7% (i.e. 14.5% vs 7.9% requiring one dose reduction for FDA regimen vs ITF regimen in Table 97).

After additional discussions, the review team determined that the (b) (4) Dose B values for subjects ≥40 kg in the "FDA Revised" regimen and the "ITF Revised" regimen (40 mg twice-daily for subjects 40 kg to <60 kg, and 50 mg twice-daily for subjects ≥60 kg) should be reduced even further to mitigate thrombocytopenia risk. The Dose B values recommended for labeling are 35 mg twice daily for subjects 40 kg to <60 kg, and 45 twice daily for subjects ≥60 kg.

Please refer to IV. Interdisciplinary Assessment, Section 6.1.2 "Optimization of the Dosing Regimen" for additional details.

14.5.5. Exposure-Response Modeling

14.5.5.1. Applicant's Analyses

The objective of E-R modeling is to graphically explore and model the E-R relationships with endpoints of clinical efficacy and clinical safety based on data collected in Study 48, with the aim to support the choice of the clinical dose in subjects with DMD:

- Clinical efficacy: the time course of change from baseline of the following endpoints was graphically explored: i) time of 4 standard stairs climb (4SC, primary endpoint of the study), ii) time to rise from floor (RFF); iii) six-minute walking test (6MWT); iv) physical function assessed by North Star Ambulatory Assessment (NSAA), total score and cumulative loss of function; and v) vastus lateralis muscle fat fraction (VL MFF) based on magnetic resonance spectroscopy (MRS), vi) knee extension and vii) elbow flexion strengths, measured using handheld myometry. Modeling was performed on the Month 18 change from baseline 4SC, time to RFF, MRS-VL MFF, NSAA (both as total score and cumulative loss of function), knee extension and elbow flexion strengths. Clinical efficacy was assessed in the Target population (subjects with a baseline MRS-VL MFF in the range >5% and ≤30%).
- Clinical safety: the following endpoints were considered for graphical exploration and modeling:
    - Incidence of diarrhea of any grade,
    - Triglycerides concentrations change from baseline (and incidence of triglycerides increases of any grade),
    - Change from baseline of forced vital capacity ([FVC] % predicted),
    - Change from baseline of peak expiratory flow (PEF% predicted).

Modeling of clinical safety endpoints had to be pursued only in the presence of a signal from the exploratory graphical analysis. Clinical safety was assessed in the overall (i.e., both on- and Off-Target) population.

Data were available from 179 subjects (118 receiving givinostat and 61 receiving placebo). Systemic exposure and clinical efficacy data were evaluable for a total of 75 givinostat subjects. Clinical safety analyses were based on the ITT population (n = 118). After Applicant removed subjects without exposures, without baseline values as applicable, and without Month 18 data as applicable (or a combination thereof), the analysis was performed on 111 subjects for diarrhea as a binary endpoint, 117 subjects for triglycerides as a binary endpoint, 106 subjects for triglycerides change from baseline at 18 months, 94 subjects for FVC1 %predicted change from baseline at 18 months, and 91 for PEF %predicted change from baseline at 18 months.

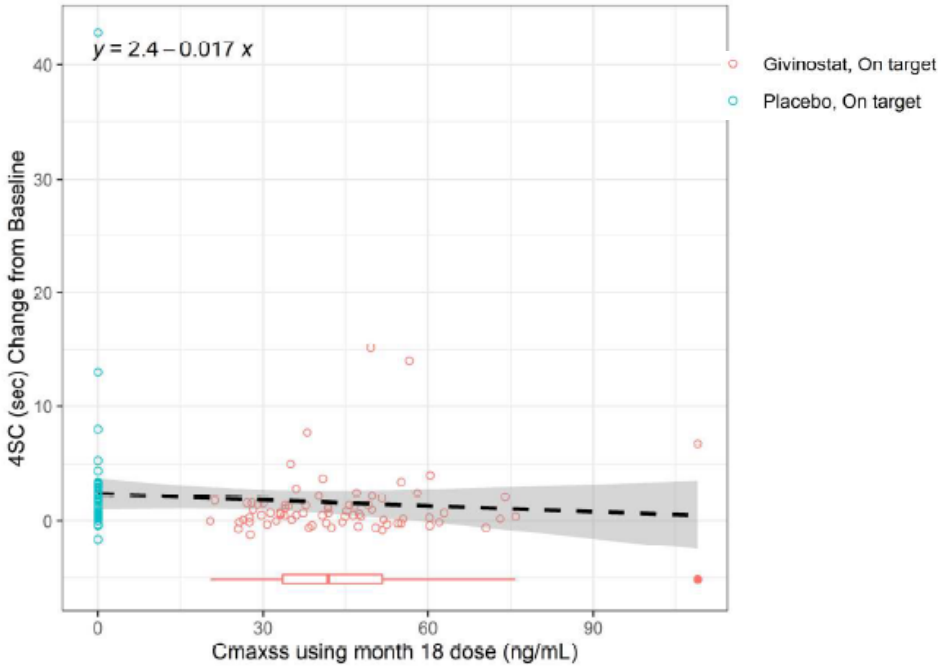
A Type C/End of Phase 3 meeting was held with the FDA on August 25, 2022. A follow-up meeting was granted for February 3, 2023, to further discuss dose rationale and other topics. In Question 1, the Applicant asked if the Agency agrees that the safety, efficacy, and pharmacometrics data support the proposed labeling. In the Written Responses Applicant

received by the Applicant on January 27, 2023, OCP responded to this question by indicating that it is not clear whether the pharmacometrics model relating average concentration at steady state with the 4SC accounted for dose modifications and time effect. OCP also recommended that data from the placebo group should be included to derive an estimate of drug effect slope. Dose modifications and time effect should also be considered for exposure-safety analyses. The Applicant included an updated modeling and simulation report in sequence 0003 module 5351, dsc-14-2357-48 folder (Appendix 1 in response-to-fda-type-c-meeting-comments.pdf) which addressed these comments from OCP. The Applicant indicated that the dose modifications and time of dose modifications were considered in the analyses by using different metrics of givinostat systemic exposure at steady-state, including an average concentration at steady-state term that considered the actual individual dosing history (by computing the mean value of C_{avss} across all dosing intervals for an individual subject).

14.5.5.1.1. Efficacy

The Applicant utilized a linear model to relate givinostat exposure to various endpoints. The models include placebo data and various covariates. The Applicant's final exposure-response model for 4SC is presented in Figure 53.

Figure 53. Relationship Between the 4SC Time Change From Baseline and Steady-State Maximal Concentration at Month 18 (Target Population, Placebo Included)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 48
Note: Colored dots represent individual model-predicted exposures and associated observed response, the horizontal box indicate the distribution of the metrics of systemic exposure (line: median; box 25th and 75th percentiles; whiskers: 1.5 interquartile distance; filled red symbols: outliers). The dashed black line is a loess fit with 95% CI. At the top of the plot the linear regression equation is reported.
Abbreviations: 4SC, 4-stair climb test (time); CI, confidence interval; $C_{max,ss}$, maximal concentration at steady state (using Month 18 dose level)

A summary of the final E-R model for 4SC is presented in Table 97.

Table *97*. Parameters of the Complete Regression Model of 4SC Time Change From Baseline and Steady-State Maximal Concentration at Month 18 (Target Population, Placebo Included)

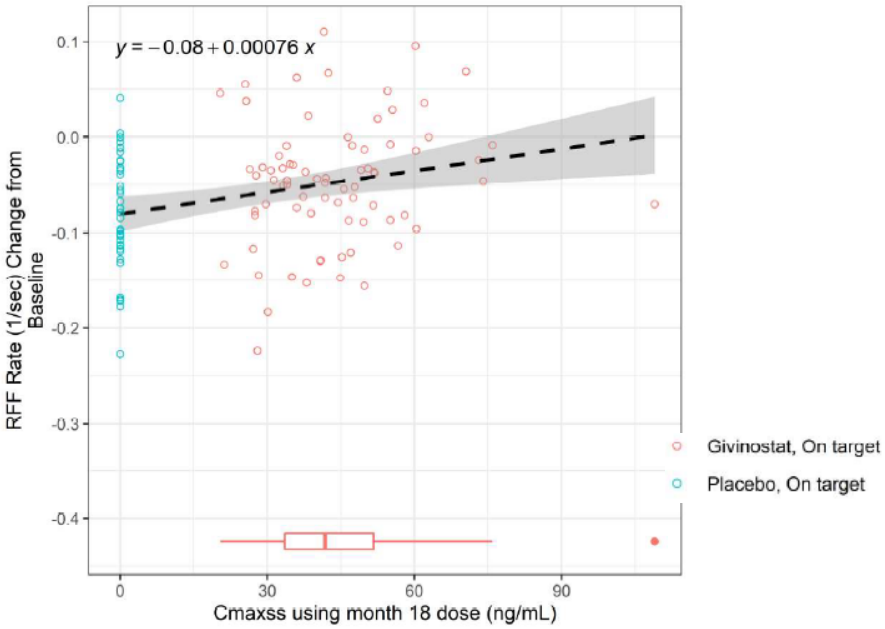
Parameter	Estimate	RSE%	p-value
Intercept	-4.97	68.30%	0.1464
CMAX18	-0.0207	85.30%	0.2427
Baseline 4SC	1.65	24.50%	<0.0001
Age	0.299	96.70%	0.3036
Baseline weight	-0.0479	132.80%	0.4531
Baseline steroid=Daily non-deflazacort steroid	0.141	1031.70%	0.9228
Baseline steroid=Intermittent deflazacort	-1.15	140.30%	0.4756
Baseline steroid=Intermittent non-deflazacort steroid	-0.28	622.20%	0.8724
Starting Dose=Lower Starting Dose	0.858	104.30%	0.3397

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 48-49
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Staring Dose.
Abbreviations: 4SC, 4-stair climb test (time); CMAX18, maximal concentration at steady state (using Month 18 dose level); RSE, relative standard error

The Applicant conducted an additional analysis using $C_{avg,ss}$ as the predictor instead of $C_{max,ss}$. The results for the model using $C_{avg,ss}$ (not shown in this review) are similar to the results for the model with $C_{max,ss}$ in terms of relative standard error % and p-value. In the subsequent exposure-response analyses for other efficacy endpoints, this review will present the final exposure-response model for each efficacy endpoint.

The Applicant conducted similar analyses for additional efficacy endpoints. The relationship of time to RFF endpoint to givinostat exposure was assessed by the Applicant. The Applicant also analyzed a transformation of RFF by computing rate=1/(RFF time). Applicant reports that the RFF rate provides a better fit than RFF. As such, the E-R analyses of RFF will not be further discussed. Applicant's final exposure-response model for RFF rate is presented in Figure 54.

