The NEW ENGLAND JOURNAL of MEDICINE

VOL. 357 NO. 26

ESTABLISHED IN 1812 DECEMBER 27, 2007 WWW.NEJM.ORG

of patients with a history of myocardial infarction are at risk for ventricular tachyarrhythmias and sudden cardiac arrest, and all available therapies have risks and substantial expense. The reputation of the fields of cardiology and cardiac electrophysiology, advanced by rigorous adherence to the important principle of evidence-based medicine, is also at risk. More important, the health and well-being of our patients with ICDs who have a history of myocardial infarction are at stake.

Dr. Estes reports receiving lecture fees from Medtronic, St. Jude Medical, and Boston Scientific. No other potential conflict of interest relevant to this article was reported.

From the Tufts University School of Medicine, New England Cardiac Arrhythmia Center, Boston.

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Skipping toward Personalized Molecular Medicine

Eric P. Hoffman, Ph.D.

"Personalized molecular medicine." As with other catchy terms for big ideas, such as "reversing global warming" and "renewable energy," the concept of personalized molecular medicine is certainly important, but the path to achieving it is far from clear. When such phrases are considered, definitions are important. Does personalized molecular medicine mean the tailoring of drugs for the individual patient, an approach that evokes images of Bones on *Star Trek* making instantaneous diagnoses with his Tricorder followed by loud pneumatic injections of customized drugs? Such a concept would place the realization of this technology in the same time frame as the achievement of "warp drive" that hurtled the *Enterprise* into new galaxies.

On the contrary, personalized molecular medicine appears to be at our doorstep. In this issue of the *Journal*, van Deutekom and colleagues report on a proof-of-concept clinical trial involving four boys with Duchenne's muscular dystrophy, the most common of the inherited childhood disorders.[1] The investigators chose to create a small antisense oligonucleotide that, in principle, would enable the cellular machinery specifically to "skip over" an exon in the mutated *DMD* gene by blocking its inclusion during splicing. For this trial, they designed a small, modified nucleic acid drug (PRO051) consisting of 2′-*O*-methyl–modified ribose molecules with a full-length phosphorothioate backbone (2OMePS) that targeted the patients' genetic mutation and hybridized to the

