A Phase I/II trial of MYO-029 in Adult Subjects with Muscular Dystrophy

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Objective: Myostatin is an endogenous negative regulator of muscle growth and a novel target for muscle diseases. We conducted a safety trial of a neutralizing antibody to myostatin, MYO-029, in adult muscular dystrophies (Becker muscular dystrophy, facioscapulohumeral dystrophy, and limb-girdle muscular dystrophy).

Methods: This double-blind, placebo-controlled, multinational, randomized study included 116 subjects divided into sequential dose-escalation cohorts, each receiving MYO-029 or placebo (Cohort 1 at 1mg/kg; Cohort 2 at 3mg/kg; Cohort 3 at 10mg/kg; Cohort 4 at 30mg/kg). Safety and adverse events were assessed by reported signs and symptoms, as well as by physical examinations, laboratory results, echocardiograms, electrocardiograms, and in subjects with facioscapulohumeral dystrophy, funduscopic and audiometry examinations. Biological activity of MYO-029 was assessed through manual muscle testing, quantitative muscle testing, timed function tests, subject-reported outcomes, magnetic resonance imaging studies, dual-energy radiographic absorptiometry studies, and muscle biopsy.

Results: MYO-029 had good safety and tolerability with the exception of cutaneous hypersensitivity at the 10 and 30mg/kg doses. There were no improvements noted in exploratory end points of muscle strength or function, but the study was not powered to look for efficacy. Importantly, bioactivity of MYO-029 was supported by a trend in a limited number of subjects toward increased muscle size using dual-energy radiographic absorptiometry and muscle histology.

Interpretation: This trial supports the hypothesis that systemic administration of myostatin inhibitors provides an adequate safety margin for clinical studies. Further evaluation of more potent myostatin inhibitors for stimulating muscle growth in muscular dystrophy should be considered.

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Muscular dystrophies are a diverse set of distinct, inherited disorders that commonly manifest with progressive skeletal muscle weakness and wasting. Despite substantial progress in understanding the pathophysiological basis of these diseases, no pharmacological therapies have been identified that increase muscle strength, other than corticosteroids, which provide a modest benefit to some patients with these disorders.[1] For muscular dystrophies that present in adulthood, there have been only a few small clinical trials, and none involved a novel therapeutic agent.[2–6] This article describes a clinical trial of a novel agent, an inhibitor of myostatin, designed to increase muscle mass and strength in several of the most common forms of adult muscular dystrophy.

Myostatin, a member of the transforming growth

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factor-β superfamily, is an endogenous inhibitor of muscle growth.[7] The function of myostatin is conserved in all animals examined, and the absence of myostatin results in muscle growth approximately two to three times greater than normal.[7–11] Importantly, the function of myostatin is also conserved in humans, as was determined by the identification of a myostatin splice-site mutation leading to the loss of myostatin protein in a hypermuscular family.[12]

In the absence of myostatin, muscle regeneration has been shown to occur earlier and more robustly after acute and chronic injury. In the *mdx* mouse model of muscular dystrophy, animals lacking myostatin had increased muscle mass and strength and decreased fibrosis.[13,14] Furthermore, postnatal inhibition of myostatin with a neutralizing monoclonal antibody to myostatin also ameliorated disease features in the *mdx* mouse.[15]

Given these preclinical results, myostatin has been considered a therapeutic target for the treatment of muscular dystrophy. MYO-029 is a recombinant human antibody that binds with a high affinity to myostatin and inhibits its activity.[16] This myostatin-neutralizing antibody has previously been shown to increase muscle mass in immunodeficient mice by approximately 30% over 3 months, similar to the biological response demonstrated for other myostatin-neutralizing antibodies.[15–17] The primary objective of this double-blind, placebo-controlled, multinational, randomized trial was to evaluate the safety of ascending doses of MYO-029 in adult subjects with Becker muscular dystrophy (BMD), facioscapulohumeral dystrophy (FSHD), and limb-girdle muscular dystrophy (LGMD). A secondary goal was to assess exploratory end points of clinical and biological activity of MYO-029 through analysis of muscle strength, mass, and composition. The prospective hypothesis was that MYO-029 would be well tolerated.

Subjects and Methods

Study Design

The study was a randomized, double-blind, placebo-controlled, ascending dose, safety study of MYO-029 approved by regulatory agencies and the local institutional review boards in 10 participating centers in the United States and the United Kingdom. Subjects were randomly assigned using a computerized randomization and enrollment system. The randomization technology was provided through a centralized telephone software system that provides sites with the ability to perform various subject enrollment functions, including screening, randomization, and emergency unblinding. Sites were able to access the randomization system by secure Internet access or by telephone.

Approximately 136 subjects were planned to be divided into sequential dose cohorts, each compared with placebo. There was an equal number of subjects with BMD, FSHD, or LGMD in each cohort with MYO-029 dose escalation: Cohort 1 received 1mg/kg; Cohort 2 received 3mg/kg; and Cohort 3 received 10mg/kg. Within each cohort, subjects were randomly assigned to receive the test drug or placebo in a 3:1 ratio. Test article was administered intravenously every 2 weeks for 6 months (total of 13 doses). After the last dose, subjects were followed for 3 months. After the start of the study, safety data from a multiple ascending dose study in healthy subjects became available, permitting an amendment to add a fourth cohort, at 30mg/kg.

Subjects

Informed consent was given by all subjects before participation in the trial. All subjects were at least 18 years old and had a clinical and confirmed molecular diagnosis of BMD, FSHD, or one of the following forms of LGMD: 2A, 2B, 2C, 2D, 2E, or 2I.[18,19] Eligibility required independent ambulation; muscle strength of $\geq 3-$ and $\leq 4+$ on manual muscle testing (MMT) in at least 8 of 16 muscle groups[2,3,20] on initial evaluation and confirmed at visit 2 by strength within 2 steps (eg, 4 vs 5−) in at least 10 of the 16 muscles; a forced vital capacity ≥60% of the predicted value; and ejection fraction greater than 40% by echocardiogram. A negative urinary pregnancy test was required for women at risk. All subjects of childbearing potential agreed to use two reliable methods of birth control for the duration of the study.

