PPMO Results in Widespread Muscle Delivery and Efficacy in Mice and Nonhuman Primates: A Therapeutic Platform for Duchenne Muscular Dystrophy

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BACKGROUND

Duchenne muscular dystrophy (DMD)

- DMD is a rare, progressive, fatal degenerative neuromuscular disease with X-linked recessive inheritance[1,2]
- The disease affects approximately 1 in every 3,500–5,000 males born worldwide[3,4] and there are an estimated 9,000–12,000 patients with DMD in the US[5]
- Sarepta is at the forefront of developing precision genetic medicines for central nervous system disorders, which include RNA and gene therapy for DMD
  - The focus of this presentation is the preclinical development of peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) for treatment of DMDD

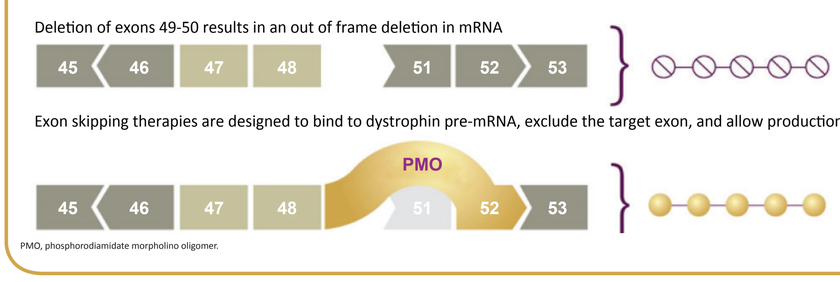
Dystrophin and DMD

- The dystrophin protein is 427 kDa and anchors the cytoskeletal system with the extracellular matrix via the dystrophin-associated glycoprotein complex[6]
- The *DMD* gene that encodes the dystrophin protein consists of 79 exons[6]
- Mutations in the *DMD* gene result in disruption of the messenger ribonucleic acid (mRNA) open-reading frame, leading to incomplete translation of an unstable protein that is degraded
- Absence of dystrophin leads to loss of functional abilities, including loss of ambulation in the early to mid teens, the need for diurnal and nocturnal ventilation, and premature death, usually by age 30[7,8]

Exon-skipping approach

- Exon skipping is a treatment strategy for DMD that involves the use of antisense oligonucleotides to restore the mRNA reading frame and facilitate production of an internally shortened dystrophin protein (**Figure 1**)[9,10]
- Published data suggest that approximately 80% of DMD patients have genotypes amenable to exon skipping[11]

Figure 1. Example of a genotype amenable to PMO treatment



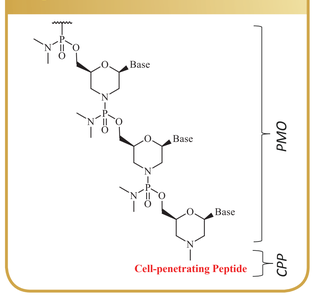
PMO, phosphorodiamidate morpholino oligomer.

METHODS

PPMOs for DMD next-generation antisense platform

- PPMOs are composed of a cell-penetrating peptide (CPP) conjugated to the 3′-end of a PMO (**Figure 2**)
  - PPMOs serve as a platform technology that may be tailored to target any organ or tissue
- CPP conjugation has the potential to provide:
  - Improved delivery and subsequent increased dystrophin production *in vivo*
  - More efficient dosing

Figure 2. PPMO structure



PPMO administration in *mdx* mice

- *mdx* mice and wild-type mice were housed with ad libitum access to food and water
- A proprietary CPP was conjugated to a mouse PMO sequence to form the PPMO that was administered to *mdx* mice; this PPMO specifically targets exon 23
- PPMO persistence of effect
  - Single intravenous (IV) dose of PPMO 40 mg/kg was administered to *mdx* mice (n=6 mice per group)
    - Additional groups of *mdx* and wild-type mice (n=8, each group) received 200 µL saline (vehicle controls)
  - Dystrophin expression was measured using immunohistochemistry (IHC) and Western blot at 7, 30, 60, and 90 days post-injection
- PPMO dose response
  - Single IV doses of PPMO 10, 20, 40, or 80 mg/kg were administered to *mdx* mice (n=6 mice per group)
  - Dystrophin expression was measured using IHC and Western blot 30 days post-injection

