

## ORIGINAL ARTICLE

# Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy

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## ABSTRACT

**BACKGROUND**

Local intramuscular administration of the antisense oligonucleotide PRO051 in patients with Duchenne's muscular dystrophy with relevant mutations was previously reported to induce the skipping of exon 51 during pre-messenger RNA splicing of the dystrophin gene and to facilitate new dystrophin expression in muscle-fiber membranes. The present phase 1–2a study aimed to assess the safety, pharmacokinetics, and molecular and clinical effects of systemically administered PRO051.

**METHODS**

We administered weekly abdominal subcutaneous injections of PRO051 for 5 weeks in 12 patients, with each of four possible doses (0.5, 2.0, 4.0, and 6.0 mg per kilogram of body weight) given to 3 patients. Changes in RNA splicing and protein levels in the tibialis anterior muscle were assessed at two time points. All patients subsequently entered a 12-week open-label extension phase, during which they all received PRO051 at a dose of 6.0 mg per kilogram per week. Safety, pharmacokinetics, serum creatine kinase levels, and muscle strength and function were assessed.

**RESULTS**

The most common adverse events were irritation at the administration site and, during the extension phase, mild and variable proteinuria and increased urinary  $\alpha_1$ -microglobulin levels; there were no serious adverse events. The mean terminal half-life of PRO051 in the circulation was 29 days. PRO051 induced detectable, specific exon-51 skipping at doses of 2.0 mg or more per kilogram. New dystrophin expression was observed between approximately 60% and 100% of muscle fibers in 10 of the 12 patients, as measured on post-treatment biopsy, which increased in a dose-dependent manner to up to 15.6% of the expression in healthy muscle. After the 12-week extension phase, there was a mean ( $\pm$ SD) improvement of  $35.2\pm 28.7$  m (from the baseline of  $384\pm 121$  m) on the 6-minute walk test.

**CONCLUSIONS**

Systemically administered PRO051 showed dose-dependent molecular efficacy in patients with Duchenne's muscular dystrophy, with a modest improvement in the 6-minute walk test after 12 weeks of extended treatment. (Funded by Prosensa Therapeutics; Netherlands National Trial Register number, NTR1241.)

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**D**UCHENNE'S MUSCULAR DYSTROPHY IS an X-linked recessive muscle disorder, affecting 1 in 3500 newborn boys.<sup>1</sup> Patients have severe, progressive muscle wasting, leading to early death.<sup>2,3</sup> The disease is caused by mutations in the dystrophin gene (*DMD*),<sup>4,5</sup> leading to disruption of the open reading frame, dystrophin deficiency at the myofiber membrane, and continued fiber degeneration.<sup>6-8</sup> Mutations in the same gene cause Becker's muscular dystrophy, but the open reading frame is maintained, permitting the production of semifunctional dystrophin proteins and a typically milder phenotype and longer life span.<sup>6-9</sup>

A promising therapeutic strategy involves antisense oligonucleotides that induce specific exon skipping during pre-messenger RNA (mRNA) splicing,<sup>10</sup> aimed at reading-frame correction and production of transcripts like those in patients with Becker's muscular dystrophy.<sup>11</sup> Although the functionality of the resulting protein may vary, this treatment could delay or even stop disease progression and improve function in the remaining muscle.<sup>12,13</sup> The antisense oligonucleotides are chemically modified to resist nucleases and promote RNA binding and are designed to have high sequence specificity. In studies in the *mdx* mouse model, oligonucleotides with chemical properties similar to those of 2'-*O*-methyl phosphorothioate RNA were taken up in dystrophin-deficient muscle up to 10 times as much as in healthy muscle tissue, most likely owing to increased permeability of the muscle myofiber membrane.<sup>14</sup> In addition, 4 to 8 weeks' subcutaneous delivery of the oligonucleotides resulted in a steady increase in oligonucleotide levels, exon skipping, and dystrophin levels.<sup>14</sup>

Exon skipping provides a mutation-specific, and thus potentially personalized, therapeutic approach for patients with Duchenne's muscular dystrophy. Since mutations cluster around exons 45 to 55 of *DMD*, the skipping of one specific exon may be therapeutic for patients with a variety of mutations. The skipping of exon 51 affects the largest subgroup of patients (approximately 13%), including those with deletions of exons 45 to 50, 48 to 50, 50, or 52.<sup>15</sup>

PRO051, a 2'-*O*-methyl phosphorothioate oligoribonucleotide that induces exon 51 skipping, was previously tested in patients with Duchenne's muscular dystrophy by means of local intramuscular administration of a single dose.<sup>16</sup> The com-

pound produced sarcolemmal dystrophin in 64 to 97% of myofibers. The amount of dystrophin ranged from 17 to 35% of control levels. The current dose escalation and follow-up extension study assessed the safety, tolerability, pharmacokinetics, and molecular and clinical effects of subcutaneously administered PRO051.

