Pentoxifylline as a rescue treatment for DMD

A randomized double-blind clinical trial

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ABSTRACT

Objective: To determine whether pentoxifylline (PTX) slows the decline of muscle strength and function in ambulatory boys with Duchenne muscular dystrophy (DMD).

Methods: This was a multicenter, randomized, double-blinded, controlled trial comparing 12 months of daily treatment with PTX or placebo in corticosteroid-treated boys with DMD using a slow-release PTX formulation (~20 mg/kg/day). The primary outcome was the change in mean total quantitative muscle testing (QMT) score. Secondary outcomes included changes in QMT subscales, manual muscle strength, pulmonary function, and timed function tests. Outcomes were compared using Student *t* tests and a linear mixed-effects model. Adverse events (AEs) were compared using the Fisher exact test.

Results: A total of 64 boys with DMD with a mean age of 9.9 ± 2.9 years were randomly assigned to PTX or placebo in 11 participating Cooperative International Neuromuscular Research Group centers. There was no significant difference between PTX and the placebo group in total QMT scores ($p = 0.14$) or in most of the secondary outcomes after a 12-month treatment. The use of PTX was associated with mild to moderate gastrointestinal or hematologic AEs.

Conclusion: The addition of PTX to corticosteroid-treated boys with DMD at a moderate to late ambulatory stage of disease did not improve or halt the deterioration of muscle strength and function over a 12-month study period.

Classification of evidence: This study provides Class I evidence that treatment with PTX does not prevent deterioration in muscle function or strength in corticosteroid-treated boys with DMD.

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GLOSSARY

AE = adverse event; **CINRG** = Cooperative International Neuromuscular Research Group; **CTCAE** = Common Terminology Criteria for Adverse Events; **DMD** = Duchenne muscular dystrophy; **MMT** = manual muscle testing; **NCI** = National Cancer Institute; **PFA** = platelet function assay; **PFT** = pulmonary function test; **PT** = prothrombin time; **PTT** = partial thromboplastin time; **PTX** = pentoxifylline; **QMT** = quantitative muscle testing; **TGF- β** = transforming growth factor- β ; **TNF- α** = tumor necrosis factor- α .

Duchenne muscular dystrophy (DMD) is the most common type of muscular dystrophy of childhood caused by mutations involving the dystrophin gene. The loss of dystrophin leads to muscle membrane fragility, altered calcium homeostasis, and increased oxidative stress, which in turn triggers a cascade of pathologic events that ultimately results in muscle necrosis, fibrosis, and impaired muscle regeneration.^{1,2} Corticosteroids are currently the only available disease-modifying therapies for DMD, by prolonging independent ambulation and delaying the onset of secondary complications.³⁻⁵ However, the use of chronic high-dose corticosteroids for DMD is frequently associated with significant side effects and does not halt disease progression. An

Supplemental data at www.neurology.org

Supplemental Data

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effective treatment for DMD may require a combination of therapies, including pharmacologic agents and gene or cell-based approaches targeting different pathways involved in muscle necrosis and degeneration.^{6,7}

Pentoxifylline (PTX) is a phosphodiesterase inhibitor with potential ability to counteract the complex pathology in DMD; it improves calcium homeostasis and diminishes inflammation, fibrosis, and oxidative stress.^{8,9} Preclinical studies showed that PTX reduced muscle strength deterioration by 51% in the exercised *mdx* mouse.¹⁰ The anti-inflammatory effect of PTX is mediated primarily through the inhibition of tumor necrosis factor- α (TNF- α) production by adenylyl cyclase activation and increased cellular cyclic AMP.^{11–13} In addition, PTX decreases fibrosis by altering the metabolism of metalloproteinases and collagen through the transforming growth factor- β (TGF- β) pathways that are upregulated in DMD.^{14–16} In addition, PTX has antioxidant effects by inhibiting polymorphonucleated cell degranulation and improves peripheral circulation by increasing red blood cell deformability, reducing blood viscosity, decreasing platelet aggregation, and increasing blood glucose supply.¹⁷ The combination of PTX plus corticosteroids had a synergistic and profound immunomodulatory effect on stimulated human peripheral blood mononuclear cells.¹⁸ However, the clinical benefits of PTX in children with DMD remained unknown.

