Durable Exon Skipping and Dystrophin Production With Endosomal Escape Vehicle (EEV™)–Oligonucleotide Conjugates in Preclinical Models of Duchenne Muscular Dystrophy

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INTRODUCTION

- Currently approved phosphorodiamidate morpholino oligomer (PMO) therapies for Duchenne muscular dystrophy (DMD) were designed to restore the mRNA reading frame and produce dystrophin by exon skipping, but have shown modest improvements.[1,2]
  - Intracellular delivery of oligonucleotides is challenging because of poor cell entry and limited escape from the endosome in the target cell.[3,4]
- To enhance PMO delivery to target tissues, we designed a family of proprietary cyclic cell-penetrating peptides that form the core of the Endosomal Escape Vehicle (EEV™) platform[5] (**Figure 1**).
  - Results of preliminary studies in *mdx* and D2-*mdx* mice demonstrated that EEV-PMO constructs dosed monthly produce dystrophin in skeletal and cardiac muscle by exon skipping.[6,7]

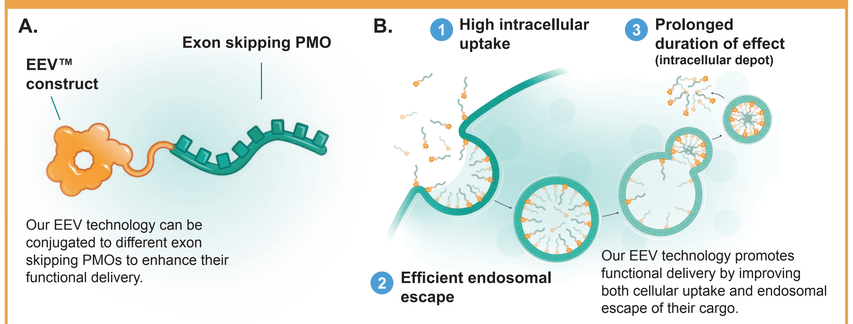
OBJECTIVE

- To assess the durable efficacy and therapeutic potential of exon-skipping EEV-PMO constructs with less frequent dosing in preclinical models of DMD.

MATERIALS AND METHODS

- EEV-PMO-23, a DMD exon 23 skipping PMO conjugated to the EEV platform, was administered intravenously (IV) every 6 weeks (Q6W) to assess exon skipping, dystrophin production, and muscle contractility in D2-*mdx*[8] mice (**Figures 2** and **3**). These mice carry a nonsense mutation in exon 23.
- del44hDMD.*mdx* are human dystrophin (hDMD)-expressing mice engineered with a deletion in the DMD exon 44 transgene on the *mdx* background, resulting in an exon 45 skip–amenable mouse line. These mice were treated with a human exon 45 skipping EEV-PMO construct (EEV-PMO-45) (**Figure 4**).
- Dystrophin restoration was evaluated by Simple Western Jess (Bio-Techne, Minneapolis, MN) and immunofluorescence. Exon-skipping efficiency was analyzed by either reverse-transcriptase polymerase chain reaction (PCR) and LabChip (Perkin Elmer, Santa Clara, CA) (**Figure 2**) or reverse-transcriptase digital droplet PCR (**Figure 4**).

Figure 1. EEV-PMO Construct Structure and Mechanism of Action.



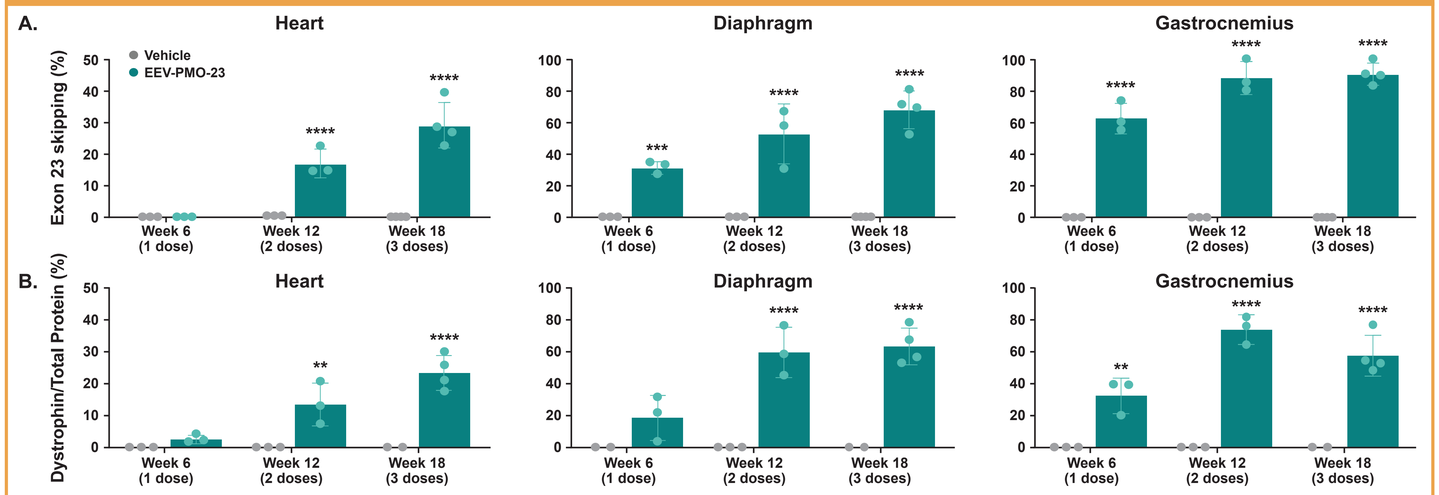
(A) Structure of EEV-PMO construct consisting of a cyclic cell-penetrating peptide-based EEV-construct and exon skipping PMO. **(B)** Mechanism of EEV construct-mediated delivery of PMOs. EEV, Endosomal Escape Vehicle; PMO, phosphorodiamidate morpholino oligomer.