## METHODS

### PATIENTS

We recruited patients with Duchenne's muscular dystrophy who were 5 to 16 years of age and had mutations that could be corrected by means of inducing exon 51 skipping. Inclusion and exclusion criteria were similar to those in the previous study.<sup>16</sup> Briefly, patients with no evidence of dystrophin in 5% or more of fibers on previous diagnostic muscle biopsy were eligible to participate in the study. Concurrent glucocorticoid treatment was permitted. Eligibility criteria also included an estimated life expectancy of 6 months or more, no serious preexisting medical conditions, and no dependency on assisted ventilation (or a forced expiratory volume in 1 second or forced vital capacity of 60% or less of the predicted value). Additional details are given in the Supplementary Appendix (available with the full text of this article at NEJM.org). Written informed consent was obtained from all patients over 12 years of age or, for younger patients, from their parents.

### STUDY DESIGN

In this open-label, dose-escalation, phase 1-2a study, 12 patients were to receive weekly abdominal subcutaneous injections of PRO051 (from 0.5 to 10 mg per kilogram of body weight, with 3 patients receiving each dose) for 5 weeks. The specific increases in dose were determined after analysis of safety and dystrophin levels in muscle-biopsy specimens. Since early increases in dystrophin levels were observed in patients receiving 0.5, 2.0, and 4.0 mg per kilogram of body weight (3 patients in each dose cohort), the maximum study dose was set at 6.0 mg per kilogram of body weight (which was the dose the last cohort of 3 patients received).

Assessments of safety (the primary outcome) and pharmacokinetics and molecular and clinical effects (secondary outcomes) were made at regular intervals. Tibialis anterior muscle biopsy was performed at baseline and 2 weeks after the last

dose of PRO051 in the 0.5-mg group and at 2 and 7 weeks after the last dose in the three other groups. After an interval of 6 to 15 months after the last dose, each patient restarted treatment at 6.0 mg per kilogram of body weight per week, with close monitoring of safety and clinical-efficacy measures. The current report includes data through 12 weeks of restarted treatment (with biopsy not conducted at 12 weeks). No formal statistical testing was performed, owing to the small number of patients. Data are presented for individual patients and are also summarized.

The study was sponsored by Prosensa Therapeutics (Leiden, the Netherlands) and performed in compliance with Good Clinical Practice guidelines, the provisions of the Declaration of Helsinki, the European Directive 2001/20/EC, and local regulations in Belgium and Sweden. The studies were approved by the local independent ethics committees and authorized by the Competent Authorities of Belgium and Sweden. The study was conducted in accordance with the protocol (available at NEJM.org). All authors contributed to the study design, participated in the collection and analysis of the data, had complete and free access to the data, jointly wrote the manuscript, and vouch for the completeness and accuracy of the data and analyses presented.

#### STUDY DRUG

The antisense oligonucleotide PRO051 (GSK2402968) (5'-UCAAGGAAGAUGGCAUUUCU-3') with full-length 2'-O-methyl-substituted ribose moieties and phosphorothioate internucleotide linkages,<sup>16</sup> was provided in 0.5-ml glass vials in sodium phosphate-buffered saline (100 mg per milliliter). Non-clinical safety data are provided in the Supplementary Appendix.

#### SAFETY AND TOLERABILITY

Safety was monitored as described previously.<sup>16</sup> Changes in aspartate aminotransferase and alanine aminotransferase levels were interpreted in relation to changes in creatine kinase levels for the evaluation of hepatotoxicity. Urine was monitored for  $\alpha_1$ -microglobulin, proteinuria, and hematuria. Creatinine clearance was not measured, since plasma creatinine levels change with changes in muscle mass in patients with Duchenne's muscular dystrophy. Complement activation, coagulation profiles, and inflammatory responses were monitored. For detection of putative immuno-

globulin G antibodies against dystrophin, serum samples obtained 120 days before and 120 days after treatment were analyzed.<sup>17</sup>

#### PHARMACOKINETIC ASSESSMENTS

We assessed plasma levels of PRO051 during the dose-escalation phase of the study, by using a validated hybridization ligation assay adapted from Yu and colleagues<sup>18</sup> (see the Supplementary Appendix).