Figure 54. Relationship Between the RFF Rate Change From Baseline and Steady-State Maximal Concentration at Month 18 (Target Population, Placebo Included)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 54
Note: Colored dots represent individual model-predicted exposures and associated observed response, the horizontal box indicate the distribution of the metrics of systemic exposure (line: median; box 25th and 75th percentiles; whiskers: 1.5 interquartile distance; filled red symbols: outliers). The dashed black line is a loess fit with 95% CI. At the top of the plot the linear regression equation is reported.
Abbreviations: CI, confidence interval; $C_{max,ss}$, maximal concentration at steady state (using Month 18 dose level); RFF, rise from floor (time)

A summary of the final E-R model for RFF rate is presented in Table 98.

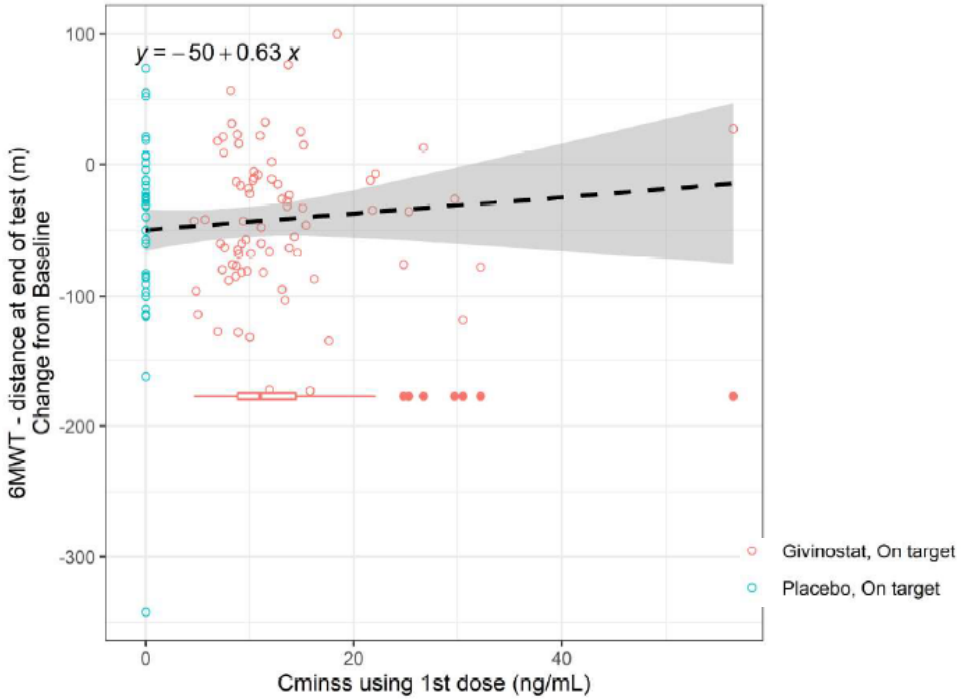
Table *98*. Parameters of the Complete Regression Model of RFF Rate Change From Baseline and Steady-State Maximal Concentration at Month 18 (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	-0.074800	54.4%	0.0689
CMAX18	0.000790	31.7%	0.0021
Baseline value	0.000856	388.3%	0.7973
Age (year)	-0.003340	119.9%	0.4057
Baseline weight (kg)	0.000732	122.9%	0.4179
Baseline steroid=Daily non-deflazacort steroid	-0.024400	83.9%	0.2365
Baseline steroid=Intermittent deflazacort	0.010800	211.5%	0.6367
Baseline steroid=Intermittent non-deflazacort steroid	-0.037700	66.4%	0.1356
Starting Dose=Lower Starting Dose	0.005600	227.9%	0.6615

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 54-55
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: CMAX18, maximal concentration at steady state (using Month 18 dose level); RFF, rise from floor (rate); RSE, relative standard error.

Applicant's final exposure-response model for 6MWT is presented in Figure 55.

Figure 55. Relationship Between the 6MWT Change From Baseline and Steady-State Minimal Concentration at the Starting Dose (Target Population, Placebo Included)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 56
Note: Colored dots represent individual model-predicted exposures and associated observed response, the horizontal box indicate the distribution of the metrics of systemic exposure (line: median; box 25th and 75th percentiles; whiskers: 1.5 interquartile distance; filled red symbols: outliers). The dashed black line is a loess fit with 95% CI. At the top of the plot the linear regression equation is reported.
Abbreviations: 6MWT, 6-minute walking test (distance); CI, confidence interval; $C_{min,ss}$, minimal concentration at steady state (using the first dose level)

A summary of the final E-R model for 6MWT is presented in Table 99.

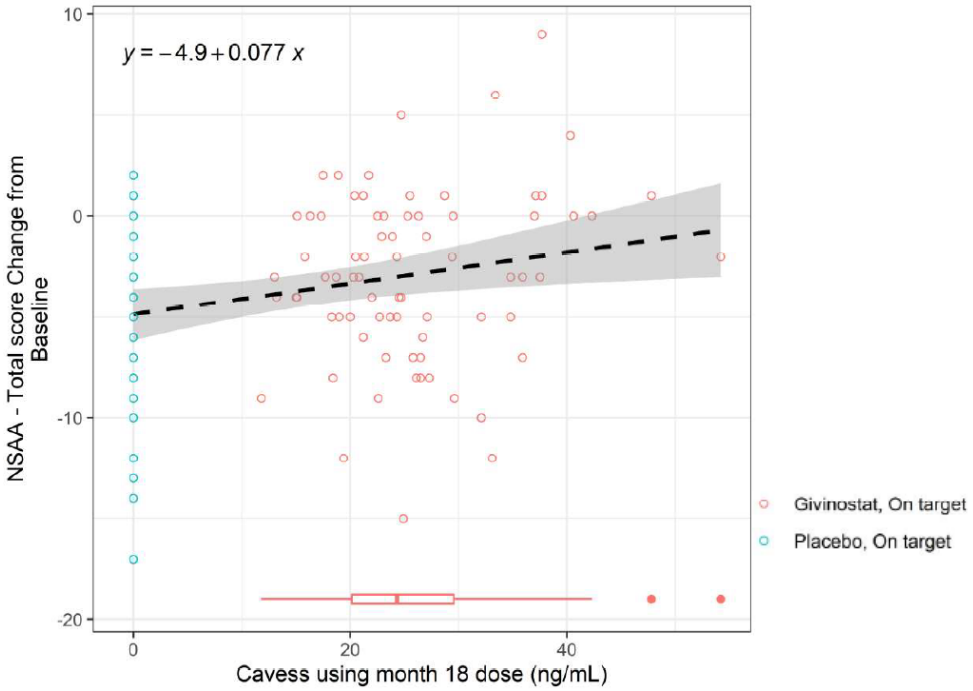
Table *99*. Parameters of the Complete Regression Model of 6MWT Change From Baseline and Steady-State Minimal Concentration at the Starting Dose (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	-4.2600	1119.5%	0.9290
CMIN1	0.5360	122.2%	0.4148
Baseline value	-0.1280	69%	0.1512
Age (year)	0.7720	494.6%	0.8402
Baseline weight (kg)	0.0848	1015%	0.9217
Baseline steroid=Daily non-deflazacort steroid	-34.5000	56.7%	0.0801
Baseline steroid=Intermittent deflazacort	14.1000	156.2%	0.5239
Baseline steroid=Intermittent non-deflazacort steroid	-27.8000	84%	0.2363
Starting Dose=Lower Starting Dose	2.5600	469.9%	0.8321

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 57
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: 6MWT, 6-minute walking test (distance); CMIN1, minimal concentration at steady state (using first dose level); RSE, relative standard error

Applicant's final exposure-response model for NSAA total score is presented in Figure 56.

Figure 56. Relationship Between the NSAA Total Score Change From Baseline and Steady-State Average Concentration at Month 18 (Target Population, Placebo Included)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 58
Note: Colored dots represent individual model-predicted exposures and associated observed response, the horizontal box indicate the distribution of the metrics of systemic exposure (line: median; box 25th and 75th percentiles; whiskers: 1.5 interquartile distance; filled red symbols: outliers). The dashed black line is a loess fit with 95% CI. At the top of the plot the linear regression equation is reported.
Abbreviations: $C_{ave,ss}$, minimal concentration at steady state (using the first dose level); CI, confidence interval; NSAA, North Star Ambulatory Assessment total score (score)

A summary of the final E-R model for NSAA total score is presented in Table 100.

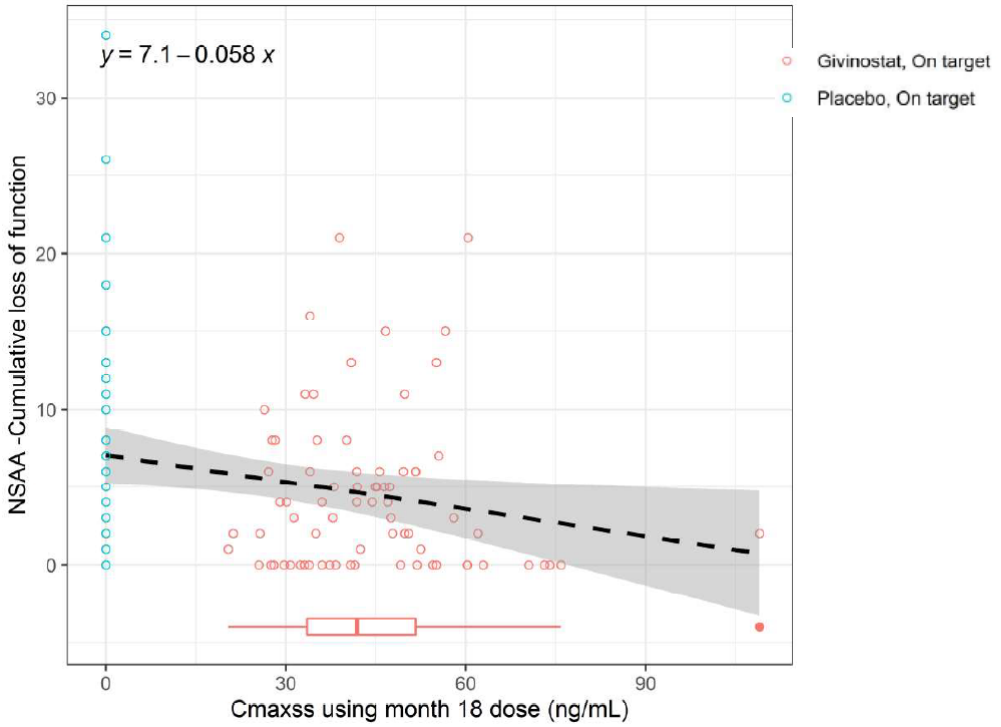
Table *100*. Parameters of the Complete Regression Model of NSAA Total Score Change From Baseline Steady-State Average Concentration at Month 18 (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	-4.8000	58.1%	0.0881
CAVE18	0.0833	33.4%	0.0035
Baseline value	-0.0588	146.3%	0.4955
Age (year)	-0.1090	243.4%	0.6823
Baseline weight (kg)	0.0744	77.3%	0.1985
Baseline steroid=Daily non-deflazacort steroid	-1.2400	106.1%	0.3463
Baseline steroid=Intermittent deflazacort	2.6200	55.9%	0.0763
Baseline steroid=Intermittent non-deflazacort steroid	-5.6500	27.6%	0.0005
Starting Dose=Lower Starting Dose	0.7310	110.8%	0.3689

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 59
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: CAVE18, average concentration at steady state (using Month 18 dose level); NSAA, North Star Ambulatory Assessment total score (score); RSE, relative standard error

Applicant's final exposure-response model for NSAA cumulative loss of function is presented in Figure 57.

