ORIGINAL ARTICLE

Local Dystrophin Restoration with Antisense Oligonucleotide PRO051

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ABSTRACT

BACKGROUND

Duchenne's muscular dystrophy is associated with severe, progressive muscle weakness and typically leads to death between the ages of 20 and 35 years. By inducing specific exon skipping during messenger RNA (mRNA) splicing, antisense compounds were recently shown to correct the open reading frame of the *DMD* gene and thus to restore dystrophin expression in vitro and in animal models in vivo. We explored the safety, adverse-event profile, and local dystrophin-restoring effect of a single, intramuscular dose of an antisense oligonucleotide, PRO051, in patients with this disease.

METHODS

Four patients, who were selected on the basis of their mutational status, muscle condition, and positive exon-skipping response to PRO051 in vitro, received a dose of 0.8 mg of PRO051 injected into the tibialis anterior muscle. A biopsy was performed 28 days later. Safety measures, composition of mRNA, and dystrophin expression were assessed.

RESULTS

PRO051 injection was not associated with clinically apparent adverse events. Each patient showed specific skipping of exon 51 and sarcolemmal dystrophin in 64 to 97% of myofibers. The amount of dystrophin in total protein extracts ranged from 3 to 12% of that found in the control specimen and from 17 to 35% of that of the control specimen in the quantitative ratio of dystrophin to laminin $\alpha 2$.

CONCLUSIONS

Intramuscular injection of antisense oligonucleotide PRO051 induced dystrophin synthesis in four patients with Duchenne's muscular dystrophy who had suitable mutations, suggesting that further studies might be feasible.

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Duchenne's muscular dystrophy is a severely debilitating childhood neuromuscular disease that affects 1 in 3500 newborn boys.[1] Progressive weakness of the skeletal muscles, cardiomyopathy, and respiratory failure are the most prominent features, but the brain can also be affected.[2,3] Virtually all patients are wheelchair-dependent by the age of 12 years, and most die in early adulthood. Improved ventilation techniques and glucocorticoid treatment have substantially improved fitness and muscle strength, prolonged mobility, and extended the expected lifespan from less than 20 years to 25 to 35 years.[4-6] However, there has been no treatment to prevent the eventual fatal outcome.

Duchenne's muscular dystrophy is caused by deletions (approximately 72%) and duplications (approximately 7%) of one or more exons or point mutations (20%) in the 2.4-Mb *DMD* gene,[7] which encodes a protein, dystrophin, that is crucial for sarcolemmal integrity.[8-12] In patients with the disease, such mutations disrupt the open reading frame and abrogate dystrophin synthesis. In contrast, mutations in the same gene that conserve the reading frame but lead to internally truncated or slightly altered dystrophins[8-10] often cause the milder Becker's muscular dystrophy, which is characterized by a life expectancy that is longer than that of patients with Duchenne's muscular dystrophy.[9,11]

Up to 50% of patients with Duchenne's muscular dystrophy show evidence of rare, dystrophin-positive fibers (revertant fibers) caused by spontaneous, clonal, frame-restoring skipping of stretches of exons.[13-16] This finding has prompted the investigation of the potential for therapeutic conversion of Duchenne's muscular dystrophy into its nearest in-frame counterpart (i.e., Becker's muscular dystrophy) with the use of antisense techniques. Because of their capacity to skip an exon specifically by blocking its inclusion during splicing,[12] antisense oligonucleotides can correct the reading frame of *DMD* transcripts, yielding internally truncated dystrophins such as those associated with Becker's muscular dystrophy (Fig. 1A and 1B). Although in principle such a process is mutation-specific, the skipping of particular exons is theoretically therapeutic in a series of different mutations. Thus, a judicious choice of 10 exons may eventually correct more than 85% of mutations in patients with Duchenne's muscular dystrophy.

Two main types of compounds are being investigated for antisense-induced exon skipping: 2′-*O*-methyl–modified ribose molecules with a full-length phosphorothioate backbone (2OMePS) and phosphorodiamidate morpholino oligomers. Preclinical proof-of-concept has been obtained with both types of molecules in cultured muscle cells from a series of patients with Duchenne's muscular dystrophy with various mutations,[13,14,17-19] as well as in the *mdx* mouse model and the golden retriever muscular dystrophy (*GRMD*) dog model.[15,16,20] In the *mdx* mouse, which carries a nonsense mutation in exon 23, systemic delivery of exon-23–skipping antisense compounds restored up to 50% of dystrophin expression in various muscle groups.[15,16] This treatment led to improved muscle force and reduced creatine kinase levels without tissue toxicity.[15,16]

This recent preclinical progress has led to initiatives toward preliminary clinical studies in patients. Our previous studies in cultured cells from patients showed that an intraexonic 2OMePS antisense oligonucleotide, PRO051, efficiently induced specific exon-51 skipping.[19] On the basis of the frequency of mutations in patients with Duchenne's muscular dystrophy in the Leiden database,[7] we concluded that PRO051 might correct the reading frame in 16% of all patients with the disease — in other words, 25% of deletions, including exon 50 (Fig. 1), exon 52, exons 45 to 50, exons 48 to 50, and exons 49 to 50. Obviously, the actual therapeutic benefit would depend on the functionality of the resulting modified dystrophin.