#### ASSESSMENTS OF RNA AND PROTEIN

Details of the RNA and protein analyses are given in the Supplementary Appendix. To detect exon-51 skipping, total RNA was isolated from 10 to 15 mg of muscle tissue and analyzed by means of reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay and sequencing, as reported previously.<sup>16,19,20</sup> For detection of new dystrophin expression, immunofluorescence analysis of serial 8- $\mu$ m cross sections and Western blot analysis of total protein extracts isolated from 20 to 30 mg of muscle tissue were performed according to methods described previously.<sup>16,20</sup>

#### CLINICAL ASSESSMENTS

Muscle strength assessments included both quantitative testing of 10 muscle groups according to the quantitative measuring system of the Cooperative International Neuromuscular Research Group<sup>21,22</sup> and manual testing according to the averaged Medical Research Council score of 34 muscle groups. In addition, timed functional tests (10-m walk, 4-stair climb, and time to rise from floor), the 6-minute walk test, and pulmonary-function tests were performed.

## RESULTS

#### PATIENTS

Twelve patients were prescreened with the use of an in vitro cell-based PRO051 assay.<sup>16</sup> The specific mutation and a positive response to PRO051 were confirmed by means of RNA and sequence analysis. The 12 patients had a mean age of 9.2 years (range, 5 to 13). All 12 met the inclusion criteria, received PRO051 treatment, completed the dose-escalation phase, and entered the extension phase. For 7 of the 12 patients, a prestudy diagnostic biopsy was available, showing less than 5% "revertant" (dystrophin-positive) muscle fibers. Baseline characteristics of the 12 patients are presented in

**Table 1. Adverse Events That Occurred in More Than 2 Patients during the 12-Week Extension Phase.**

Event	No. of Patients
Proteinuria	12
Elevated urinary $\alpha_1$ -microglobulin levels	11
Injection site	
Erythema and inflammation	9
Hematoma or bruising	6
Tenderness	5
Irritation or itching	3
Moderate pain during injection	4
Common cold	4
Gastroenteritis	4
Pain*	3

\* Pain was in the stomach in 1 patient, in the foot in 1, and in the arm after immunization in 1.

Table 1 in the Supplementary Appendix. All patients had been receiving a stable dose of glucocorticoids for at least 1 year at the time of enrollment.

#### SAFETY AND ADVERSE EVENTS

No patients withdrew from the dose-escalation or extension phases of the study, and no serious adverse events were reported. After the 12 weeks of extended treatment with PRO051 (6.0 mg per kilogram of body weight per week in all 12 patients), a total of 120 adverse events of mild or moderate intensity were reported. The most common events (Table 1) considered to be definitely or probably causally related to the study drug were mild reactions at the injection site and increased urinary  $\alpha_1$ -microglobulin levels. Proteinuria, defined as a protein level above the upper limit of the normal range of 0.15 g per liter, was observed in all 12 patients (mean [ $\pm$ SD] protein level,  $0.078 \pm 0.038$  at baseline and  $0.206 \pm 0.119$  at week 12 of the extension phase). This may represent an adaptive process within muscle tubules, which may absorb oligonucleotides; thus, this finding warrants further monitoring. Pain in the lower leg, exanthema, dry skin, and stomach pain were also reported. None of these events led to changes in the injection schedule or treatment discontinuation.

No clinically significant changes were observed on physical examination, in vital signs, or on electrocardiograms, as compared with baseline data. No drug-related decreases in platelet counts or prolonged activated partial-thrombo-

plastin time values were observed. None of the patients showed liver-enzyme changes suggesting hepatotoxicity. No dystrophin antibodies were detected in serum samples.

#### PHARMACOKINETIC PROFILE

PRO051 was rapidly absorbed and distributed, with peak levels occurring between 2 and 3 hours after administration (Fig. 1A and 1B in the Supplementary Appendix) and a decline in plasma levels to less than 15% of the maximal level observed at 24 hours. In contrast to peak plasma levels, the predosing trough levels increased with increasing numbers of injections, as anticipated.<sup>23,24</sup> The overall terminal plasma half-life, as ascertained over the 13-week period after the end of the 5-week dose-escalation phase, ranged from 19 to 56 days (geometric mean, 29 days) (Fig. 1C in the Supplementary Appendix).

#### EFFECTS ON RNA

Muscle-biopsy samples were analyzed at 2 weeks and 7 weeks after the end of the dose-escalation phase. No effect of PRO051 on RNA level was detected in any of the three patients receiving a dose of 0.5 mg per kilogram of body weight (Fig. 1A). In the higher-dose cohorts, however, exon-51 skipping was observed at both time points in one patient receiving 2.0 mg per kilogram of body weight (Fig. 1B) and in all six patients receiving 4.0 or 6.0 mg per kilogram of body weight, albeit at variable levels (Fig. 1C and 1D). Exon-51 skipping was still detectable in these seven patients at 7 weeks after the dose-escalation phase. The specificity of exon-51 skipping was confirmed by means of sequence analysis. No unanticipated drug-induced splicing events were detected in overlapping RT-PCR fragments throughout the full-length DMD transcript.