Exclusion criteria included heart disease related to ischemia, congestive failure, or use of antiarrhythmic or anticoagulant medication within 12 weeks before randomization, glucocorticosteroids within 6 months before randomization and for the duration of the study, and pharmacological treatment potentially affecting muscle function within 4 weeks before randomization and for the duration of the study. Strengthening exercises and decline in endurance were not permitted within 8 weeks before randomization, and strengthening exercises were excluded during the study. Pregnant or lactating women were disallowed. Also excluded were subjects with a history of sensitivity to monoclonal antibodies or protein pharmaceuticals.

Blinding

MYO-029 was provided in vials containing a lyophilized form to be reconstituted with 1ml sterile water, USP. After reconstitution with 1ml sterile water, each vial delivered 0.9ml MYO-029 at a concentration of 70mg/ml. Identical placebo vials were provided, which contained a lyophilized formulation containing only the excipients. At each participating site, the responsibility for test article preparation was assigned to an unblinded pharmacist who did not participate in the evaluation of study subjects.

Assessments

SAFETY. Safety and tolerability of MYO-029 in adult subjects with muscular dystrophy were the primary outcome measures. Analyses included incidence and severity of adverse events (AEs) assessed by reported signs and symptoms, as well as physical examinations, vital signs measurements, laboratory results (excluding enzyme levels increased by muscle disease),[20] echocardiograms, electrocardiograms, and in subjects with FSHD, fundoscopic and audiometry examinations.

AEs were graded according to the World Health Organization Toxicity Scale.

BIOLOGICAL ACTIVITY. Biological activity was assessed through MMT, quantitative muscle testing (QMT), timed function tests (TFTs), pulmonary function tests, and subject-reported outcomes.

Muscle strength was assessed by MMT of 21 bilateral limb muscles, as well as neck and abdominal muscles. A modified MMT score was generated on each muscle group using an 11-point system, as published previously.[21] Composite total MMT scores were calculated by averaging the converted MMT scores across all muscle groups, as well as composite upper body scores, which include neck muscles, and lower body scores, which include abdominal muscles.

At 9 of the 10 participating centers, muscle strength was also measured using QMT to assess maximum voluntary isometric force of 12 limb-muscle groups.[22] The maximum force from three attempts was used in analysis. In addition to total muscle strength assessments, separate analyses were conducted for total upper and lower extremities.

TFTs included time to traverse 9m, climb four stairs, and stand from a seated position. Pulmonary function tests included sitting and supine forced vital capacity. Subjects with FSHD were permitted to use a face mask during spirometry.

A direct assessment of a biological effect on muscle was assessed via changes in muscle mass, myostatin levels, and muscle histology. Changes in muscle mass were assessed through dual-energy x-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI) scans. DEXA and MRI scans were performed on all subjects at pretreatment baseline and at week 26. Total body DEXA was performed to estimate lean mass from the trunk, arms, and legs. For this study, lean tissue was presumed to be muscle.

Proton-density MRI scans were performed on each upper arm and on both thighs for three different image data sets, using overlapping acquisitions. Volumes were calculated via segmentation of the three-dimensional reconstructed MRI data of the extremities by VirtualScopics® (Rochester, NY), as shown in Figure 1. Muscle was automatically separated from subcutaneous fat using the large signal differences of muscle boundaries from fat tissue.[23] A semiautomated system was then used to separate intermuscular fat from subcutaneous fat. Normal and abnormal muscle volumes were then segmented from one another using a maximum likelihood pixel classification algorithm.[24] The algorithm was trained using the signal intensities derived from selected tissue samples of the normal muscle that determined well-defined ranges for normal muscle and fat. The numbers of pixels of each type were then summed across slices to determine the total volume of normal and abnormal muscle; 13 cases were excluded from analysis because of metal artifact, excessive motion, or mispositioning of limbs.

SUBJECT-REPORTED OUTCOMES. Subject-reported outcomes were assessed using Version 1.0 of the Short Form (SF)-36, which has established validity as a measure of function and well-being.[25,26] Subjects self-administered the SF-36 during three visits. Scoring of the SF-36 Physical Health and Mental Health components used a norm-based approach.[27]

MYOSTATIN LEVELS. Both free and total myostatin levels were assessed in subject serum and muscle biopsy samples using a validated enzyme-linked immunosorbent assay (ELISA) that the Wyeth Biological Technologies group developed. The ELISA capture antibody, RK35, is a mouse monoclonal antibody that binds the putative receptor (activin receptor IIB) binding site in myostatin.[28] The detector antibody, RK22, is a mouse monoclonal antibody specific for myostatin that binds the ALK4/5 binding site near the amino terminus (unpublished data). This assay detects free myostatin but does not effectively measure latent myostatin or myostatin bound to MYO-029. Therefore, an acid dissociation step was incorporated into the assay to convert latent myostatin to free myostatin and to dissociate MYO-029 from myostatin, thereby enabling the measurement of both endogenous free and total myostatin in serum and muscle samples. All clinical samples were analyzed in the Wyeth Biomarker Laboratory (Collegeville, PA), which also conducted a thorough analytical validation of the ELISA assay. Free and total serum myostatin levels were assessed during three visits (baseline, week 6, and week 36). Free and total myostatin levels at the two later visits were compared with baseline to determine whether alterations could be detected in myostatin levels over time and in response to escalating doses of MYO-029. Muscle myostatin levels were assessed before (baseline) and after (week 26) dose administration to determine a response to MYO-029. It should be noted that serum and muscle biopsy samples were collected and stored at ≤70°C

