Development of a Novel, EEV-Conjugated PMO for Duchenne Muscular Dystrophy

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INTRODUCTION

- Duchenne muscular dystrophy (DMD) is an X-linked, progressive disease caused by mutations in the *DMD* gene, resulting in the production of nonfunctional dystrophin protein.[1-2]
- Currently approved phosphorodiamidate morpholino oligomer (PMO) therapies were designed to restore the reading frame and produce dystrophin by exon skipping (**Figure 1**) but have shown modest improvements.[3-4]
  - Limited exposure and poor endosomal escape in target tissue after systemic administration of antisense oligonucleotides (ASOs) remain significant hurdles for the treatment of patients with DMD and results in insufficient dystrophin protein restoration, especially in non-skeletal muscle tissues such as the heart.
- To improve cellular uptake and enhance endosomal escape of oligonucleotides and other biologics, we developed an Endosomal Escape Vehicle (EEV) delivery platform based on cyclic cell-penetrating peptides (cCPPs).[5-6]

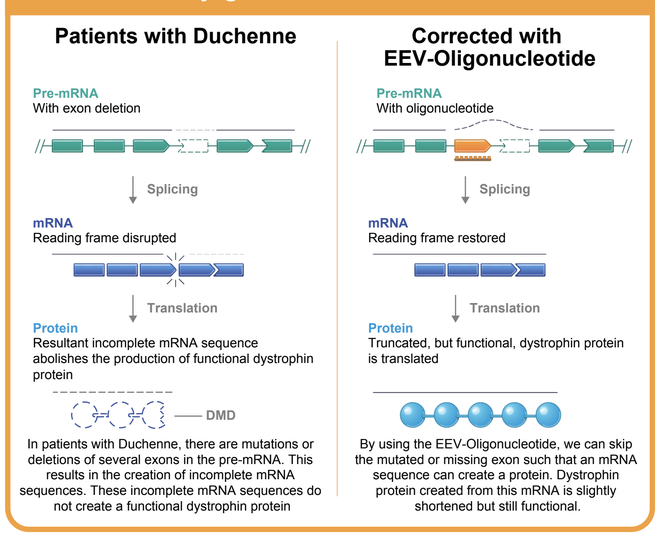
OBJECTIVES

- Evaluate a proprietary Endosomal Escape Vehicle (EEV) platform as a novel antisense oligonucleotide delivery system in preclinical models of DMD
- Assess ENTR-601-44 as a potential therapeutic candidate for patients with exon 44 skip-amenable DMD

MATERIALS AND METHODS

- EEV-PMO-23, a *DMD* exon 23 skipping PMO conjugated to our EEV platform, was administered intravenously (IV) to assess exon skipping and dystrophin production in D2-*mdx*[7] mice. These mice contain a nonsense mutation in *DMD* exon 23 and exhibit a more robust DMD-like pathology than other models.
- ENTR-601-44 is a *DMD* exon 44 skipping PMO conjugated to our EEV platform. hDMD[8] mice expressing full length human *DMD* gene and non-human primates (NHPs, Macaca fascicularis) were treated by IV to evaluate exon 44 skipping efficacy. ENTR-601-44 was also evaluated in patient-derived skeletal muscle cells for exon skipping and dystrophin protein restoration.
- Exon skipping efficiency was analyzed by RT-PCR and LabChip. Dystrophin restoration was evaluated by Simple Western Jess and immunofluorescence. Serum creatine kinase (CK) activity was measured to assess muscle membrane integrity.

Figure 1. Mechanism of Exon Skipping with EEV-PMO conjugates.



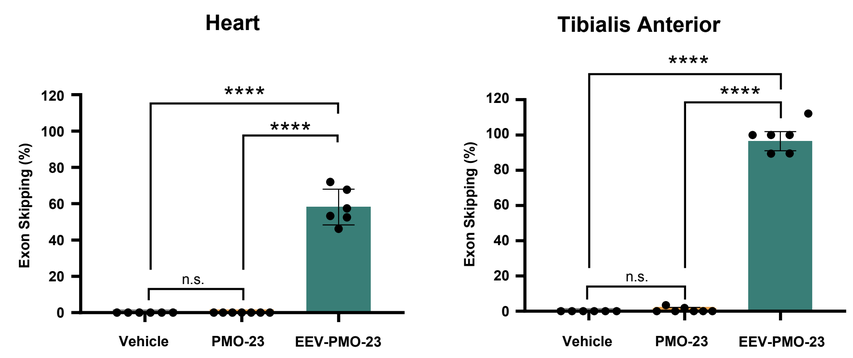
RESULTS

EEV-PMO-23 in D2-*mdx* Mice

EEV Conjugation Enhances Exon Skipping in D2-*mdx* Mice

Administration of EEV-PMO-23 produced robust exon 23 skipping in cardiac and skeletal muscles of D2-*mdx* mice after four monthly IV doses (**Figure 2**)

Figure 2. Exon Skipping with EEV-PMO-23 in D2-*mdx* mice.