PPMO dose response in nonhuman primates

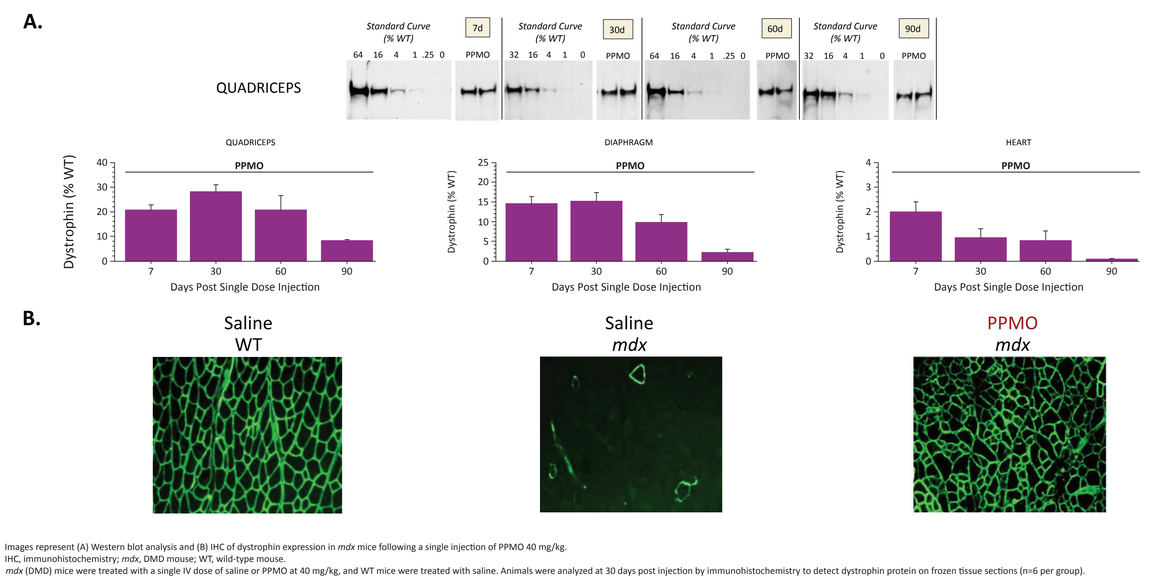
- Healthy cynomolgus monkeys were housed with ad libitum access to food and water
- Monkeys were administered 4 weekly IV low, medium, and high doses of PPMO as 30-minute infusions
- Exon skipping was measured using reverse transcription polymerase chain reaction (RT-PCR) at 48 hours after final dose
  - The PPMOs consisted of a proprietary CPP conjugated to a PMO sequence targeting exons 51 and 53

RESULTS

PPMO persistence of effect in *mdx* Mice (Figure 3)

- A single 40-mg/kg injection of PPMO increased levels of exon 23 skipping and dystrophin production in the quadriceps, diaphragm, and heart of *mdx* mice

Figure 3. A single 40-mg/kg injection of PPMO increased levels of exon 23 skipping and dystrophin production in the quadriceps, diaphragm, and heart of mdx mice



Images represent (A) Western blot analysis and (B) IHC of dystrophin expression in *mdx* mice following a single injection of PPMO 40 mg/kg.
IHC, immunohistochemistry; *mdx*, DMD mouse; WT, wild-type mouse.
mdx (DMD) mice were treated with a single IV dose of saline or PPMO at 40 mg/kg, and WT mice were treated with saline. Animals were analyzed at 30 days post injection by immunohistochemistry to detect dystrophin protein on frozen tissue sections (n=6 per group).

Repeated PPMO dosing (Figure 4)

Figure 4. Repeat administration of PPMO doses increased and sustained high levels of widespread dystrophin production in muscle

Images represent (A) Western blot analysis and (B) IHC of dystrophin expression in *mdx* mice following repeated dosing with PPMO 40 mg/kg. The images in panel B show dystrophin expression in the quadricepts at 3 months.
mdx (DMD) mice were treated with 3 monthly IV doses of PPMO at 40 mg/kg per dose, or a single IV dose of PPMO at 40 mg/kg (n=6 per time point per group)
Animals were analyzed 3 months after the initial dose by immunohistochemistry to detect dystrophin protein on tissue sections. Control age-matched *mdx* (DMD) and WT mice treated with saline are also shown.

