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PN-Containing Oligonucleotides Yield High Levels of Exon Skipping and Dystrophin Protein Restoration in Preclinical Models for DMD

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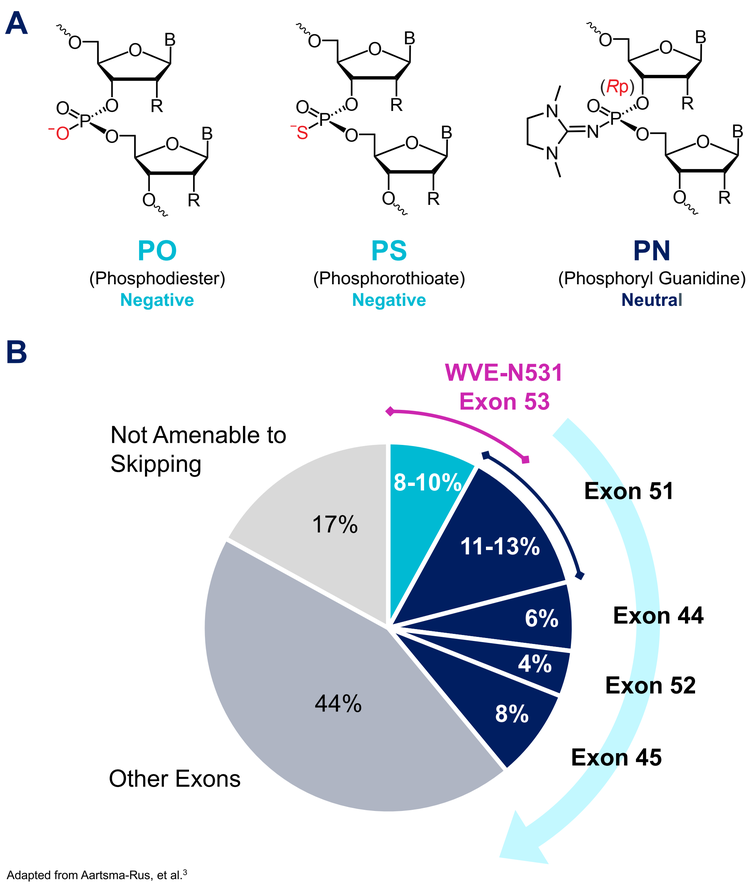
SUMMARY

- Duchenne muscular dystrophy (DMD) is one of the most common and severe congenital myopathies that occurs due to the absence of functional dystrophin.[1]
- We evaluated the *in vitro* exon skipping activity and dystrophin protein restoration of phosphoryl guanidine (PN)-containing oligonucleotides designed to skip exons 52, 51, and 44, as well as the *in vivo* exon skipping activity of a surrogate oligonucleotide designed to target mouse exon 23.
- PN-containing oligonucleotides designed to skip exons 52, 51, or 44 yielded high levels of exon skipping and dystrophin protein restoration in patient-derived myoblasts.
  - Mean exon skipping was dose-dependent for all exons evaluated. At low doses (2.5 μM for Exons 52 and 44; 1.1 μM for Exon 51), we observed 50% exon skipping, whereas at high doses (10 μM), we observed 95% exon skipping.
  - Dystrophin protein restoration ranged from 65% to near healthy control levels across all exons assessed.
- After administration of a single dose of surrogate PN-containing oligonucleotide in mdx23 mice, we observed exon skipping at week one with dystrophin protein accumulation observed at day 28 in both skeletal and cardiac tissues. After administering three biweekly (every other week) doses, a long duration of action of the surrogate was observed.
- These preclinical data highlight the promise of PN-containing exon skipping oligonucleotides as potential investigational treatments for patients with DMD amenable to exon skipping beyond exon 53.

INTRODUCTION

- Exon-skipping oligonucleotides are designed to bind to pre-mRNA, causing cellular machinery to bypass exon(s) during splicing to restore the translational reading frame and protein production for out-of-frame mutations.[2]
- Ongoing research aims to continually improve the pharmacological properties of oligonucleotides for DMD.
- In this regard, we have developed stereopure antisense oligonucleotides featuring chimeric phosphodiester (PO), phosphorothioate (PS) and PN-containing backbones for patients with a variety of mutations amenable to exon skipping (**Figure 1**).
- WVE-N531, a PN-containing oligonucleotide designed for exon 53 skipping, has demonstrated promising results in preclinical studies and a Phase 1b/2 study for patients with DMD.
- To further investigate the impact of PN chemistry, we evaluated the exon skipping activity and dystrophin protein restoration of stereopure PN-containing oligonucleotides designed to skip human exons 51, 52, 44, and mouse exon 23.