As part of an initial exploratory study, our group conducted an open-label pilot study of an immediate-release oral formulation of PTX at 20 mg/kg/day in 17 corticosteroid-naïve boys between 4 and 8 years of age with DMD.¹⁹ Although there was no observable deterioration in strength or motor function over a 12-month treatment period, the immediate-release formulation of PTX was poorly tolerated because of significant gastrointestinal side effects and/or neutropenia, thus precluding adequate assessment of efficacy. The lack of deterioration in that small group of patients with DMD, however, was encouraging and prompted the current study. To cir-

cumvent the gastrointestinal side effects, we used a Food and Drug Administration–approved slow-release formulation of PTX in this study as an add-on treatment to corticosteroids to determine the benefits of combination therapies for boys with DMD over those of corticosteroids alone. We also included additional laboratory monitoring as safety measures to watch for early signs of neutropenia or other adverse events (AEs) related to PTX. We hypothesized that a 12-month treatment of daily oral PTX in corticosteroid-treated boys with DMD would result in significantly increased muscle strength as measured by their total quantitative muscle testing (QMT) scores compared with scores of those treated with corticosteroids alone.

METHODS Trial design. This was a randomized, multicenter, double-blinded, placebo-controlled trial conducted in 11 academic institutions that are members of the Cooperative International Neuromuscular Research Group (CINRG) from September 2005 to January 2008. Ambulant boys with DMD were randomized in a parallel equal (1:1 ratio) allocation to either oral PTX or placebo.

Study participants. Ambulant boys ages 7 or older with a confirmed diagnosis of DMD were recruited. Participants were required to be taking a stable dose of corticosteroids (either prednisone or deflazacort) for at least 12 months before screening, with normal blood clotting ability based on platelet function assays (PFAs). In addition, participants had to demonstrate consistent muscle testing efforts between screening visits (maximum 7 days apart) with no more than 15% variation in a unilateral biceps QMT score before enrollment. Participants were excluded from participation if they were currently participating in another clinical trial, had a recent cerebral or retinal hemorrhage, or had a history of significant concomitant illnesses including renal or hepatic impairment, bleeding diathesis, or gastric ulcer.

Standard protocol approvals, registrations, and patient consents. All participating clinical research centers obtained approval from their local institutional research board or ethics review board. This study was registered at the NIH Web site (accessed via www.clinicaltrials.gov, registration number NCT00243789). Each participant gave assent, and the parents provided written informed consent as consistent with local institutional policy before the conduct of any study-related assessments.

Interventions. Study arms. Participants were randomly assigned to receive either daily oral PTX or placebo while continuing preexisting corticosteroid therapy at a stable dose. With the exception of multivitamins, vitamin D, and calcium, use of nutritional supplements was not permitted during the study.

Study drug. The study drug was PTX (Trental; Sanofi-Aventis U.S. LLC, Bridgewater, NJ) tablets, an FDA-approved pharmaceutical that is available for oral administration as 400-mg oblong tablets. Both the study drug PTX and placebo were overencapsulated by Capsugel (Pfizer Inc.), a clinical trial

Figure 1 Participant flow through the trial

grade opaque gelcap that is supplied by Fischer Pharmaceuticals. Each PTX capsule contained one 400-mg time-release PTX tablet and inert filler. The placebo capsules contained only inert filler.

Dosing. Participants received 1 of 3 dosing regimens based on their weight at the screening visit: those weighing less than 30 kg received 400 mg once/day; those weighing between 30 and 50 kg received 400 mg twice/day; and those weighing greater than 50 kg received 400 mg 3 times per day, not to exceed 20 mg/kg/day or total dose of 1,200 mg/day, according to available safety information in the pediatric population.^{20–23}

Criteria for dose reduction. During the study, a dose reduction by 400 mg/day was performed if participants experienced AEs; doses were not re-escalated. Criteria for dose reductions included an increase in prothrombin time (PT), partial thromboplastin time (PTT), or PFA over the upper limit of normal, or any grade 4 severe AE as defined by National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) grading criteria (version 3.0, available via <http://ctep.cancer.gov>). Safety data and all AEs were reviewed by the CINRG Data and Safety Monitoring Board on a regular basis.

Study procedures and evaluation. Participants had a total of 9 study visits. At screening, 2 QMT assessments were performed (up to 7 days apart for reliability inclusion), then a safety blood draw at day 15, and further evaluations at study treatment months 1, 3, 6, 9, and 12. Participants had a final QMT assessment 1 week after the end of treatment (figure 1). At each study treatment visit, clinical and safety evaluations included review of medical history, medication administration records, pill counts, AE collections, physical examination, and strength and function assessments as measured by the CINRG quantitative measurement system, platelet function assessment (PFA-epinephrine and PFA-adenosine diphosphate), and standard laboratory panels including complete blood count, PT, PTT, serum chemistry, creatine kinase, electrolytes, glucose, total cholesterol, renal, and liver function tests.