#### EFFECTS ON PROTEIN EXPRESSION

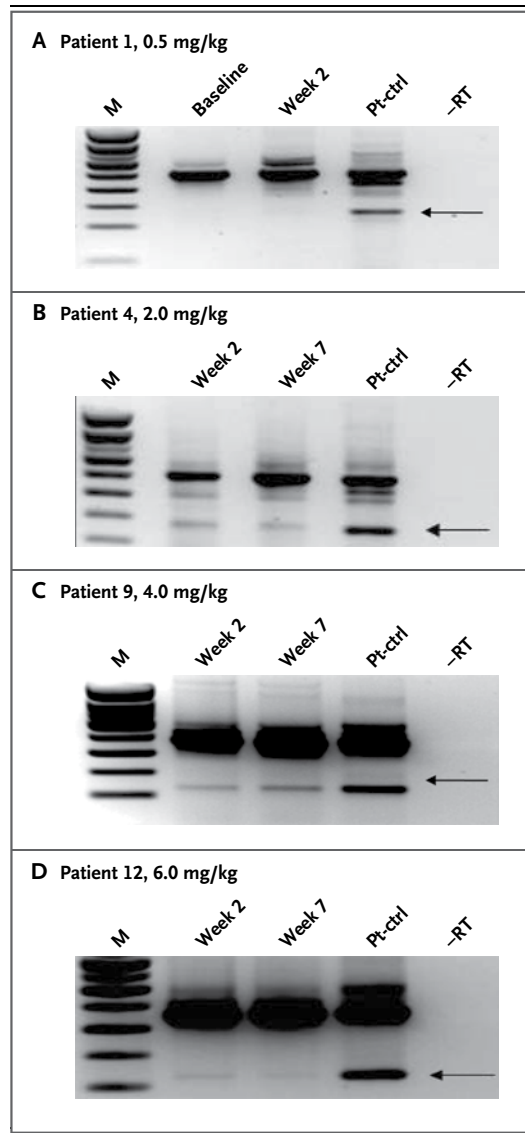
Essentially no dystrophin expression was observed on immunofluorescence analysis of muscle-tissue sections obtained at baseline in the group receiving 0.5 mg per kilogram of body weight, although two patients showed a few dystrophin-positive ("revertant") fibers<sup>25-27</sup> (Fig. 2A). In all three patients in this group, new dystrophin expression was first observed at 2 weeks after the end of treatment, with 20 to 88% of fibers positive for dystrophin and slightly higher dystrophin signal intensities than seen in baseline samples (Table 2).

**Figure 1. Effect of PRO051 on RNA Processing at 2 Weeks and 7 Weeks after the Last Administration in the Dose-Escalation Phase.**

Results of reverse-transcriptase (RT)–polymerase-chain-reaction (PCR) analysis of RNA isolated from muscle-biopsy specimens from the patients are shown for one patient per dose cohort: Patient 1 (Panel A) and Patient 4 (Panel B), whose mutations result in the deletion of exon 52 in the dystrophin gene (*DMD*); and Patient 9 (Panel C) and Patient 12 (Panel D), whose mutations result in the deletion of exons 45 to 50 in *DMD*. A positive control specific to each patient was derived from the *in vitro* PRO051 prescreening procedure (Pt-ctrl). The arrows indicate the transcript fragment anticipated if exon 51 were skipped during splicing in the given patient. In the negative-control samples, no reverse transcriptase was added (–RT). DNA size-marker samples (M) are also shown. Because of the small amount of the patients' samples, high-sensitivity PCR conditions were used, which renders inaccurate the quantification of skipping efficiencies.

Both the average number of dystrophin-positive fibers and the average dystrophin signal intensity increased with increasing dose, with similar values at 2 weeks and 7 weeks after treatment (Fig. 2A and Table 2). The proportions of dystrophin-expressing fibers were between 80 and 100% in six patients and between 56 and 75% in four others; the remaining two patients had muscle-biopsy specimens of relatively poor quality, in which only up to 20% of positive fibers were seen, which hindered accurate dystrophin signal detection. The average dystrophin signal intensity was highest in the groups receiving 4.0 mg per kilogram and 6.0 mg per kilogram at 2 weeks after the end of treatment, with a maximal signal of 15.6% of that observed in a control sample (Table 2). Plotting the average of the dystrophin signal intensities (vs. control), detected in the three patients per cohort, pooled per visit (baseline, week 2 or week 7 post-treatment), showed a dose-dependent effect of PRO051 (Fig. 2B).

