Safety and efficacy of givinostat in boys with Duchenne muscular dystrophy (EPIDYS) a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial

Mercuri, E.; Vilchez, J.J.; Boespflug-Tanguy, O.; Zaidman, C.M.; Mah, J.K.; Goemans, N.; ... ; EPIDYS Study Grp

Safety and efficacy of givinostat in boys with Duchenne muscular dystrophy (EPIDYS): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial

*Eugenio Mercuri, Juan J Vilchez, Odile Boespflug-Tanguy, Craig M Zaidman, Jean K Mah, Nathalie Goemans, Wolfgang Müller-Felber, Erik H Niks, Ulrike Schara-Schmidt, Enrico Bertini, Giacomo P Comi, Katherine D Mathews, Laurent Servais, Krista Vandenborne, Jessika Johannsen, Sonia Messina, Stefan Spinty, Laura McAdam, Kathryn Selby, Barry Byrne, Chamindra G Laverty, Kevin Carroll, Giulia Zardi, Sara Cazzaniga, Nicoletta Coceani, Paolo Bettica, Craig M McDonald, and the EPIDYS Study Group**

Summary

Background Duchenne muscular dystrophy, the most common childhood muscular dystrophy, is caused by dystrophin deficiency. Preclinical and phase 2 study data have suggested that givinostat, a histone deacetylase inhibitor, might help to counteract the effects of this deficiency. We aimed to evaluate the safety and efficacy of givinostat in the treatment of Duchenne muscular dystrophy.

Methods This multicentre, randomised, double-blind, placebo-controlled, phase 3 trial was done at 41 tertiary care sites in 11 countries. Eligible participants were ambulant, male, and aged at least 6 years, had a genetically confirmed diagnosis of Duchenne muscular dystrophy, completed two four-stair climb assessments with a mean of 8 s or less (≤1 s variance), had a time-to-rise of at least 3 s but less than 10 s, and had received systemic corticosteroids for at least 6 months. Participating boys were randomly assigned (2:1, allocated according to a list generated by the interactive response technology provider) to receive either oral givinostat or matching placebo twice a day for 72 weeks, stratified by concomitant steroid use. Boys, investigators, and site and sponsor staff were masked to treatment assignment. The dose was flexible, based on weight, and was reduced if not tolerated. Boys were divided into two groups on the basis of their baseline vastus lateralis fat fraction (VLFF; measured by magnetic resonance spectroscopy): group A comprised boys with a VLFF of more than 5% but no more than 30%, whereas group B comprised boys with a VLFF of 5% or less, or more than 30%. The primary endpoint compared the effects of givinostat and placebo on the change in results of the four-stair climb assessment between baseline and 72 weeks, in the intention-to-treat, group A population. Safety was assessed in all randomly assigned boys who received at least one dose of study drug. When the first 50 boys in group A completed 12 months of treatment, an interim futility assessment was conducted, after which the sample size was adapted using masked data from the four-stair climb assessments. Furthermore, the starting dose of givinostat was reduced following a protocol amendment. This trial is registered with ClinicalTrials.gov, NCT02851797, and is complete.

Findings Between June 6, 2017, and Feb 22, 2022, 359 boys were assessed for eligibility. Of these, 179 were enrolled into the study (median age 9·8 years [IQR 8·1–11·0]), all of whom were randomly assigned (118 to receive givinostat and 61 to receive placebo); 170 (95%) boys completed the study. Of the 179 boys enrolled, 120 (67%) were in group A (81 givinostat and 39 placebo); of these, 114 (95%) completed the study. For participants in group A, comparing the results of the four-stair climb assessment at 72 weeks and baseline, the geometric least squares mean ratio was 1·27 (95% CI 1·17–1·37) for boys receiving givinostat and 1·48 (1·32–1·66) for those receiving placebo (ratio 0·86, 95% CI 0·745–0·989; p=0·035). The most common adverse events in the givinostat group were diarrhoea (43 [36%] of 118 boys *vs* 11 [18%] of 61 receiving placebo) and vomiting (34 [29%] *vs* 8 [13%]); no treatment-related deaths occurred.

Interpretation Among ambulant boys with Duchenne muscular dystrophy, results of the four-stair climb assessment worsened in both groups over the study period; however, the decline was significantly smaller with givinostat than with placebo. The dose of givinostat was reduced after an interim safety analysis, but no new safety signals were reported. An ongoing extension study is evaluating the long-term safety and efficacy of givinostat in patients with Duchenne muscular dystrophy.

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*Members listed in the appendix (pp 3–5)

Pediatric Neurology, Department of Woman and Child Health and Public Health, Child Health Area, Universita Cattolica del Sacro Cuore, Rome, Italy (Prof E Mercuri MD); **Centro Clinico Nemo Fondazione Policlinico Gemelli IRCCS, Rome, Italy** (Prof E Mercuri); **Servicio de Neurología, Neuromuscular Unit, CIBERER, EURO-RN-NMD, Hospital Universitario y Politécnico La Fe Valencia, Valencia, Spain** (Prof J J Vilchez MD); **I-Motion, Institut de Myologie, Hôpital Armand-Trousseau, APHP, Sorbonne Université, Paris, France** (Prof O Boespflug-Tanguy MD); **Université Paris Cité UMR INSERM 1141, Hôpital Robert Debré, Paris, France** (Prof O Boespflug-Tanguy); **Washington University School of Medicine, St Louis, MO, USA** (C M Zaidman MD); **Division of Pediatric Neurology, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada** (Prof J K Mah MD); **Department of Child Neurology, University Hospitals Leuven, Leuven, Belgium** (N Goemans MD); **LMU Munich, University Hospital, Hauner Children's Hospital, Pediatric Neurology and Developmental Medicine, Munich, Germany** (Prof W Müller-Felber MD); **Department of Neurology, Leiden University Medical Center, Leiden, Netherlands**

Introduction

Duchenne muscular dystrophy is the most common childhood muscular dystrophy.[1] An X-linked disorder caused by mutations of the dystrophin gene, this condition results in an absence of functional dystrophin, part of the dystrophin–glycoprotein complex that helps to preserve cell membrane integrity after mechanical stress following muscle contraction.[1,2] This deficiency damages muscles