Our exploratory, open-label, single-center study involved four patients with Duchenne's muscular dystrophy, each of whom received a single injection of PRO051 into the tibialis anterior muscle. The primary outcome of this trial was adverse events in the four subjects; secondary outcomes were specific exon-51 skipping and dystrophin expression.

METHODS

PATIENTS AND STUDY DESIGN

Patients with Duchenne's muscular dystrophy who were between the ages of 8 and 16 years were eligible to participate in the study. All patients had deletions that were correctable by exon-51 skipping and had no evidence of dystrophin on previous diagnostic muscle biopsy. Concurrent

glucocorticoid treatment was allowed. Written informed consent was obtained from the patients or their parents, as appropriate. During the prescreening period (up to 60 days), each patient's mutational status and positive exon-skipping response to PRO051 in vitro were confirmed, and the condition of the tibialis anterior muscle was determined by T_1-weighted magnetic resonance imaging (MRI).[21] For patients to be included in the study, fibrotic and adipose tissue could make up no more than 50% of their target muscle.

During the baseline visit, safety measures were assessed. In each patient, the leg that was to be injected was fixed with a tailor-made plastic mold and its position was recorded. A topical eutectic mixture of local anesthetics (EMLA) was used to numb the skin. Four injections of PRO051 were given along a line measuring 1.5 cm running between two small skin tattoos with the use of a 2.5-cm electromyographic needle (MyoJect Disposable Hypodermic Needle Electrode, TECA Accessories) to ensure intramuscular delivery. The volume of each injection was 200 μl containing 200 μg of PRO051, which was dispersed in equal portions at angles of approximately 30 degrees.

At day 28, safety measures were assessed again. The leg that had been injected was positioned with the use of the patient's own mold, and a semiopen muscle biopsy was performed between the tattoos under local anesthesia with a forceps with two sharp-edged jaws (Blakesley Conchotoma, DK Instruments).[22] The biopsy specimen was snap-frozen in 2-methylbutane cooled in liquid nitrogen.

Patients were treated sequentially from May 2006 through March 2007 and in compliance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki. The study was approved by the Dutch Central Committee on Research Involving Human Subjects and by the local institutional review board at Leiden University Medical Center. 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.

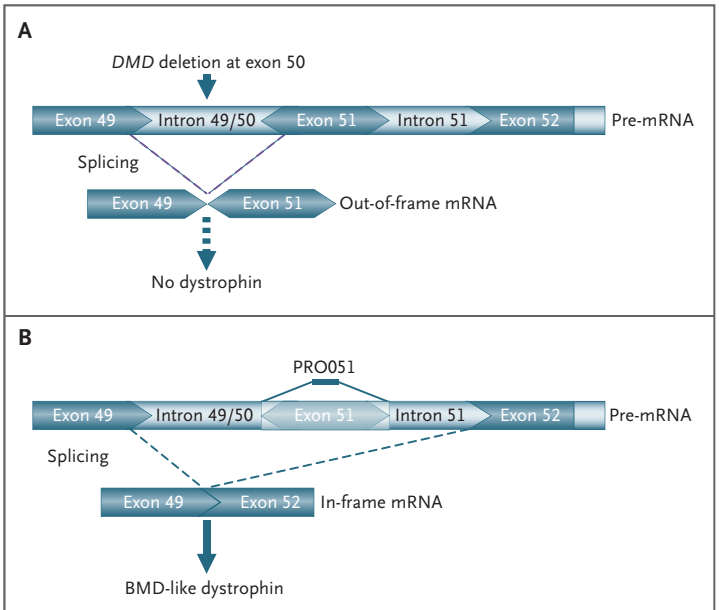


Figure 1. Schematic Representation of Exon Skipping.