Immunofluorescence findings were confirmed by analyses of total muscle-protein extracts on Western blotting (Fig. 2B and 2C and Table 2). In biopsy specimens from patients receiving 0.5 mg per kilogram of body weight, low levels of dystrophin were detected at baseline (Fig. 2C), consistent with the small numbers of dystrophin-positive (“revertant”) fibers visualized on immunofluorescence analyses for two patients. No increase in dystrophin levels was observed in either of these patients at 2 weeks after the



last dose of PRO051 during the escalation phase. In the higher-dose groups, the dystrophin-signal intensities at 2 weeks and 7 weeks after the last dose during the escalation phase were typically greater than the average intensity among the three baseline specimens from the lowest-dose group (Fig. 2C). Quantitative analysis of signal intensity, normalized for the variable levels of muscle-fiber content (as represented by dysferlin levels), suggested that the patients receiving the two highest doses of PRO051 (4.0 and 6.0 mg per kilogram) had dystrophin expression that was 1.5 times to 8.2 times greater, respectively, than baseline levels (i.e., the average signal intensity of 2.5 with the dose of 0.5 mg per kilogram)

(Table 2). Plotting the dystrophin-signal intensities detected on average in the three patients per group per visit indicated a dose-dependent effect of PRO051 on dystrophin expression (Fig. 2B), similar to findings from immunofluorescence studies.

#### CLINICAL FINDINGS

In the dose-escalation phase, 5 weeks of treatment with PRO051 resulted in increased dystrophin levels but did not induce clear, clinically relevant differences in muscle strength, timed functional tests, and pulmonary-function tests, either between or within the dose groups. The average distance walked in 6 minutes (Table 2 and Fig. 3A), the distance walked per minute, and creatine kinase levels were variable, consistent with historical data for the age group of our patients.<sup>29</sup> However, after 12 weeks of treatment in the extension phase, there was improvement in the distance walked in 6 minutes (mean [ $\pm$ SD] improvement, 35.2 $\pm$ 28.7 m); three patients (Patients 1, 2, and 7) showed an improvement of 65 m or more (Table 2 and Fig. 3B). This contrasts with the mean 37-m decrease in the 6-minute walking distance seen between the start of the dose-escalation study and the start of the extension study (time interval, 6 to 15 months) and to the expected decline in these patients during this study period (based on a previous report of a decline of 115 m over a total of 52 weeks in a 7-year period<sup>30</sup>). No increase in specific muscle force was observed. There was minimal effect on serum levels of creatine kinase, but the sample size was small, and the clinical disease stage was heterogeneous.

#### DISCUSSION

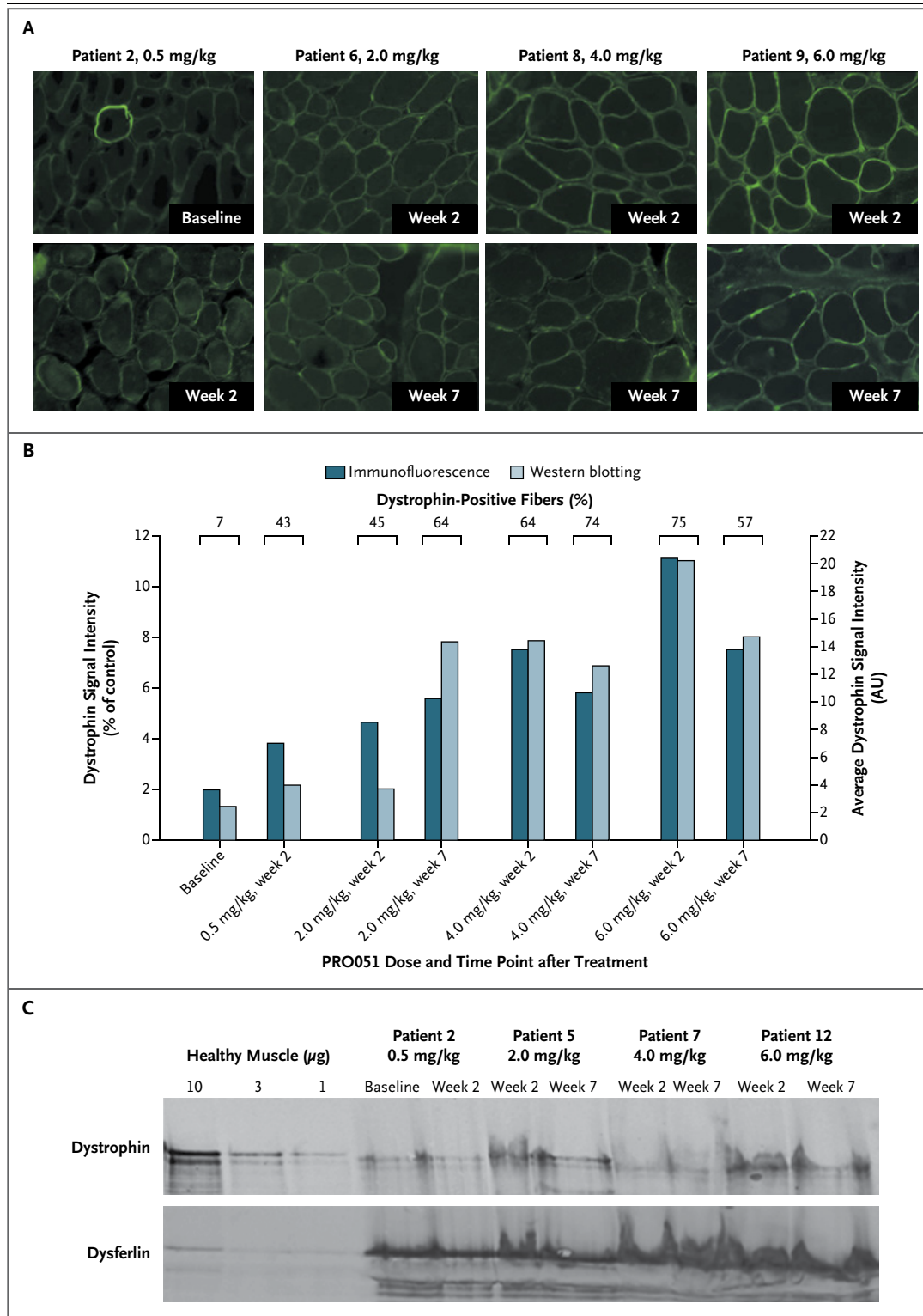
Our phase 1–2a study, consisting of a dose-escalation phase and a follow-up extension phase, reports the molecular and clinical effects of systemic weekly administration of an antisense oligonucleotide in patients with Duchenne's muscular dystrophy. No serious adverse events were reported, but receipt of PRO051 was associated with local skin reactions of mild to moderate intensity, although none led to treatment discontinuation. All patients had elevated urinary  $\alpha_1$ -microglobulin levels by week 12 of therapy, and all had variable proteinuria. However, given the decreased muscle mass in these patients, urinary protein:creatinine ratios are difficult to assess.