RESULTS

Exon Skipping and Dystrophin Production With EEV-PMO-23 in D2-mdx Model of DMD

- Significant exon 23 skipping (**Figure 2A**) and dystrophin production (**Figure 2B**) was observed in cardiac and skeletal muscle following three Q6W doses of EEV-PMO-23.
  - Improvements over vehicle treated mice were observed after the first dose in skeletal muscle and after the second dose in cardiac muscle.

Figure 2. Exon 23 Skipping and Dystrophin Production With EEV-PMO-23 in D2-*mdx* Mice.

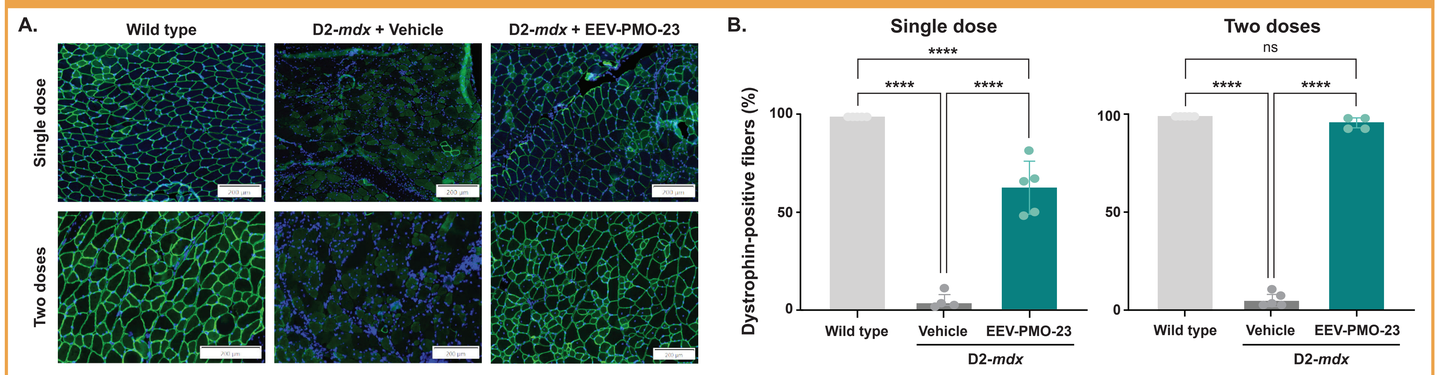


D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Exon skipping **(A)** and dystrophin production **(B)** were analyzed 6 weeks after each dose. Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; **p<0.01, ***p<0.001, ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; EEV, Endosomal Escape Vehicle; PMO, phosphorodiamidate morpholino oligomer; Q6W, every 6 weeks; SD, standard deviation.

Dystrophin Restoration With EEV-PMO-23 in the D2-mdx Mouse Model of DMD

- Two Q6W doses of EEV-PMO-23 significantly increased dystrophin expression in the skeletal muscle of D2-*mdx* mice to similar levels observed in wild type mice (**Figure 3**).

Figure 3. Dystrophin Expression With EEV-PMO-23 in D2-*mdx* Mice.