Figure 57. Relationship Between the NSAA Cumulative Loss of Function Change From Baseline and Steady State Maximal Concentration at Month 18 (Target Population, Placebo Included)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 61
Note: Colored dots represent individual model-predicted exposures and associated observed response, the horizontal box indicate the distribution of the metrics of systemic exposure (line: median; box: 25th and 75th percentiles; whiskers: 1.5 interquartile distance; filled red symbols: outliers). The dashed black line is a loess fit with 95% CI. At the top of the plot the linear regression equation is reported.
Abbreviations: CI, confidence interval; $C_{max,ss}$, maximal concentration at steady state (using month 18 dose level), NSSA, North Star Ambulatory Assessment cumulative loss of function (score)

A summary of the final E-R model for NSAA Cumulative Loss of Function is presented in Table 101.

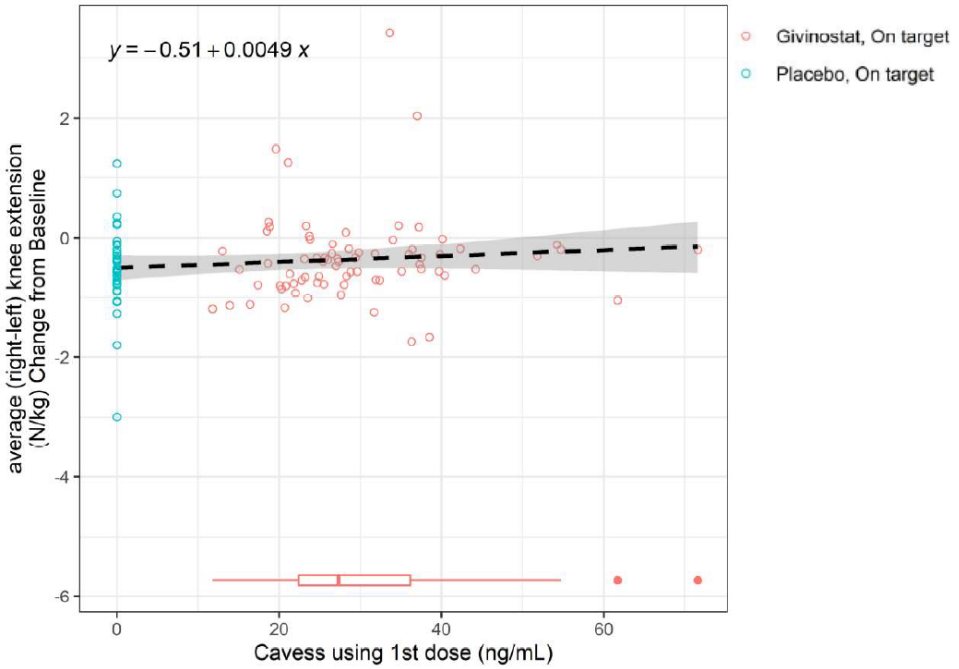
Table *101*. Parameters of the Complete Regression Model of NSAA Cumulative Loss of Function Change From Baseline and Steady State Maximal Concentration at Month 18 (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	3.9500	79.4%	0.2099
CMAX18	-0.0637	33.2%	0.0032
Age (year)	0.6440	52.4%	0.0594
Baseline weight (kg)	-0.1330	57.1%	0.0817
Baseline steroid=Daily non-deflazacort steroid	3.2300	53.3%	0.0631
Baseline steroid=Intermittent deflazacort	0.2650	728.2%	0.891
Baseline steroid=Intermittent non-deflazacort steroid	12.9000	15.9%	<0.0001
Starting Dose=Lower Starting Dose	-0.4340	244.5%	0.6836

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 61-62
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: CI, confidence interval; CMAX18, maximal concentration at steady state (using Month 18 dose level); NSAA, North Star Ambulatory Assessment cumulative loss of function (score); RSE, relative standard error.

Applicant's final exposure-response model for Knee Extension Strength is presented in Figure 58.

Figure 58. Regression of Knee Extension Strength and Average Concentration at Steady State Using the Initial Dose Level (Target Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 63
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: $C_{ave,ss}$, average concentration at steady state (using the first dose level); CI, confidence interval

A summary of the final E-R model for knee extension strength is presented in Table 102.

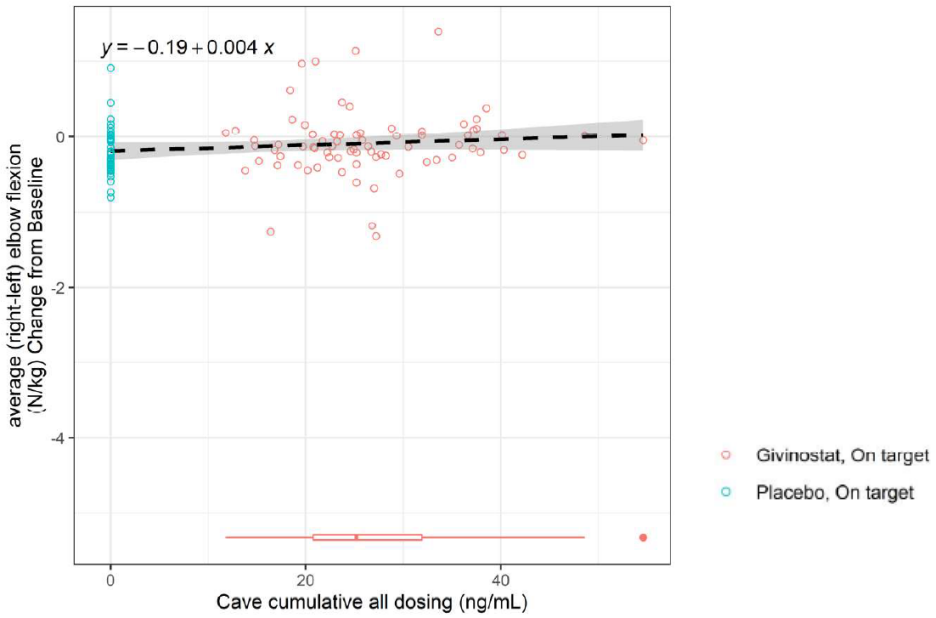
Table *102*. Parameters of the Complete Regression Model of Knee Extension Strength Change From Baseline and Steady State Average Concentration Considering the Actual Dosing History (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	0.270000	137%	0.4679
CAVECUM	0.005470	68.4%	0.147
Baseline value	-0.546000	12.4%	<0.0001
Age (year)	0.030200	117.5%	0.3964
Baseline weight (kg)	0.000498	1623.8%	0.951
Baseline steroid=Daily non-deflazacort steroid	-0.069900	258.4%	0.6994
Baseline steroid=Intermittent deflazacort	0.033000	616.9%	0.8715
Baseline steroid=Intermittent non-deflazacort steroid	-0.404000	54%	0.0665
Starting Dose=Lower Starting Dose	-0.099700	112.5%	0.3761

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 63-64
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: CAVECUM, average concentration at steady state (using the actual dosing history); RSE, relative standard error

Applicant's final exposure-response model for Elbow Flexion Strength is presented in Figure 59.

Figure 59. Regression of Elbow Flexion Strength and Average Concentration at Steady State Considering the Actual Dosing History (Target Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 65
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: C_{ave}, average concentration at steady state (using the actual dosing history); CI, confidence interval.

A summary of the final E-R model for Elbow Flexion Strength is presented in Table 103.

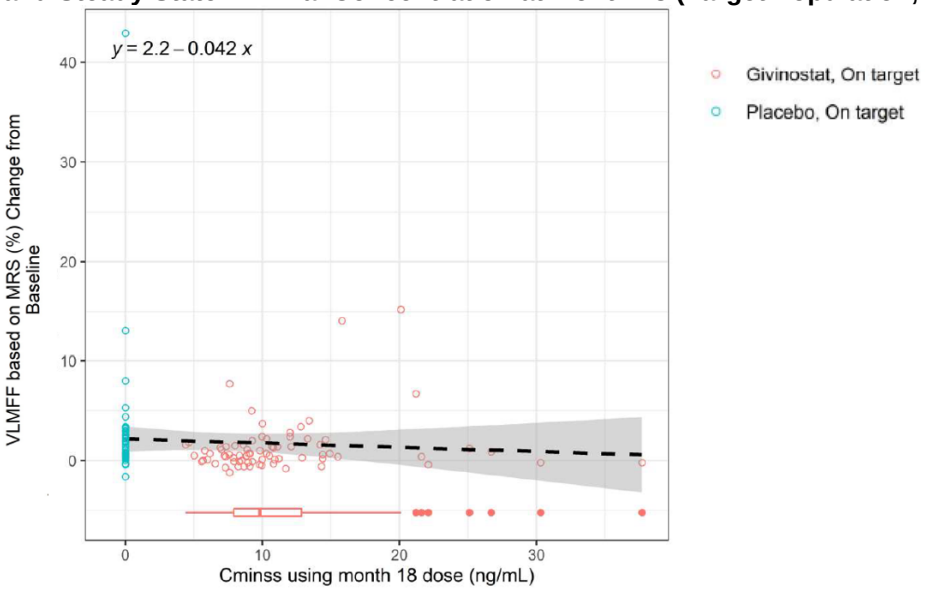
Table *103*. Parameters of the Complete Regression Model of Elbow Flexion Strength Change From Baseline and Steady State Average Concentration Considering the Actual Dosing History (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	0.30100	72.8%	0.1731
CAVECUM	0.00252	87.5%	0.2558
Baseline value	-0.47900	14.1%	<0.0001
Age (year)	0.04190	50.6%	0.0508
Baseline weight (kg)	-0.00866	58.4%	0.0894
Baseline steroid=Daily non-deflazacort steroid	-0.07230	149.8%	0.5061
Baseline steroid=Intermittent deflazacort	0.14000	85%	0.2437
Baseline steroid=Intermittent non-deflazacort steroid	-0.00980	1290.1%	0.9384
Starting Dose=Lower Starting Dose	-0.04460	146.9%	0.4972

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 65-66
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: CAVECUM, average concentration at steady state (using the actual dosing history); RSE, relative standard error.