Study outcomes. The primary endpoint was the change in mean QMT total scores from baseline to the end of treatment. The total QMT score (measured in pounds) is the average force generated from 10 muscle groups (including bilateral elbow flex-

ors, elbow extensors, knee flexors, knee extensors, and hand grip), as measured by the CINRG quantitative measurement system.^{24,25}

The secondary endpoints included change in mean QMT scores for arm, leg, grip,^{24,25} elbow extensors, elbow flexors, knee extensors, and knee flexors. Other secondary endpoints included change in velocity of timed function tests to walk 10 m, climb 4 stairs, and stand from supine, manual muscle testing (MMT) scores,²⁶ Brooke upper extremity²⁶ and Vignos lower extremity²⁷ functional rating scales, goniometry measurements,²⁶ and pulmonary function tests (PFTs).²⁸ Pediatric health-related quality of life was assessed using the PedsQL version 4.0 parent-report form.^{29,30} Surrogate outcome measurements of TGF- β and TNF- α as markers of fibrosis and inflammation were also included as exploratory endpoints. TGF- β 1 and TNF- α cytokines were measured using ELISA kits (88-7344 and 88-7346; eBioscience, San Diego, CA).

Statistical methods. Sample size estimation. The sample size was calculated based on the mean total QMT scores obtained from 50 boys aged 4–10 years with DMD who were enrolled in a previous CINRG trial.¹⁹ Twenty-eight participants in each arm allowed us to detect a difference in total QMT score between groups of 1.0 (SD 1.3) pounds, which was considered clinically significant, with 80% power and a 2-sided α of 0.05. The sample size was increased to 32 participants in each of the 2 arms to allow for up to 20% participant withdrawals or incomplete follow-up and for one interim analysis at 6 months.

Randomization and blinding. Patients were randomly assigned using a randomly permuted balanced blocks of variable size (2 and 4) approach. The CINRG coordinating center generated the randomization schedule and provided it to the central pharmacist. The central pharmacist was responsible for dispensing and shipping the supply of investigational drug to the study sites for the duration of treatment. Each site dispensed the study medications to the participants and kept an accurate medication log. All participants, site principal investigators, clinical evaluators, and study coordinators were blinded to the group assignments.

Statistical analysis. Baseline participants' characteristics were summarized using means and SDs. The two-sided Student *t* test was used to compare the changes in the total QMT scores at 12 months between PTX and placebo groups. All randomly assigned participants who received one or more doses of study medication were included in accordance with the modified intention-to-treat analysis; only participants who withdrew before receiving any treatment were not included. To carry out the intention-to-treat approach, a single imputation was used to impute the 12-month values if the assessments were not done at that time because of dropout or any other reasons except for disease progression. Participants who were unable to perform selective components of the QMT because of disease progression were assigned a total QMT score of 0. To investigate the change in QMT scores and timed function tests over time, a linear mixed-effects model was constructed to account for the correlation between the observations due to the clustered structure of the data.³¹ Comparisons between the components of the linear evolutions (intercepts and slopes) were performed using the *F*-test.

The secondary outcomes between the 2 study arms including changes in arm, leg, grip, elbow extensors, elbow flexors, knee extensors, and knee flexors QMT as well as the total MMT, velocity of timed tests, Brooke and Vignos functional rating scales, degree of joint contractures, PedsQL scores, and PFTs were compared using 2-sample *t* tests.

The frequency, body system, severity, and relationship to drug of AEs were tabulated for each group according to CTCAE categories and compared using the Fisher exact test. Analyses were performed using SAS/STAT software 9.1; *p* < 0.05 was considered to be statistically significant.

RESULTS Recruitment and baseline data. Eleven participating CINRG institutions screened a total of 73 boys, of whom 65 were eligible and 64 were enrolled and randomly assigned in equal numbers to the 2 study arms. Although 32 boys were randomly assigned to the PTX group, 2 (6%) withdrew from the study before receiving the first dose; thus, only 30 boys received PTX. Another 32 boys were randomly assigned to the placebo group, and they all began treatment (figure 1). Recruitment took place from November 2005 to December 2006. Participants attended clinic visits at the time of randomization and at protocol-specified intervals for 1 year. Baseline characteristics of study participants are summarized in table 1. The mean age of the study participants was 9.9 (SD 2.9) years in the PTX group and 10.2 (SD 2.8) years in placebo group. Fifty-six (90%) of the participants were Caucasian, 2 (3%) were Asian, and 4 (7%) were of other ethnic origins. Other than corticosteroids, calcium, vitamin D, and multivitamins, none of the participants were taking regular medications or nutritional supplements.