Figure 1. Potential for Wave to address up to 40% of DMD population with investigational antisense oligonucleotides with PN backbone chemistry

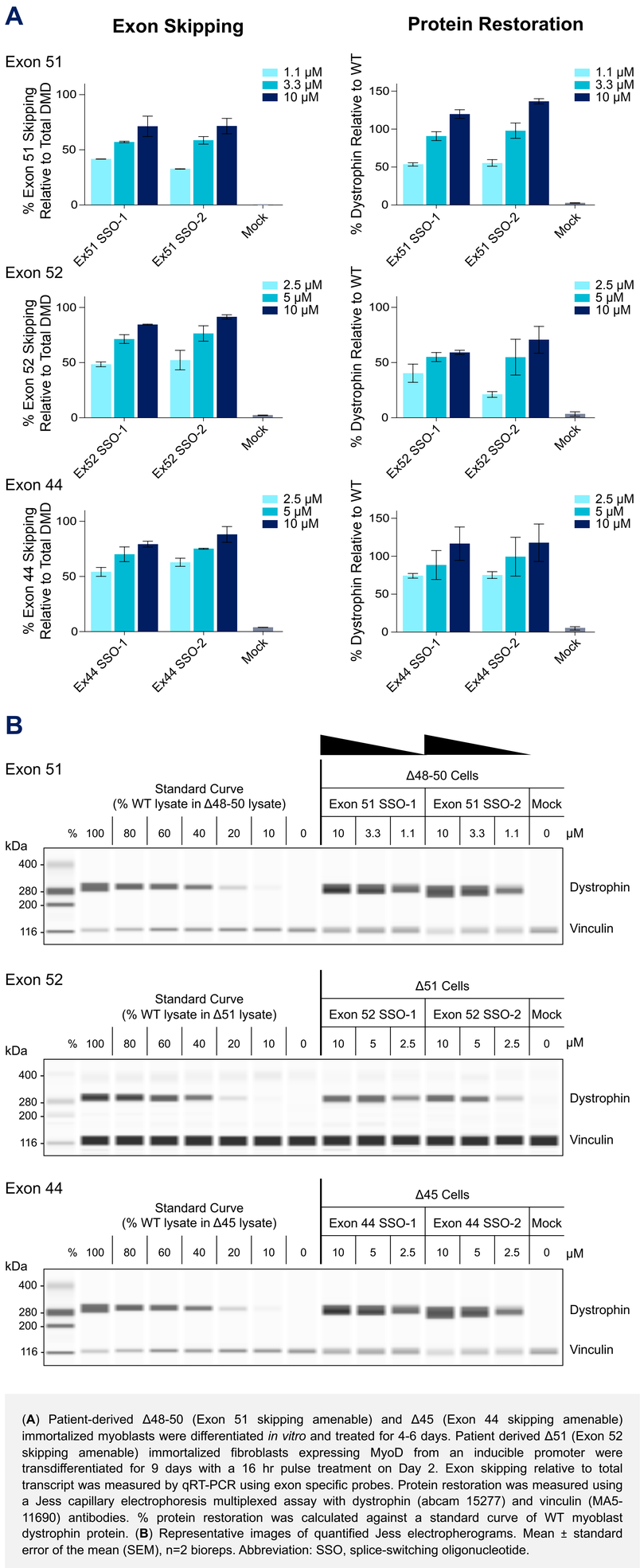


Adapted from Aartsma-Rus, et al.[3]