Applicant's final exposure-response model for vastus lateralis muscle fat fraction is presented in Figure 60.

Figure 60. Relationship Between the Vastus Lateralis Muscle Fat Fraction Change From Baseline and Steady State Minimal Concentration at Month 18 (Target Population, Placebo Included)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 67
Note: Colored dots represent individual model-predicted exposures and associated observed response, the horizontal box indicate the distribution of the metrics of systemic exposure (line: median; box 25th and 75th percentiles; whiskers: 1.5 interquartile distance; filled red symbols: outliers). The dashed black line is a loess fit with 95% CI. At the top of the plot the linear regression equation is reported.
Abbreviations: CI, confidence interval; $C_{min,ss,}$ minimal concentration at steady state (using month 18 dose level); MRS, magnetic resonance spectroscopy; VL MFF, vastus lateralis muscle fat fraction

A summary of the final E-R model for vastus lateralis muscle fat fraction is presented in Table 104.

Table *104*. Parameters of the Complete Regression Model of Vastus Lateralis Muscle Fat Fraction Change From Baseline and Steady State Minimal Concentration at Month 18 (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	10.300	37.5%	0.0088
CMIN18	-0.220	39.9%	0.0139
Baseline value	0.251	41.5%	0.0176
Age (year)	-0.999	42.6%	0.0207
Baseline weight (kg)	0.139	68.7%	0.1493
Baseline steroid=Daily non-deflazacort steroid	5.790	37%	0.0080
Baseline steroid=Intermittent deflazacort	-2.900	82.7%	0.2300
Baseline steroid=Intermittent non-deflazacort steroid	9.080	28%	0.0005
Starting DoseLower Starting Dose	0.445	294.4%	0.7348

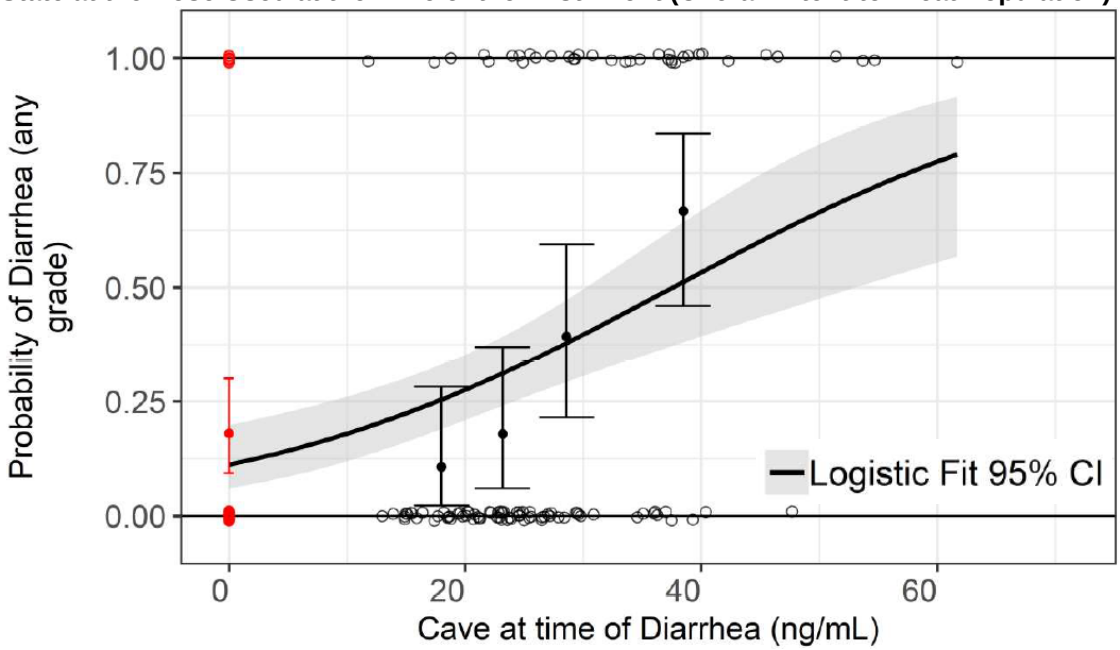
Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 68
Note: Reference strata are Baseline steroid=Daily deflazacort; Starting Dose=High Starting Dose.
Abbreviations: CMIN18, minimal concentration at steady state (using Month 18 dose level); RSE, relative standard error.

The Applicant's conclusions are found in Section 14.5.5.1.3.

14.5.5.1.2. Safety

The Applicant used a logistic regression model to relate a givinostat PK metric to the probability of the first instance of diarrhea (any grade). The Applicant utilized simulated PK at the time of the AE using the subjects individual dosing history. The Applicant's final exposure-response model for diarrhea is presented in Figure 61.

Figure 61. Logistic Regression of Diarrhea (Any Grade) and Average Concentration at Steady State at the Dose Used at the Time of the First Event (Overall Intent-to-Treat Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 70
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: C_{ave}, average concentration at steady state (at the dose used at the time of event); CI, confidence interval.

The parameter estimates for the final E-R model for diarrhea are presented in Table 105.

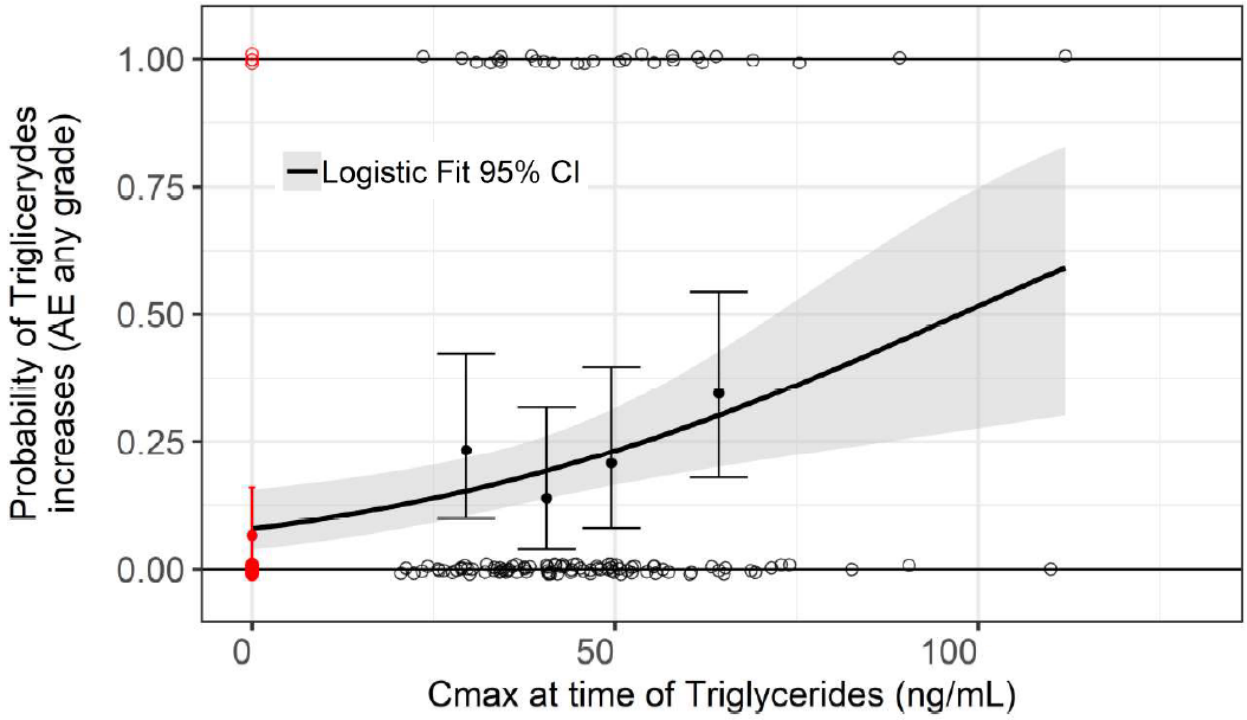
Table *105*. Parameters of the Complete Regression Model of Diarrhea Occurrence Any Grade) and Average Concentration at Steady State at the Dose Used at the Time of the First Event (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value	Odds Ratio (95%CI)
Intercept	-1.8200	53.5%	0.0610	
CAVEDIAR	0.0507	25.8%	0.0001	1.052 (1.0265,1.081)
Baseline age	-0.0635	199.2%	0.6158	0.9358 (0.7284, 1.199)
Baseline weight	0.0252	103.4%	0.3338	1.0255 (0.97413,1.0798)
Starting DoseLower Starting Dose	-0.6450	58.7%	0.0887	0.52476 (0.24774, 1.1018)

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 71
Note: Reference stratum; Starting Dose=High Starting Dose.
Abbreviations: CAVEDIAR, average concentration at steady state (computed using the dose used at the time of event); CI, confidence interval; RSE, relative standard error.

A logistic regression model was applied to relate givinostat PK to the probability of the first instance of triglyceride elevation (any grade). Simulated PK at the time of the AE (using the subject's individual dosing history) was used the exposure metric in the analyses. The Applicant's final exposure-response model for triglyceride elevation is presented in Figure 62.

Figure 62. Logistic Regression of Triglycerides Elevation Occurrence (Any Grade) and Maximal Concentration at Steady State at the Dose Used at the Time of the First Event (Overall Intent-to-Treat Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 72
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: AE, adverse event; CI, confidence interval; C_{max}, maximal concentration at steady state (computed using the dose used at the time of event)

The parameter estimates for the final E-R model for triglyceride elevation occurrence are presented in Table 106.