In a patient with Duchenne's muscular dystrophy who has a deletion of exon 50, an out-of-frame transcript is generated in which exon 49 is spliced to exon 51 (Panel A). As a result, a stop codon is generated in exon 51, which prematurely aborts dystrophin synthesis. The sequence-specific binding of the exon-internal antisense oligonucleotide PRO051 interferes with the correct inclusion of exon 51 during splicing so that the exon is actually skipped (Panel B). This restores the open reading frame of the transcript and allows the synthesis of a dystrophin similar to that in patients with Becker's muscular dystrophy (BMD).

DESCRIPTION OF PRO051

PRO051 is a synthetic, modified RNA molecule with sequence 5′-UCAAGGAAGAUGGCAUUUCU-3′.[23] It carries full-length 2′-*O*-methyl–substituted ribose molecules and phosphorothioate internucleotide linkages. The drug was provided by Prosensa B.V. in vials of 1 mg of freeze-dried material with no excipient. It was dissolved and administered in sterile, unpreserved saline (0.9% sodium chloride). PRO051 was not found to be mutagenic by bacterial Ames testing. In regulatory Good Laboratory Practice safety studies, rats that received a single administration of up to 8 mg per kilogram of body weight intramuscularly and 50 mg per kilogram intravenously showed no adverse effects; monkeys receiving PRO051 for 1 month appeared to tolerate doses up to 16 mg per kilogram per week when the drug was administered by intravenous 1-hour infusion or by subcutaneous injection, without clinically relevant adverse effects.

IN VITRO PRESCREENING

A preexisting primary myoblast culture[19] was used for the prescreening of Patient 4. For the other three patients, fibroblasts were converted into

myogenic cells after infection with an adenoviral vector containing the gene for the myogenic transcription factor (MyoD) as described previously.[19,24,25] Myotube cultures were transfected with PRO051 (100 nM) and polyethylenimine (2 μl per microgram of PRO051), according to the manufacturer's instructions for ExGen500 (MBI Fermentas). RNA was isolated after 48 hours. Reverse transcriptase–polymerase chain reaction (RT-PCR), immunofluorescence, and Western blot analyses were performed as reported previously.[19,23] PCR fragments were analyzed with the use of the 2100 Bioanalyzer (Agilent) and isolated for sequencing by the Leiden Genome Technology Center.

SAFETY ASSESSMENT

At baseline and at 2 hours, 1 day, and 28 days after injection, all patients received a full physical examination (including the measurement of vital signs) and underwent electrocardiography. In addition, plasma and urine were obtained to determine renal and liver function, electrolyte levels, complete cell counts, the activated partial-thromboplastin time, and complement activity values in the classical (CH50) and alternative (AP50) routes. The use of concomitant medications was recorded. At baseline and on day 28, the strength of the tibialis anterior muscle was assessed with the use of the Medical Research Council scale[26] to evaluate whether the procedures had affected muscle performance. (On this scale, a score of 0 indicates no movement and a score of 5 indicates normal muscle strength.) Since only a small area of the muscle was treated, clinical benefit in terms of increased muscle strength was not expected. At each visit, adverse events were recorded.