Baseline QMT, MMT, functional scores, and goniometry values were obtained for all participants. Because of disease severity at baseline, there were missing data from 1 (3%) boy in the placebo group who could not perform the 10 m run/walk test, 4 (13%) boys in the PTX group, and 5 (15%) in the placebo group who could not perform the 4 steps climb test and 7 (23%) boys in the PTX group and 7 (21%) in the placebo group who could not stand from supine without assistance. In addition, 2 (6%) boys each from the PTX and placebo groups were unable to cooperate fully with PFTs and total PedsQL scores could not be calculated from 2 (6%) parents in the placebo group at baseline because of incomplete responses.

At baseline, the average of all TGF- β values (both placebo and PTX combined) was 2,394 pg/mL (range 62–15,060 pg/mL). Serum TNF- α levels in the PTX and placebo groups were both nondetectable at baseline.

Outcomes at 12 months. One (3%) participant was lost to follow-up and another withdrew from the PTX group. Two (6%) participants in the placebo group also withdrew during the study (figure 1). There was no significant difference in the primary outcome measure, total QMT score, between PTX and placebo group at 12 months (*p* = 0.14, 95%

Table 1 Summary of study participants between the 2 study arms at baseline

Characteristics	PTX		Placebo		95% CI for difference	p Value ^a
	n	Mean (SD)	n	Mean (SD)		
Age, y	30	9.9 (2.9)	32	10.2 (2.8)	−0.23 (−1.67, 1.21)	0.75
Height, cm	30	124.9 (9.0)	32	124.5 (9.2)	0.44 (−4.18, 5.06)	0.85
Weight, kg	30	31.0 (11.9)	32	29.4 (10.2)	1.57 (−4.10, 7.24)	0.58
BMI, kg/m ²	30	19.3 (4.8)	32	18.6 (4.4)	0.69 (−1.64, 3.01)	0.56
QMT total score, pounds	29	10.6 (4.3)	31	10.8 (3.4)	−0.21 (−2.23, 1.81)	0.84
QMT arm score, pounds	30	7.1 (3.3)	32	7.4 (3.1)	−0.22 (−1.85, 1.41)	0.79
QMT leg score, pounds	30	11.2 (4.8)	32	11.5 (4.3)	−0.31 (−2.63, 2.01)	0.79
QMT grip score, pounds	29	15.7 (7.2)	31	16.3 (5.0)	−0.63 (−3.86, 2.61)	0.70
QMT elbow flexor, pounds	30	7.8 (3.3)	32	8.0 (3.5)	−0.24 (−1.96, 1.48)	0.78
QMT elbow extensor, pounds	30	6.5 (3.5)	32	6.7 (3.0)	−0.20 (−1.87, 1.47)	0.81
QMT knee flexor, pounds	30	11.4 (4.0)	32	12.0 (4.1)	−0.59 (−2.65, 1.47)	0.57
QMT knee extensor, pounds	30	11.0 (6.8)	32	11.0 (5.7)	−0.02 (−3.20, 3.17)	0.99
Total MMT score	30	226.8 (26.6)	32	224.0 (33.1)	2.80 (−12.41, 18.01)	0.71
Walk 10 m, m/s	30	1.46 (0.69)	31	1.67 (0.59)	−0.22 (−0.55, 0.11)	0.19
Climb 4 stairs, 4 stairs/s	26	0.23 (0.14)	27	0.27 (0.13)	−0.04 (−0.12, 0.03)	0.25
Stand from supine, stands/s	23	0.18 (0.10)	25	0.20 (0.10)	−0.02 (−0.08, 0.03)	0.42
FT upper extremity	30	1.2 (0.4)	32	1.2 (0.5)	−0.04 (−0.27, 0.19)	0.75
FT lower extremity	30	2.1 (1.3)	32	2.0 (1.4)	0.08 (−0.59, 0.75)	0.81
FVC, % predicted	28	82.6 (23.2)	28	94.6 (23.5)	−11.98 (−24.50, 0.54)	0.06
FEV1, % predicted	28	84.3 (23.2)	28	94.4 (22.5)	−10.11 (−22.34, 2.13)	0.10
PEFR, % predicted	28	75.2 (20.8)	28	83.4 (19.8)	−8.21 (−19.10, 2.68)	0.14
MIP, cm H ₂ O	29	48.4 (14.7)	32	45.4 (16.6)	2.97 (−5.06, 11.00)	0.46
MEP, cm H ₂ O	30	45 (16)	32	44 (15)	0.70 (−7.16, 8.56)	0.86
PF cough, L/min	29	168.8 (50.1)	32	162.7 (49.8)	6.14 (−19.50, 31.78)	0.63
Wrist extension, degrees	30	73.7 (21.6)	32	79.2 (18.2)	−5.55 (−15.75, 4.65)	0.28
Elbow extension, degrees	30	0.7 (6.7)	32	3.3 (6.4)	−2.61 (−5.95, 0.72)	0.12
Knee extension, degrees	30	−0.4 (5.9)	32	0.4 (5.0)	−0.81 (−3.60, 1.99)	0.57
Ankle dorsiflexion, degrees	30	−0.5 (13.0)	32	−1.6 (9.0)	1.14 (−4.59, 6.87)	0.69
Pediatric QOL total score	30	58.5 (15.2)	30	57.1 (15.7)	1.38 (−6.62, 9.38)	0.73
Pediatric QOL physical score	30	50.1 (21.1)	30	49.9 (21.0)	0.21 (−10.68, 11.10)	0.97
Pediatric QOL emotional score	30	65.9 (14.7)	30	65.3 (15.5)	0.58 (−7.24, 8.41)	0.88
Pediatric QOL social score	30	60.9 (18.5)	30	55.3 (19.7)	5.58 (−4.31, 15.48)	0.26
Pediatric QOL school score	30	62.1 (19.1)	30	62.3 (14.3)	−0.17 (−8.91, 8.58)	0.97

