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

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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* mice demonstrated that EEV-PMO constructs produce dystrophin in skeletal and cardiac muscle by exon skipping.[6]
- Here, we further examined the EEV-PMO approach in multiple preclinical models of DMD.

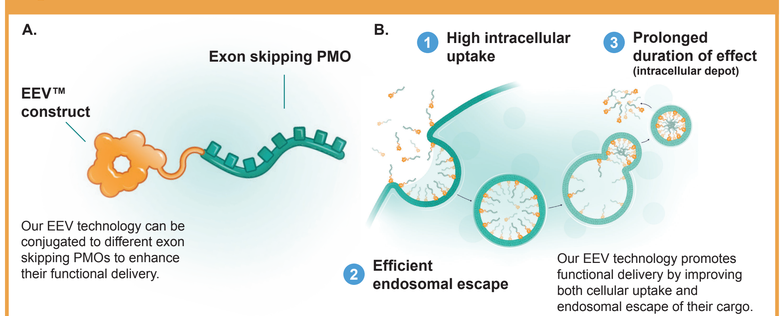
MATERIALS AND METHODS

- The efficacy of 2 EEV-PMO constructs was assessed in the following animal models:
  - 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*[7] mice (**Figures 2–4**). These mice carry a nonsense mutation in exon 23.
  - ENTR-601-44, a DMD exon 44 skipping PMO conjugated to the EEV platform, was administered IV to human dystrophin (hDMD) producing mice[8] and nonhuman primates (NHPs) to assess exon skipping in cardiac and skeletal muscles (**Figure 5**).
- Dystrophin restoration was evaluated by Simple Western Jess (Bio-Techne, Minneapolis, MN) and immunofluorescence. Exon-skipping efficiency was analyzed by reverse-transcriptase polymerase chain reaction and LabChip (Perkin Elmer, Santa Clara, CA).

OBJECTIVE

- To assess the therapeutic potential of exon-skipping EEV-PMO constructs in preclinical models of DMD.

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



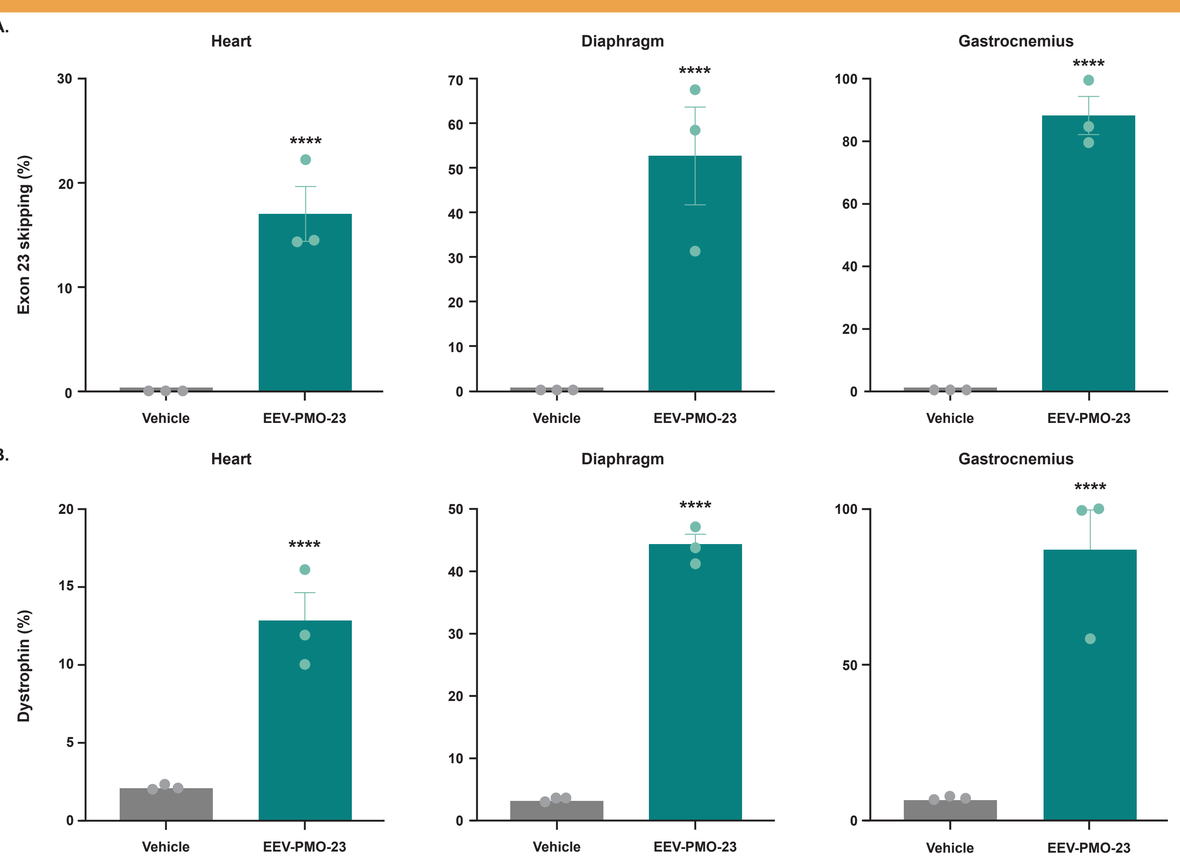
(A) Structure of EEV-PMO construct consisting of a cyclic cell-penetrating peptide-based EEV-construct and exon skipping PMO. **(B)** Mechanism of EEV construct-mediated delivery of PMOs. EEV, endosomal escape vehicle construct; PMO, phosphorodiamidate morpholino oligomer.