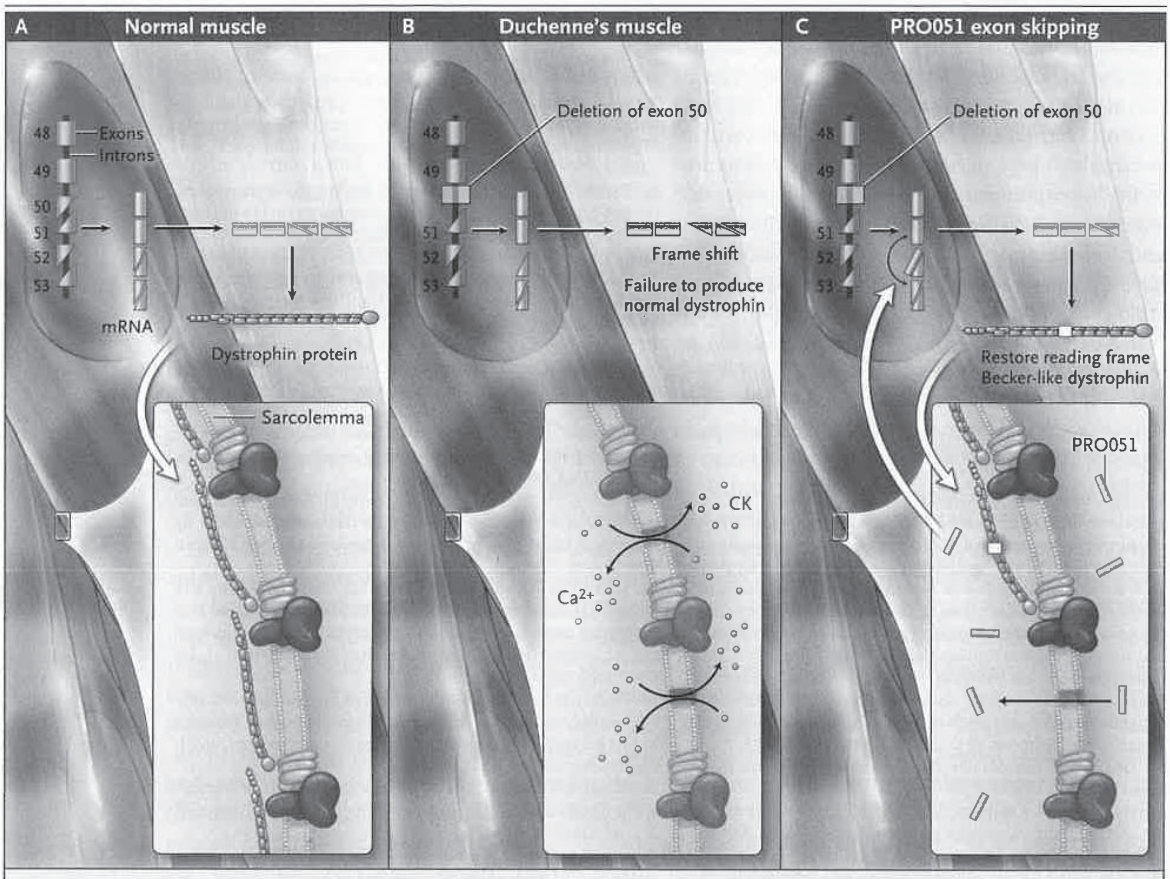

Figure 1. Mechanism of PRO051 in the Restoration of Dystrophin Expression through Exon Skipping.

Normal muscle produces dystrophin, a critical protein, in response to signals encoded by the enormous *DMD* gene, in which 79 exons are spliced together in a precise lockstep manner into messenger RNA (mRNA). The mRNA is then translated into dystrophin protein. All exons are spliced to maintain the triplet codon reading frame required for effective protein translation (Panel A). In the muscle of patients with Duchenne's muscular dystrophy, mutations in the dystrophin gene lead to the loss of one or more exons (Panel B, in which exon 50 is deleted). The mRNA splices together the remaining exons; however, the triplet reading frame is not maintained, which leads to errors in translation (frame shift) and loss of production of the dystrophin protein. The loss of dystrophin at the plasma membrane leads to the secondary loss of other associated membrane cytoskeleton structures. This, in turn, leads to membrane fragility, an abnormal influx of calcium ions (Ca^{2+}), and the efflux of creatine kinase (CK) into the patient's blood. Intramuscular injection of the small modified DNA molecule called PRO051 probably enters the Duchenne's muscle through abnormal muscle membranes; it then enters the nucleus and binds to the dystrophin mRNA in a sequence-specific manner (Panel C). In hybridization to the mRNA, PRO051 blocks the splicing machinery and prevents the inclusion of an additional exon (exon 51 in this example). The skipping of this additional exon restores the reading frame of the mRNA, allowing new production of dystrophin. The dystrophin that is produced is not normal but probably retains considerable function, as evidenced by the condition of patients with clinically milder Becker's muscular dystrophy who have similar or identically modified dystrophins.

messenger RNA (mRNA) in such a way that the cellular machinery was instructed to "skip over" the mutation in the boys' muscles (Fig. 1). The authors observed partial restoration of the expression of dystrophin protein in muscle into which the drug had been injected. In one patient, the restoration reached a level that would be expected to permit partial recovery of muscle function (at least for the very small area of muscle that was studied).

If we make the two reasonable assumptions — that the drug could be delivered systemically for all muscles and that various drugs could be designed for the hundreds of different mutations

in boys affected by Duchenne's muscular dystrophy — then the study by van Deutekom et al. might herald the dawn of personalized molecular medicine. It is very possible to imagine a series of drugs designed to overcome the specific mutations in each boy with Duchenne's muscular dystrophy and perhaps in patients with a host of other disorders. However, many hurdles must be overcome before the techniques described by van Deutekom et al. can be used therapeutically.