#### Figure 2 (facing page). Effect of PRO051 on Dystrophin Expression Levels.

Panel A shows the results of immunofluorescence analysis involving staining with the MANDYS106 dystrophin antibody,<sup>28</sup> with increased dystrophin expression found in the membranes of the muscle fibers after treatment with PRO051 in all patients (only one patient per group is shown here). Few dystrophin-positive "revertant" fibers<sup>25–27</sup> were observed in biopsy specimens obtained at baseline, illustrated here for Patient 2. Panel B shows the dystrophin signal intensity in cross sections of muscle-tissue specimens, averaged for the three patients in each dose group at each time point after the end of treatment. Intensities are shown in two ways: as measured by the percentage of the control value (set at 100%) for the immunofluorescence analysis (after correction for the phosphate-buffered saline, with results also averaged across three to six nonoverlapping images per cross section) and as measured with the use of Western blotting of protein extracts after normalization for the varying density and quality of muscle fiber (as indicated by the signal intensities for reference protein dysferlin, calculated against average baseline sample intensities and reported in arbitrary units [AU]). In addition, the percentage of dystrophin-positive fibers averaged across three to six nonoverlapping images per cross section is indicated across the top of the graph. Panel C (top) shows results of Western blot analysis (involving the dystrophin monoclonal antibody NCL-DYS1) of total protein extracts (300 to 500  $\mu$ g loaded, depending on tissue quality) isolated from the patients' biopsy specimens (with results shown for one patient per dose). Patient 2 has a positive dystrophin signal in the baseline biopsy specimen, consistent with the presence of few revertant fibers. Panel C (top) also shows, for comparison, blotting of 1 to 10  $\mu$ g of total protein from a healthy gastrocnemius muscle-tissue sample. All samples were cohybridized with a dysferlin antibody to normalize for the variable levels of muscle fiber content (see bottom). Because of the relatively high total-protein loading required for dystrophin signal detection in our patients, quantitative comparison with a control sample was not considered accurate.

Given the presence of dystrophin isoforms and revertant fibers, the risk for cell-mediated immunity to the new dystrophin was considered limited but should be evaluated further.