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 two Q6W doses of EEV-PMO-23.

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

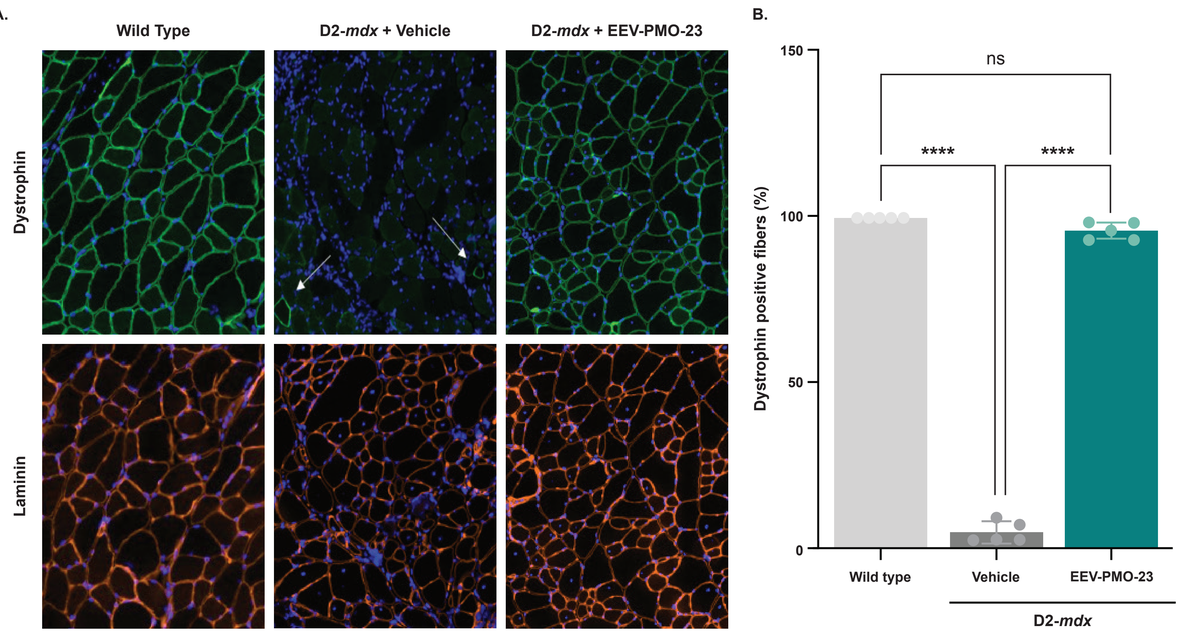


D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Exon skipping **(A)** and dystrophin production **(B)** were analyzed 6 weeks after the second dose. Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; EEV,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

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

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

Figure 3. Exon 23 Skipping and Dystrophin Production With EEV-PMO-23 in D2-*mdx* mice.