(E H Niks MD); **Duchenne Center Netherlands, Netherlands** (E H Niks); **Department of Pediatric Neurology, Children's University Hospital Essen, University of Duisburg-Essen, Essen, Germany** (Prof U Schara-Schmidt MD); **Research Unit of Neuromuscular and Neurodegenerative Disorders, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy** (E Bertini MD); **Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Milan, Italy** (Prof G P Comi MD); **Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy** (Prof G P Comi); **Department of Pediatrics** (Prof K D Mathews MD) **and Department of Neurology** (Prof K D Mathews), **University of Iowa Carver College of Medicine, Iowa City, IA, USA; MDUK Oxford Neuromuscular Centre** (Prof L Servais MD) **and NIHR Oxford Biomedical Research Centre** (Prof L Servais), **University of Oxford, Oxford, UK; Neuromuscular Reference Center, Department of Paediatrics, University and University Hospital of Liege, Belgium** (Prof L Servais); **ImagingDMD** (K Vandenborne PhD), **Department of Physical Therapy** (K Vandenborne), **and Child Health Research Institute, Department of Pediatrics** (B Byrne MD), **University of Florida, Gainesville, FL, USA; University Medical Center Hamburg-Eppendorf, Department of Pediatrics, Hamburg, Germany** (J Johannsen MD); **Department of Clinical and Experimental Medicine, Unit of Neurodegenerative Diseases, AOU Policlinico G Martino, University of Mesina, Messina, Italy** (Prof S Messina MD); **Department of Paediatric Neurology, Alder Hey Children's Hospital NHS Trust, Liverpool, UK** (S Spinty MD); **Holland Bloorview Kids Rehabilitation Hospital, Bloorview Research Institute, Department of Pediatrics, University of Toronto, Toronto, ON, Canada** (L McAdam MD); **The University**

Research in context

Evidence before this study

We searched PubMed for articles, published in any language, from database inception until Feb 13, 2024, using the search term "Duchenne muscular dystrophy" AND therapy, limiting the results to articles reporting on clinical trials. Of the 314 records retrieved, 132 reported on clinical trials that evaluated the efficacy of interventions in Duchenne muscular dystrophy. 31 manuscripts assessed the efficacy of either prednisone or deflazacort, with a wide range of doses (one study used a complex tapering regimen for the prednisone dose), regimens (from daily to weekend-only), and durations of administration. In head-to-head studies, both corticosteroids were similarly effective in slowing the progression of Duchenne muscular dystrophy, but some studies suggested that deflazacort had a better safety profile. Most other previous studies did not show any treatment benefit (eg, from allopurinol or calcium antagonists) or focused on specific aspects of Duchenne muscular dystrophy. For example, the use of angiotensin Converting enzyme inhibitors, β blockers, a potassium-sparing diuretic, or an aldosterone inhibitor had beneficial effects on cardiac function; the antioxidant idebenone slowed the loss of pulmonary function; and phosphodiesterase 5 inhibition with tadalafil or sildenafil normalised skeletal muscle blood flow, although did not lessen the decline in ambulatory ability. However, all of these treatments subsequently failed in phase 3 trials as an addition to corticosteroid treatment. The first gene-targeted therapy to show an effect on the progression of Duchenne muscular dystrophy was eteplirsen; however, this drug—as well as ataluren, drisapersen, golodirsen, and viltolarsen—is effective only in patients with specific subtypes of the disease. Newer treatments that have been investigated for use in people with Duchenne muscular dystrophy include the dissociative steroidal anti-inflammatory drug vamorolone, which was associated with the maintenance of muscle strength and function in a 30-month study, with efficacy similar to that of corticosteroids. Furthermore, the NF-κB inhibitor edasalonexent showed some evidence of a delay in disease progression, although a phase 3 trial of the drug did not meet the primary endpoint. Finally, in a phase 2 study, the micro-dystrophin gene-transfer therapy delandistrogene moxeparvovec showed stabilisation in disease progression as measured by the North Star Ambulatory Assessment, although did not in a subsequent Phase 3 study.

Added value of this study

In a broad population of ambulant boys with Duchenne muscular dystrophy, unselected for specific disease subtypes and who were receiving a stable dose and regimen of systemic corticosteroids, givinostat delayed disease progression compared with placebo and had a predictable safety profile, with a high proportion of participants completing the study. To our knowledge, the 72-week, double-blind follow-up period makes our study unique in Duchenne muscular dystrophy to date.

Implications of all the available evidence

Historically, the treatment of Duchenne muscular dystrophy has involved either non-targeted disease modification with systemic corticosteroids, resulting in a range of unacceptable side-effects, or therapy aimed at relieving individual symptoms. Subsequently, targeted therapies that can affect disease progression have become available; however, unlike givinostat, many are suitable only for specific subtypes of the disease. The available evidence suggests that the care of boys with Duchenne muscular dystrophy is improving. Future studies should evaluate the long-term implications of these therapies, especially when used in clinical practice.

and impairs repair, with muscle fibre being replaced by fatty tissue, fibrosis, muscle wasting, loss of ambulation, and death.[3,4] The dystrophin–glycoprotein complex also regulates histone deacetylase activity, with increased activity in Duchenne muscular dystrophy reducing the expression of genes involved in muscle regeneration.[5]

Systemic corticosteroids are the mainstay of treatment for Duchenne muscular dystrophy[3] and delay loss of muscle strength and function;[6–8] however, they have undesirable effects that often result in dose reduction.[6,7,9] Despite studies evaluating a range of doses, regimens, and durations of therapy,[10–12] the optimal dose and regimen of corticosteroids are unclear, although daily prednisone or deflazacort appear preferable to intermittent prednisone.[13] Subsequently, several gene-targeted therapies—including eteplirsen,[14] ataluren,[15] drisapersen,[16] golodirsen,[17] and viltolarsen[18]—have been approved in some countries for use in specific subgroups of people with Duchenne muscular dystrophy, and the micro-dystrophin gene-transfer therapy delandistrogene moxeparvovec is approved in the USA for ambulatory patients aged 4–5 years.[19]

Givinostat is a pan-histone deacetylase inhibitor that could help to counteract the pathogenic events downstream of dystrophin deficiency. In a mouse model of Duchenne muscular dystrophy, givinostat increased cross-sectional myofibre area and decreased inflammatory infiltrate and fibrotic scars,[20] and in a phase 2 trial, the drug increased the fraction of muscle tissue and reduced the fraction of fibrosis, necrosis, and fatty replacement in brachial biceps biopsy samples.[21] We therefore did the Epigenetic Rescue of Dystrophin Dysfunction (EPIDYS) trial to evaluate the safety and efficacy of givinostat in ambulant boys with Duchenne muscular dystrophy.

Methods

Study design

This multicentre, randomised, double-blind, placebo-controlled, phase 3 trial was done at 41 tertiary care sites in 11 countries (Belgium, Canada, France, Germany,

Israel, Italy, the Netherlands, Serbia, Spain, the UK, and the USA; for a full list of sites, see appendix pp 3–5). The study was approved by independent ethics committees at each institution, and was done in accordance with the Declaration of Helsinki and Good Clinical Practice.