RNA ASSESSMENT

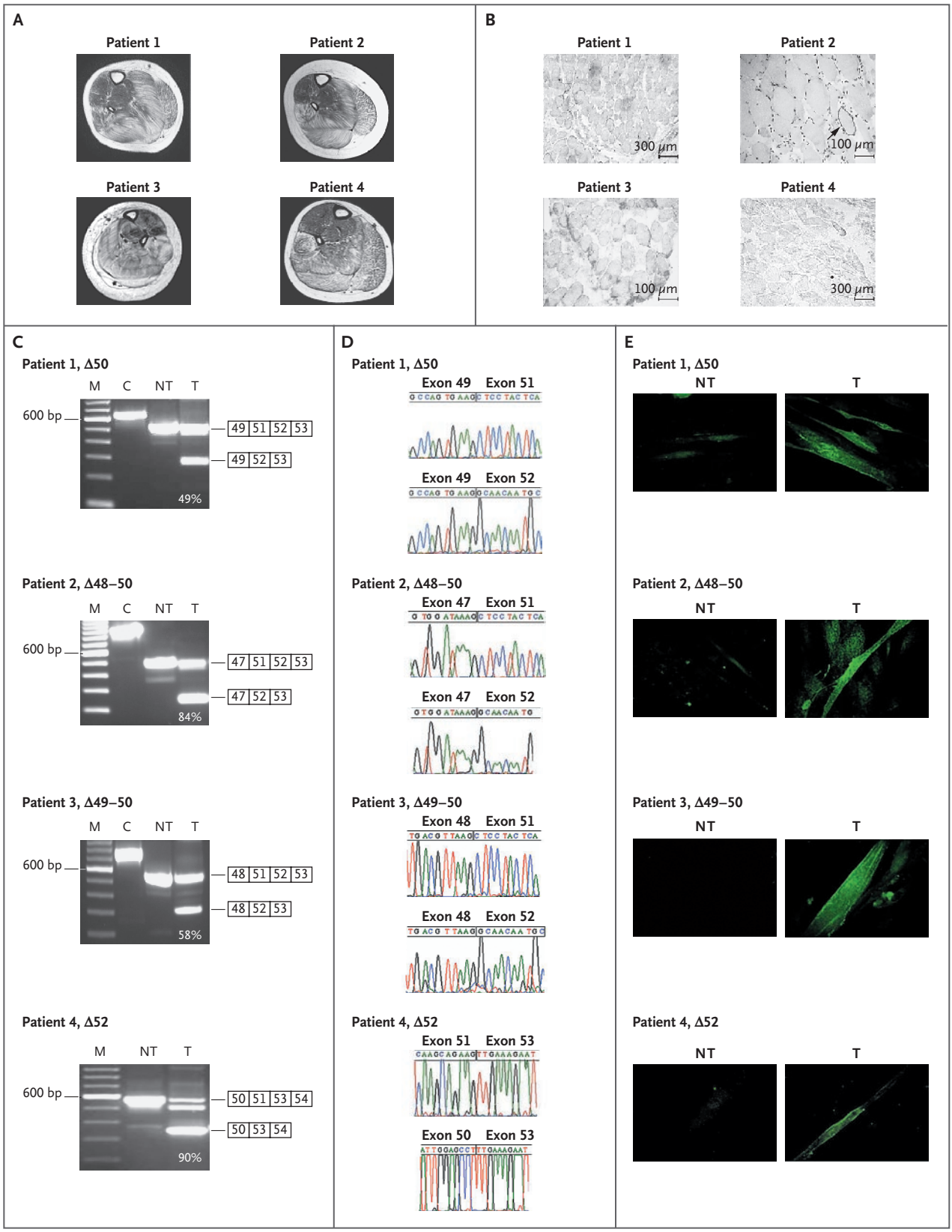
Serial sections (50 μm) of the frozen muscle-biopsy specimen were homogenized in RNA-Bee solution (Campro Scientific) and MagNA Lyser Green Beads (Roche Diagnostics). Total RNA was isolated and purified according to the manufacturer's instructions. For complementary DNA, synthesis was accomplished with Transcriptor reverse transcriptase (Roche Diagnostics) with the use of 500 ng of RNA in a 20-μl reaction at 55°C for 30 minutes with human exon 53 or 54 specific reverse primers. PCR analyses were performed as described previously.[19,23] Products were analyzed on 2% agarose gels and sequenced. In addition, RT-PCR with the use of a primer set for the protein-truncation test[27] was used to rapidly screen for aspecific aberrant splicing events throughout the *DMD* gene.

Figure 2 (facing page). Prescreening Studies of the Four Patients.

Magnetic resonance images of the lower legs of the four patients (the left leg of Patient 3 and right legs of the other three patients) show the adequate condition of the tibialis anterior muscle (less than 50% fat infiltration and fibrosis) (Panel A). The diagnosis of Duchenne's muscular dystrophy in these patients was confirmed by diaminobenzidine tetrahydrochloride staining of cross sections of biopsy specimens obtained previously from the quadriceps muscle (Panel B). No dystrophin expression was observed, with the exception of one dystrophin-positive, or revertant, fiber in Patient 2 (arrow). Reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analysis of the transcript region flanking the patients' mutations and exon 51 confirmed both the individual mutations in nontreated myotubes (NT) and the positive response to PRO051 (i.e., exon 51 skipping) in treated myotubes (T) on the RNA level (Panel C). The efficiencies of exon skipping were 49% for Patient 1, 84% for Patient 2, 58% for Patient 3, and 90% for Patient 4. A cryptic splice site within exon 51 is sometimes activated by PRO051 in cell culture, resulting in an extra aberrant splicing product, as seen in the treated sample from Patient 4. Lane M shows a 100-bp size marker, and lane C RNA from healthy control muscle. Sequence analysis of the RT-PCR fragments from treated and untreated myotubes identified the precise skipping of exon 51 for each patient (Panel D). The new in-frame transcripts led to substantial dystrophin synthesis, as detected by immunofluorescence analysis of treated myotubes with the use of monoclonal antibody NCL-DYS2 (Panel E). No dystrophin was detected before treatment.