RESULTS

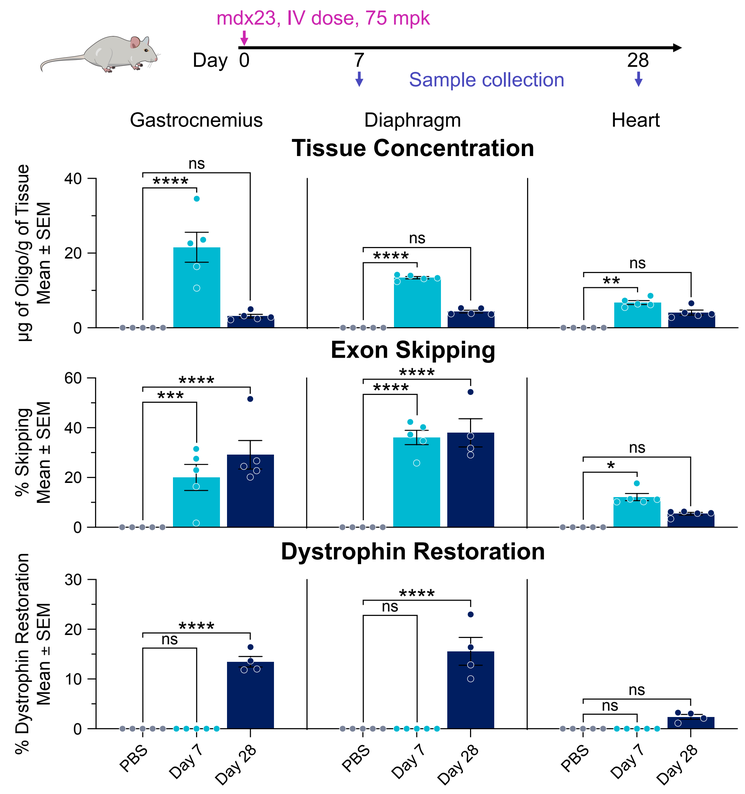
- Our results demonstrated that PN-containing oligonucleotides designed to skip exons 52, 51, or 44 yielded high levels of exon skipping and dystrophin protein restoration *in vitro*.
- In patient-derived myoblasts, we observed substantial dystrophin protein restoration ranging from 65% to near healthy control levels across all exons assessed. Exon skipping was dose-dependent, with a mean of 50% skipping at low doses (2.5 μM for Exons 52 and 44; 1.1 μM for Exon 51) and 95% skipping at high doses (10 μM) (**Figure 2A,B**).
- In mdx23 mice, we observed exon skipping one week after administration of a single dose of surrogate oligonucleotide (inducing skipping of mouse exon 23), with dystrophin protein accumulation observed at day 28 in both skeletal and cardiac muscles (**Figure 3**).
- After three biweekly doses, the surrogate induced durable dystrophin protein restoration in mice, reaching levels up to 40% of wild-type (WT) in skeletal muscle and 8% in cardiac muscle (**Figure 4**).

Figure 2. PN-containing oligonucleotides designed to skip exons 52, 51, or 44 yielded high levels of exon skipping and dystrophin protein restoration in patient-derived myoblasts