Participants

Participants were recruited by the site staff from among the patients at that site. Eligible participants were ambulant male patients aged at least 6 years with genetically confirmed Duchenne muscular dystrophy, who completed two four-stair climb assessments with a mean of 8 s or less (≤1 s variance), had a time-to-rise of at least 3 s but less than 10 s, and had received stable systemic corticosteroids for at least 6 months (with a reasonable expectation that corticosteroid dose and regimen would not change over the duration of the study). Recruitment was prespecified in two groups: group A consisting of participants with baseline vastus lateralis fat fraction (VLFF) of more than 5% but no more than 30%, and group B consisting of those with a VLFF of 5% or less, or more than 30%. Group A was intended to comprise patients who were not at risk of sudden, complete loss of ambulation but who, if receiving placebo, would show sufficient decline over the study duration in the function, strength, and fat fraction endpoints being tested. These criteria were based on expert opinion (KV), were subsequently published,[22–24] and are consistent with the recommendations of a consensus workshop on Duchenne muscular dystrophy outcome measures.[25] Group B was recruited so that the safety of givinostat could be evaluated in a broader population of patients with Duchenne muscular dystrophy. The main exclusion criteria were any surgery or medication change in the previous 3 months that could affect muscle strength or function, or a loss of ankle plantar flexion of 30° or more. Full inclusion and exclusion criteria are listed in the appendix (pp 6–8). All boys and their parent or legal guardian provided written informed consent before screening assessments.

Randomisation and masking

Eligible participants were randomly assigned 2:1 to receive oral givinostat or matching placebo, with the allocation according to a randomisation list generated by the statistician of the interactive response technology provider (accessed by site staff telephoning the provider's system to receive a packaging number), stratified by concomitant corticosteroid use in four strata according to corticosteroid type (deflazacort or other corticosteroids) and regimen (daily or intermittent). The 2:1 randomisation ratio was chosen because of the rapidly progressive nature of Duchenne muscular dystrophy as well as the rarity of the disease, as this ratio maximised the number of boys exposed to active treatment while maintaining study integrity. Boys, investigators, and site and sponsor staff were masked to treatment assignment, with the placebo suspension indistinguishable in appearance and taste to givinostat. Because reductions in platelet count are observed after the administration of givinostat, the personnel who did the efficacy analyses were different from those who recorded the safety results; all were masked to treatment assignment, although the success of masking was not assessed.

Procedures

The initial doses of givinostat were based on a dose-ranging evaluation in a mouse model,[20] in addition to the results of the previous phase 2 trial in boys with Duchenne muscular dystrophy[21] and several studies of other disorders. A flexible treatment regimen was used, in which a high starting dose of givinostat was given to maximise efficacy but dose reductions were prespecified if adverse events occurred, such as platelet count reduction or diarrhoea (appendix pp 8–10). The weight-based starting dose was initially 20–70 mg oral givinostat twice a day, with a reduced dose of 13–47 mg twice a day (regimen 1; appendix p 9).

Eight amendments were made to the protocol after recruitment commenced, only three of which affected study conduct. The first amendment, applying to the USA and Canada, permitted the use of deflazacort; this was an investigational drug in these countries when the study started, although was subsequently approved. The second amendment, applying to all countries in the study and following the masked safety review in June, 2018, reduced the starting dose to regimen 2 for participants who were recruited after the amendment (regimen 2: starting dose 13–47 mg twice a day, with a reduced dose of 11–37 mg twice a day; in light of laboratory findings; appendix pp 8–9) and broadened the quadriceps manual muscle test inclusion criterion. The third amendment, applying only to France, moved all participants to regimen 2. The statistical analysis plan was amended five times, with all amendments completed before unmasking. Most amendments were minor and were made for consistency with the associated protocol; the only major amendments were to add baseline covariates for efficacy analyses (based on emergent literature showing that baseline values of function tests predict subsequent changes) and to include analyses of velocity for timing endpoints.

Participants attended study site visits every 12 weeks for 72 weeks. At baseline and at every visit, participants completed a four-stair climb,[26,27] the North Star Ambulatory Assessment (NSAA,[28–30] including time-to-rise from the floor), a 6-min walk test,[26,31] and muscle strength assessment (knee extension and elbow flexion, by standardised hand-held myometry).[26] All functional and strength assessments were evaluated by trained physiotherapists masked to treatment and other assessments, with all baseline and 72-week visits recorded and reviewed by an expert, independent team of physiotherapists, not otherwise involved in the study, for quality assurance. Magnetic resonance spectroscopy (MRS) of the right

of British Columbia, Children's and Women's Health Centre, Vancouver, BC, Canada (K Selby MD); **Department of Neuroscience, University of California, San Diego, San Diego, CA, USA** (Prof C G Laverty MD); **KJC Statistics, Cheshire, UK** (K Carroll PhD); **Alira Health, Milan, Italy** (G Zardi MSc); **Italfarmaco, Milan, Italy** (S Cazzaniga MSc, N Coceani, P Bettica MD); **University of California Davis Health, Sacramento, CA, USA** (Prof C M McDonald MD)

Correspondence to:
Prof Eugenio Mercuri, Pediatric Neurology, Department of Woman and Child Health and Public Health, Child Health Area, Università Cattolica del Sacro Cuore, Rome 00168, Italy
eugeniomaria.mercuri@policlinicogemelli.it

See **Online** for appendix

upper leg was done at screening and after 48 and 72 weeks (appendix p 10), with the images centrally read by an independent team of MRS experts, not otherwise involved in the study, for the calculation of VLFF.[32] Permitted visit windows were prespecified in the protocol as no more than 7 days before or after visits. Drug compliance was assessed using diary data, and was calculated as the total number of expected doses (duration of exposure in days×2) minus the total number of missed doses, divided by the total number of expected doses×100 to give the total percentage compliance.

Outcomes

The primary endpoint compared the effect of givinostat and placebo on disease progression, as measured by the change in the results of the four-stair climb assessment between baseline and 72 weeks, and was not assessed centrally. The key secondary endpoints were the change from baseline after 72 weeks in NSAA total score, NSAA cumulative loss-of-function (appendix pp 10–11), time-to-rise, 6-min walk test, knee extension, elbow flexion, and VLFF. Safety was assessed throughout the study in terms of adverse events, haematology and blood chemistry parameters, vital signs, electrocardiogram, and pulmonary function.