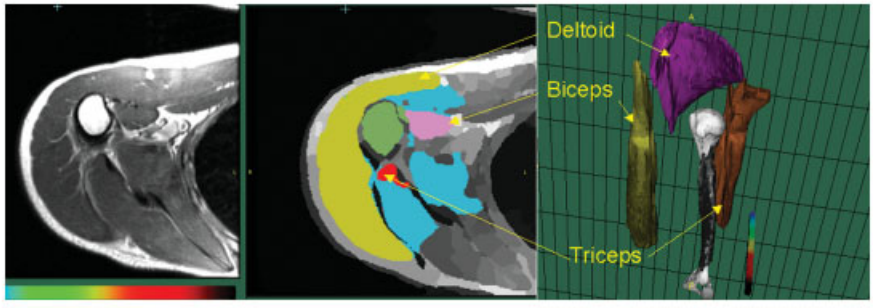

Fig 1. Segmentation and three-dimensional reconstruction of proximal arm muscle by magnetic resonance imaging. Axially acquired images (left) were obtained through the entire lengths of the extremities. Individual muscles were then segmented into cross-sectional areas (center) and volumes summed from the segmented areas (right).

for a period of up to 2 years in some cases. The long-term stability of myostatin has not been characterized, and the effects of long-term storage are therefore unknown.

MUSCLE HISTOLOGY. An open muscle biopsy was an optional procedure, and specimens were obtained from 26 subjects at baseline and at week 26. Baseline biopsies were obtained from muscles having MMT grades of 4 or 4+, and the same or contralateral muscle underwent biopsy at week 26. Fiber necrosis, inflammation, and regeneration were qualitatively scored as none/minimal, mild, moderate, or severe by a muscle pathologist blinded to prestudy/poststudy status, diagnosis, and treatment group. Central nucleated fibers and fiber diameter were quantitatively determined on all muscle fibers up to 500 fibers per biopsy using OpenLab software (Improvision®, Lexington, MA).

Statistical Analysis

Statistical analysis of safety end points is reported using the intent-to-treat population, defined as all randomly assigned subjects who received at least one dose of placebo or MYO-029. Analysis of biological activity was based on a modified intent-to-treat analysis, which included all subjects who had results from baseline and visit 15 for a particular end point. Because the primary objective of the study was assessment of safety and tolerability, sample sizes were determined by clinical rather than statistical considerations. However, for 9 (or 6 BMD subjects in Cohorts 3 and 4) MYO-029 subjects with 1 dystrophy in 1 cohort, the probabilities of detecting at least 1 AE were calculated to be 0.09 (0.06), 0.61 (0.47), 0.77 (0.62), 0.87 (0.74), and 0.96 (0.88) when the rates are 1, 10, 15, 20, and 30, respectively.

The MMT, QMT, TFT, DEXA, and SF-36 percentage changes from baseline measurements were summarized by treatment group using mean and standard errors within a disease cohort by week. The mean percentage change from baseline response of subjects treated in each dose cohort was compared with the mean percentage change from baseline of the placebo group at week 26 via Dunnett's Multiple-Comparisons Procedure. The adjusted p values of these comparisons were reported and considered to be statistically significant if less than 0.05.

The MRI percentage changes from baseline measurements were evaluated for adequacy of statistical assumptions (ie, symmetry of the response about the group mean). Because skewness was observed among the MRI responses, the percentage changes from baseline measurements were summarized via the treatment group medians within disease cohort by week. The median percentage changes from baseline response of subjects treated with either 1.0, 3.0, or 10.0mg/kg MYO-029 were compared with the median percentage change from baseline of the placebo group at week 26, via Dunn's Multiple-Comparisons Procedure. The adjusted p values of these comparisons were reported and considered to be statistically significant if less than 0.05.

Mean muscle fiber diameters pretreatment and posttreatment were compared using a paired t test for each dosing cohort. Analysis of variance was used to evaluate the effect of dose on the percentage change in mean fiber diameter between the pretreatment and posttreatment samples. Myostatin levels were compared using a paired t test. Significance was set at $p < 0.05$ for both analyses.

Results

A total of 116 subjects in 4 dosing cohorts with muscular dystrophies (36 with BMD, 42 with FSHD, and 38 with LGMD) were included in the study. Enrollment in Cohort 4 (30mg/kg) was discontinued during the study. There were no significant differences between groups in any demographic characteristic, as shown in Table 1. Figure 2 depicts subject disposition.

Safety

Safety assessments, including vital signs, laboratory tests, and physical examination showed no significant differences between treatment and placebo groups, and were not dose limiting. Twenty-seven subjects discontinued from the study as depicted in Figure 2. This included four subjects who were withdrawn after experiencing hypersensitivity reactions (three with urticaria). Two of the subjects experiencing hypersensitivity reactions were in the 10mg/kg cohort and two were in the 30mg/kg cohort. A decision was made to terminate Cohort 4 (30mg/kg) after the occurrence of hypersensitivity reactions and a case of unconfirmed aseptic meningitis in the 10mg/kg group. In the one unconfirmed case of "aseptic meningitis" in a subject in the 10mg/kg cohort, symptoms included diplopia and headache. Cerebrospinal fluid showed increased protein without cells. MRI showed an area of meningeal enhancement. This subject's symptoms resolved and cerebrospinal fluid findings normalized on repeat study without treatment.

A total of 109 subjects reported AEs, of which 104 were considered to be treatment-emergent adverse events. Table 2 shows treatment-emergent adverse events that occurred in at least 5% of any group and were more common in any treatment cohort than in the placebo group. The only treatment-emergent adverse event more common in the treatment groups than placebo that was statistically significant was accidental injury ($p = 0.026$).

Rash, with or without pruritus, and urticaria were seen in 12 subjects. Three of the 12 had urticaria only (all occurring in the 10mg/kg cohort), believed to be drug related. Other rashes were also considered to be forms of cutaneous hypersensitivity reactions, based on the temporal relation to drug infusion. The number of these skin reactions according to treatment regimen was as follows: placebo group had 2 reactions in 29 placebo-treated subjects, 1mg/kg cohort had 3 in 27 subjects, 3mg/kg cohort had 1 in 27 subjects, 10mg/kg cohort had 4 in 27 subjects, and 30mg/kg cohort had 2 in 6 subjects.