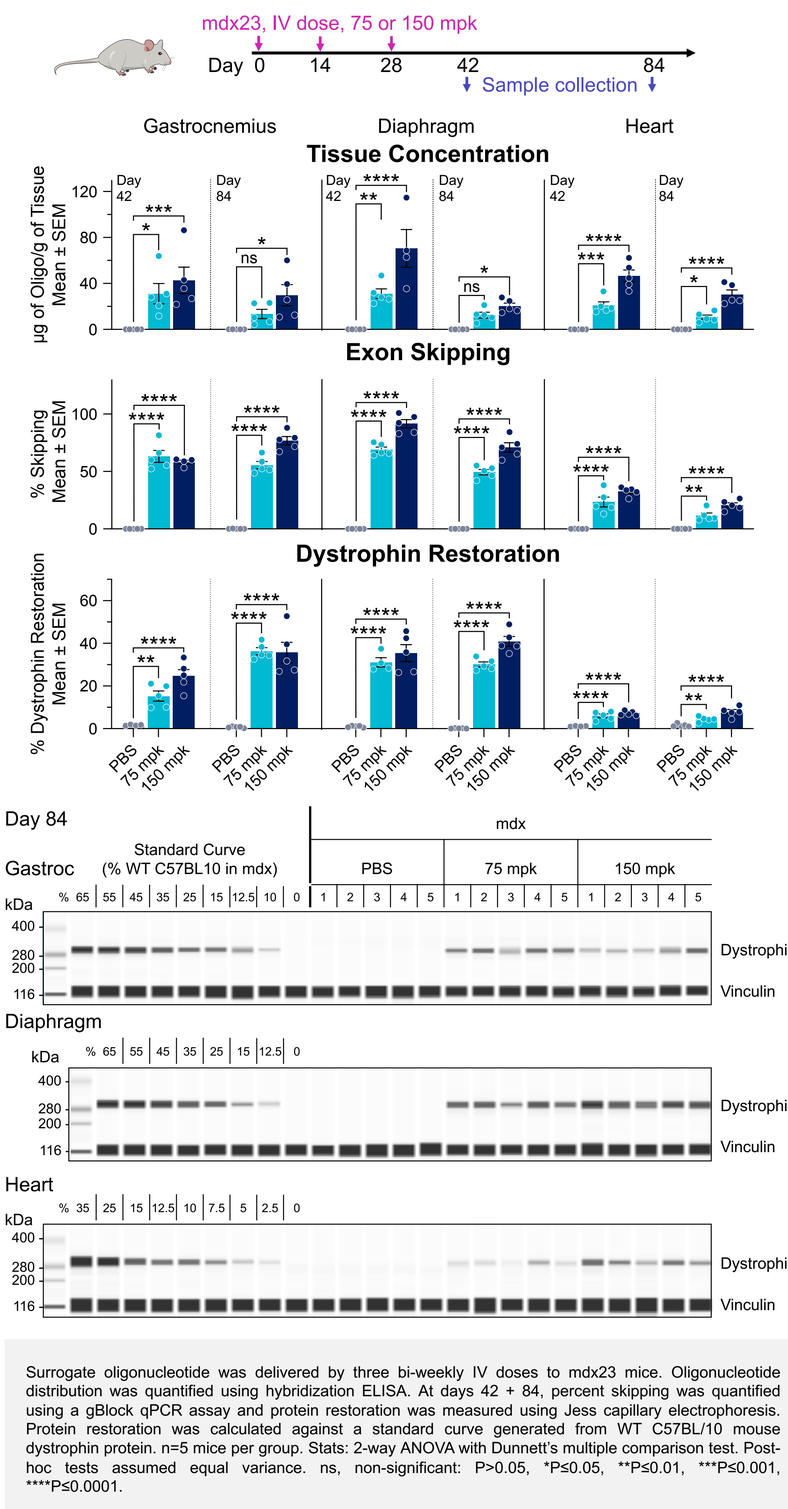
(**A**) Patient-derived Δ48-50 (Exon 51 skipping amenable) and Δ45 (Exon 44 skipping amenable) immortalized myoblasts were differentiated *in vitro* and treated for 4-6 days. Patient derived Δ51 (Exon 52 skipping amenable) immortalized fibroblasts expressing MyoD from an inducible promoter were transdifferentiated for 9 days with a 16 hr pulse treatment on Day 2. Exon skipping relative to total transcript was measured by qRT-PCR using exon specific probes. Protein restoration was measured using a Jess capillary electrophoresis multiplexed assay with dystrophin (abcam 15277) and vinculin (MA5-11690) antibodies. % protein restoration was calculated against a standard curve of WT myoblast dystrophin protein. (**B**) Representative images of quantified Jess electropherograms. Mean ± standard error of the mean (SEM), n=2 bioreps. Abbreviation: SSO, splice-switching oligonucleotide.

Figure 3. A single dose of surrogate oligonucleotide in mdx23 mice results in high skipping and dystrophin protein accumulation by day 28



Surrogate oligonucleotide was delivered by a single 75 mg/kg intravenous (IV) dose to mdx23 mice. Oligonucleotide distribution was quantified using hybridization ELISA. At days 7 + 28, percent skipping was quantified using a gBlock qPCR assay and protein restoration was measured using Jess capillary electrophoresis. Protein restoration was calculated against a standard curve of WT C57BL/10 mouse dystrophin protein. n=5 mice per group. Stats: 2-way ANOVA with Dunnett's multiple comparison test. Post-hoc tests assumed equal variance. ns, non-significant: P>0.05, *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

Figure 4. Administration of surrogate oligo in mdx23 mice results in high levels of dystrophin restoration and durability 3 months post-biweekly dosing



Surrogate oligonucleotide was delivered by three bi-weekly IV doses to mdx23 mice. Oligonucleotide distribution was quantified using hybridization ELISA. At days 42 + 84, percent skipping was quantified using a gBlock qPCR assay and protein restoration was measured using Jess capillary electrophoresis. Protein restoration was calculated against a standard curve generated from WT C57BL/10 mouse dystrophin protein. n=5 mice per group. Stats: 2-way ANOVA with Dunnett's multiple comparison test. Post-hoc tests assumed equal variance. ns, non-significant: P>0.05, *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

References: 1. Blake DJ, et al. *Physiol Rev.* 2002 Apr;82(2):291-329; 2. Hoffman EP and McNally EM. *Sci Transl Med.* 2014 Apr;6(230):230fs14; 3. Aartsma-Rus A, et al. *Hum Mutat.* 2009;30(3):293-299. **Acknowledgments:** For development of this poster, the authors thank Amy Donner and Alexander Lin (Wave Life Sciences) for medical writing support and Eric Smith for graphics support. This work was funded by Wave Life Sciences.