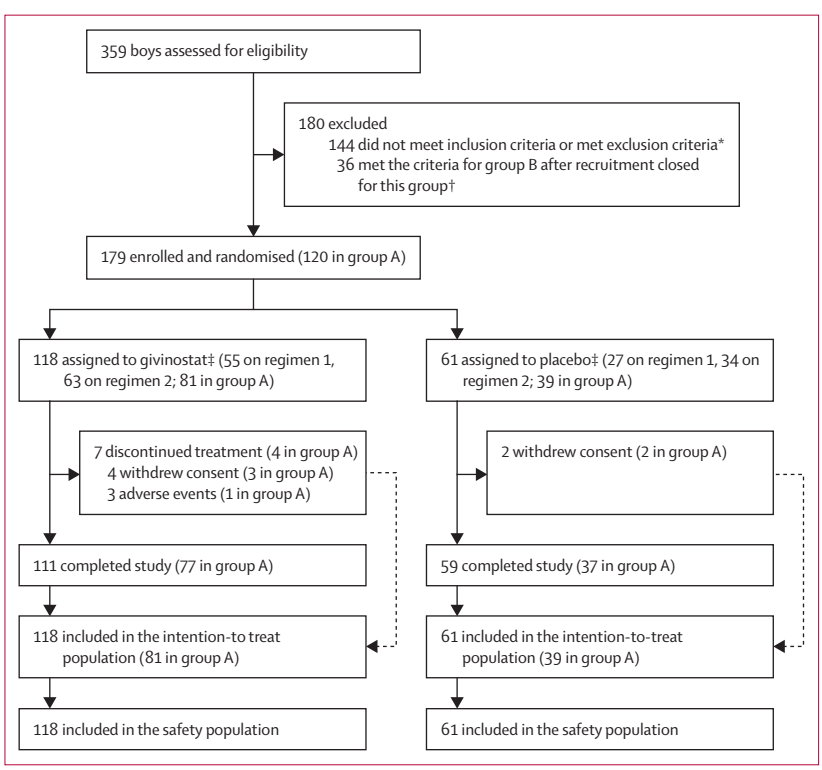

Figure 1: **Trial profile**
See appendix pp 8–9 for details on regimens 1 and 2. Of the 118 boys in the givinostat group, 77 were included in the MRS cohort; of the 61 in the placebo group, 37 were included in the MRS cohort. *Reasons for exclusion were as follows (boys can be counted under more than one criterion): could not give informed assent or consent (n=1); did not complete two four-stair climb screening assessments (n=8); mean of two four-stair climb screening assessments >8 s (n=3); time-to-rise from floor of <3 s or ≥10 s (n=25); manual muscle testing of quadriceps grade <3 (n=7); use of systemic corticosteroids did not meet criterion (n=79); not willing to use adequate contraception (n=1); use of other pharmacological treatment (n=1); loss of ≥30° of plantar flexion (n=20); diagnosis of other uncontrolled neurological diseases or somatic disorders (n=1); platelet, white blood cell, and haemoglobin counts below lower limits of normal (n=4); symptomatic cardiomyopathy or heart failure or left ventricular ejection fraction <50% (n=3); triglyceride concentration >3·42 mmol/L at fasting (n=2); positive hepatitis B antigen, hepatitis C antibody, or HIV test (n=2); risk factors for torsades de pointes (n=1); unable to understand and comply with the muscle function tests or study procedures (n=2); contraindications to magnetic resonance spectroscopy (n=2). †These boys were not included in any analyses. ‡All patients were also receiving systemic corticosteroids, in a dose and regimen that was to remain unchanged over the follow-up period.

Statistical analysis

An independent data monitoring committee (appendix p 5) reviewed safety findings every 3 months and oversaw an interim futility analysis in January, 2020, in which the sponsor and investigators were masked to the results. Data from this interim analysis then informed a masked sample size re-estimation when the first 50 participants in group A completed 12 months of study treatment. No other changes to the study conduct were made as a result of this analysis and there was no bias adjustment. On the basis of the SD of 3·094 s in this masked sample size reassessment, 102 participants would provide 90% power, with a one-sided alpha of 2·5%, to detect a difference of 2 s in the results of the four-stair climb between the givinostat and placebo groups at 72 weeks in group A (with the 2 s difference confirmed as clinically relevant based on analyses by Wong and colleagues,[33] which were subsequently substantiated by data from the Cooperative International Neuromuscular Research Group natural history database [data on file]). With an estimated drop-out of 8%, 110 participants would need to be assigned to group A. Up to 50 participants (35% of the overall population) were to be recruited into group B. The original sample size calculation is given in the appendix (p 11).

The primary endpoint was analysed using an ANCOVA. As prespecified in the statistical analysis plan, because blinded four-stair climb data were not normally distributed they were log-transformed before analysis. The dependent variable was log change in four-stair climb results from baseline to 72 weeks, with log four-stair climb, time-to-rise, time-to-run or walk 10 m, and 6-min walk test as baseline covariates, and randomised treatment, corticosteroid use, and age as independent class variables. Key secondary endpoints were analysed using a similar ANCOVA (without log transformation), except for cumulative loss-of-function, which was analysed using negative binomial regression (appendix pp 10–11). Because the key secondary endpoints were of equal clinical relevance, they were prospectively adjusted for multiplicity using the Hochberg procedure[34] rather than a hierarchy. In this procedure, p values are ordered from least to most significant: if the largest one-sided p-value is less than or equal to 0·025 then all endpoints are significant; otherwise, the endpoint with the largest p value is deemed non-significant and the next largest p value is considered in relation to 0·025/2=0·0125. If the nth ordered one-sided p value reaches ≤0·025/n, treatment

effects for that and subsequent endpoints are significant; otherwise, the nth p value is deemed non-significant. Because the Hochberg procedure does not account for correlation between endpoints, a post-hoc permutation test was done to assess the overall probability of efficacy while accounting for this correlation (appendix p 14).[35,36] The handling of missing data is described in the appendix (pp 11–12). All analyses were done in SAS 9.4. Safety data were prespecified to be analysed descriptively only.

The intention-to-treat population included all randomly assigned boys who received at least one dose of study drug, and had at least one non-missing post-baseline four-stair count measure or missing post-baseline four-stair count measure due to being either non-ambulatory or otherwise physically unable to take part in the assessment, with treatment group assignment according to initial randomisation. The intention-to-treat, group A population (ie, those with baseline vastus lateralis fat fraction >5% to 30%) was used for the prespecified efficacy analyses; supportive analyses were also done post-hoc in the overall intention-to-treat population. The safety population comprised all randomly assigned boys who received at least one dose of study drug, with treatment group assignment defined by the treatment actually received, and was used for safety evaluations. The MRS cohort comprised all randomly assigned participants in group A who completed at least one post-baseline assessment, and was used for the VLFF analyses.

This trial is registered at ClinicalTrials.gov, NCT02851797.

Role of the funding source

The funder of the study was responsible for the design of the study and for data analysis, oversaw the study conduct (including data collection), funded the data analyses (which were conducted by a contract research organisation), and was responsible for preparation of the report. Employees of the funder were involved, as authors, in data interpretation and in the preparation of this manuscript.