ASSESSMENT OF PROTEIN LEVEL

For immunofluorescence analysis, acetone-fixed sections were incubated for 90 minutes with monoclonal antibodies against the central rod domain (MANDYS106, Dr. G. Morris, United Kingdom) at a dilution of 1:60, the C-terminal domain (NCL-DYS2, Novocastra Laboratories) at a dilution of 1:30, or (as a reference) laminin α2 (Chemicon International), a basal lamina protein that is unaffected by dystrophin deficiency, at a dilution of 1:150, followed by Alexa Fluor 488 goat anti-mouse IgG (H+L) antibody (Molecular Probes) at a dilution of 1:250 for 1 hour. Sections were mounted with Vectashield Mounting Medium (Vector Laboratories). ImageJ software (W. Rasband, National Institutes of Health, http://rsb.info.nih.gov/ij) was used for quantitative image analysis



as described previously.[28,29] Entire cross sections were subdivided into series of 6 to 10 adjacent images, depending on the size of the section. To ensure reliable measurements, staining of the sections and recording of all images were performed during one session with the use of fixed exposure settings and the avoidance of pixel saturation. The lower-intensity threshold was set at background for Duchenne's muscular dystrophy, and positive fluorescence was quantified for each section (area percentage), both for dystrophin and laminin $\alpha 2$.

Western blot analysis was performed as described previously[19] with the use of pooled homogenates from sets of four serial 50-μm sections throughout the biopsy specimen. For each patient, two amounts of total protein — 30 μg and 60 μg — were applied, and for the control sample, 3 μg. The Western blot was incubated overnight with dystrophin monoclonal antibody NCL-DYS1 (Novocastra Laboratories) at a dilution of 1:125, followed by horseradish-peroxidase–labeled goat antimouse IgG (Santa Cruz Biotechnology) at a dilution of 1:10,000 for 1 hour. Immunoreactive bands were visualized with the use of the ECL Plus Western blotting detection system (GE Healthcare) and Hyperfilm ECL (Amersham Biosciences). Signal intensities were measured with the use of ImageJ software.

RESULTS

PRESCREENING OF PATIENTS

The study was planned to include four to six patients. Six patients were invited to participate, and one declined. The remaining five patients were prescreened. First, the condition of the tibialis anterior muscle was evaluated on MRI. The muscle condition of four patients was deemed to be adequate for the study (Fig. 2A), and the absence of dystrophin was confirmed in the patients' original biopsy specimens (Fig. 2B). Second, the mutational status and positive exon-skipping response to PRO051 of these four patients were confirmed in fibroblast cultures. PRO051 treatment generated a novel, shorter fragment of messenger RNA for each patient, representing 46% (in Patient 4) to 90% (in Patient 1) of the total RT-PCR product (Fig. 2C). Precise exon-51 skipping was confirmed by sequencing (Fig. 2D). No other transcript regions were found to be altered. Immunofluorescence analyses showed a preponderance of dystrophin-positive myotubes (Fig. 2E), a finding that was confirmed by Western blot analysis (not shown). Thus, the four patients were judged to be eligible for PRO051 treatment. Their baseline characteristics are shown in Table 1.

SAFETY AND ADVERSE EVENTS

All patients had one or more adverse events. However, only one patient reported mild local pain at the injection site, which was considered to be an adverse event related to the study drug. Other events included mild-to-moderate pain after the muscle biopsy. Two patients had blistering under the bandages used for wound closure. In the period between injection and biopsy, two patients reported a few days of flulike symptoms, and one patient had mild diarrhea for 1 day. At baseline, the muscle-strength scores of the treated tibialis anterior muscle in Patients 1, 2, 3, and 4 were 4, 2, 3, and 4, respectively, on the Medical Research

Table 1. Baseline Characteristics of the Patients.

Variable	Patient 1	Patient 2	Patient 3	Patient 4
Age (yr)	10	13	13	11
Deletion	Exon 50	Exons 48–50	Exons 49–50	Exon 52
Age at loss of ambulation (yr)	9	11	7	10
Previous glucocorticoid treatment	Yes	Yes	No	Stopped 8 months before study
Previous diagnostic biopsy				
Age at time of biopsy (yr)	4	8	4	4
Type of muscle specimen	Quadriceps	Quadriceps	Quadriceps	Quadriceps
Dystrophin-positive fibers in biopsy specimen (%)	0	<1	0	0

Council scale. None of the patients showed changes in the strength of this muscle during the study or significant alterations in standard laboratory measures or increased measures of complement split products or activated partial-thromboplastin time. No local inflammatory or toxic response was detected in the muscle sections of the patients (data not shown). Patient 3 successfully underwent preplanned surgery for scoliosis in the month after the study was completed.