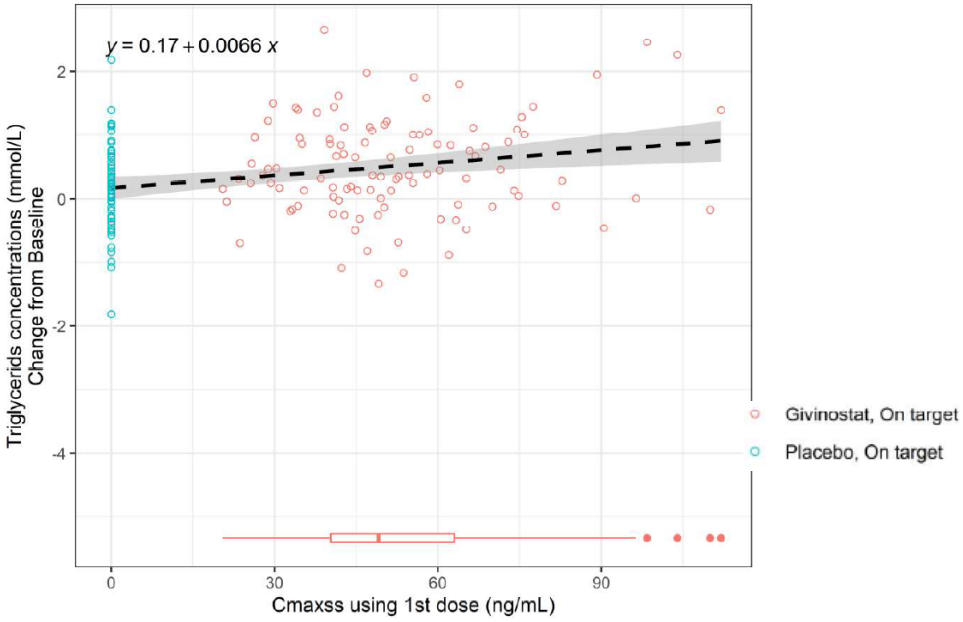
Table *106*. Parameters of the Complete Regression Model of Triglycerides Elevation Occurrence (Any Grade) and Maximal Concentration at Steady State at the Dose Used at the Time of the First Event (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value	Odds Ratio (95%CI)
Intercept	-2.2800	50.6%	0.0476	
CMAXTG	0.0306	30.3%	0.0010	1.031 (1.0133, 1.0512)
Baseline age	-0.1750	83.7%	0.2334	0.83987 (0.62309, 1.1104)
Baseline weight	0.0255	117.3%	0.3947	1.0258 (0.96644, 1.0879)
Starting DoseLower Starting Dose	0.8050	58.9%	0.0894	2.236 (0.90806, 5.9065)

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 72-73
Reference stratum; Starting Dose=High Starting Dose.
Abbreviations: CI, confidence interval; CMAXTG, maximal concentration at steady state (at the time of first occurrence); RSE, relative standard error.

Applicant also conducted exposure-response analyses where triglycerides concentrations were treated as a continuous variable. The Applicant's final exposure-response model for triglyceride concentration is presented in Figure 63.

Figure 63. Regression of Triglycerides Concentrations and Maximal Concentration at Steady State for the Initial Dose Level (Overall Intent-to-Treat Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 74
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: CI, confidence interval; $C_{max,ss}$, maximal concentration at steady state computed using the initial dose level

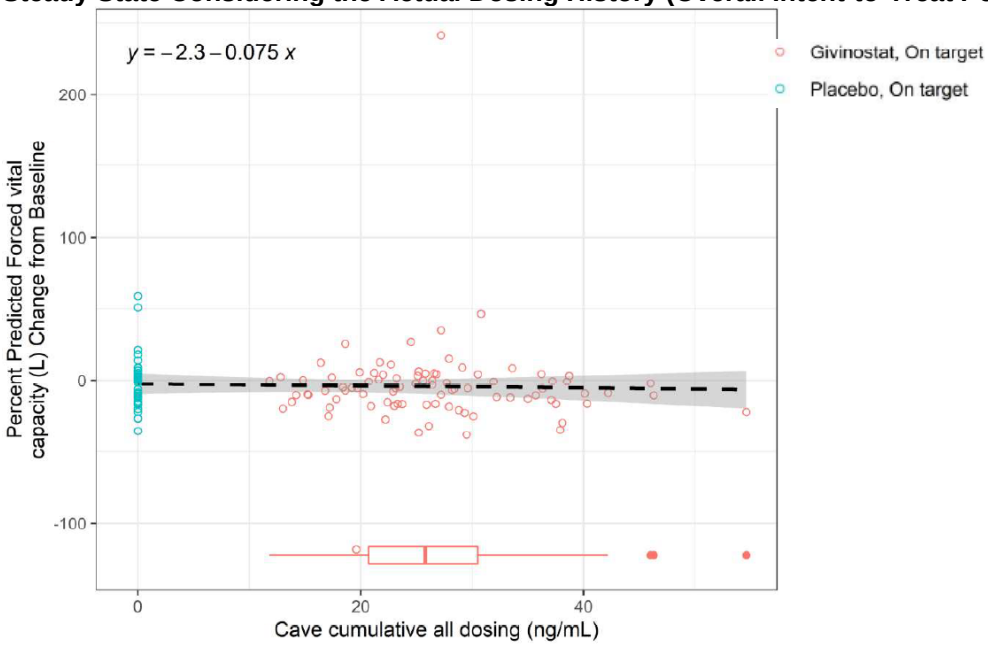
Table *107*. Parameters of the Complete Regression Model of Triglycerides Concentrations and Maximal Concentration at Steady State for the Initial Dose Level (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	0.9399	30.4%	0.0012
CMAX1	0.00763	24.8%	<0.0001
Baseline age	-0.06420	53.9%	0.0654
Baseline weight	0.01150	66.7%	0.1366
Baseline triglycerides	-0.4200	21.7%	<0.0001
Starting DoseLower Starting Dose	0.11300	99.4%	0.3177

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 75
Note: Reference stratum; Starting Dose=High Starting Dose.
Abbreviations: CI, confidence interval; CMAX1, maximal concentration at steady state (computed using the initial dose level); RSE, relative standard error.

The Applicant's final model for predicted forced vital capacity is presented in Figure 64.

Figure 64. Regression of Percent Predicted Forced Vital Capacity and Average Concentration at Steady State Considering the Actual Dosing History (Overall Intent-to-Treat Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 76
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: C_{ave}, average concentration at steady state (using the actual dosing history); CI, confidence interval.

The parameter estimates for the final E-R model for predicted forced vital capacity are presented in Table 108.

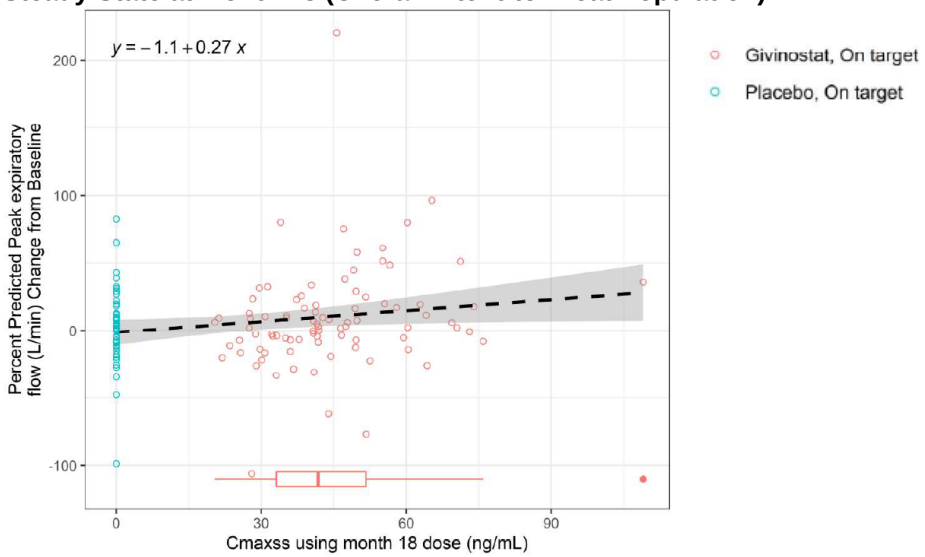
Table *108*. Parameters of the Complete Regression Model of Percent Predicted Forced Vital Capacity and Average Concentration at Steady State Considering the Actual Dosing History (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	75.5	22.1%	<0.0001
CAVECUM	-0.1130	141%	0.4805
Baseline age	-2.1200	68.4%	0.1464
Baseline weight	-0.0312	935.8%	0.915
Baseline FVC	-0.5340	22.2%	<0.0001
Starting DoseLower Starting Dose	-1.8500	248.9	0.6882

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 76-77
Note: Reference stratum; Starting Dose=High Starting Dose.
Abbreviations: CAVECUM, average concentration at steady state (using the actual dosing history); CI, confidence interval; FVC, forced vital capacity; RSE, relative standard error.

The Applicant's final model for predicted peak expiratory flow is presented in Figure 65.

Figure 65. Regression of Percent Predicted Peak Expiratory Flow and Maximal Concentration at Steady State at Month 18 (Overall Intent-to-Treat Population)



Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 78
Note: Open symbols represent individual model-predicted exposures and associated observed response (0=no event, 1=event). The solid black line indicates the model-predicted proportion, and the grey area represents the 95% CI of the predictions. Closed symbols represent observed incidence by quartile of systemic exposure with 95%CI.
Abbreviations: CI, confidence interval; $C_{max,ss}$, maximal concentration at steady state using Month 18 dose level

The parameter estimates for the final E-R model for percent predicted peak expiratory flow are presented in Table 109.

Table *109*. Parameters of the Complete Regression Model of Percent Predicted Peak Expiratory Flow and Maximal Concentration at Steady State at Month 18 (Target Population, Placebo Included)

Parameter	Estimate	RSE%	p-value
Intercept	35.500	42.1%	0.0191
CMAX18	0.181	56.2%	0.0781
Baseline age	3.250	48.5%	0.0413
Baseline weight	-0.254	130.8%	0.4456
Baseline PEF	-0.702	11.9%	0.0001
Starting DoseLower Starting Dose	-10.500	47.9%	0.0379

Source: Sequence 0003, module 5351, dsc-14-2357-48, response-to-fda-type-c-meeting-comments.pdf, page 79
Note: Reference stratum; Starting Dose=High Starting Dose.
Abbreviations: CMAX18, maximal concentration at steady state (at Month 18); PEF, peak expiratory flow; RSE, relative standard error.

The Applicant's conclusions regarding these analyses are found in Section 14.5.5.1.3.