	Overall population		Group A	
	Givinostat* (n=118)	Placebo* (n=61)	Givinostat* (n=81)	Placebo* (n=39)
Age, years	9·8 (8·1–11·0)	9·9 (8·3–11·4)	9·8 (8·0–10·9)	9·6 (8·2–11·4)
Race†				
White	106 (90%)	57 (93%)	74 (91%)	36 (92%)
Asian	4 (3%)	2 (3%)	3 (4%)	1 (3%)
Black	3 (3%)	0	0	0
Other	5 (4%)	2 (3%)	4 (5%)	2 (5%)
Country				
Belgium	6 (5%)	3 (5%)	4 (5%)	2 (5%)
Canada	9 (8%)	6 (10%)	7 (9%)	5 (13%)
France	9 (8%)	5 (8%)	5 (6%)	2 (5%)
Germany	13 (11%)	2 (3%)	9 (11%)	1 (3%)
Israel	1 (1%)	0	1 (1%)	0
Italy	24 (20%)	13 (21%)	17 (21%)	9 (23%)
Netherlands	5 (4%)	3 (5%)	2 (2%)	2 (5%)
Serbia	0	1 (2%)	0	1 (3%)
Spain	17 (14%)	6 (10%)	13 (16%)	4 (10%)
UK	10 (8%)	3 (5%)	6 (7%)	2 (5%)
USA	24 (20%)	19 (31%)	17 (21%)	11 (28%)
BMI, kg/m²	19·7 (4·10); 12·4–30·6	19·9 (4·40); 13·3–31·4	19·5 (3·83); 12·4–30·6	20·1 (4·52); 13·3–31·3
Time since diagnosis, years	5·4 (3·3–7·4)	5·3 (3·9–7·1)	5·5 (3·9–7·5)	5·3 (3·7–8·3)
Dystrophin mutation				
Deletion	83 (70%)	40 (66%)	58 (72%)	28 (72%)
Duplication	17 (14%)	14 (23%)	13 (16%)	9 (23%)
Point mutation	18 (15%)	7 (11%)	10 (12%)	2 (5%)
Corticosteroid regimen				
Deflazacort, daily regimen	84 (71%)	39 (64%)	60 (74%)	25 (64%)
Deflazacort, intermittent regimen	7 (6%)	6 (10%)	6 (7%)	4 (10%)
Other corticosteroid, daily regimen	15 (13%)	9 (15%)	9 (11%)	6 (15%)
Other corticosteroid, intermittent regimen	12 (10%)	7 (11%)	6 (7%)	4 (10%)

Data are median (IQR), n (%), or mean (SD); range. The group A population was used for the prespecified efficacy analyses; supportive efficacy analyses were conducted post-hoc in the overall population, which was also used for the safety evaluations. *All patients were also receiving systemic corticosteroids, in a dose and regimen that was to remain unchanged over the follow-up period. †Race was self-reported.

Table 1: **Baseline demographics and disease characteristics of the intention-to-treat population**

Results

Between June 6, 2017, and Feb 22, 2022, we assessed 359 male patients for eligibility, 179 of whom were enrolled into the study. All 179 participants were randomly assigned to receive either givinostat (n=118) or placebo (n=61), and 170 (95%) completed the study. On the basis of VLFF, 120 (67%) of the 179 participants were in group A (114 [95%] completed the study) and 59 (33%) were in group B (56 [95%] completed the study; figure 1). Baseline characteristics were similar in the givinostat and placebo groups (median age 9·8 years [IQR 8·1–11·0]; table 1). The mean treatment duration was 493 days and was similar in all groups (givinostat, placebo, group A, and group B); mean compliance was greater than 98% in all groups (98·3% for givinostat and 98·1% for placebo overall; 98·7% for givinostat and 98·0% for placebo in group A). Study treatment was temporarily interrupted for 25 patients (19 receiving givinostat [15 owing to adverse events] and six receiving placebo [three owing to adverse events]; mean interruption duration was 34·4 days [28·7 days with givinostat, 52·7 days with placebo]), and dose was reduced for 57 (48%) of 118 boys in the givinostat group compared with seven (11%) of 61 in the placebo group. Dosing was changed to regimen 2 after 82 (46%) patients had been randomly assigned (appendix pp 8–10). One boy, who was receiving placebo, lost ambulation at week 60.

The geometric least squares mean ratios for the log-transformed four-stair climb results at 72 weeks versus

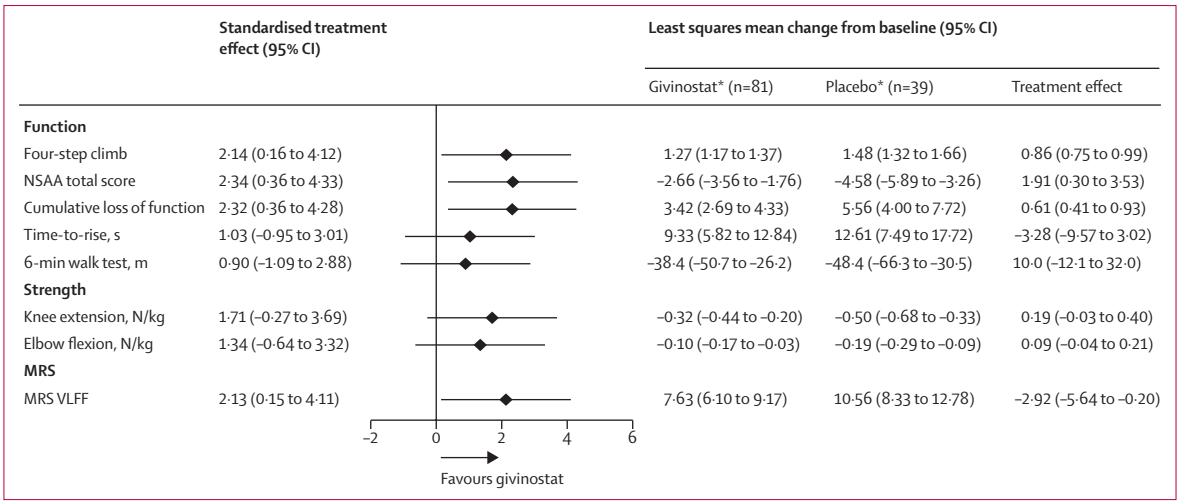

Figure 2: **Forest plot of primary and secondary endpoints at week 72**
Assessed in the group A part of the intention-to-treat population. For the MRS VLFF assessment, n=77 for givinostat and n=37 for placebo. The treatment effect estimates and CIs were standardised for presentation on the same scale, with each estimate, upper and lower CI divided by its related SE. The CIs have not been adjusted for multiplicity and should not be used for hypothesis testing. Four-stair climb results were analysed on the log scale and cumulative loss of function was analysed via negative binomial regression. Direction of interpretation has been fixed accordingly. MRS=magnetic resonance spectroscopy. NSAA=North Star Ambulatory Assessment. VLFF=vastus lateralis fat fraction. *All patients were also receiving systemic corticosteroids, in a dose and regimen that was to remain unchanged over the follow-up period.