Abbreviations: BMI = body mass index; FEV1 = force expiratory volume in 1 minute; FT = functional test; FVC = force vital capacity; MEP = maximum expiratory pressure; MIP = maximum inspiratory pressure; MMT = manual muscle testing; PEFR = peak expiratory flow rate; PTX = pentoxifylline; QMT = quantitative muscle testing; QOL = quality of life; PF cough = peak cough flow.

^a p Values are based on a two-sided Student t test.

confidence interval 0.63 [0.21, 1.48]) (table 2). Similarly, a trend analysis obtained from the linear mixed-effects model showed no significant difference in the fitted mean of the total QMT scores between the study arms (figure 2). The secondary outcomes also failed to detect any significant differences between the 2 groups for the mean QMT subgroup scores, MMT, functional grading, PFTs, degree of contractures, timed function test, and PedsQL scores

except for the timed 10-m run/walk test. The PTX group showed significantly less decline in the velocity to perform the 10-m timed run/walk test after 12 months of treatment than placebo (−0.1 m/second vs −0.3 m/second, respectively; $p = 0.03$, 95% confidence interval 0.16 [0.01, 0.31]). There was no difference between treatment groups in longitudinal trends for total QMT score (figure 2) or any secondary outcomes (data not shown).

Table 2 Comparison between study arms for changes from baseline to month 12

Characteristics	Change from baseline to month 12					
	PTX		Placebo		95% CI for difference	p Value ^a
	n	Mean (SD)	n	Mean (SD)		
QMT total score, pounds	29	−0.8 (1.9)	30	−1.4 (1.3)	0.63 (−0.21, 1.48)	0.14
QMT arm score, pounds	30	−0.6 (1.4)	32	−1.1 (1.6)	0.45 (−0.32, 1.22)	0.24
QMT leg score, pounds	30	−1.5 (2.9)	32	−1.6 (1.7)	0.11 (−1.12, 1.35)	0.86
QMT grip score, pounds	29	0.1 (3.0)	30	−1.4 (2.9)	1.50 (−0.02, 3.03)	0.05
QMT elbow flexor, pounds	30	−0.5 (1.6)	32	−1.2 (1.7)	0.70 (−0.15, 1.54)	0.10
QMT elbow extensor, pounds	30	−0.7 (1.4)	32	−0.9 (1.8)	0.27 (−0.54, 1.07)	0.51
QMT knee flexor, pounds	30	−1.4 (2.8)	32	−1.3 (1.7)	−0.17 (−1.37, 1.03)	0.78
QMT knee extensor, pounds	30	−1.6 (4.1)	32	−2.1 (2.4)	0.51 (−1.24, 2.25)	0.56
Total MMT score	26	−11.1 (20.1)	30	−15.0 (23.3)	3.88 (−7.74, 15.50)	0.51
Walk 10 m, m/s	30	−0.13 (0.30)	31	−0.29 (0.27)	0.16 (0.01, 0.31)	0.03
Climb 4 stairs, 4 stairs/s	26	−0.05 (0.07)	27	−0.05 (0.06)	−0.001 (−0.04, 0.03)	0.94
Stand from supine, stands/s	23	−0.06 (0.05)	25	−0.05 (0.03)	−0.01 (−0.03, 0.02)	0.51
FT upper extremity	27	0.1 (0.5)	30	0.1 (0.3)	0.06 (−0.17, 0.30)	0.59
FT lower extremity	27	0.4 (0.8)	30	0.3 (1.0)	0.04 (−0.46, 0.53)	0.89
FVC, % predicted	25	−2.5 (14.8)	26	−6.7 (9.8)	4.19 (−2.94, 11.33)	0.24
FEV1, % predicted	25	−2.4 (17.1)	26	−7.8 (11.4)	5.39 (−2.84, 13.62)	0.19
PEFR, % predicted	25	2.4 (22.2)	26	−3.0 (16.4)	5.46 (−5.59, 16.51)	0.33
MIP, cm H ₂ O	27	4.4 (14.3)	30	3.1 (12.9)	1.36 (−5.89, 8.62)	0.71
MEP, cm H ₂ O	28	5.5 (11.8)	30	2.5 (13.6)	3.04 (−3.65, 9.72)	0.37
PF cough, L/min	27	−2.0 (47.3)	30	6.1 (33.9)	−8.12 (−30.27, 14.03)	0.46
