Pediatric Neurology 51 (2014) 607–618

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Topical Review

Gene Therapy for Muscular Dystrophy: Moving the Field Forward

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ABSTRACT

Gene therapy for the muscular dystrophies has evolved as a promising treatment for this progressive group of disorders. Although corticosteroids and/or supportive treatments remain the standard of care for Duchenne muscular dystrophy, loss of ambulation, respiratory failure, and compromised cardiac function is the inevitable outcome. Recent developments in genetically mediated therapies have allowed for personalized treatments that strategically target individual muscular dystrophy subtypes based on disease pathomechanism and phenotype. In this review, we highlight the therapeutic progress with emphasis on evolving preclinical data and our own experience in completed clinical trials and others currently underway. We also discuss the lessons we have learned along the way and the strategies developed to overcome limitations and obstacles in this field.

Keywords: gene therapy, muscular dystrophy, exon skipping

Pediatr Neurol 2014; 51: 607-618

Introduction

The revolution in genetics, witnessed over the past few decades, has transformed the practice of medicine. Gene therapy exemplifies the move toward personalized medicine, where molecular-based therapies are tailored to the individual's genetic disorder. Approaches to gene therapy include direct gene replacement or gene repair or indirectly through a surrogate gene that enhances cellular performance. The promising findings from preclinical proof-of-concept studies in Duchenne muscular dystrophy (DMD) and the limb-girdle muscular dystrophies (LGMDs) have moved the field forward for the treatment of neuromuscular disorders.

In this review, we highlight the progress and lessons learned with an emphasis on our own experience in clinical trials that are underway or have been completed.

Gene repair through mutation-specific therapies in DMD (nonviral vectors)

Exon skipping using antisense oligonucleotides

Mutations in the *DMD* gene disrupt the open reading frame and result in an incomplete translation of the dystrophin protein. Exon skipping is an approach to gene repair that targets the pre-messenger RNA transcript, introducing alternative splice sites that result in skipping one or more targeted exons with resultant restoration of the dystrophin reading frame. Antisense oligonucleotides (AONs) are synthetically modified strands of nucleic acids, typically 20-30 nucleotides in length, composed of complementary sequences to dystrophin pre-messenger RNA. This treatment approach was inspired by evidence of restoration of the open reading frame of the dystrophin gene expressed as "revertant fibers" by intrinsic alternative splicing appearing in a high percentage of DMD boys.[1] The result is small clusters of dystrophin-positive fibers, too few to provide clinical efficacy. Synthetically designed AONs were designed to simulate naturally occurring alternative splicing, with the intention of achieving this in a higher percentage of muscle fibers that could attain a clinically meaningful result. It is an approach that could apply to approximately 83% of all DMD patients.[2] The early emphasis for clinical trials has been to target the hot spot region for deletions between exons 42 and 55 where mutations are generally amenable to exon skipping. Exon 51 skipping is applicable to the largest group of all DMD patients (13%) inclusive of deletions: 45-50, 47-50, 48-50, 49-50, 50, 52, or 52-63.[2] Preclinical efficacy of AONs has been demonstrated in the *mdx*, dystrophin and/or utrophin knockout (KO) mouse, and the dystrophin-deficient dog using 2′O-methyl-ribo-oligonucleoside-phosphorothioate

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http://dx.doi.org/10.1016/j.pediatrneurol.2014.08.002

and phosphorodiamidate morpholino oligomers (PMOs).[3-5] These two oligomers share a common mechanism of action but differ in their biochemical structure, stability against endonucleases, and toxicity profiles.

Two phase 1, proof-of-principle, clinical trials using a 2′O-methyl-ribo-oligonucleoside-phosphorothioate oligomer (PRO051/GSK2402968; Prosensa Therapeutics) or a PMO (AVI-4658/Eteplirsen; Sarepta Therapeutics) targeting exon 51 were completed by a direct muscle injection, both demonstrating expression of dystrophin fibers.[6,7] Safety profiles were comparable, with neither revealing adverse events. These studies led to subsequent phase 2a open-label trials, in which higher doses of systemically delivered AONs were given to study participants. In the PRO051 trial, 12 patients participated in a dose-escalation 5-week trial of product given by subcutaneous injection.[8] Dystrophin expression was observed in approximately 60-100% of muscle fibers reaching 15.6% of normal. A 12-week extension study improved the 6-minute walk distance by about 35 m. Complications included injection site reactions and proteinuria without the loss of renal function. In the phase 2, 12-week, open-label, dose-escalation PMO study (dose range, 0.5-20.0 mg/kg), dystrophin expression at the sarcolemma was modestly increased in seven of 19 patients to a mean of 8.9-16.4%.[9] One patient reached 55% dystrophin positive fibers after treatment with 20 mg/kg. No drug-related adverse events were encountered.