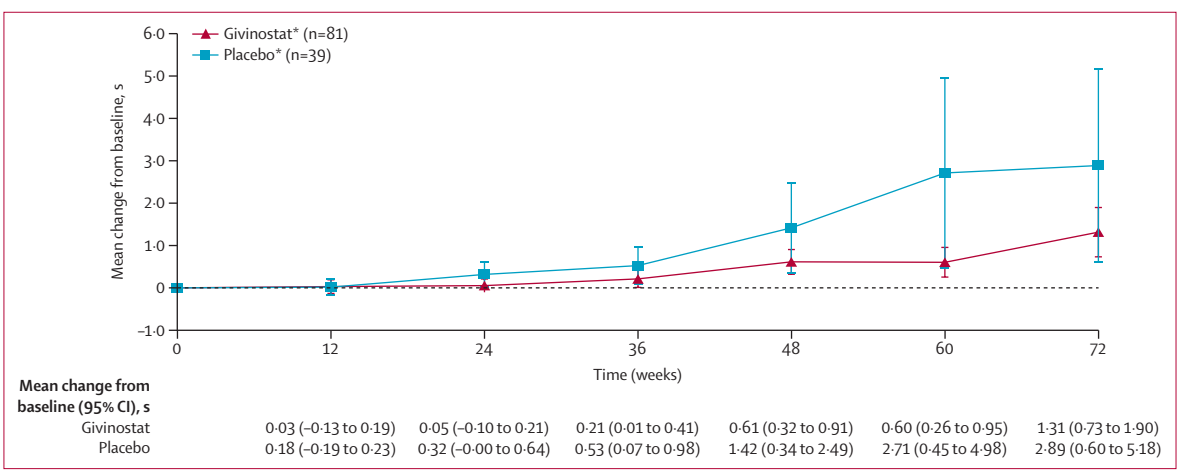

Figure 3: **Mean change in results of the four-stair climb assessment between baseline and 72 weeks**
Assessed in the group A part of the intention-to-treat population. Data are mean (95% CI). The CIs have not been adjusted for multiplicity and should not be used for hypothesis testing. Baseline mean values were 3·39 s for the givinostat group and 3·48 s for the placebo group. *All patients were also receiving systemic corticosteroids, in a dose and regimen that was to remain unchanged over the follow-up period.

baseline were 1·27 (95% CI 1·17 to 1·37) in the givinostat group and 1·48 (1·32 to 1·66) in the placebo group (ratio 0·86 [95% CI 0·75 to 0·99, p=0·035; figure 2). Using non-log-transformed data at 72 weeks, mean four-stair climb changes from baseline were 1·25 s (0·31 to 2·18) in the givinostat group compared with 3·03 s (1·67 to 4·39) in the placebo group—a 1·78 s smaller decline with givinostat (least squares mean difference –1·78 s [95% CI –3·46 to –0·11]; p=0·037). The greater worsening in four-stair climb results with placebo than with givinostat was apparent from week 48 (mean changes from baseline: givinostat, ranging between 0·60 s and 1·31 s; placebo, 1·42 s to 2·89 s; figure 3). When analysed as velocity, four-stair climb results at week 72 were 0·243 tasks per second in the givinostat group and 0·209 tasks per second in the placebo group, a least-squares-mean difference of 0·034 (0·004 to 0·065) tasks per second (p=0·029). A post-hoc ANCOVA analysis suggested that changing from regimen 1 to regimen 2 was unlikely to have affected the results (appendix p 20). Analyses of the primary endpoint by subgroup are in the appendix (p 15). No patients withdrew from the study because of COVID-19. Six patients (four in the givinostat group and two in the placebo group) missed assessments owing to COVID-19, and eight patients (four in each group) had delayed assessments for this reason. However, the study was not

	Givinostat group* (n=118)	Placebo group* (n=61)
Adverse events	112 (95%)	57 (93%)
Diarrhoea	43 (36%)	11 (18%)
Decreased platelet count or thrombocytopenia	38 (32%)	0
Vomiting	34 (29%)	8 (13%)
Nasopharyngitis	31 (26%)	19 (31%)
Headache	28 (24%)	14 (23%)
Increased blood triglyceride concentration or hypertriglyceridaemia	27 (23%)	4 (7%)
Abdominal pain	25 (21%)	9 (15%)
Upper abdominal pain	17 (14%)	7 (11%)
Fall	15 (13%)	13 (21%)
Pyrexia	15 (13%)	5 (8%)
Cough	13 (11%)	9 (15%)
Pain in extremity	8 (7%)	7 (11%)
Upper respiratory tract infection	7 (6%)	8 (13%)
Back pain	6 (5%)	8 (13%)
Rhinitis	6 (5%)	7 (11%)
Serious adverse events	8 (7%)	2 (3%)
Gastroenteritis	1 (1%)	2 (3%)
Fatal adverse events	0	0
Treatment-related adverse events	81 (69%)	17 (28%)
Decreased platelet count or thrombocytopenia	37 (31%)	0
Increased blood triglyceride concentration or hypertriglyceridaemia	26 (22%)	4 (7%)
Diarrhoea	25 (21%)	2 (3%)
Abdominal pain	16 (14%)	2 (3%)
Upper abdominal pain	9 (8%)	1 (2%)
Vomiting	7 (6%)	0
(Continued from previous column)		
Adverse events leading to treatment interruption	16 (14%)	4 (7%)
Increased blood triglyceride concentration or hypertriglyceridaemia	11 (9%)	3 (5%)
Adverse events leading to treatment discontinuation	4 (3%)	0
Increased blood triglyceride concentration or hypertriglyceridaemia	3 (3%)	0
Adverse events leading to study withdrawal	3 (3%)	0
Increased blood triglyceride concentration or hypertriglyceridaemia	2 (2%)	0
Adverse events leading to dose reduction	42 (36%)	1 (2%)
Thrombocytopenia	17 (14%)	0
Platelet count decreased	16 (14%)	0
Severe adverse events	5 (4%)	1 (2%)
Vomiting	2 (2%)	0

Data are number of boys (%). Events are listed by percentage of boys. Data are grouped by treatment received at baseline. We show MedDRA preferred terms that occurred in ≥10% boys in either group for adverse events or adverse events leading to dose reduction; ≥5% boys in either group for treatment-related adverse events; and ≥2 boys in either group for adverse events leading to treatment interruption or withdrawal, serious, and severe adverse events. The thresholds were selected for clarity of reporting and to highlight the most commonly reported MedDRA preferred terms only. The severity of the adverse events was assessed and graded by the investigators according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (June 14, 2010). MedDRA=Medical Dictionary for Regulatory Activities. *All patients were also receiving systemic corticosteroids, in a dose and regimen that was to remain unchanged over the follow-up period.