14.5.5.1.3. Applicant Conclusions

The Applicant concludes the following:

Efficacy

- The analyses of the clinical efficacy endpoints demonstrated that givinostat treatment and/or the increasing systemic exposure of givinostat led to trends to improvements or significant improvements of 4SC, RFF (rate), and NSAA scores (as both total score and cumulative loss of function), and VL MFF.
- No significant E-R relationships were observed for RFF (time), 6MWT, and strength endpoints.
- When subjects receiving placebo were removed from the E-R analyses, none of the clinical efficacy endpoints showed a significant association with the metrics of givinostat systemic exposure, suggesting that the beneficial effects highlighted in the present assessment are plateauing in the conditions used in the pivotal trial.

Safety

- The E-R analyses evidenced a significant association between increased givinostat systemic exposure and increased incidence of diarrhea and triglycerides elevation.
- Triglyceride concentrations (continuous endpoint) also showed a significant increase with increasing givinostat systemic exposure.
- The effect of givinostat exposure was not significant for FVC and a trend toward a significant relationship was observed for PEF in the multivariate analyses.

Dosing

Overall, it is felt that the [redacted] (b) (4) givinostat dosing regimens, starting with a dose aiming to maximize the chances of the therapeutic benefit that may be subsequently decreased based on e emerging toxicities, are able to optimize the benefit:risk ratio of givinostat therapy in patients with DMD. The review team assessed the Applicant's above conclusions and made the following determinations.

- The review team agrees with the Applicant's E-R analyses for safety. In general, the risk of AEs analyzed increase with increasing exposure to givinostat.
- For E-R for efficacy, the Applicant determined that givinostat exposure is a significant predictor of efficacy only when placebo was included. The Applicant concludes that, since there was no statistically significant E-R relationship for efficacy when excluding placebo, that the exposure range for the dosing used in Study 48 must be on the "plateau" (that it, the response has reached a practical peak and cannot be expected to increase with further increases in exposure). Overall, though the exposure-response relationship was not always statistically significant, the direction of the slope estimates favors a givinostat exposure-response relationship (for example, higher exposures tend to be associated with less worsening from baseline than lower exposures or placebo).
- Among the various exposure metrics for givinostat PK, the Applicant assessed the mean value of C_{avg} across all dosing intervals over time for a subject (accounting for dose changes and missed doses) in their exposure-response analyses. However, the Applicant's final E-R models for each efficacy endpoints utilized the PK metric that provided the best model fit for that endpoint. The result is that the final models for various efficacy endpoints used different PK metrics. For the majority of the efficacy endpoints assessed in the exposure-response analyses in Section 14.5.5.1.1 Efficacy, the Applicant's final model includes a PK metric that reflects either a single timepoint ((i.e. C_{maxs}s for 4SC, C_{minss} for 6MWT) or a single dosing interval (i.e. C_{avess} for NSAA total). Considering the variability in dosing history across patients over the 12-month treatment duration in Study 48 (see Figure 27), the individual dosing history for each subject may be an important factor affecting efficacy results.
- To further explore exposure-response relationships for efficacy, the reviewer conducted independent analyses and assessed cumulative area under the givinostat plasma concentration-time curve as a function of time. This approach can provide insight into whether the cumulative exposure to givinostat, accounting for dose adjustments, missed dose, and temporary dose breaks, is related to efficacy at various timepoints aside from Month 18. Please see Section 14.5.5.1.2 for details.

14.5.5.2. Reviewer's Analyses

The reviewer conducted exposure-response modeling for Month 18 4SC, 6MWT, and NSAA total.

14.5.5.2.1. Exposure-Response Modeling

The reviewer utilized the Applicant's popPK model (see Section 14.5.1 for details) to simulate the PK data based on the individual dosing history for subjects in Study 48 (see Section 14.5.2

for details). The cumulative AUC, as a function of time after first dose, was computed for each subject starting from the first administration until the time of the last efficacy assessment (~ 18 months). The relationship between the cumulative AUC at Month 18 and various efficacy endpoints at Month 18 was assessed. In addition to the Month 18 timepoint used in the primary efficacy analyses, the Month 12 timepoint was also assessed due to the variations in dosing history across patients (different timing of dose reductions, instances where one daily dose is missed, and temporary dose pausing). Key variables expected to have a relevant effect on outcomes were included in the analyses. A linear model was used to relate the cumulative AUC and covariates with change from baseline in various endpoints. Analyses were conducted using subjects that are in both the ITT and in the Target population.

The final model for change from baseline 4SC at Month 12 and at Month 18 is presented graphically in Module 3, Section 6.3.3.

After adjusting for baseline 4SC and concomitant medication use, cumulative AUC is a predictor of Month 12 4SC but not a predictor of Month 18 4SC. However, the slope of the cumulative AUC coefficient at Month 18 is negative which is favorable to givinostat. The parameter estimates for the final exposure-response models for 4SC are presented in Table 110 and Table 111.

Table *110*. Parameter Estimates for the Model Relating Between the Month 12 4SC Change From Baseline and Month 12 Cumulative AUC (Target Population, Placebo Included)

Variable	Estimate	Standard Error	p-value
(Intercept)	-0.698	0.702	0.323
Cumulative AUC	-0.0036	0.00179	0.0472
Co-med: deflazacort daily regimen	---	---	---
Co-med: deflazacort intermittent regimen	-1.501	0.766	0.0531
Co-med: other steroids daily regimen	-1.278	0.743	0.892
Co-med: other steroids intermittent regimen	-1.426	0.776	0.0693
Baseline endpoint value	0.725	0.197	0.000371

Source: Reviewer's analyses
Note: "Deflazacort daily regimen" is one of four categories of concomitant medications and serves as the reference category.
Note: Units for the slope of cumulative AUC in this table are points (seconds) / (hr*ug/mL). In the plot in Module 3 Section 6.3.1, the units are converted to (seconds)/(1000 hr*ug/mL).
Abbreviations: 4SC, 4-stair climb; AUC, area under the curve

Table *111*. Parameter Estimates for the Model Relating Between the Month 18 4SC Change From Baseline and Month 18 Cumulative AUC (Target Population, Placebo Included)

Variable	Estimate	Standard Error	p-value
(Intercept)	-1.07	0.840	0.207
Cumulative AUC	-0.00007	0.00145	0.962
Co-med: deflazacort daily regimen	---	---	---
Co-med: deflazacort intermittent regimen	-1.28	0.886	0.153
Co-med: other steroids daily regimen	0.561	0.862	0.517
Co-med: other steroids intermittent regimen	0.0298	0.982	0.976
Baseline endpoint value	0.766	0.236	0.00162

Source: Reviewer's analyses
Note: "Deflazacort daily regimen" is one of four categories of concomitant medications and serves as the reference category.
Note: Units for the slope of cumulative AUC in this table are points (seconds) / (hr*ug/mL). In the plot in Module 3 Section 6.3.1, the units are converted to (seconds)/(1000 hr*ug/mL).
Abbreviations: 4SC, 4-stair climb; AUC, area under the curve

The final model for change from baseline NSAA total score at Month 12 and at Month 18 is presented graphically in Module 3, Section 6.3.1.

After adjusting for baseline NSAA total score and concomitant medication use, cumulative AUC is a predictor of NSAA total score at both Month 12 and Month 18. The slope of the cumulative AUC coefficient is positive which supports a relationship between improving NSAA total score with increasing cumulative givinostat AUC for both Month 12 and Month 18. The parameter estimates for the final exposure-response models for NSAA total are presented in Table 112 and Table 113.

Table *112*. Parameter Estimates for the Model Relating Between the Month 12 NSAA Total Score Change From Baseline and Month 12 Cumulative AUC (Target Population, Placebo Included)

Variable	Estimate	Standard Error	p-value
(Intercept)	-0.809	2.01	0.688
Cumulative AUC	0.0104	0.00287	0.000454
Co-med: deflazacort daily regimen	---	---	---
Co-med: deflazacort intermittent regimen	1.38	1.21	0.256
Co-med: other steroids daily regimen	-0.664	1.17	0.572
Co-med: other steroids intermittent regimen	-2.48	1.19	0.0402
Baseline endpoint value	-0.101	0.0727	0.168

Source: Reviewer's analyses
Note: "Deflazacort daily regimen" is one of four categories of concomitant medications and serves as the reference category.
Note: Units for the slope of cumulative AUC in this table are points (NSAA total) / (hr*ug/mL). In the plot in Module 3 Section 6.3.1 the units are converted to (NSAA total)/(1000 hr*ug/mL).
Abbreviations: AUC, area under the concentration-time curve; NSAA, North Star Ambulatory Assessment

Table *113*. Parameter Estimates for the Model Relating Between the Month 18 NSAA Total Score Change From Baseline and Month 18 Cumulative AUC (Target Population, Placebo Included)

Variable	Estimate	Standard Error	p-value
(Intercept)	-4.34	2.44	0.0780
Cumulative AUC	0.00736	0.00231	0.00187
Co-med: deflazacort daily regimen	---	---	---
Co-med: deflazacort intermittent regimen	2.53	1.40	0.0735
Co-med: other steroids daily regimen	-1.54	1.31	0.244
Co-med: other steroids intermittent regimen	-5.94	1.50	0.000143
Baseline endpoint value	-0.0132	0.0879	0.881

Source: Reviewer's analyses
Note: "Deflazacort daily regimen" is one of four categories of concomitant medications and serves as the reference category.
Note: Units for the slope of cumulative AUC in this table are points (NSAA total) / (hr*ug/mL). In the plot in Module 3 Section 6.3.1, the units are converted to (NSAA total)/(1000 hr*ug/mL).
Abbreviations: AUC, area under the concentration-time curve; NSAA, North Star Ambulatory Assessment

The final model for change from baseline 6MWT distance at Month 12 and at Month 18 is presented graphically in Module 3, Section 6.3.1.

After adjusting for baseline 6MWT distance and concomitant medication use, cumulative AUC is a predictor of 6MWT at Month 12 but not at Month 18. However, the slope of the cumulative AUC coefficient for Month 18 is positive which is favorable to givinostat. The parameter estimates for the final exposure-response models for 6MWT are presented in Table 114 and Table 115.