The first, double-blind, randomized, controlled trial of exon skipping using a PMO (eteplirsen; Sarepta Therapeutics) took place at Nationwide Children's Hospital in Columbus, Ohio.[10] Enrollment included 12 DMD boys, ages 7 to 13 years with confirmed of frame *DMD* gene deletions potentially correctable by skipping exon 51 that were randomized to one of three eteplirsen-treated cohorts: cohort 1, received 30 mg/kg/wk; cohort 2, 50 mg/kg/wk; and cohort 3, placebo treated. At week 25, cohort 3 switched to open-label treatment, either 30 or 50 mg/kg/wk, thereafter referred to as "placebo delayed" (Fig 1). All were on a stable dose of corticosteroids for 6 months minimum before enrollment. Outcome measures included dystrophin production, both percentage of muscle fibers and intensity at the sarcolemma. Muscle biopsies were performed at baseline, at 12 weeks for the 50 mg/kg cohort and 24 weeks for the 30 mg/kg cohort. Two blinded placebo-treated patients were biopsied at 12 weeks and two at week 24. At the week 25, the placebo-treated patients (n = 4) received weekly eteplirsen dosing, two received 30 mg/kg and two received 50 mg/kg. All patients had a third biopsy at week 48. The 6-minute walk test (6MWT) was the primary functional outcome, performed pretreatment and posttreatment through week 48.

The results of dystrophin production at all time points are illustrated in (Fig 2). At 12 weeks after treatment, no significant dystrophin was produced (50 mg/kg cohort and two placebo treated). Dystrophin production could be observed after 24 weeks of eteplirsen except for the two placebo-treated patients (as expected). At week 48, all patients, including the placebo-delayed cohort, produced significant dystrophin. These findings are a clear indication that dystrophin production started sometime after week 12 and was more influenced by duration of treatment than dose. We also found restoration of the sarcoglycans and nNOS-binding site accompanying dystrophin production.

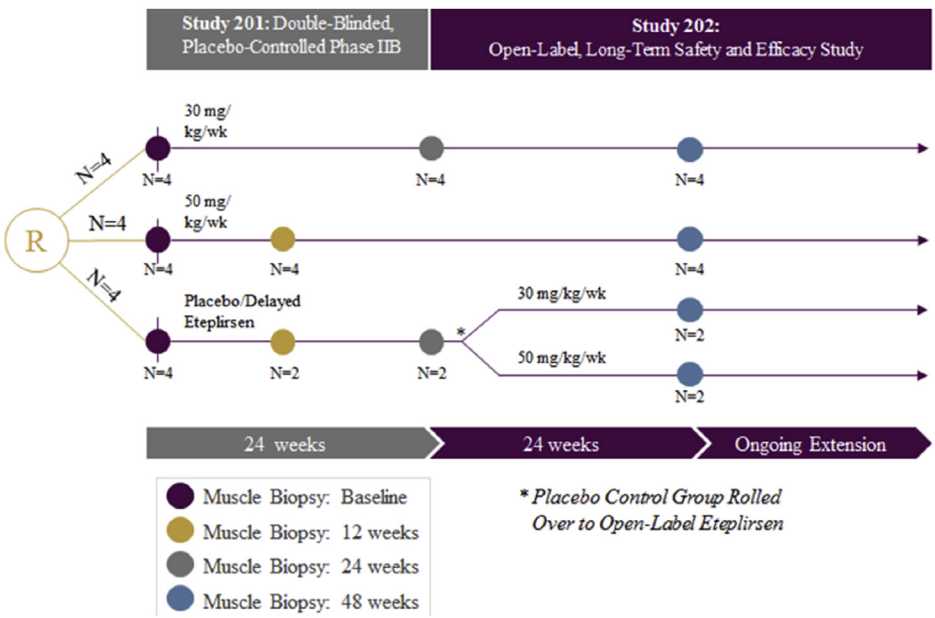

FIGURE 1.
Eteplirsen exon skipping study design. Twelve patients with Duchenne muscular dystrophy were randomized (R) to one of three eteplirsen-treated cohorts in study 201: cohort 1, received 30 mg/kg/wk; cohort 2, 50 mg/kg/wk; and cohort 3, placebo treated. At week 25, cohort 3, switched to open-label treatment, either 30 or 50 mg/kg/wk; thereafter referred to as "placebo delayed". Patients were maintained on the same starting dose of eteplirsen under the open-label extension study 202. Biceps biopsies were obtained on all patients at baseline and deltoid biopsies at week 48 for analysis of dystrophin expression. At week 12, biceps biopsies were obtained from patients in cohort 2 (50 mg/kg/wk) and two placebo-treated patients in cohort 3. At week 24, biceps biopsies were obtained from patients in cohort 1 (30 mg/kg/wk) and two placebo-treated patients in cohort 3. Reprinted from Mendell, et al.[10]