Table 2: **Adverse events in the safety population**

modified as a result of the COVID-19 pandemic, and a supportive analysis of four-stair climb data that excluded patients who completed the 72-week assessment outside of the specified window showed similar results to the main analysis (givinostat-to-placebo ratio 0·83 [0·70 to 0·99]).

The key secondary endpoints did not differ between groups after multiplicity adjustment using the Hochberg procedure (figure 2). However, there was evidence to suggest that the decrease in NSAA total score from baseline might be lower (ie, less decline) with givinostat than with placebo, both over 72 weeks (least squares mean difference 1·91 [95% CI 0·295 to 3·533]; figure 2) and at all timepoints (appendix p 16). Cumulative loss-of-function also seemed to be lower with givinostat than with placebo (2·14 fewer items failed over 72 weeks; 3·42 *vs* 5·56; ratio 0·61 [95% CI 0·41 to 0·93]), with fewer items failed at each timepoint (appendix p 16). Similarly, the change from baseline in time-to-rise was numerically lower over 72 weeks with givinostat, with a least squares mean difference between groups of –3·28 (–9·57 to 3·02), with a numerically lower mean change from baseline with givinostat at all timepoints (appendix p 17). When analysed as velocity (1/time-to-rise), the least mean squares difference between givinostat and placebo at 72 weeks was 0·03 s^{-1} (0·007 to 0·055). The 6-min walk test decline over 72 weeks seemed to be slower with givinostat than placebo, with a least squares mean difference in distance covered of 10·0 m (–12·1 to 32·0), with lower mean change from baseline with givinostat at all timepoints (appendix p 17). Furthermore, changes from baseline in elbow flexion and knee extension over 72 weeks were seemingly lower with givinostat than placebo, with least squares mean differences between givinostat and placebo of 0·09 N/kg (–0·04 to 0·21) for elbow flexion and 0·19 N/kg (–0·03 to 0·40) for knee extension. Finally, MRS evidence suggested that there was less fat infiltration in the vastus lateralis at 72 weeks with givinostat than with placebo (LSM difference in fat fraction –2·92% [–5·64 to –0·20]), and the change from baseline was numerically lower in the givinostat group at all timepoints (appendix p 18).

Because the Hochberg procedure does not account for the correlation between the endpoints tested, a post-hoc permutation test was done to assess the overall probability of efficacy while accounting for this correlation.[35,36] The

probability of observing this overall constellation of results under the null hypothesis of no true treatment effect was less than 0·01% (appendix p 14). The results of the supportive post-hoc analyses of the primary and key secondary endpoints in the overall intention-to-treat population were broadly consistent with the prespecified analyses (appendix p 18).

Similar proportions of boys had adverse events in both groups (112 [95%] of 118 receiving givinostat *vs* 57 [93%] of 61 receiving placebo; table 2). The most common adverse events with givinostat were diarrhoea and vomiting (both in more than twice as many boys as placebo), nasopharyngitis, headache, and abdominal pain; the only severe adverse event in two or more boys was vomiting. Treatment-related adverse events and adverse events leading to dose reduction were more common with givinostat than with placebo, although were typically those known to be associated with givinostat (eg, diarrhoea, thrombocytopenia, and hypertriglyceridaemia). None of the severe or serious adverse events was treatment-related or resulted in study withdrawal.

A reduction in mean platelet count between baseline and week 72 was observed in boys receiving givinostat but not in those receiving placebo, although platelet counts were highly variable (appendix p 20; for reference ranges see appendix p 21). In the givinostat group, 67 (57%) of 118 boys had a shift from normal to low platelet counts during the study (compared with three [5%] of 61 in the placebo group); in 33 (28%) of these 118, the dose of givinostat was reduced owing to decreased platelet count (23 [42%] of 55 on regimen 1 and ten [16%] of 63 on regimen 2). Decreased platelet count (reported as an adverse event) or thrombocytopaenia occurred only with givinostat; no events resulted in study withdrawal and none was associated with clinical signs such as excessive bleeding. Mean triglyceride concentrations increased from baseline to week 72; 70 (59%) of 118 boys receiving givinostat had a shift from normal to high triglyceride concentrations during this time, compared with 30 (49%) of 61 boys receiving placebo (appendix p 20). The adverse events of increased blood triglyceride concentrations or hypertriglyceridaemia were more common with givinostat (27 [23%] of 118 boys) than with placebo (four [7%] of 61 boys); two such events, each in a boy receiving givinostat, resulted in study withdrawal. Only minor changes were observed in other haematological and blood chemistry parameters. Vital signs, electrocardiogram, and pulmonary function were similar in the givinostat and placebo groups.

Discussion

Although the primary endpoint, four-stair climb, worsened from baseline to the end of the study in both groups, the decline was significantly smaller with givinostat than with placebo, with a difference of –1·78 s. This smaller decline is potentially meaningful to patients, because slower four-stair climb correlates with reduced participation in physical and social activities in daily life[37] and predicts loss of stair-climbing ability and ambulatory capacity.[38] Moreover, the velocity difference between the givinostat and placebo groups of 0·034 tasks per second is similar to the minimal clinically important difference reported by Duong and colleagues (0·035 tasks per second; derived using a questionnaire-anchored approach).[39] Because no test alone evaluates Duchenne muscular dystrophy in all muscle groups, we also assessed key secondary endpoints, which provided initial evidence to suggest an effect in favour of givinostat over placebo—although the differences were not significant after multiplicity adjustment. However, multiplicity corrections make reaching significance difficult, especially with sample size limitations imposed by the rarity of Duchenne muscular dystrophy, and the totality of the primary and key secondary endpoint data show a grouping of effects that consistently lend support to the efficacy of givinostat.