RNA AND PROTEIN LEVEL

At day 28, a biopsy of the treated area was performed in each patient. Total muscle RNA was isolated from serial sections throughout the biopsy specimen. In all patients, RT-PCR identified a novel, shorter fragment caused by exon-51 skipping, as confirmed by sequencing (Fig. 3). Further transcript analysis showed no other alterations (data not shown). Immunofluorescence analyses of sections throughout the biopsy specimen of each patient showed clear sarcolemmal dystrophin signals in the majority of muscle fibers (Fig. 4A and 4B). Dystrophin antibodies proximal and distal to the deletions that were used included MANDYS106 (Fig. 4A and 4B) and NCL-DYS2 (similar to MANDYS106, not shown). The fibers in each section were manually counted after staining for laminin $\alpha 2$.[30] The individual numbers varied, consistent with the size of the biopsy specimen and the quality of the muscle. In the largest sections, Patient 2 had 726 fibers, of which 620 were dystrophin-positive, whereas Patient 3 had 120 fibers, of which 117 were dystrophin-positive (Fig. 4A and 4C). The dystrophin intensities were typically lower than those in a healthy muscle biopsy specimen (Fig. 4B). The single fibers with a more intense dystrophin signal in Patients 2 and 3 could well be revertant fibers (Fig. 4B).

Western blot analysis confirmed the presence of dystrophin in varying amounts (Fig. 4E). The dystrophin signals were scanned and correlated

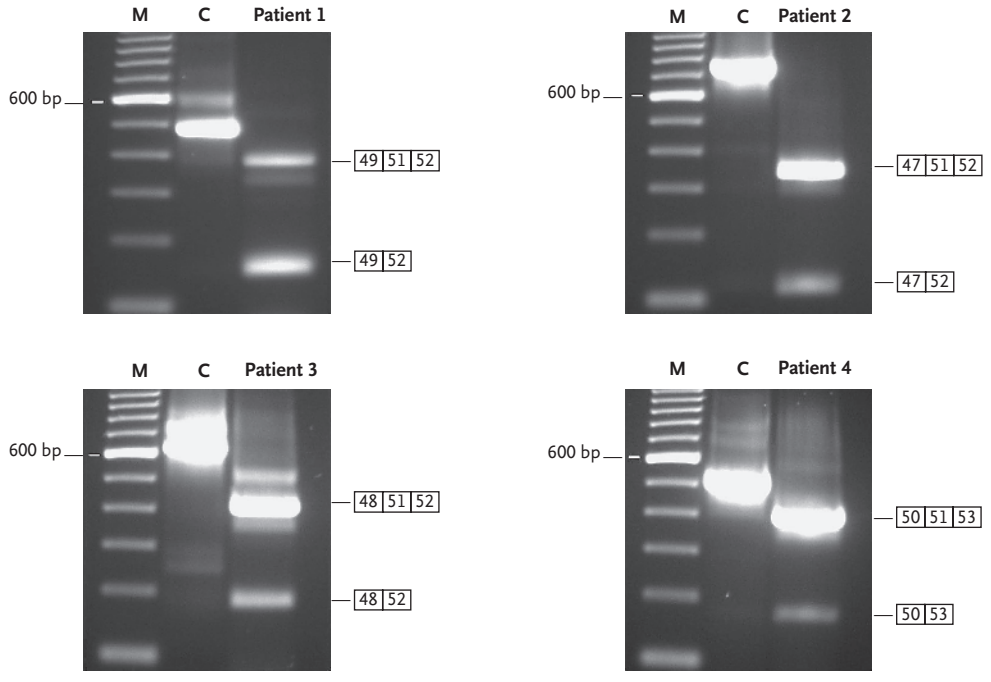


Figure 3. RT-PCR Analysis of RNA Isolated from Serial Sections of Biopsy Specimens from the Patients.

After treatment with PRO051, reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analysis shows novel, shorter transcript fragments for each patient. Both the size and sequence of these fragments confirm the precise skipping of exon 51. No additional splice variants were observed. At 28 days, still significant in-frame RNA transcripts were detected, suggesting prolonged persistence of PRO051 in muscle. Owing to the small amount of section material, high-sensitivity PCR conditions were used; this process precluded the accurate quantification of skipping efficiencies and the meaningful correlation between levels of RNA and protein. M denotes size marker, and C control.

to the control (per microgram of total protein). The amounts varied from 3% in Patient 3, who had the most-dystrophic muscle, to 12% in Patient 2, who had the best-preserved muscle. Since such comparison on the basis of total protein does not correct for the varying amounts of fibrotic and adipose tissue in patients with Duchenne's muscular dystrophy, we also quantified the dystrophin fluorescence signal (Fig. 4A and 4B) relative to that of the similarly located laminin $\alpha 2$ in each section by ImageJ analysis. When the ratio of dystrophin to laminin $\alpha 2$ was set at 100 for the control section, Patients 1, 2, 3, and 4 had ratios of 33, 35, 17, and 25, respectively (Fig. 4D).