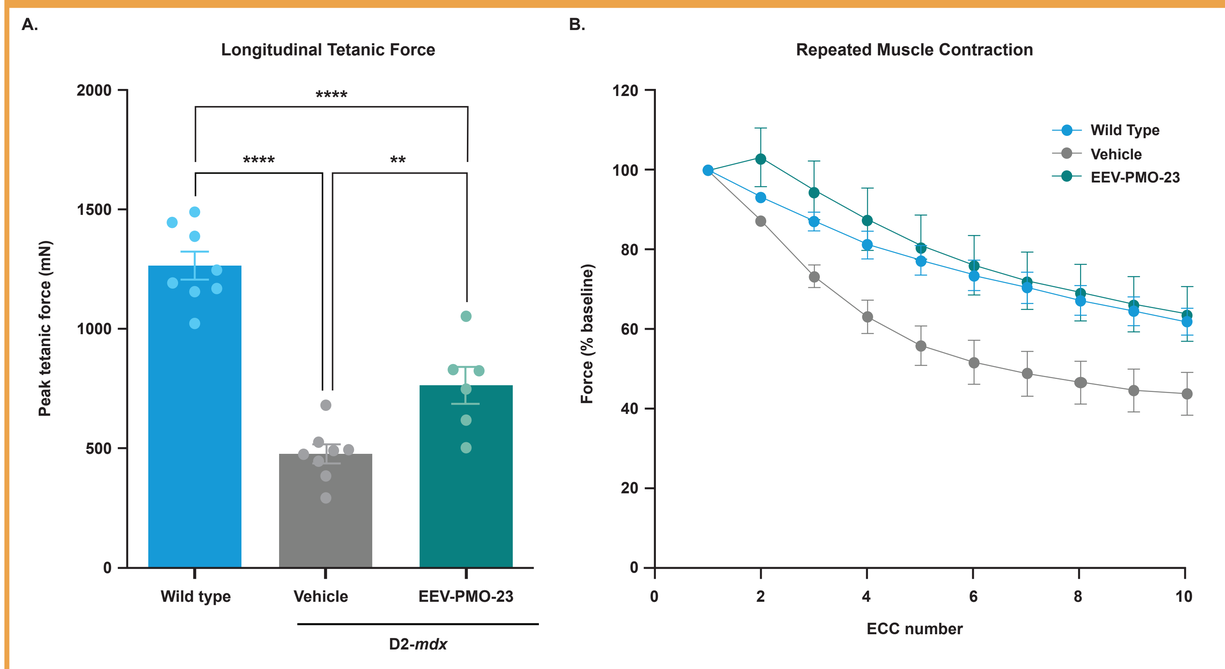


D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Gastrocnemius muscle was assessed for dystrophin expression 6 weeks after the second dose. **(A)** Immunofluorescence showing dystrophin in blue and laminin in red. **(B)** Quantification of dystrophin positive fibers. Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; EEV, endosomal escape vehicle; ns, not significant; PMO, phosphorodiamidate morpholino oligomer; Q6W, every 6 weeks.

EEV-PMO-23 Improves Muscle Contractile Function in D2-mdx Model of DMD

- Skeletal muscle contractile function was significantly increased following two Q6W doses of EEV-PMO-23 **(Figure 4)**.

Figure 4. Muscle Contractility With EEV-PMO-23 in D2-*mdx* mice.