A total of 7 (6%) subjects reported serious AEs, including 2 of 29 (6.9%) in the placebo group (dyspnea

Table 1. **Demographic and Baseline Characteristics**

Characteristics	Treatment Group					
	Placebo (n = 29)	MYO-029				Total (n = 116)
		1mg/kg (n = 27)	3mg/kg (n = 27)	10mg/kg (n = 27)	30mg/kg (n = 6)	
Mean age, yr (SD)	39.3 (13.3)	37.2 (9.5)	37.1 (13.6)	40.2 (11.5)	44.3 (10.2)	38.8 (12.0)
Sex, n (%)						
Female	8 (27.6)	7 (25.9)	5 (18.5)	7 (25.9)	1 (16.7)	28 (24.1)
Male	21 (72.4)	20 (74.1)	22 (81.5)	20 (74.1)	5 (83.3)	88 (75.9)
Race, n (%)						
White	26 (89.7)	25 (92.6)	25 (92.6)	27 (100)	6 (100)	109 (94.0)
American Indian	0	1 (3.7)	0	0	0	1 (0.9)
Asian	1 (3.4)	0	0	0	0	1 (0.9)
Black	2 (6.9)	1 (3.7)	1 (3.7)	0	0	4 (3.4)
Hispanic	0	0	1 (3.7)	0	0	1 (0.9)
Mean weight, kg (SD)	73.4 (18.0)	82.5 (20.3)	75.6 (19.8)	79.0 (17.6)	87.2 (19.4)	78.0 (19.1)
Mean height, cm (SD)	174.2 (10.9)	174.4 (9.6)	173.2 (8.6)	173.1 (8.9)	179.7 (13.1)	174.0 (9.7)
Mean body mass index, kg/m^2 (SD)	23.9 (3.7)	26.9 (5.4)	25.0 (5.0)	26.3 (4.9)	26.9 (5.0)	25.6 (4.9)
Mean body surface area (SD)	1.9 (0.3)	2.0 (0.3)	1.9 (0.3)	1.9 (0.2)	2.1 (0.3)	1.9 (0.3)

SD = standard deviation.

and upper respiratory infection in 1 and unintended pregnancy in 1), 2 of 27 (7.4%) in the 3mg/kg group (1 with dementia and 1 with depression followed by a suicide attempt), and 3 of 27 (11.1%) in the 10mg/kg cohort (1 with diplopia and unconfirmed aseptic meningitis, 1 with diarrhea, and 1 with chest pain). No serious AEs were reported in the 1 (n = 27) and 30mg/kg (n = 6) cohorts. No deaths were reported in this study.

There were no clinically significant changes in electrocardiograms, echocardiograms, audiometry, and eye examinations in the review of changes from baseline in treatment groups compared with placebo groups.

Biological Activity

Results of strength, as measured by MMT, at baseline and end of treatment (week 26), together with the percentage change from baseline, are provided in Table 3. No improvement in total, upper body, or lower body strength was seen for any dystrophy subgroup at any dose. Note that there were fewer subjects completing testing at week 26 in the 10mg/kg dose cohort as a result of the subject discontinuations described in the safety section. Based on several analyses, QMT followed a pattern similar to MMT, without demonstrable improvement. No improvement in QMT was seen for any of the subgroups with muscular dystrophy. Based on the hypothesis that stronger muscle groups might respond better to MYO-029, the QMT scores of muscle groups with MMT scores of grade 4 (4+, 4, 4−) were evaluated in each of the dystrophy subgroups at each dosing level. This approach also failed to demonstrate any improvement in QMT for any of the diseases under study (data not shown).

No improvement in TFTs was seen for any of the groups at any dosing regimen (data not shown). There was also no evidence of perceived improvement via analysis of the SF-36 Physical Health or Mental Health component in the total subject population or in individual disease states.

Percentage change in lean body mass, as measured by DEXA, was -0.07 ± 0.7 in control subjects during the study period. Changes in the 1.0, 3.0, and 10mg/kg cohorts were 0.9 ± 0.9, 2.4 ± 0.7, and 1.4 ± 0.7, respectively. The changes were not significantly different in treatment groups versus placebo groups at the 1.0 and 10mg/kg doses ($p = 0.6427$ and 0.4863, respectively), and approached significance in the 3.0mg/kg group ($p = 0.0514$).

Percentage change in muscle volume of the arms and legs, as measured by MRI, was 0.7 ± 0.8 in control subjects during the study period. Changes in the 1.0, 3.0, and 10mg/kg cohorts were -0.6 ± 0.9, 2.1 ± 1.0, and 1.2 ± 1.1, respectively. These changes were