Wrist extension, degrees	28	2.9 (11.9)	30	2.3 (8.1)	0.61 (−4.80, 6.02)	0.82
Elbow extension, degrees	28	0.5 (5.4)	30	−0.3 (5.4)	0.79 (−2.06, 3.63)	0.58
Knee extension, degrees	28	−1.8 (5.8)	30	0.8 (4.7)	−2.62 (−5.44, 0.20)	0.07
Ankle dorsiflexion, degrees	28	−4.1 (14.8)	30	−1.3 (5.8)	−2.77 (−8.84, 3.29)	0.36
Pediatric QOL total score	28	−1.7 (14.1)	28	3.0 (13.5)	−4.68 (−12.08, 2.71)	0.21
Pediatric QOL physical score	28	−3.0 (20.3)	28	5.2 (23.7)	−8.22 (−20.05, 3.61)	0.17
Pediatric QOL emotional score	28	−2.8 (18.3)	28	2.5 (17.5)	−5.27 (−14.87, 4.34)	0.28
Pediatric QOL social score	28	−1.5 (21.6)	28	0.7 (18.0)	−2.23 (−12.89, 8.42)	0.68
Pediatric QOL school score	26	3.9 (17.0)	28	1.9 (15.3)	2.07 (−6.81, 10.94)	0.64

Abbreviations: FEV1 = force expiratory volume in 1 minute; FT = functional test; FVC = force vital capacity; MEP = maximum expiratory pressure; MIP = maximum inspiratory pressure; MMT = manual muscle testing; PEFR = peak expiratory flow rate; PTX = pentoxifylline; QMT = quantitative muscle testing; QOL = quality of life; PF cough = peak cough flow. ^a p Values are based on a two-sided Student t test.

AEs. AEs by study arms are summarized in table 3. There were no withdrawals due to medication non-adherence or dose reductions. Mild to moderate AEs were significantly higher in the PTX group as seen by the difference in the proportion of boys with reported gastrointestinal (13 of 30 vs 5 of 32, $p = 0.02$) or hemorrhage/bleeding (8 of 30 vs 1 of 32, $p = 0.01$) AEs. There were no significant differences in EKG, PFA, or other safety laboratory measures between the 2 study arms.

DISCUSSION Our study found that the addition of PTX to corticosteroid-treated ambulant boys with

DMD failed to slow the decline in overall muscle strength and function after a 12-month treatment period. A number of factors may have contributed to the lack of effect of PTX in this study, despite the positive preclinical results in the *mdx* mouse. First, although the mechanism of action of PTX is through modulation of the TGF- β and TNF- α pathways, the TNF- α pathway was already significantly reduced in corticosteroid-treated boys with DMD, as shown by nondetectable baseline serum TNF- α levels among our study participants. In addition, their serum TGF- β levels were very variable at baseline, which differed from previous studies showing a significant

Figure 2 Trend analysis of quantitative muscle testing (QMT) total score

The fitted mean is the prediction obtained from the linear mixed model. Observations were displayed with sample mean and SD. PTX = pentoxifylline; STD = standard.

increase in plasma TGF- β with DMD.^{14,32} It is possible that the increases in the tissue levels of TGF- β and TNF- α may not be reflected in serum, and chronic high-dose corticosteroid treatment may have inhibitory effects on both TGF- β and TNF- α pathways. In addition, our cohort was in the mid to late ambulatory stage, wherein some of the potential effects of PTX on early pathologic events in DMD might be lost.