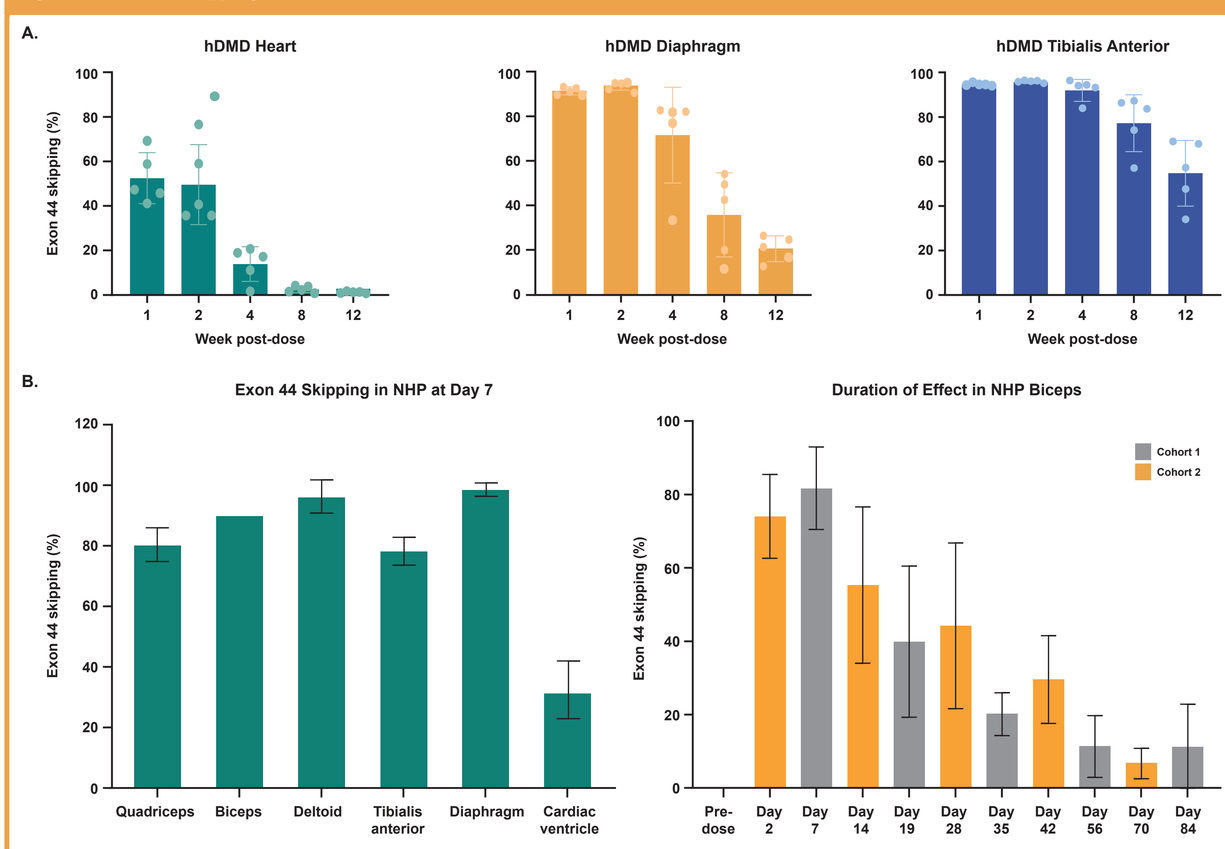


D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Muscle contractility of the tibialis anterior muscle was assessed 6 weeks after the second dose via **(A)** isometric force and **(B)** ECC generated by repeated tetanic contraction. Data shown as mean ± SEM. One-way ANOVA was used for statistical comparison; **p<0.01, ****p<0.0001. ANOVA, analysis of variance; ECC, eccentric force; EEV, endosomal escape vehicle; PMO, phosphorodiamidate morpholino oligomer; Q6W, every 6 weeks; SEM, standard error of the mean.

Exon Skipping and Durable Efficacy of ENTR-601-44 in a Murine Model of DMD and NHPs

- A single IV dose of ENTR-601-44 resulted in high and durable levels of exon 44 skipping across major muscle groups in hDMD mice (**Figure 5A**) and NHPs (**Figure 5B**) for at least 12 weeks.

Figure 5. Exon 44 Skipping With ENTR-601-44 in hDMD Mice and NHPs.



(A) hDMD mice were treated with a single 60 mg/kg IV dose of ENTR-601-44. **(B)** NHPs were treated with a single IV dose of 35 mg/kg ENTR-601-44 and analyzed 7 days later (left) or up to 12 weeks post-infusion (right). Data shown as mean ± SD. hDMD, human dystrophin; IV, intravenous; NHP, nonhuman primate; PMO, phosphorodiamidate morpholino oligomer.

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CONCLUSIONS

- The results presented here demonstrate that the EEV platform efficiently delivers exon skipping oligonucleotides to skeletal and cardiac muscle in preclinical models of DMD.
- ENTR-601-44 showed robust exon skipping efficacy in animal models.
- Together, these findings support the potential for further study in patients with Duchenne.
  - A phase 1 clinical trial of ENTR-601-44 in healthy volunteers is ongoing with an estimated completion date in the second half of 2024.