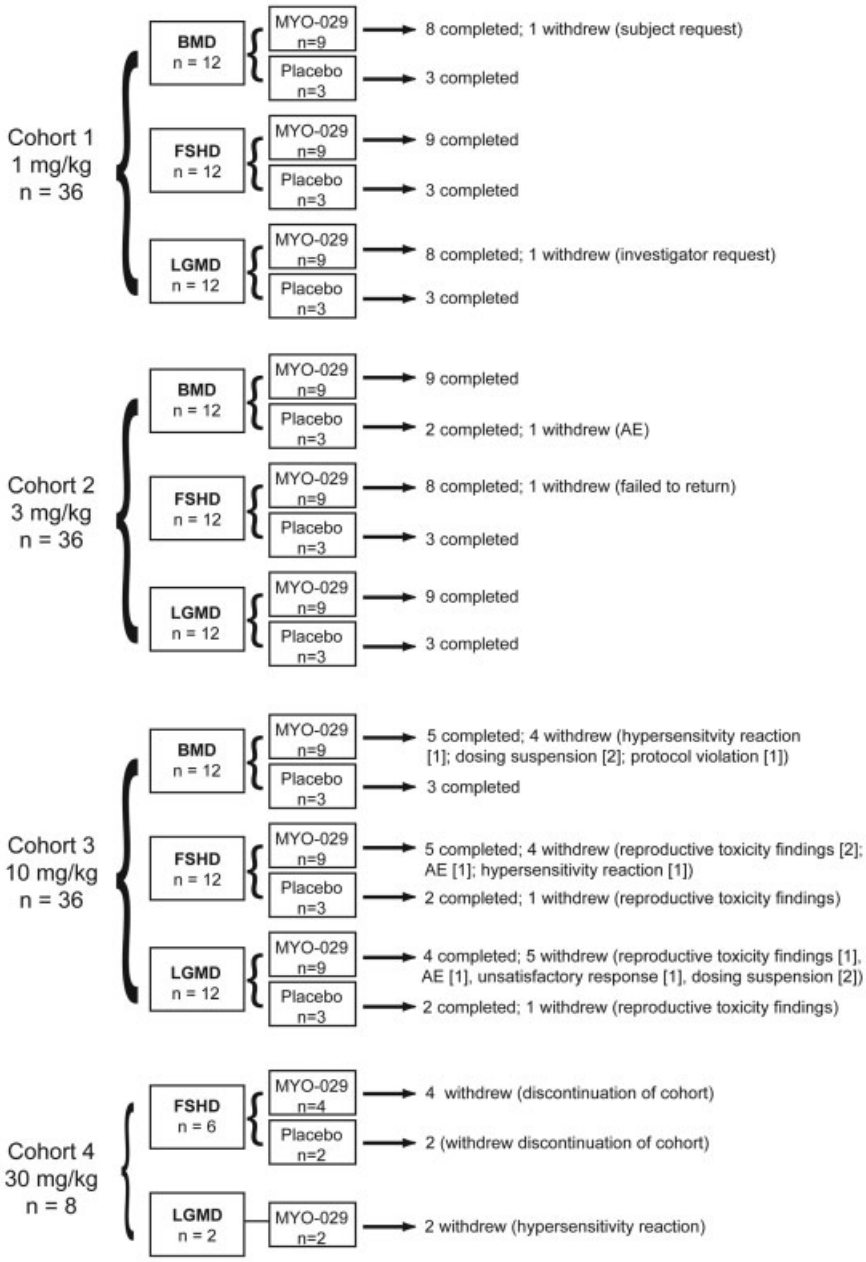

Fig 2. Trial profile shows the breakdown of enrolled subjects by disease, including the total number per group and the number completing the trial. AE = adverse event; BMD = Becker's muscular dystrophy; FSHD = facioscapulohumeral dystrophy; LGMD = limb-girdle muscular dystrophy.

not significantly different from control subjects in any treatment group.

Of the 296 serum myostatin samples received for testing (representing baseline, week 6, and week 36) from 119 subjects, all had a total myostatin concentration within the limits of quantitation (0.147–37.5ng/ml) by the ELISA, whereas 189 samples (64%) had a free myostatin concentration below the lower limit of quantitation reflecting interference in the assay from specific binding of MYO-029 to myostatin in those samples. No observable changes could be detected in total myostatin levels or measurable free myostatin levels in serum (data not shown) at either week 6 or 36 compared with baseline in any group.

Determination of changes in muscle myostatin levels was limited by the number of biopsies received and the level of sensitivity of the ELISA assay. Of the 59 biopsy samples received from 33 subjects, 12 had a total myostatin concentration below the lower limit of quantitation (<44pg/ml), and 42 samples had a free myostatin concentration below the lower limit of quantitation. In subjects for whom total myostatin levels were

Table 2. **Number (%) of subjects experiencing adverse events in descending order of incidence for events occurring in ≥5% of subjects in any group, including the total group (intent-to-treat population)**