Table *114*. Parameter Estimates for the Model Relating Between the Month 12 6MWT Change From Baseline and Month 12 Cumulative AUC (Target Population, Placebo Included)

Variable	Estimate	Standard Error	p-value
(Intercept)	35.5	27.3	0.197
Cumulative AUC	0.0725	0.0332	0.0316
Co-med: deflazacort daily regimen	---	---	---
Co-med: deflazacort intermittent regimen	9.40	15.0	0.532
Co-med: other steroids daily regimen	-12.1	14.0	0.390
Co-med: other steroids intermittent regimen	-10.5	13.7	0.445
Baseline endpoint Value	-0.158	0.0637	0.0148

Source: Reviewer's analyses
Note: "Deflazacort daily regimen" is one of four categories of concomitant medications and serves as the reference category.
Note: Units for the slope of cumulative AUC in this table are points (meters) / (hr*ug/mL). In the plot in Module 3 Section 6.3.1, the units are converted to (meters)/(1000 hr*ug/mL).
Abbreviations: 6MWT, 6-minute walk time; AUC, area under the curve

Table *115*. Parameter Estimates for the Model Relating Between the Month 18 6MWT Change From Baseline and Month 18 Cumulative AUC (Target Population, Placebo Included)

Variable	Estimate	Standard Error	p-value
(Intercept)	6.48	33.2	0.846
Cumulative AUC	0.0137	0.0281	0.626
Co-med: deflazacort daily regimen	---	---	---
Co-med: deflazacort intermittent regimen	6.32	17.2	0.715
Co-med: other steroids daily regimen	-48.0	17.0	0.00584
Co-med: other steroids intermittent regimen	-30.7	17.5	0.0822
Baseline endpoint value	-0.111	0.0765	0.151

Source: Reviewer's analyses
Note: "Deflazacort daily regimen" is one of four categories of concomitant medications and serves as the reference category.
Note: Units for the slope of cumulative AUC in this table are points (meters) / (hr*ug/mL). In the plot in Module 3 Section 6.3.1, the units are converted to (meters)/(1000 hr*ug/mL).
Abbreviations: 6MWT, 6-minute walk time; AUC, area under the curve

The exposure-response analyses were conducted using change from baseline efficacy data measured at Month 18 as this was the time of the primary efficacy assessment. Due to the variations in dosing history across patients in Study 48 (different timing of dose reductions, instances where one daily dose is missed, and temporary dose pausing), the Month 12 timepoint was also assessed. Analyses were performed in subjects that are ITT and in the Target population.

After adjusting for concomitant medication use and baseline NSAA total value, the estimate for cumulative AUC as a predictor of Month 12 NSAA total is 0.0104 NSAA total score / (1000 hr*ug/mL) (20.5% standard error, $p<0.000454$) and the estimate for cumulative AUC as a predictor of Month 18 NSAA total is 0.00736 NSAA total score / (1000 hr*ug/mL) (31.4% standard error, $p<0.00187$). Overall, the data support a relationship such that increases in cumulative AUC are associated with improvements NSAA total score at Month 12 as well as Month 18 over the range of cumulative AUC values observed at these timepoints in the Target population Study 48.

At Month 12, after adjusting for concomitant medication use and baseline efficacy value, the estimate for cumulative AUC as a predictor of Month 12 4SC is -0.0036 seconds / (1000 hr*ug/mL) (49.7% standard error, p<0.0472) and the estimate for cumulative AUC as a predictor of Month 12 6MWT distance is 0.0725 meters / (1000 hr*ug/mL) (45.8% standard error, p< 0.0316). At Month 18, the analyses do not support cumulative AUC as a predictor of 4SC (>2000 % standard error, p-value 0.96) nor 6MWT (>200 % standard error, p-value 0.63). However, the signs of these slope estimates for 4SC and 6MWT favor givinostat in the Target population at Month 18.

14.5.6. Potential Molecular Markers

In Study 48, the Applicant collected a few molecular markers (i.e., LAP-TGFβ1, IL-1b, TNF-a, IL18bp, IP-10, Osteopontin, Leptin, FABP3), related to some areas involved in the pharmacological activity of givinostat. As the clinical meaningfulness of these molecular markers to DMD is largely unknown, these data are not explored by the review team.

14.6. Physiologically Based Pharmacokinetic Modeling

Executive Summary

This review evaluates the adequacy of the Applicant's PBPK analysis to support the proposed labeling claims: (b) (4)

Based on the review of the applicant's PBPK modeling report (TR2278/E) and modeling and simulations files, the Division of Pharmacometrics concluded the following:

- The PBPK analysis is inadequate to support the Applicant's conclusion about oral bioavailability of givinostat to inform labeling in the absence of adequate clinical data.

14.6.1. Applicant's Modeling Effort

14.6.1.1. Methods

(b) (4)

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(b) (4)

14.6.2. FDA Assessment

The review team considered the Applicant's modeling analysis inadequate to predict the human oral bioavailability of givinostat.

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Reference ID: 5351242

(b) (4)

14.7. Pharmacogenetics

Not applicable.

15. Study/Trial Design

Not applicable

16. Efficacy

Not applicable.

17. Clinical Safety

Not applicable.

18. Clinical Virology

Not applicable.

19. Clinical Microbiology

Not applicable.

20. Mechanism of Action/Drug Resistance

Not applicable.

21. Other Drug Development Considerations

None.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

For complete review, please see OSI consult by Dr. Alfaro. Briefly, Mercuri (Site #15), Vilchez (Site #23), Zaidman (Site #30), and the contract research organization (CRO), (b) (4) were inspected in support of this NDA covering Study 48. Based on the inspection results, the study appears to have been conducted adequately, and the data generated by these sites and submitted by the Applicant appear acceptable in support of the respective indication.

Primary efficacy data, the 4-stair climb (4SC) test, and key secondary efficacy data, North Star Ambulatory Assessment (NSAA), Time to Rise from floor (TTR), and 6 Minute Walk Test (6MWT) were reviewed at the clinical investigator sites. There were no discrepancies identified for the 4SC or TTR data. Minor discrepancies in NSAA and 6MWT data were identified in 4 of 10 randomized subjects at Site #23 only. These discrepancies were due to transcription errors and did not appear to have impact on the overall efficacy results. Two unreported adverse events, fall and resulting right femur fracture, were identified for one subject at Site #23 only. These adverse events should be included in the overall assessment of efficacy and safety.

In the NDA submission, the Applicant had included a Serious Breach document describing good clinical practice (GCP) protocol deviations. The most significant deviation was that for 4 of 10 subjects randomized at Site #23, the back-up physiotherapists who performed the functional assessments (i.e., 4SC, NSAA, TTR, 6MWT) at some study visits were also the study coordinators. Per protocol, physiotherapists were to be blinded and not have access to subject data, specifically laboratory data, which could unblind the physiotherapist. During the clinical investigator and CRO inspections, there was no evidence that other subjects at other sites had

similar protocol deviations. None of the functional assessments performed by the back-up physiologists involved the time points of interest for the efficacy analyses in the target population.

23. Labeling: Key Changes and Considerations

This Prescribing Information (PI) review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the Applicant's draft PI (Table 118). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

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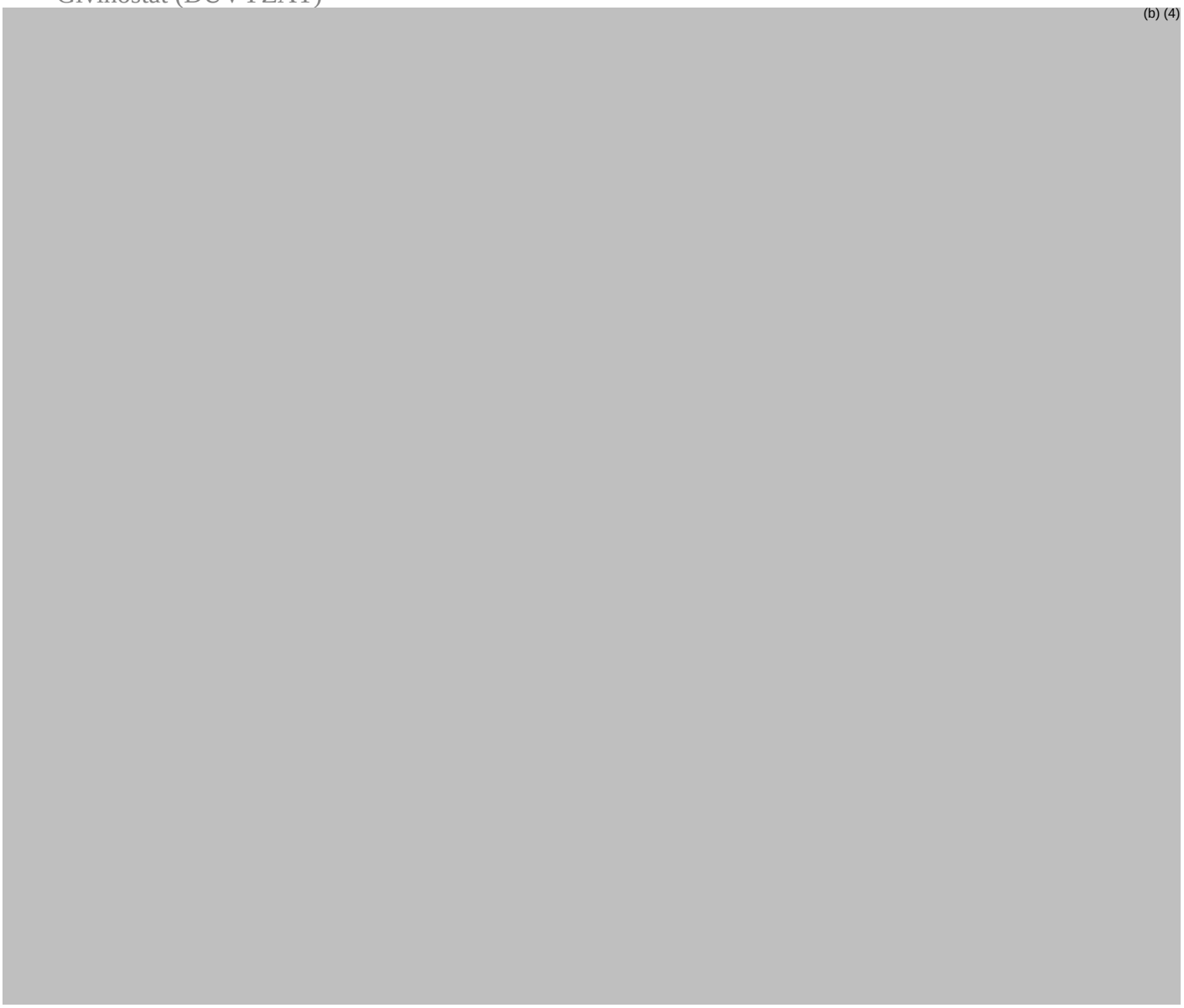

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved:

- Prescribing Information
- Medication Guide
- Instructions for Use
- Carton
- Container

24. Postmarketing Requirements and Commitments

The following postmarketing requirements (PMRs) and postmarketing commitments will be imposed:

PMR 4575-1

Conduct a carcinogenicity study of givinostat in mouse.