Figure 4 (facing page). Dystrophin-Restoring Effect of a Single Intramuscular Dose of PRO051.

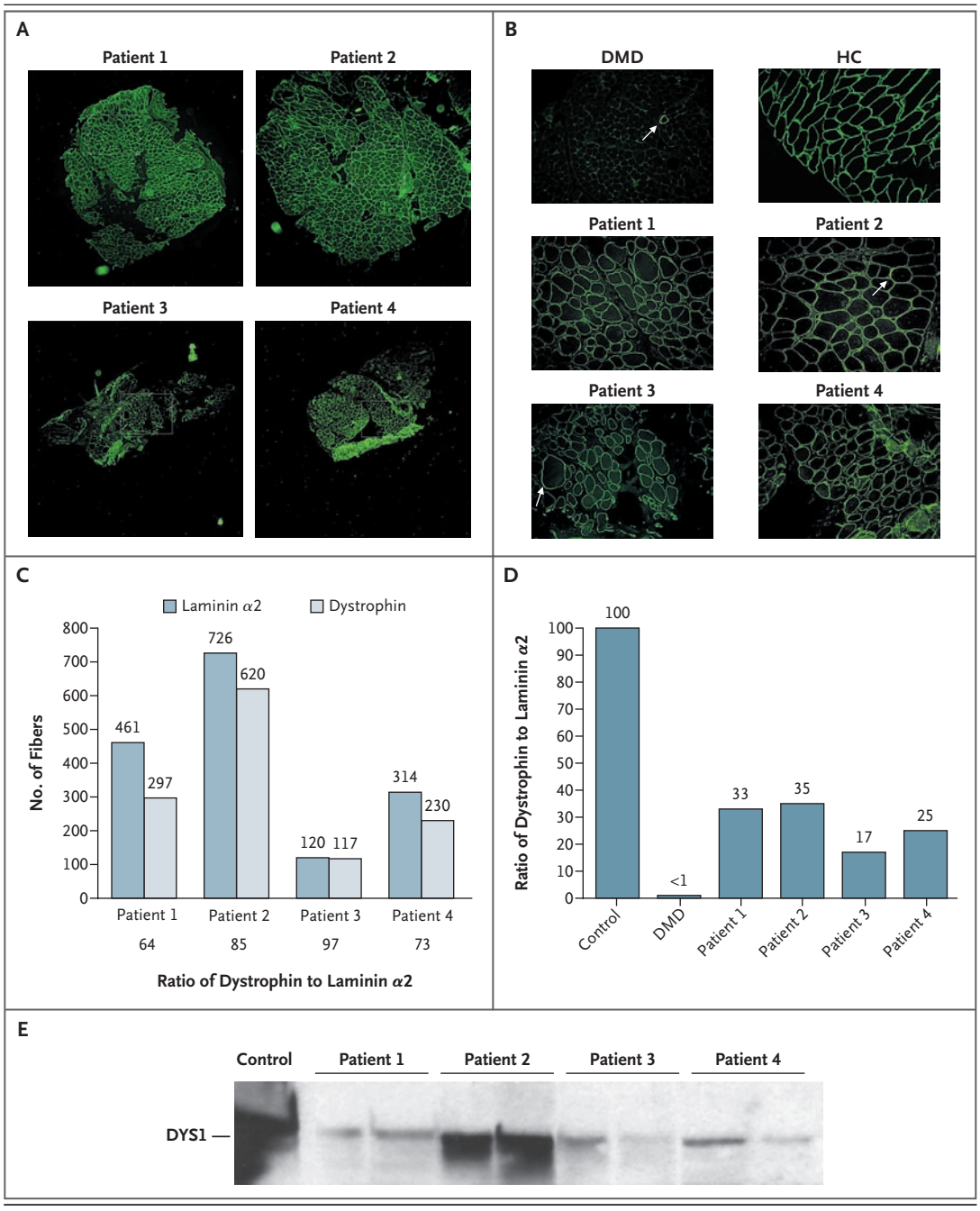
Immunofluorescence analysis with the use of the dystrophin antibody MANDYS106 clearly shows dystrophin expression at the membranes of the majority of fibers throughout the biopsy specimen obtained from each patient (Panel A). The areas indicated by the squares are shown in higher magnification in Panel B. For comparison, a sample from an untreated patient with Duchenne's muscular dystrophy (DMD) and a healthy control sample from gastrocnemius muscle (HC) are included with the samples from the patients. Putative revertant fibers are indicated by arrows. The total number of muscle fibers that contained dystrophin and laminin $\alpha 2$ were counted manually and the ratios of dystrophin to laminin $\alpha 2$ were plotted (Panel C). Western blot analysis of total protein extracts isolated from the patients' biopsy specimens with the use of NCL-DYS1 antibody show restored dystrophin expression in all patients (Panel E). For each patient, 30 μg (right lane) and 60 μg (left lane) were loaded; for comparison, 3 μg of total protein from a healthy gastrocnemius muscle sample was also loaded (to avoid overexposure). Because of the relatively small deletions in the *DMD* gene of these patients, no differences were observed in protein sizes. In Patient 1, a transfer irregularity disturbed signal detection in the 60-μg lane. To correct for the varying density of muscle fibers in the different cross sections, the total fluorescent dystrophin signal (area percentage) in each section was plotted as a ratio to the area percentage of laminin $\alpha 2$ (Panel D).