		----------MYO-029----------										
	Overall *p*-value	**Placebo n=29 (%)**		**1mg/kg n=27 (%)**		**3mg/kg n=27 (%)**		**10mg/kg n=27 (%)**		**30mg/kg n=6 (%)**		**Total n=116 (%)**	
Any Adverse Event	0.129	28	(96.6)	25	(92.6)	25	(92.6)	22	(81.5)	4	(66.70)	104	(89.7)
Accidental Injury	0.026*	8	(27.6)	13	(48.1)	11	(40.7)	4	(14.8)	0		36	(31.0)
Infection	0.221	6	(20.7)	4	(14.8)	9	(33.3)	3	(11.1)	0		22	(19.0)
Back Pain	0.749	4	(13.8)	6	(22.2)	6	(22.2)	3	(11.1)	1	(16.70)	20	(17.2)
Pain	0.727	5	(17.2)	5	(18.5)	4	(14.8)	2	(7.4)	0		16	(13.8)
Arthralgia	0.547	3	(10.3)	6	(22.2)	4	(14.8)	2	(7.4)	1	(16.70)	16	(13.8)
Dizziness	1.000	4	(13.8)	4	(14.8)	3	(11.1)	4	(14.8)	0		15	(12.9)
Nausea	0.052	1	(3.4)	4	(14.8)	1	(3.7)	7	(25.9)	0		13	(11.2)
Myalgia	0.260	2	(6.9)	6	(22.2)	1	(3.7)	3	(11.1)	0		12	(10.3)
Upper Respiratory	0.267	4	(13.8)	0		4	(14.8)	3	(11.1)	0		11	(9.5)
Diarrhea	0.430	3	(10.3)	5	(18.5)	1	(3.7)	2	(7.4)	0		11	(9.5)
Neck Pain	0.915	2	(6.9)	3	(11.1)	2	(7.4)	1	(3.7)	0		8	(6.9)
Insomnia	0.809	1	(3.4)	2	(7.4)	3	(11.1)	2	(7.4)	0		8	(6.9)
Flue Syndrome	0.097	0		1	(3.7)	4	(14.8)	1	(3.7)	1	(16.70)	7	(6.0)
Dyspepsia	0.541	2	(6.9)	2	(7.4)	3	(11.1)	0		0		7	(6.0)
Pharyngitis	0.080	1	(3.4)	5	(18.5)	1	(3.7)	0		0		7	(6.0)
Sinusitis	0.072	0		1	(3.7)	5	(18.5)	1	(3.7)	0		7	(6.0)
Rash	0.268	2	(6.9)	3	(11.1)	1	(3.7)	0		1	(16.70)	7	(6.0)
Injection Site Pain	0.449	1	(3.4)	3	(11.1)	0		2	(7.4)	0		6	(5.2)
Musculoskeletal	0.599	0		2	(7.4)	2	(7.4)	2	(7.4)	0		6	(5.2)
Cough Increased	1.000	2	(6.9)	2	(7.4)	1	(3.7)	1	(3.7)	0		6	(5.2)
Chest Pain	0.95	1	(3.4)	2	(7.4)	1	(3.7)	1	(3.7)	0		5	(4.3)
Fever	0.95	1	(3.4)	1	(3.7)	1	(3.7)	2	(7.4)	0		5	(4.3)
Injection Site Pain	0.667	1	(3.4)	2	(7.4)	0		2	(7.4)	0		5	(4.3)
Anxiety	0.467	1	(3.4)	3	(11.1)	0		1	(3.7)	0		5	(4.3)
Peripheral Edema	0.811	2	(6.9)	2	(7.4)	1	(3.7)	0		0		5	(4.3)
Hypokalemia	0.796	1	(3.4)	1	(3.7)	2	(7.4)	0		0		4	(3.4)
Dyspenea	0.796	1	(3.4)	2	(7.4)	0		1	(3.7)	0		4	(3.4)
Rhinitis	0.236	1	(3.4)	0		3	(11.1)	0		0		4	(3.4)
Allergic Reaction Other	0.236	1	(3.4)	3	(11.1)	0		0		0		4	(3.4)
Depression	0.346	0		2	(7.4)	0		2	(7.4)	0		4	(3.4)
Muscle Spasms	0.566	0		1	(3.7)	2	(7.4)	1	(3.7)	0		4	(3.4)
Abdominal Pain	0.142	0		1	(3.7)	0		1	(3.7)	1	(16.70)	3	(2.6)
Vasodilation	0.422	0		1	(3.7)	0		2	(7.4)	0		3	(2.6)
Muscle Cramp	0.198	1	(3.4)	1	(3.7)	0		0		1	(16.70)	3	(2.6)
Sinus Congestion	0.542	1	(3.4)	0		2	(7.4)	0		0		3	(2.6)
Urticaria	0.076	0		0		0		3	(11.1)	0		3	(2.6)
Migraine	0.542	1	(3.4)	2	(7.4)	0		0		0		3	(2.6)
Allergic Reaction	0.259	0		2	(7.4)	0		0		0		2	(1.7)
Pruritic Rash	0.075	0		0		0		1	(3.7)	1	(16.70)	2	(1.7)
Taste Loss	0.259	0		0		0		2	(7.4)	0		2	(1.7)
Urine Abnormality	0.259	0		0		2	(7.4)	0		0		2	(1.7)
Hypotonia	0.259	0		0		2	(7.4)	0		0		2	(1.7)
Hernia	0.259	0		0		0		2	(7.4)	0		2	(1.7)
Constipation	0.259	0		0		2	(7.4)	0		0		2	(1.7)
Tachycardia	0.052	0		0		0		0		1	(16.70)	1	(0.9)
Hyperlipemia	0.052	0		0		0		0		1	(16.70)	1	(0.9)

p-Value: Fisher exact test (2 tailed). *$p<0.05$

detectable in both predose and postdose samples, the high variability and low sample number precluded detection of meaningful changes in myostatin levels relative to baseline for all treatment groups.

Based on 26 available pairs of biopsy specimens, treatment with MYO-029 had no observable adverse effect on muscle pathology as assessed by standard histological analysis. Specifically, there was no change in inflammation or fiber necrosis in treated versus untreated subjects. There was also no significant change in fibrosis, fiber regeneration, or the percentage of central nucleated fibers, a marker of degeneration and subsequent regeneration of muscle fibers.

Morphometric analysis demonstrated a dose-dependent increase in fiber size diameter. Muscle fiber diameter after treatment, expressed as percentage of baseline, is shown in Figure 3. Increased muscle fiber diameters were seen in the 10 (median = +15.2%