- Final Protocol Submission: 12/2022 (submitted)
- Study Completion: 12/2024
- Final Report Submission: 12/2025

PMR 4575-2

Conduct a 2-year carcinogenicity study of givinostat in rat.

- Final Protocol Submission: 12/2022 (submitted)
- Study Completion: 12/2024
- Final Report Submission: 12/2025

PMR 4575-3

Conduct a prospective observational registry for a minimum of 5 years to characterize the incidence, frequency, and severity of thrombocytopenia and incidence, frequency, and severity of serious events of bleeding in patients with Duchenne muscular dystrophy exposed to givinostat. For each case of thrombocytopenia and severe bleeding identified, provide detailed case narratives that include, but are not limited to, information on concomitant medications, complete blood count results at baseline, and during and after the event, and disposition (e.g., was the givinostat dose reduced in response to the event, discontinued temporarily or permanently, and outcomes if the dose was reduced).

- Draft Protocol Submission: 09/2024
- Final Protocol Submission: 07/2025
- Study Completion: 09/2031
- Final Report Submission: 03/2032

PMR 4575-4

Conduct a clinical trial to evaluate the effect of hepatic impairment on the exposure of givinostat relative to that in subjects with normal hepatic function after oral administration. Please refer to the Guidance for Industry Pharmacokinetics in Patients with Impaired Hepatic Function: Study

Design, Data Analysis, and Impact on Dosing and Labeling (https://www.fda.gov/media/71311/download).

- Draft Protocol Submission: 07/2024
- Final Protocol Submission: 12/2024
- Trial Completion: 06/2025
- Final Report Submission: 12/2025

PMC 4575-5

Perform experimental activities to evaluate if the drug product matrix can be suppressed (e.g., drug product dilutions or interfering matrix precipitation/liquid extraction with recovery evaluation).

- Final Report Submission: 07/2024

PMC 4575-6

If matrix interference can be suppressed: Test the three drug product submission batches at their 36 months stability endpoint at 25°C/60%RH.

In addition, to assess any trending, test the first three postapproval batches at multiple time points - from release through the end of proposed shelflife. [Results from the postapproval batches will be submitted in the NDA annual reports.]

- Final Protocol Submission: 07/2024
- Final Report Submission: 12/2024

PMR 4575-7

If matrix interference cannot be suppressed: Update regarding the ongoing leachable study with simulant solvent and provide an overall risk assessment to evaluate if there is any significant risk associated to presence of leachables in the drug product.

25. Financial Disclosure

Table 119. Covered Clinical Studies: [Study 48]

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 497		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): None		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Enter text here. Significant payments of other sorts: Enter text here. Proprietary interest in the product tested held by investigator: Enter text here. Significant equity interest held by investigator: Enter text here. Sponsor of covered study: Enter text here.		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): Enter text here.		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

Abbreviation: CFR, Code of Federal Regulations; FDA, Food and Drug Administration

26. References

26.1. Literature

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26.2. Guidances

Draft Guidance for Industry *Pharmacokinetics in Patients with Impaired Renal Function – Study Design, Data Analysis, and Impact on Dosing* (September 2020)

26.3. Other

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 (Italfarmaco 2019)

27. Review Team

Table 120. Reviewers of Integrated Assessment

Role	Names
Regulatory project manager	Annie Nguyen/IA; Heather Bullock/IA (CPMS)
Nonclinical reviewer	David Carbone/IA; J. Edward Fisher/IA
Nonclinical team leader	Lois Freed/IA
OCP reviewers	Min Li/IA, Michael Bewernitz/IA, Manuela Grimstein/IA
OCP team leaders	Bilal AbuAsal/IA, Atul Bhattaram/IA, Yuching Yang/IA
Clinical reviewer	Peggy Lazerow/IA
Clinical team leader	Emily Freilich/IA
Biometrics reviewer	Tristan Massie/IA
Biometrics team leader	John Lawrence/IA
Cross-disciplinary team leader	Emily Freilich/IA
Division director (pharm/tox)	Lois Freed/IA
Division director (OCP)	Mehul Mehta/IA
Division director (OB)	Jim Hung/IA
Division director (clinical)	Emily Freilich/IA
Office director (or designated signatory authority)	Teresa Buracchio/IA

Abbreviations: CPMS, Chief Project Management Staff; IA, Interdisciplinary Assessment; OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology

Table 121. Additional Reviewers of Application

Office or Discipline	Names
OPQ	Mariappan Chelliah (DP)/Martha Heimann (TL)/IA; Zhixing Shan (DS)/Donna Christner Miral Patel (CMC Facilities)/ Shu-Wei Yang (TL); Hansong Chen (Biopharm)/ Ta-Chen Wu (TL)
Microbiology	Helen Ngai (CMC Micro)/Elizabeth Bearr (TL)
OPDP	Lindsay McCann/ Aline Moukhtara (TL)
OSI	Cara Alfaro/ Phillip Kronstein (TL)
OSE/DEPI	Danielle Abraham/ Catherine Callahan (Acting TL)/ Kira Leishear (Associate Director RWE&Oncology)
OSE/DMEPA	Rina Patel/Stephanie DeGraw (TL)
OSE/DRISK	Donnella Fitzgerald/Jacqueline Sheppard (TL)
Other	CSS: Neil Varshneya/ Chad Ressig (TL) Clinical Data Analyst: William Quarles/DeAngelo McKinley (TL) Medical Editors: Noah Benjamin Whiteman; Katharine Bradley, Shannon Hayes, Kristen Lee Decision Support Team: Leila Lackey; Blair Coleman CDRH: Dan Kraniak DDS: Sally Jo Yasuda ADL: Tracy Peters DMPP: Lonice Carter/Marcia Williams (TL)

Abbreviations: ADL, Associate Director of Labeling; CDRH, Center for Devices and Radiological Health; CMC, Chemistry Manufacturing and Controls; CSS, Controlled Substance Staff; DDS, Deputy Director of Safety; DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DMPP, Division of Medical Policy Programs; DP, Drug Product; DRISK, Division of Risk Management; DS, Drug Substance; IA, Interdisciplinary Assessment; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations; RWE, Real World Evidence; TL, Team Leader

27.1. Reviewer Signatures

Table 27-122 Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Secondary Reviewer	Emily Freilich ON DNI	Sections: 1-7	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Emily Freilich			**Digitally signed by Emily Freilich** **Date: 3/21/2024 4:58 PM EDT** **GUID: 2024321205835**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline Primary Reviewer	Anhtu Nguyen ORO DRON	Sections: 12	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Anhtu Nguyen			**Digitally signed by Anhtu Nguyen** **Date: 3/21/2024 5:00 PM EDT** **GUID: 202432121017**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline Secondary Reviewer	Heather Bullock ORO DRON	Sections: Regulatory Section	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Heather Bullock				**Digitally signed by Heather Bullock** **Date: 3/21/2024 5:00 PM EDT** **GUID: 202432121025**

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Primary Reviewer	Peggy Lazerow ON DNI	Sections: Sections 1-7	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Peggy Lazerow				**Digitally signed by Peggy Lazerow** **Date: 3/21/2024 5:03 PM EDT** **GUID: 202432121329**

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Secondary Reviewer	Martha Heimann OPQAII DPQAVIII	Sections: 9, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Martha Heimann			**Digitally signed by Martha Heimann** **Date: 3/21/2024 5:05 PM EDT** **GUID: 202432121556**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Secondary Reviewer	Lois Freed ON DPTN	Sections: 5, 7.1, and 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Lois Freed			**Digitally signed by Lois Freed** **Date: 3/21/2024 5:05 PM EDT** **GUID: 202432121559**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Bilal Abuasal OCP DNP	Sections: 6.1, 6.3.2.2	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Bilal Abuasal			**Digitally signed by Bilal Abuasal** **Date: 3/21/2024 5:06 PM EDT** **GUID: 202432121619**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Primary Reviewer	Martha Heimann OPQAII DPQAVIII	Sections: 9, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Martha Heimann			**Digitally signed by Martha Heimann** **Date: 3/21/2024 5:06 PM EDT** **GUID: 202432121632**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Primary Reviewer	Anhtu Nguyen ORO DRON	Sections: 5.1, 8.4 and 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Anhtu Nguyen				**Digitally signed by Anhtu Nguyen Sign on behalf of David Carbone Date: 3/21/2024 5:10 PM EDT GUID: 2024321211049**

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Tertiary Reviewer	Mehul Mehta OCP DNP	Sections: 6.1, 6.3.2.2	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Mehul Mehta				**Digitally signed by Mehul Mehta Date: 3/21/2024 5:10 PM EDT GUID: 2024321211059**

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Secondary Reviewer	John Lawrence OB DBI	Sections: 6	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☒ Deficiencies preclude approval. ☐ Not applicable.	Insufficient evidence of efficacy.

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: John Lawrence			**Digitally signed by John Lawrence** **Date: 3/21/2024 5:11 PM EDT** **GUID: 2024321211151**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director of Labeling Discipline Secondary Reviewer	Anhtu Nguyen ORO DRON	Sections: 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Anhtu Nguyen			**Digitally signed by Anhtu Nguyen** **Sign on behalf of Tracy Peters** **Date: 3/21/2024 5:12 PM EDT** **GUID: 2024321211219**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Primary Reviewer	Tristan Massie OB DBI	Sections: 3	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Tristan Massie			**Digitally signed by Tristan Massie** **Date: 3/21/2024 5:14 PM EDT** **GUID: 2024321211411**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Reviewer	Manuela Grimstein OCP DPM	Sections: 14.6	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Manuela Grimstein			**Digitally signed by Manuela Grimstei** **Date: 3/21/2024 5:16 PM EDT** **GUID: 2024321211645**	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Tertiary Reviewer	Hsien Ming Hung OB DBI	Sections: 6.2	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Signature: Hsien Ming Hung				**Digitally signed by Hsien Ming Hung** **Date: 3/21/2024 5:22 PM EDT** **GUID: 2024321212248**

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Bilal Abuasal OCP DNP	Sections: 6.1, 6.3.2.2	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Bilal Abuasal				**Digitally signed by Bilal Abuasal** **Sign on behalf of Venkatesh Bhattara** **Date: 3/21/2024 5:23 PM EDT** **GUID: 2024321212314**

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

EMILY R FREILICH
03/21/2024 05:39:48 PM

TERESA J BURACCHIO
03/21/2024 05:50:13 PM