Our pharmacokinetic studies indicate that PRO051 is rapidly absorbed and distributed, which limits the peak plasma levels and the potential for acute adverse reactions. The fact that plasma trough levels increased with repeated oligonucleotide administration suggests that tissue, including muscle, gradually increases levels of PRO051. The terminal elimination half-life (ranging from 19 to 56 days) is similar to that of other second-generation phosphorothioate oligo-



nucleotides.<sup>23,24</sup> The half-life range suggests that the terminal half-life in tissues may vary among patients, with resultant variable tissue exposures during long-term treatment. Analysis of biopsy

specimens obtained after the dose-escalation study showed that PRO051 was effective in inducing detectable, specific exon-51 skipping in muscle. Immunofluorescence analyses indicated that

**Table 2. Data from Muscle-Biopsy Analyses and the 6-Minute Walk Test, According to Dose and Weeks after Last Dose.\***

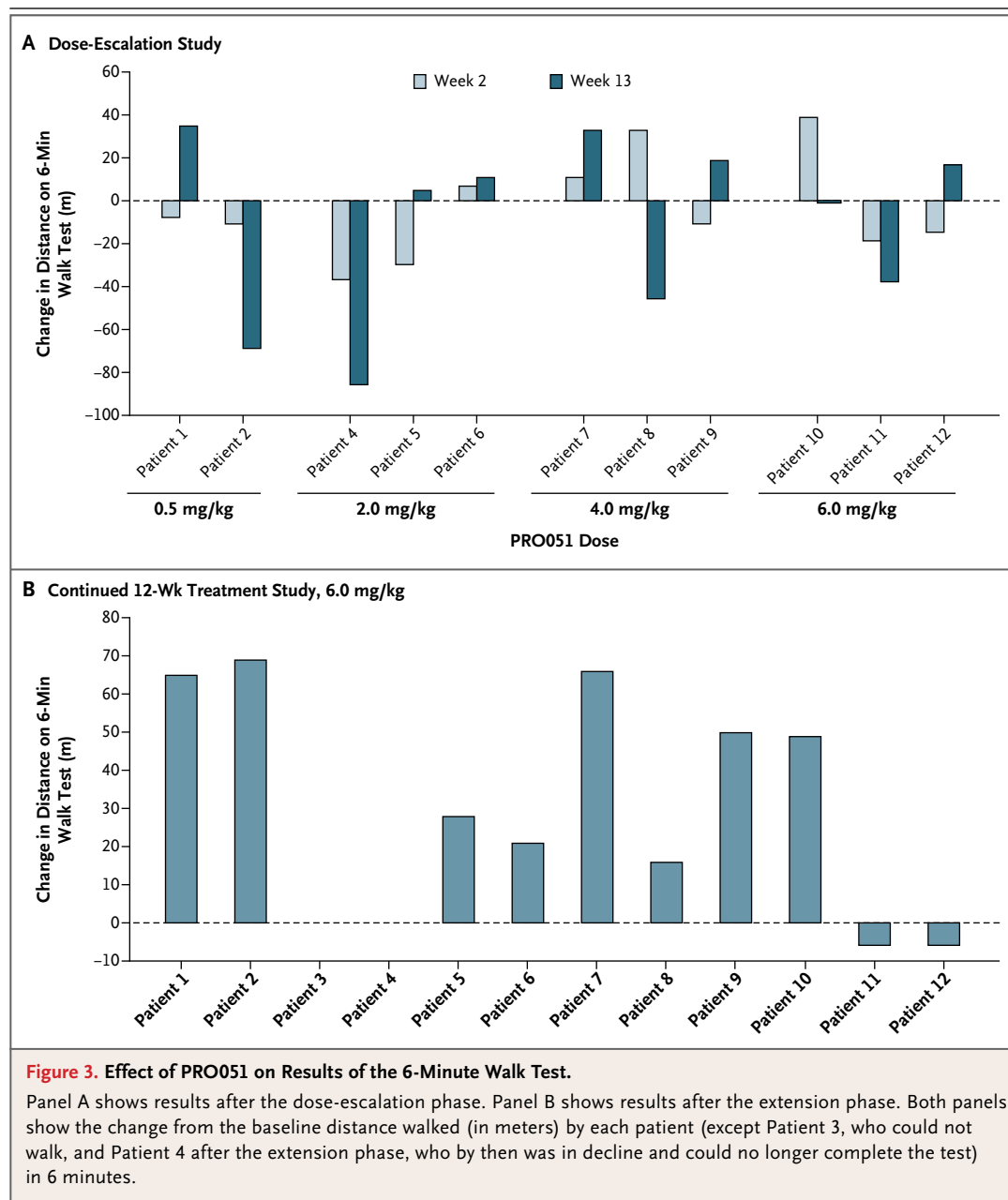
Patient No.	Immunofluorescence Analysis						Western Blotting			6-Minute Walk Test		
	Baseline		2 Weeks		7 Weeks		Baseline	2 Weeks	7 Weeks	2 Weeks	13 Weeks	End of 12-Wk Extension Phase
	% positive fibers	mean signal intensity	% positive fibers	mean signal intensity	% positive fibers	mean signal intensity	dystrophin	signal intensity	signal intensity	meters walked in 6 min vs. baseline distance		
0.5 mg/kg												
1	4	2.9	88	2.2	NT	NT	0.5	4.0	NT	-8	35	65
2	10	1.7	56	6.3	NT	NT	1.8	0.8	NT	-11	-69	69
3	NQ	1.4	20	3.0	NT	NT	5.1	7.2	NT	NA	NA	NA
2.0 mg/kg												
4	NT	NT	40	4.6	80	7.8	NT	5.4	8.6	-37	-86	NA
5	NT	NT	50	6.7	91	5.5	NT	2.8	23.5	-30	5	28
6	NT	NT	NQ	2.7	20	3.5	NT	3.0	11.0	7	11	21
4.0 mg/kg												
7	NT	NT	64	5.2	65	4.6	NT	7.4	11.4	11	31	66
8	NT	NT	76	15.6	85	9.2	NT	13.5	6.5	33	-46	16
9	NT	NT	51	1.8	71	3.7	NT	22.5	20.0	-11	19	50
6.0 mg/kg												
10	NT	NT	100	13.2	75	10.1	NT	28.5	22.5	39	-1	49
11	NT	NT	89	15.5	20	6.9	NT	19.6	9.5	-19	-38	-6
12	NT	NT	34	4.7	75	5.6	NT	12.6	12.2	-15	17	-6