D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg unconjugated PMO or 20 mg/kg PMO equivalent of EEV-PMO, and the data were collected ~4 weeks after the last dose. Data shown as mean ± standard deviation. ****p<0.0001; n.s., not significant.

Dystrophin Restoration in Skeletal and Cardiac Muscle

Broad dystrophin expression and restoration of skeletal and cardiac muscle integrity were observed with EEV-PMO-23 compared to unconjugated PMO-23 (**Figure 3**)

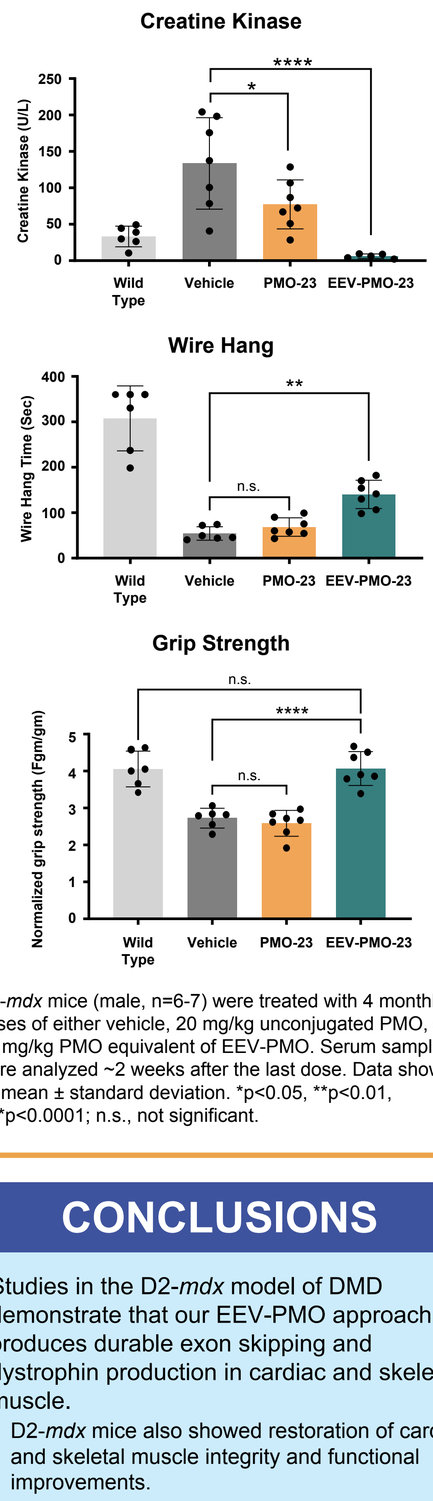
Figure 3. Dystrophin Restoration with EEV-PMO-23 in skeletal and cardiac muscles of D2-*mdx* mice.

D2-*mdx* mice (male, n=6-7) were treated with 4 monthly IV doses of either vehicle, 20 mg/kg unconjugated PMO-23, or 20 mg/kg PMO equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose. Dystrophin in green and DAPI in blue.

CK Correction and Functional Improvement in D2-*mdx* Mice

EEV-PMO-23 administration normalized serum CK levels and showed significant improvements in muscle function when compared to unconjugated PMO after four monthly IV doses in D2-*mdx* mice (**Figure 4**)

Figure 4. Creatine Kinase Correction and Functional Improvement in D2-*mdx* Mice.



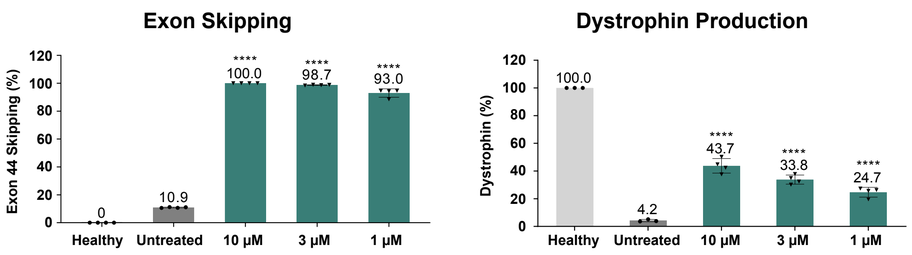
D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg unconjugated PMO, or 20 mg/kg PMO equivalent of EEV-PMO. Serum samples were analyzed ~2 weeks after the last dose. Data shown as mean ± standard deviation. *p<0.05, **p<0.01, ****p<0.0001; n.s., not significant.