Table 3. **Strength by Manual Muscle Testing (Average of All Muscles Tested)**

Diagnosis	Treatment	Baseline Mean (SE)	Week 26 Mean (SE)	Week 26 Mean Percentage Change (SE)	Adjusted p[a]
BMD	Placebo	8, 3.29 (0.29)	8, 3.59 (0.17)	8, 0.66 (1.41)	
	MYO-029 1.0mg/kg	8, 3.90 (0.10)	8, 3.97 (0.11)	8, 3.67 (1.23)	0.5503
	MYO-029 3.0mg/kg	9, 3.81 (0.12)	9, 3.89 (0.15)	9, 3.99 (2.54)	0.4490
	MYO-029 10.0mg/kg	5, 3.77 (0.15)	5, 3.84 (0.20)	5, 2.45 (1.97)	0.8871
FSHD	Placebo	8, 3.70 (0.05)	8, 3.74 (0.07)	8, 2.87 (1.33)	
	MYO-029 1.0mg/kg	9, 3.70 (0.08)	9, 3.73 (0.13)	9, 1.99 (2.71)	0.9894
	MYO-029 3.0mg/kg	8, 3.88 (0.12)	8, 3.91 (0.14)	8, 2.22 (1.67)	0.9959
	MYO-029 10.0mg/kg	5, 3.87 (0.15)	5, 3.99 (0.20)	5, 5.28 (5.31)	0.8896
LGMD	Placebo	8, 3.15 (0.32)	8, 3.43 (0.12)	8, 2.25 (2.77)	
	MYO-029 1.0mg/kg	8, 3.74 (0.13)	8, 3.75 (0.10)	8, 2.00 (1.87)	0.9997
	MYO-029 3.0mg/kg	9, 3.30 (0.17)	9, 3.25 (0.15)	9, −0.40 (1.86)	0.7325
	MYO-029 10.0mg/kg	5, 3.44 (0.27)	5, 3.45 (0.34)	5, 0.09 (2.93)	0.8847
Total	Placebo	24, 3.38 (0.15)	24, 3.59 (0.07)	24, 1.93 (1.09)	
	MYO-029 1.0mg/kg	25, 3.78 (0.06)	25, 3.81 (0.07)	25, 2.53 (1.17)	0.9746
	MYO-029 3.0mg/kg	26, 3.65 (0.09)	26, 3.67 (0.10)	26, 1.93 (1.21)	1.0000
	MYO-029 10.0mg/kg	15, 3.69 (0.12)	15, 3.76 (0.15)	15, 2.61 (2.05)	0.9763

[a] p values represent the comparison of group means with the placebo group mean via Dunnett's test. The resultant p values reflect an adjustment for multiple comparisons of means with the placebo group mean.
SE = standard error; BMD = Becker muscular dystrophy; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb-girdle muscular dystrophy.

change from baseline) and 3mg/kg groups (+14.4%) compared with the 1mg/kg treatment (−0. 93%) and placebo groups (+2.7%). The sample sizes in each group are small (see Fig 3), and these differences did not reach statistical significance.

Discussion

Few therapeutic trials have been conducted in adults with muscular dystrophy. Small clinical trials in FSHD have included glucocorticoid steroids (prednisone),[24] β-adrenergic agonists (albuterol),[3,29] and most recently, calcium-channel blockade (diltiazem).[2] Therapeutic studies of adult BMD have been limited to inclusion of a few subjects in a Phase I study of dystrophin plasmid-based gene therapy in DMD/BMD[31] and in a study of creatine monohydrate in multiple muscular dystrophies.[5] LGMD includes more than 15 distinct diseases, some of which have been studied with glucocorticoid steroids and creatine.[5,31–33] None of these studies has resulted in the accepted use of a therapeutic agent in adult muscular dystrophy.

In this first-ever study of a myostatin inhibitor, the primary objective was safety. MYO-029 was well tolerated in a diverse group of muscular dystrophies with varying pathogenic mechanisms. No target-related side effects were identified; that is, no side effects to skeletal, smooth, or cardiac muscle were found. The most significant agent-related AEs were hypersensitivity skin reactions. Urticaria was seen in three subjects in the 10mg/kg cohort. Immune-mediated side effects, such as hypersensitivity reactions, are anticipated in biological agents and account for the majority of type B reactions, unrelated to pharmacological activity of the drug.[34] Hypersensitivity reactions to MYO-029 limited dose escalation to more than 10mg/kg and represent a potential restrictive factor in achieving efficacy with MYO-029.

This trial also examined muscle mass and strength as measures of biological activity of MYO-029, without intent to address the specific pathophysiology of the various muscular dystrophies. These exploratory outcome measures were found to be feasible in all disease populations studied. No increase in strength by MMT or QMT or improvement in function by TFTs could be demonstrated in this 9-month trial of MYO-029 (6 months of dosing, 3 months of follow-up).

Because statistically significant changes were not observed for MMT or QMT, the investigators performed a retrospective power analysis for a small subset (n = 24) of MMT and QMT measurement by disease co-

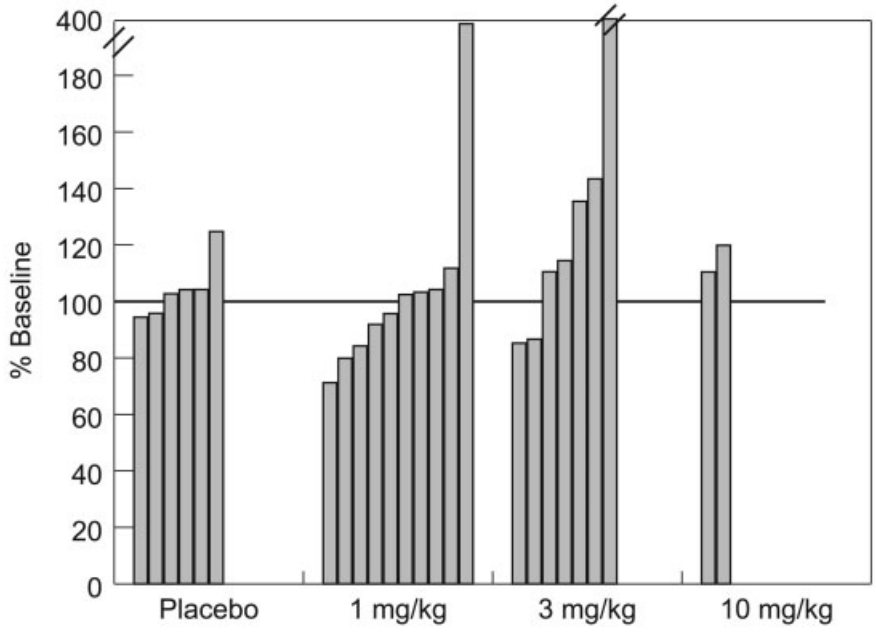

Fig 3. Muscle fiber diameters show the percentage change in muscle fiber diameter before and after treatment. Each vertical bar represents one patient. There was an increase in muscle fiber diameters in the 10 (median = +15.2% change from baseline) and 3mg/kg groups (+14.4%) compared with the 1mg/kg treatment (−0.93%) and placebo groups (+2.7%). A trend toward larger fibers with increasing dose (differences did not reach statistical significance) is shown; only two patients in the 10mg/kg group had muscle biopsies.