\* PRO051 was given for 5 weeks and then stopped for a period of 6 to 15 months, during which muscle-biopsy data were collected 2 weeks and 7 weeks after the last dose. The 6-minute walk test was performed 2 weeks and 13 weeks after the last dose. Treatment was then restarted for a 12-week period, at a dose of 6.0 mg per kilogram in all 12 patients. The 6-minute walk test was conducted once more, at the end of the extension period. Immunofluorescence analysis was performed on cross sections of muscle-biopsy specimens, with the percentage of positive fibers calculated for each cross section (in three to six images, depending on image size and quality), and the mean signal intensity reported relative to that of control samples (set at 100%). Western blotting was conducted with the use of total muscle-protein extracts, measuring dystrophin signal intensity relative to the average intensity of baseline samples. Baseline data for the 6-minute walk test are given in Table 1 in the Supplementary Appendix. NA denotes not assessed, NQ not quantifiable, and NT not tested.

even at the lowest dose of 0.5 mg per kilogram of body weight, dystrophin was detectable at the membrane of 20 to 88% of muscle fibers. The mean percentages and average signal intensities of dystrophin-positive fibers increased in a dose-dependent manner (with 100% positive fibers in Patient 10 and a signal intensity of 15.6% of the control intensity in Patient 8). Although Western blot analyses for dystrophin typically lack sensitivity, and the results are difficult to quantify because of great variation in the quality of biopsy or tissue quality, Western blotting confirmed the results of immunofluorescence analyses in our study.

The duration of the dose-escalation study and the highest dose were chosen on the basis of the potential for additive effects of repeated dosing on molecular effects in muscle and our aim to minimize the inherent risks of systemic admin-

istration of a new compound to young patients. Most patients had similar dystrophin expression at 2 weeks and 7 weeks after treatment, suggesting a prolonged effect of the oligonucleotide, consistent with its *in vivo* stability and the improved stability of the *in-frame* transcript of the dystrophin protein at the cell membrane. Considering the pharmacokinetic profile of PRO051 and previous data on a chemically identical surrogate compound in *mdx* mice,<sup>14</sup> we anticipated that the pharmacodynamic effect would continue to increase during the extension phase. Indeed, an increase in the distance walked in 6 minutes was observed in most of our patients after extended treatment with 6.0 mg of PRO051 per kilogram of body weight per week, which is unusual for patients of this age with Duchenne's muscular dystrophy. Because our study was not placebo-



controlled, the findings need to be interpreted carefully. However, no improvement was observed in the initial dose-escalation phase, which suggests that the improvements seen in the extension phase of our study are related to the study drug. A possible learning effect with the 6-minute walk test was considered minimal because all patients were familiar with this test before the study began. Because no biopsy specimens were obtained at the end of the 12-week extension phase, we could not ascertain the correlation between dystrophin levels and the results of the 6-minute

walk test. The actual therapeutic benefit of PRO051 will depend on the functionality of the resulting dystrophin, which may differ depending on the patient's mutation. This is exemplified by the mild disease phenotype observed in patients with Becker's muscular dystrophy, with proteins similar to those gained during our study by 10 of 12 patients after exon-51 skipping induced by the study drug.<sup>12,13</sup>

In conclusion, systemic administration of PRO051 resulted in dose-dependent, abundant expression of dystrophin in muscle distant from the

injection sites in all our patients after 5 weeks of treatment. The modest improvement in 6-minute walking distance observed after an extended-treatment phase suggests that PRO051 may be clinically effective in the treatment of Duchenne's muscular dystrophy.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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