Duchenne's muscular dystrophy results from the loss of the critical dystrophin protein in the myofibers of patients' muscles.[2] The absence of dystrophin leads to membrane instability, and the affected myofibers tear holes in their cell walls, leading to an efflux of creatine kinase and an influx of calcium (Fig. 1B). The genetic mutations that cause the disease preclude the expression of dystrophin; most such mutations disrupt the triplet reading frame in which mRNA is turned into protein (a frame-shift mutation).

A clinically milder, yet highly variable, form of dystrophinopathy, termed Becker's muscular dystrophy, is common. Phenotypes for this disorder range from benign (an asymptomatic condition in which levels of creatine kinase are elevated but the patient is well) to only marginally less severe than Duchenne's muscular dystrophy. Some patients with clinically mild or asymptomatic Becker's muscular dystrophy have very large deletions of the dystrophin megagene, characterized by dystrophin proteins that are just half the molecular weight of normal dystrophin.[3] Muscles in patients with Becker's muscular dystrophy still produce dystrophin and retain muscle function because of in-frame mutations. Despite the large deletions, the gene can still be translated into protein, since the triplet codon reading frame is maintained. PRO051 effectively turns Duchenne's muscle into Becker's muscle. By blocking the inclusion of exons adjacent to the *DMD* mutation, the drug restores the reading frame and allows the production of a form of dystrophin with some function (Fig. 1C).

What are the hurdles lying between the research of van Deutekom et al. and the use of PRO051 to keep a child with Duchenne's muscular dystrophy out of a wheelchair? First, the single local injections that are described by van Deutekom et al. must be scaled up to a systemic delivery system (either intravenous or subcutaneous). The systemic delivery of antisense oligonucleotides such as 2OMePS and phosphorodiamidate morpholino oligomers has already proved to be highly effective in mouse models of Duchenne's muscular dystrophy by restoring dystrophin expression throughout the animal.[4,5] Second, the possibly toxic effects of delivering very high doses (grams) of PRO051 or similar sequence-specific drugs repeatedly over years must be carefully examined and overcome. Patients with Duchenne's muscular dystrophy probably have a disease-specific delivery advantage, since PRO051 might traverse the myofiber membrane through the same holes through which creatine kinase leaks out (Fig. 1B). Off-target tissue toxicity might be rare, since the drug might not easily enter other cells with intact plasma membranes.

However, the major hurdles to implementing the use of PRO051 may involve requirements regarding both the development and the regulation of the drug. PRO051 has passed toxicity tests for phase 1 and 2 studies in the Netherlands, and studies that involve similar techniques of exon-51 targeting with the use of alternative morpholino chemistry are under way in England.[6] However, this additional single exon-51 drug would treat only a minority of patients with Duchenne's muscular dystrophy; other sequences must be developed to treat other patients with different mutations. Furthermore, since not all in-frame deletions lead to clinically mild Becker's muscular dystrophy, it may take the deletions of multiple exons with the use of multiple sequence-specific drugs to attain the ideal skipped dystrophin protein.[7-9]

One can easily envision that dozens of specific sequences will be required for effectively treating the majority of patients with Duchenne's muscular dystrophy. Will each specific sequence need to go through the full gamut of regulatory hurdles required by the Food and Drug Administration (FDA)? Are standard toxicity panels in animals appropriate when the hybridizing sequences in humans and animals are different? Off-target drug effects are likely to be species-specific, and a greater burden of toxicity tests may need to be borne by patients themselves. The first real hope for patients with Duchenne's muscular dystrophy might lie in the technique of exon skipping, but realization of this hope might benefit from approval of dystrophin antisense sequences as a class of drugs (i.e., approval of the chemistry, rather than the specific sequences).

This type of approval would be a first for the FDA, but the promise of personalized molecular medicine might justify such a change in approach.

Dr. Hoffman reports receiving grant support from Pfizer. No other potential conflict of interest relevant to this article was reported.

From the Departments of Pediatrics, Neuroscience, and Biochemistry and Molecular Biology, George Washington University School of Medicine, and the Research Center for Genetic Medicine, Children's National Medical Center — both in Washington, DC.

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