hort combinations (eg, the MMT measurement for upper muscles in BMD, FSHD, LGMD, and combined cohorts) to develop some intuition about the optimal statistical testing conditions for comparing various groups. Statistical power was calculated based on the differences observed between the three MYO-029 dose group means and the placebo-treated patients' mean response, and at the same level of overall statistical significance as used in the analysis of the MMT and QMT data. Amongst these calculations, the maximum power for any comparison (adjusted for multiplicity of testing) was approximately 47%. Typically, the power for efficacy in a clinical trial is at least 80%. The results of these calculations are not surprising as a previously published report of a prospective, quantitative study of the natural history of FSHD estimated that a two-armed clinical trial with a power of 80% to detect arrest of disease progression after 1 year would require 160 subjects in each arm.[21]

Although an improvement in strength and function were not demonstrated, biological activity in another sphere was suggested by findings in some subjects. Muscle mass, as estimated by lean mass by DEXA, was found to increase by approximately 2.4% in the 3mg/kg cohort, which was statistically significant from control subjects in BMD subjects and approached significance in all treated subjects. Muscle mass alterations during the study period, as determined by MRI, were not significant in treated subjects versus control subjects. Muscle from placebo-treated subjects had stable fiber diameters in the pretreatment and posttreatment periods, whereas there was a dose-dependent increase in fiber diameter in the 3 and 10mg/kg cohorts. Sample sizes were small in all of the above analyses, and differences between populations did not reach statistical significance. However, the consistency of the response to treatment in the various measures of effects on muscle tissue suggest that MYO-029 reached its intended target, producing a modest degree of muscle fiber hypertrophy and increased muscle mass in some treated subjects.

Considering the duration of this treatment trial, it is not unexpected that strength is stable in adults with muscular dystrophy. As stated previously, the ability to detect arrest of disease progression or minimal improvements in strength would require larger sample sizes in a study designed to look at efficacy. The MYO-029 study was a safety trial, not originally designed to detect efficacy: The sample sizes chosen are not optimal for detecting statistically significant changes between MYO-029– and placebo-treated patients.

In conclusion, MYO-029 is a neutralizing antibody to myostatin that had good safety and tolerability with the exception of cutaneous hypersensitivity, especially in higher dose cohorts. This trial supports the hypothesis that systemic administration of myostatin inhibitors provides an adequate safety margin for clinical studies, and these inhibitors should be evaluated for

stimulating muscle growth in muscular dystrophy. Multiple pharmaceutical companies are evaluating other myostatin inhibitors for a variety of disorders including cachexia and sarcopenia. Further evaluation of more potent myostatin inhibitors for primary muscle disorders should be considered.

Disclosure

A.P. received compensation from Wyeth Pharmaceuticals (Collegeville, PA) to his laboratory for histological analysis. T.A., E.R.L., and J.M.W. are employed by Wyeth Pharmaceuticals (Cambridge, MA). R.A. and S.A.P. are employed by Wyeth Pharmaceuticals (Collegeville, PA). K.M. is a consultant for Wyeth Pharmaceuticals (Collegeville, PA). Under a licensing agreement between MetaMorphix and Johns Hopkins University, the university is entitled to royalty payments on sales of the growth factor, myostatin, described in this article. The university also is entitled to a share of sublicensing income from arrangements between MetaMorphix and Wyeth. The university owns MetaMorphix stock, which is subject to certain restrictions under university policy. The terms of this arrangement are being managed by Johns Hopkins University in accordance with its conflict of interest policies.

Appendix

The following people participated in this study (by site): Brigham and Women's Hospital—Ronan Walsh, MD, Lisa Krivickas, MD, Kristen McIntosh, MPH, Kristen Whiteside (Study Coordinator), and Merideth Donlan, DPT; Children's National Medical Center—Robert Leshner, MD, Paola Canelos (Study Coordinator), Katherina Parker, MSPT, PCS, and Marissa Bartczak, MSPT; Columbus Children's Research Institute—Roula al-Dahhak, MD, Karen Downing, and Cheryl Wall, RN; Johns Hopkins University School of Medicine—Leigh Warsing (Senior Laboratory Technician), Ronald Cohn, MD, PhD, Daniel B. Drachman, MD, Regina Brock-Simmons (Study Coordinator), Molly Sprung (Study Coordinator), and Hejab Imteyaz (Clinical Evaluator); University of Kansas Medical Center—April McVey, MD, Arthur Dick, MD, Victoria Watts, RN (Study Coordinator), and Laura Herbelin, BS; University of Newcastle Upon Tyne—Jane Barnes, SRN, Penny Garrood, MBChB, Michelle McCallum, and Sarah Russell, SRN; University of Rochester Medical Center–Colleen Donlin-Smith, MA (Study Coordinator), and Deborah Whalen PT, DPT, MHS; University of Texas Southwestern Medical Center—Sharon Nations, MD, Nina Gorham, MA, CCRP, Cindy Wynne-Jones, RN, and Rhonda McLin, PTA; University of Utah Hospital—Jacinda Sampson, MD, PhD, Cade Walker (Study Coordinator), Kim Hart, MS (Study Coordinator), Justine Bagley (Study Coordinator), and Eduard Gappmaier, PT, PhD; Washington University School of Medicine—Charlie Wulf, BA (Study Coordinator), Jeanine Schierbecker, PT, MHS (Study Coordinator), Betsy Malkus, PT, MHS, and Catherine Seiner, PT, MHS, GCS; Wyeth Pharmaceuticals—Christopher Corcoran, BS, Lisa A. Collins-Racie, MS, Stephen Bradley Forlow, PhD, Riyez Karim, BSc, and Lioudmila Tchistiakova, PhD.

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