DISCUSSION

Our study showed that local intramuscular injection of PRO051, a 2OMePS antisense oligoribonucleotide complementary to a 20-nucleotide sequence within exon 51, induced exon-51 skipping, corrected the reading frame, and thus introduced dystrophin in the muscle in all four patients with Duchenne's muscular dystrophy who received therapy. Dystrophin-positive fibers were found throughout the patients' biopsy specimens, indicating dispersion of the compound in the injected area. Since no delivery-enhancing excipient was used, PRO051 uptake did not seem to be a major potentially limiting factor. We cannot rule out that increased permeability of the dystrophic fiber membrane had a favorable effect. The patients produced levels of dystrophin that were 3 to 12% of the level in healthy control muscle, as shown on Western blot analysis of total protein. Since the presence of fibrosis and fat may lead to some underestimation of dystrophin in total protein extracts, we determined the ratio of dystrophin to laminin $\alpha 2$ in the cross sections, which ranged from 17 to 35, as compared with 100 in control muscle. The dystrophin-restoring effect of PRO051 was limited to the treated area, and no strength improvement of the entire muscle was observed. Future systemic treatment will require repeated administration to increase and maintain dystrophin expression at a higher level and to obtain clinical efficacy.

Because of medical-ethics regulations regarding interventions in minors, we could not obtain a biopsy specimen from the patients' contralateral muscles that had not been injected. However, the patients showed less than 1% of revertant fibers in the original diagnostic biopsy specimens obtained 5 to 9 years before the initiation of the study (Table 1 and Fig. 2B). We consider it very likely that the effects we observed were related to the nature and sequence of the PRO051 reagent rather than to a marked increase in revertant fibers. Indeed, a single, possibly revertant fiber that had an increased dystrophin signal was observed in both Patient 2 and Patient 3 (Fig. 4B).

In summary, our study showed that local administration of PRO051 to muscle in four patients with Duchenne's muscular dystrophy restored dystrophin to levels ranging from 3 to 12% or 17 to 35%, depending on quantification relative to total protein or myofiber content. Consistent with the distinctly localized nature of the treatment, functional improvement was not observed. The consistently poorer result in Patient 3, who had the most advanced disease, suggests the importance of performing clinical trials in patients at



a relatively young age, when relatively little muscle tissue has been replaced by fibrotic and adipose tissue. Our findings provide an indication that antisense-mediated exon skipping may be a potential approach to restoring dystrophin synthesis in the muscles of patients with Duchenne's muscular dystrophy.

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Dr. van Deutekom, Mrs. Janson, Dr. Ginjaar, Mrs. Frankhuizen, Dr. Aartsma-Rus, Mrs. Bremmer-Bout, Dr. den Dunnen, Dr. Koop, Dr. Verschuuren, and Dr. van Ommen report being employed by Leiden University Medical Center, which has patent applications pending in Europe and elsewhere (including the United States, Canada, and Australia) on antisense sequences and exon-skipping technology. These patents are exclusively li-

censed to Prosensa B.V., sponsor of the study, and medical center employees are not receiving financial or other benefits from this study or otherwise from the sponsor. Dr. van Deutekom, Mrs. Janson, Dr. de Kimpe, Mr. Ekhart, and Mr. Platenburg report being employed by or having an equity interest in Prosensa B.V., which has taken PRO051 into clinical development for the treatment of patients with Duchenne's muscular dystrophy. Dr. Veneker reports receiving consulting fees from Prosensa B.V. No other potential conflict of interest relevant to this article was reported.

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