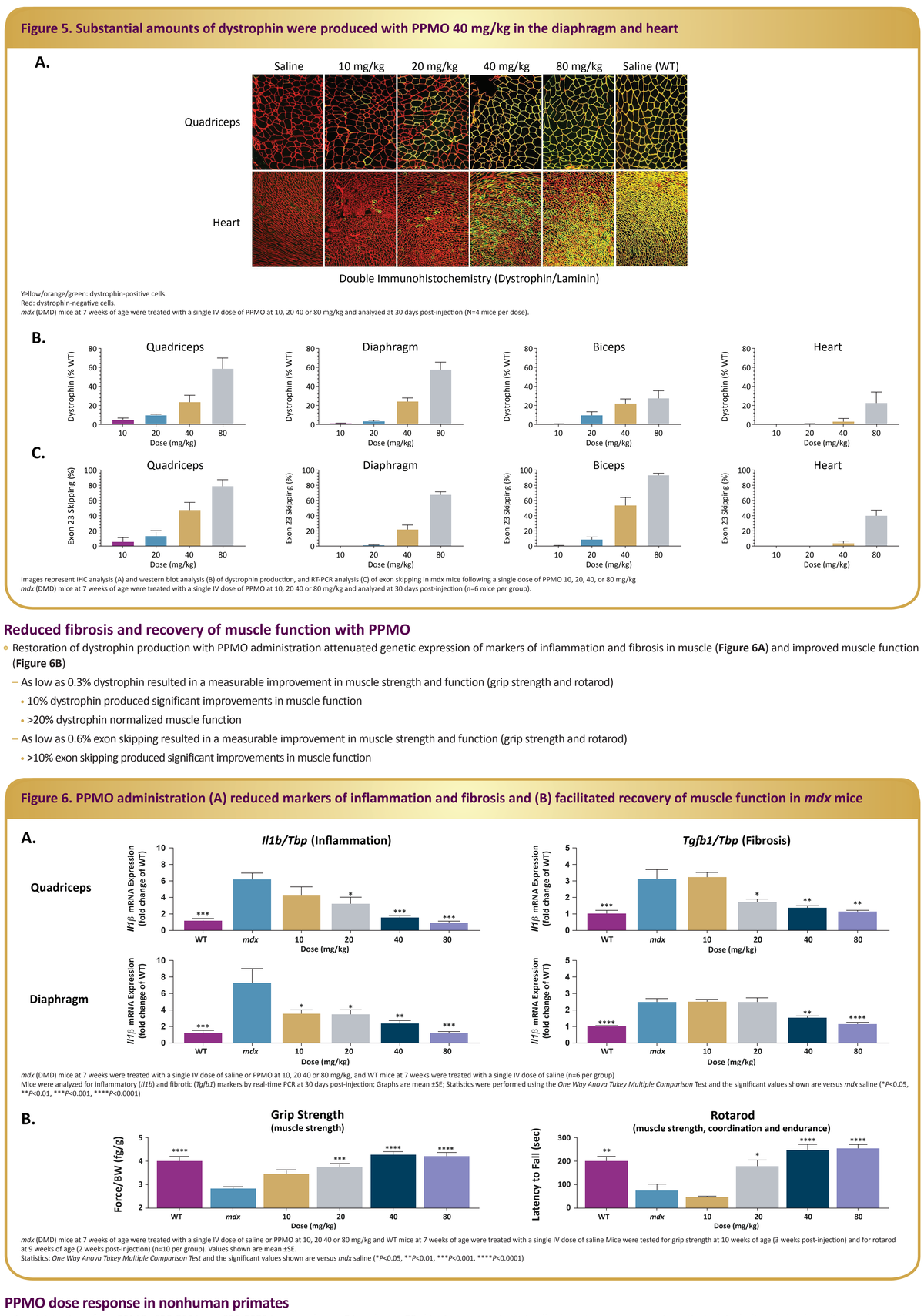
CONCLUSIONS

- PPMO is a highly potent platform for DMD, with long-lasting, robust therapeutic effects in preclinical models
- PPMO with proprietary CPP produces high levels of dystrophin in *mdx* mice
  - Therapeutic effect of a single dose of PPMO persists for at least 90 days
  - Repeat monthly dosing of PPMO maintains high levels of dystrophin in muscle
  - PPMO treatment decreases inflammatory and fibrotic markers and increases muscle function
- SRP-5051 and SRP-5053 achieve robust exon skipping in skeletal, cardiac, and smooth muscles in NHPs
  - SRP-5051 and SRP-5053 contain the same proprietary CPP used in the *mdx* mouse studies
  - SRP-5051 is the first PPMO in the clinic for DMD; a Phase 1 clinical trial has been initiated (clinicaltrials.gov: NCT03375255)

PPMO dose response in *mdx* Mouse

- PPMO administration generated a dose response in apparent levels of dystrophin production (**Figure 5**)

Figure 5. Substantial amounts of dystrophin were produced with PPMO 40 mg/kg in the diaphragm and heart



Yellow/orange/green: dystrophin-positive cells.
Red: dystrophin-negative cells.
mdx (DMD) mice at 7 weeks of age were treated with a single IV dose of PPMO at 10, 20 40 or 80 mg/kg and analyzed at 30 days post-injection (N=4 mice per dose).

Images represent IHC analysis (A) and western blot analysis (B) of dystrophin production, and RT-PCR analysis (C) of exon skipping in mdx mice following a single dose of PPMO 10, 20, 40, or 80 mg/kg
mdx (DMD) mice at 7 weeks of age were treated with a single IV dose of PPMO at 10, 20 40 or 80 mg/kg and analyzed at 30 days post-injection (n=6 mice per group).

Reduced fibrosis and recovery of muscle function with PPMO

- Restoration of dystrophin production with PPMO administration attenuated genetic expression of markers of inflammation and fibrosis in muscle (**Figure 6A**) and improved muscle function (**Figure 6B**)
  - As low as 0.3% dystrophin resulted in a measurable improvement in muscle strength and function (grip strength and rotarod)