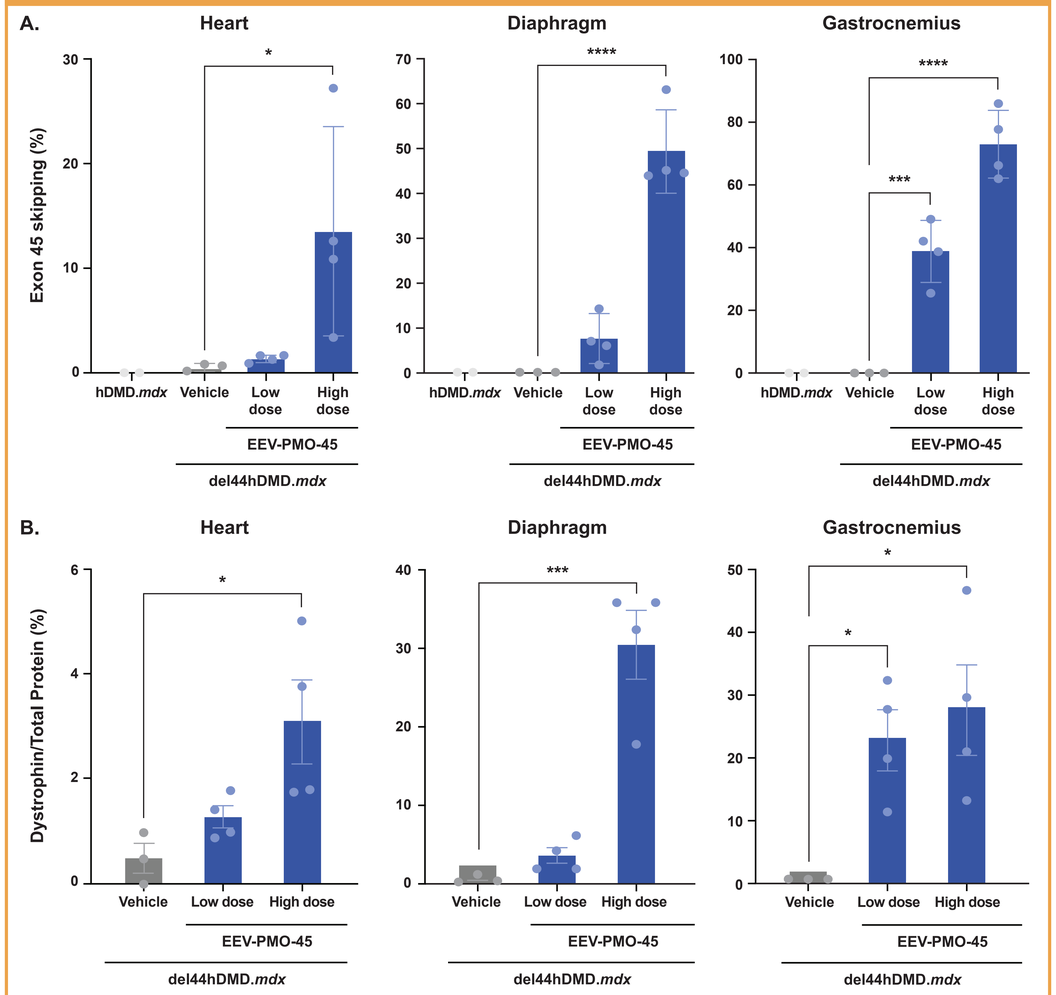


D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Gastrocnemius muscle was assessed for dystrophin expression 6 weeks after each dose. **(A)** Immunofluorescence showing dystrophin in green and DAPI in blue after one or 2 doses. **(B)** Quantification of dystrophin-positive fibers. Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; DAPI, 4′,6-diamidino-2-phenylindole; EEV, Endosomal Escape Vehicle; ns, not significant; PMO, phosphorodiamidate morpholino oligomer; Q6W, every 6 weeks; SD, standard deviation.

Exon 45 Skipping and Dystrophin Production in Exon 45 Skip–Amenable Mice

- EEV-PMO-45 produced robust exon 45 skipping and dystrophin production in del44hDMD.*mdx* mice harboring an exon 45 skip–amenable mutation (**Figure 4**).

Figure 4. Efficacy of EEV-PMO-45 in del44hDMD.*mdx* Mice With an Exon 45 Skip–Amenable Mutation.



del44hDMD.*mdx* mice were treated with a single IV injection of EEV-PMO-45 or vehicle. hDMD.*mdx* mice were used as healthy controls for dystrophin production as they contain a normal hDMD transgene on the *mdx* background. Human exon 45 skipping **(A)** and dystrophin protein expression **(B)** were analyzed in the gastrocnemius, diaphragm, and heart 2 weeks after dosing. Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; *p<0.05, ***p<0.001, ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; EEV, Endosomal Escape Vehicle; hDMD, human dystrophin; PMO, phosphorodiamidate morpholino oligomer; SD, standard deviation.

ACKNOWLEDGMENTS

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CONCLUSIONS

- EEV-PMO constructs produced robust and durable exon skipping and dystrophin production following 6-week dosing in preclinical models of DMD.
- Preliminary studies with an exon 45 skipping EEV-PMO constructs showed robust exon skipping and dystrophin production in mouse models harboring an exon 45 skip–amenable mutations, respectively.
- These findings support earlier studies demonstrating the preclinical efficacy of ENTR-601-44 and ENTR-601-45 and support further study of these EEV-PMO constructs in patients with exon 44 and 45 skip–amenable DMD, respectively.
  - A phase 1 clinical trial of ENTR-601-44 in healthy volunteers demonstrated that ENTR-601-44 showed dose-dependent exon 44 skipping with no adverse events related to study drug