Preclinical studies in the *mdx* mouse used much higher doses of PTX ranging from 50 to 100 mg/kg/day, which may have played a role in producing the beneficial results.^{7–9} In nonhuman primates, doses of 120 mg/kg, but not lower, were effective in attenuating increases of TNF- α , interferon- γ , and interleukin-2.³³ Given the paucity of published data on the safety and efficacy of PTX in children, we were limited to using the maximum safe dosing used in previous studies of 20 mg/kg/day.²⁰ Under strict monitoring, higher doses of PTX could be considered in future DMD studies. These should also include studies to assess dose-response relationships.

Twelve-month treatment with PTX might not be a sufficient period of time to allow detection of meaningful changes in muscle strength and function. Even though there was no significant difference in the mean total QMT scores after 1 year, it was intriguing to see less deterioration in the 10-m walk/

run test ($p = 0.03$) and the QMT grip scores with PTX vs placebo ($p = 0.05$). In addition, although not reaching statistical significance, the decline in most measures was less in the PTX group, a clear directionality that is unlikely to occur by chance. It would be of interest to know whether prolonged or continual treatment with PTX may lead to a more noticeable slowing in the decline of walking speed and muscle strength in selected muscle groups among boys with DMD.

Decreasing fibrosis is a novel approach in DMD, and further studies are needed to understand the appropriateness of pharmacologic modulation of inflammatory pathways using PTX or other antifibrotic treatment in this context. Because PTX was associated with an increase in the risk of mild to moderate gastrointestinal or hemorrhage/bleeding AE in the absence of significant clinical improvement, the addition of PTX to a stable dose regimen of corticosteroids in mid to late ambulant boys with DMD cannot be recommended based on the current study.

Study strengths and limitations. This study tested for the first time an antifibrotic drug as a therapeutic approach for DMD. The study was limited by the paucity of pediatric data on a well-tolerated drug formulation that would allow for more accurate dosing

Table 3 Summary of reported adverse events by study arm^a

NCI category	NCI grade									p Value ^b
	PTX				Placebo					
	1	2	3	Total	1	2	3	4	Total	
Allergy/immunology	2	1	0	3	2	0	0	0	2	0.67
Cardiac general	0	1	0	1						
Coagulation	0	0	2 (1)	2 (1)						
Constitutional symptoms	12 (9)	7 (6)	0	19 (14)	7 (5)	6 (3)	0	0	13 (7)	0.06
Dermatology/skin	14 (10)	7 (3)	0	21 (12)	8 (7)	2	0	0	10 (8)	0.21
Endocrine					0	1	0	0	1	
Gastrointestinal	21 (12)	2	0	23 (13)	5	1	0	0	6 (5)	0.02
Hemorrhage/bleeding	10 (8)	0	0	10 (8)	1	0	0	0	1	0.01
Infection	22 (13)	7 (6)	0	29 (17)	24 (13)	4	0	0	28 (16)	0.60
Metabolic/laboratory	0	2	1	3 (2)	1	0	0	0	1	0.61
Musculoskeletal/soft tissue	6	5 (4)	3 (2)	14 (11)	3	7	1	1	12 (9)	0.47
Neurology	1	4	0	5	2	4	1	0	7	0.75
Ocular/visual	1	0	0	1	1	1	0	0	2	1
Pain	34 (16)	10 (7)	1	45 (19)	25 (13)	7	0	0	32 (17)	0.42
Pulmonary/upper respiratory	17 (11)	6 (5)	0	23 (14)	11 (9)	5	0	0	16 (11)	0.32
Renal/genitourinary	1	1	0	2						
Surgery/intraoperative injury	0	0	2	2						
Total	141	53	9	203 (29)	90	38	2	1	131 (28)	

Abbreviations: NCI = National Cancer Institute; PTX = pentoxifylline.

^a Data are presented as n (m) indicating a total of n adverse events from a total of m individuals. NCI Common Terminology Criteria for Adverse Events (CTCAE) grading criteria are shown.

^b p Values are based on Fisher exact tests.

per body mass and the lack of pharmacokinetics studies that would inform the exposure/benefit relationship. In general, testing of FDA-approved compounds to accelerate the discovery of treatments for such a devastating disease is also limited by the inability to use medicinal chemistry approaches to increase specificity and potency of the drug.