    - 10% dystrophin produced significant improvements in muscle function
    - >20% dystrophin normalized muscle function
  - As low as 0.6% exon skipping resulted in a measurable improvement in muscle strength and function (grip strength and rotarod)
    - >10% exon skipping produced significant improvements in muscle function

Figure 6. PPMO administration (A) reduced markers of inflammation and fibrosis and (B) facilitated recovery of muscle function in *mdx* mice

mdx (DMD) mice at 7 weeks were treated with a single IV dose of saline or PPMO at 10, 20 40 or 80 mg/kg, and WT mice at 7 weeks were treated with a single IV dose of saline (n=6 per group)
Mice were analyzed for inflammatory (*Il1b*) and fibrotic (*Tgfb1*) markers by real-time PCR at 30 days post-injection; Graphs are mean ±SE; Statistics were performed using the *One Way Anova Tukey Multiple Comparison* Test and the significant values shown are versus *mdx* saline (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001)

mdx (DMD) mice at 7 weeks of age were treated with a single IV dose of saline or PPMO at 10, 20 40 or 80 mg/kg and WT mice at 7 weeks of age were treated with a single IV dose of saline Mice were tested for grip strength at 10 weeks of age (3 weeks post-injection) and for rotarod at 9 weeks of age (2 weeks post-injection) (n=10 per group). Values shown are mean ±SE.
Statistics: *One Way Anova Tukey Multiple Comparison Test* and the significant values shown are versus *mdx* saline (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001)

PPMO dose response in nonhuman primates

- Administration of the PPMOs SRP-5051 and SRP-5053 increased exon 51 and exon 53 skipping, respectively, in all relevant muscle groups investigated, including skeletal, cardiac, and smooth muscle (**Figures 7A and 7B**) >90% exon skipping observed in quadriceps and diaphragm at high doses

Figure 7. Exon 51 (A) and 53 (B) skipping in nonhuman primates following four weekly low, medium, and high doses of SRP-5051 or SRP-5053

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