The secondary endpoints included NSAA, a scale assessing various aspects of functional domains associated with daily activities.[28,29] Compared with placebo, givinostat seemed to slow the decline in total NSAA score over the course of the study versus placebo, and was associated with 2·14 fewer items failed. This finding is important because, in an analysis by the Collaborative Trajectory Analysis Project,[40] the loss of 2·0 NSAA items predicted clinically meaningful disease progression, loss of ambulation in the functionally declining group, and loss of ability to rise from the floor in the younger, more stable group. Moreover, complete loss of function in one NSAA item, or deterioration in 1–2 items, is perceived as an important change by patients and parents.[41]

The change in time to rise over the study period was also numerically smaller in the givinostat group than in the placebo group. Large interpatient variability is probably the reason for the non-significant p value; when velocity was analysed, variability was lower. Similarly, the decline in distance covered during the 6-min walk test over 72 weeks was numerically lower with givinostat than with placebo. The 6-min walk test decline is dependent on baseline characteristics,[42,43] but the current study had no inclusion criteria related to this test, and the baseline mean in each group was at least 350 m (appendix p 17). Boys who are able to walk these distances are likely to have stable or only slowly declining results for this test, consistent with our findings, and demonstrating a significant treatment effect would require a much larger sample size. Further, changes over time in the knee and elbow strength assessments, although seemingly favouring givinostat, were small, consistent with a study into the natural history of the disease.[44,45] Notably, a difference between the givinostat and placebo groups was also observed using muscle imaging, a technique that provides more objective evaluations than the other outcome measures. The 30% reduction in VLFF with givinostat versus placebo at week 72 (appendix p 18) is predicted to be clinically

meaningful, because VLFF correlates with daily activity[46] and predicts loss of ambulation.[24,47]

Boys received a flexible-dose regimen aimed at maximising efficacy, starting with a high dose that was reduced if treatment was not tolerated. The protocol was amended to lower the starting dose, but the post-hoc ANCOVA suggested that the treatment effect was not affected by the change in treatment regimen.

Compared with those benefitting from mutation-specific antisense therapies,[48] the study recruited a broad population of patients with Duchenne muscular dystrophy, with a wide range of baseline VLFF values, even within group A. This enriched population was expected to show sufficient decline with placebo but not to be at risk of a sudden loss of ambulation. The enrichment was effective, with only one patient (receiving placebo) losing ambulation. Safety was assessed in the overall population. The proportions of patients who had adverse events were similar in the two groups, and most adverse events were mild-to-moderate in severity. More boys receiving givinostat than placebo had adverse events related to treatment—most notably diarrhoea, decreased platelet count, and hypertriglyceridaemia. However, these are recognised adverse events of givinostat and can generally be managed by dose reduction or interruption. Indeed, adverse event monitoring in the current study (with subsequent dose adjustment) was successful—95% of participants completed the study, and treatment compliance was high.

As 48% of boys in the givinostat group (compared with 11% in the placebo group) required dose reduction—mainly due to adverse events—there was a substantial risk of unmasking of the personnel at the study site. This risk was mitigated, with the personnel who assessed efficacy differing from those who reviewed safety data. Furthermore, dose adjustments were most often in response to platelet or triglyceride abnormalities, and laboratory results were not communicated to sites until 24–48 h after these efficacy assessments.

Despite being one of the largest phase 3 clinical trials to date in Duchenne muscular dystrophy, a limitation of this study is that, given the rare nature of the disease, it was not possible to design the study to recruit sufficient patients to evaluate all of the key secondary endpoints, and the difference in primary endpoint between givinostat and placebo was smaller than assumed for the sample size calculation. The finding that our secondary endpoints did not reach formal significance after correction for multiplicity is therefore not unexpected; this is especially the case when the commonly applied alpha control mechanisms—which include the Hochberg procedure—do not account for the correlation between the endpoints included, and is supported by the post-hoc permutation test analysis,[35,36] another statistical approach to account for multiplicity. Another possible limitation of the study is that, owing to the protocol amendment—which was conducted without unmasking—a proportion of patients started the study on a lower dose of givinostat. However, this dose reduction did not affect efficacy but, in our opinion, improved overall safety, and practice guidelines recommend similar dose reductions for corticosteroids to manage adverse events in Duchenne muscular dystrophy.[7] Other limitations of the study are the exclusion of non-ambulatory patients at risk for loss of upper limb function, and the absence of cardiac and pulmonary endpoints. Furthermore, almost all participants were White and, although the study was multinational, none of the study centres was in Africa or Asia. Finally, only males were recruited into the study. Duchenne muscular dystrophy is an X-linked recessive neuromuscular disorder and so predominantly affects males;[3] however, further studies are needed to establish the possible effect of givinostat on female carriers, who can manifest the disease in rare cases and often have very different presentation to males.[49]

In summary, our study met the primary endpoint, with significantly smaller decline in four-stair climb results with givinostat than with placebo in ambulant boys with Duchenne muscular dystrophy. No new safety signals were detected, and a high proportion of boys completed the approximately 18-month follow-up. An ongoing extension study is evaluating the long-term safety and efficacy of givinostat in Duchenne muscular dystrophy.

Contributors

EM, KV, KC, SC, NC, PB, and CMM conceived and designed the study. EM, JJV, OB-T, CMZ, JKM, NG, WM-F, EHN, US-S, EB, GPC, KDM, LS, KV, JJ, SM, SS, LM, KS, BB, CGL, and CMMcD acquired the data. KC and GZ supervised the analysis. All authors were involved in data interpretation, revised the manuscript for important intellectual content, approved the final version, and agree to be accountable for all aspects of the work. All authors had access to the analysed data; EM, KV, KC, GZ, SC, NC, PB, and CMM accessed and verified the data. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

EM declares payment or honoraria for lectures and symposia from Sarepta Therapeutics, PTC Therapeutics, and Roche; and participation on advisory boards for Sarepta Therapeutics, NS Pharma, Santhera, PTC Therapeutics, Roche, Pfizer, WAVE Life Sciences, Italfarmaco, and Dyne Therapeutics, all outside the scope of this manuscript. JJV declares grants from PTC Therapeutics; consulting fees from Santhera, Sarepta Therapeutics, and PTC Therapeutics; and payment for lectures, presentations, speakers bureaus, manuscript writing or educational events from PTC Therapeutics, all outside the scope of this manuscript. OB-T declares grants to her institution from Roche, Novartis, Biogen, Genethon, and Metafora Biosystems; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Novartis; support for attending meetings and/or travel from Novartis; participation on a data safety monitoring board or advisory board for Minoryx Therapeutics; and unpaid leadership roles with AFM-Téléthon (scientific board president) and Société Francophone de Neurogenetique, all outside the scope of this manuscript. CMZ declares grants or contracts from Biogen and Novartis; consulting fees from Sarepta Therapeutics; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Sarepta Therapeutics and Optum; support for attending meetings and/or travel from Sarepta Therapeutics and Optum; and participation on a data safety monitoring board or advisory board for Sarepta Therapeutics, all outside the scope of this manuscript. JKM declares research grants to her institution from Italfarmaco, Biogen, Novartis, NS Pharma, Pfizer, PTC Therapeutics, ReveraGen Biopharma, Roche, Sarepta Therapeutics, and Alberta Children's Hospital Foundation, all outside the scope of this manuscript. NG declares payment or honoraria for lectures,

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Data sharing

The deidentified participant data from this study are available on reasonable request from the sponsor with publication of this manuscript, following submission of a valid research protocol to the corresponding author.

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