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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 α_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 (±SD) improvement of 35.2±28.7 m (from the baseline of 384±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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Duchenne's muscular dystrophy is an X-linked recessive muscle disorder, affecting 1 in 3500 newborn boys.[1] Patients have severe, progressive muscle wasting, leading to early death.[2,3] The disease is caused by mutations in the dystrophin gene (*DMD*),[4,5] leading to disruption of the open reading frame, dystrophin deficiency at the myofiber membrane, and continued fiber degeneration.[6-8] 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.[6-9]

A promising therapeutic strategy involves antisense oligonucleotides that induce specific exon skipping during pre–messenger RNA (mRNA) splicing,[10] aimed at reading-frame correction and production of transcripts like those in patients with Becker's muscular dystrophy.[11] 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.[12,13] 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.[14] In addition, 4 to 8 weeks' subcutaneous delivery of the oligonucleotides resulted in a steady increase in oligonucleotide levels, exon skipping, and dystrophin levels.[14]

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.[15]

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.[16] The compound 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.[16] 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,[16] was provided in 0.5-ml glass vials in sodium phosphate-buffered saline (100 mg per milliliter). Nonclinical safety data are provided in the Supplementary Appendix.

SAFETY AND TOLERABILITY

Safety was monitored as described previously.[16] 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 α_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 immunoglobulin G antibodies against dystrophin, serum samples obtained before and 120 days after treatment were analyzed.[17]

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[18] (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.[16,19,20] For detection of new dystrophin expression, immunofluorescence analysis of serial 8-μm 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.[16,20]

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[21,22] 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.[16] 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 α_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 α_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 [±SD] protein level, 0.078±0.038 at baseline and 0.206±0.119 at week 12 of the extension phase). This may represent an adaptive process within renal 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-thromboplastin 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.[23,24] 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[25-27] (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).

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