AUTHOR CONTRIBUTIONS

Dr. Escolar designed the clinical trial, conducted the trial as a site principal investigator (PI) and the study PI, participating in analysis and interpretation of data and writing and editing of the manuscript. She revised and approved the manuscript. A. Zimmerman participated in the protocol design, data interpretation, and manuscript drafting. She was the study's Project Manager and site study coordinator. Dr. Bertorini was a site PI. He revised and approved the manuscript. Dr. Clemens was a site PI. She participated in data interpretation and revised and edited the manuscript. Dr. Connolly was a site PI. She revised and approved the manuscript. Dr. Mesa was a site PI. She revised and approved the manuscript. Dr. Gorni was a site PI. She revised and approved the manuscript. Dr. Kornberg was a site PI. He revised and approved the manuscript. Dr. Kolski was a site PI. She revised and approved the manuscript. Dr. Kuntz was a site PI. She revised and approved the manuscript. Dr. Nevo was a site PI. He revised and approved the manuscript. Dr. Tesi-Rocha participated in protocol design and implementation. Dr. Nagaraju participated in analysis and interpretation of exploratory study outcomes. Dr. Rayavarapu participated in analysis and interpretation of exploratory study outcomes. L.P. Hache was the site study coordinator, participated in manuscript preparation and submission and was the study's backup Project

Manager. J.E. Mayhew was a study outcome trainer. She participated in analysis and interpretation of data. J. Florence was a study outcome trainer. She participated in analysis and interpretation of data. F. Hu participated in study's statistical design, data analysis, and manuscript preparation. A. Arrieta participated in protocol design and implementation. E. Henricson participated in the design of the protocol, data interpretation, and revision of the manuscript. Dr. Leshner participated in data analysis and interpretation and reviewed the manuscript. Dr. Mah was a site PI and participated in data analysis and interpretation and manuscript preparation. She revised and approved the manuscript.

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DISCLOSURE

Dr. Escolar serves on a scientific advisory board for the NIH/NINDS; serves on the speakers' bureau for and has received funding for travel and speaker honoraria from Athena Diagnostics, Inc.; serves as a consultant for Acceleron Pharma, HALO therapeutics, AVI Biopharma, Gerson Lheman Group (GLC), and Medacorp; and has received research support from the NIH, the Muscular Dystrophy Association, and the Foundation to Eradicate Duchenne (FED). A. Zimmerman serves as a consultant for Halo Therapeutics. Dr. Bertorini serves on the speakers' bureaus of Teva, Biogen Idec, Allergan, and Merck Serono and serves on scientific advisory boards for and receives speaker honoraria from Pfeiffer, Allergan, and Athena. Dr. Clemens receives research support from Genzyme Corporation, Amicus, NIH, Veterans Administration, and Department of Defense. Dr. Connolly receives research support from PTC Pharmaceutical, the Muscular Dystrophy Association and NIH and serves on scientific advisory boards for Acceleron and Halo Therapeutics. Dr. Mesa and Dr. Gorni report no disclosures. Dr. Kornberg has received funding for travel from Genzyme and Biogen Idec and serves as a Section Editor for *BMC Neurology*. Dr. Kolski receives research support from Talecris Biotherapeutics. Dr. Kuntz receives research support from Cooperative International Neuromuscular Research Group/DOD and NIH. Dr. Nevo is listed as an author on a patent re: The use of Glatiramer Acetate in muscular dystrophy and receives research support from AFM and Israeli Ministry of Health and Little Steps (Israeli Parents organization). Dr. Tesi-Rocha reports no disclosures. Dr. Nagaraju serves as an Associate Editor for the *Journal of Neurological Sciences* and is founder of Reveragen Biopharma. Dr. Rayavarapu reports no disclosures. L.P. Hache has received funding for travel and speaker honoraria from Genzyme and receives staff grant funding from NIH and Department of Defense. J.E. Mayhew has received funding for travel and speaker honoraria from Genzyme and serves as a consultant for Enobia Pharma, Inc. and Genzyme. J. Florence has served on scientific advisory boards and/or as a consultant for Prosensa, GlaxoSmithKline, Acceleron, PTC Therapeutics, and DART Therapeutics. F. Hu receives research support from US Department of Defense, NIH, US Department of Education, and the Muscular Dystrophy Association. A. Arrieta receives research support from the US Department of Defense and the Muscular Dystrophy Association. E. Henricson served as a consultant for Genzyme and PTC Therapeutics. Dr. Leshner serves on a scientific board and speakers' bureau for Genzyme and receives research support from Genzyme, Wyeth, and US Department of Defense. Dr. Mah receives research support from PTC Therapeutics, Acceleron Pharma, US Department of Defense, and US FSH Society and Muscular Dystrophy Canada.

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