ENTR-601-44: Clinical Candidate for Patients with Exon 44 Skip-Amenable DMD

Exon Skipping and Dystrophin Production in Patient Cells

Robust dose-dependent exon 44 skipping and dystrophin protein production were observed in DMD patient-derived muscle cells (DMDΔ45; with exon 45 deletion amenable to exon 44 skipping) treated with ENTR-601-44 (**Figure 5**)

Figure 5. Exon Skipping and Dystrophin Restoration with ENTR-601-44 inpatient-derived skeletal muscle cells.

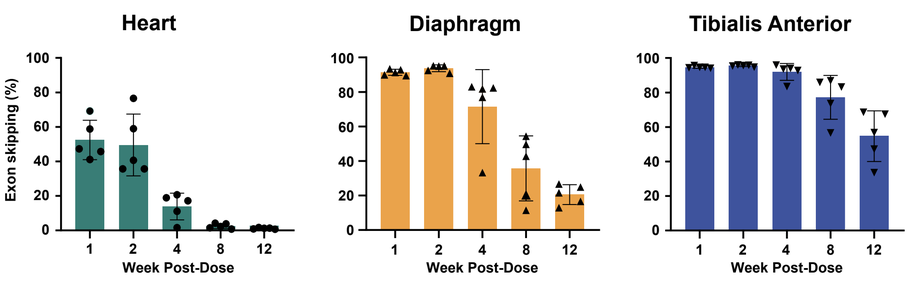


Muscle cells (DMDΔ45) treated with ENTR-601-44 were analyzed for exon skipping and protein restoration after 5 days of differentiation. Data are shown as mean ± standard deviation.****p<0.0001 vs. untreated.

Durable Exon Skipping with ENTR-601-44 in hDMD Mice

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 for at least 12 weeks (**Figure 6**)

Figure 6. Durable Exon Skipping with ENTR-601-44 in hDMD Mice.

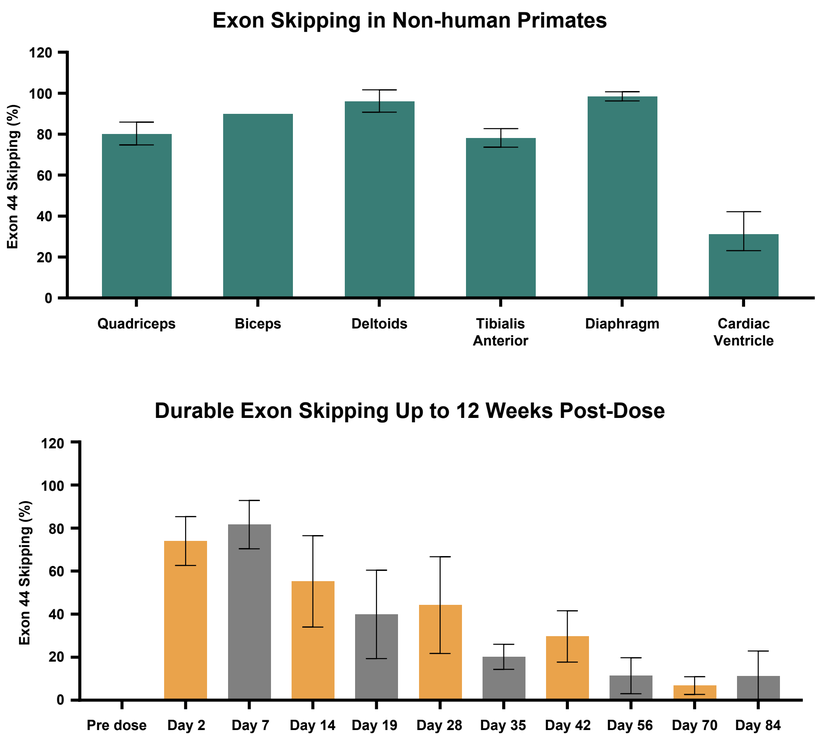


A single 60mg/kg (PMO equivalent) IV dose of ENTR-601-44 was administered to hDMDmice.

Durable Exon Skipping with ENTR-601-44 in NHP

A single dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks (**Figure 7**)

Figure 7. Durable Exon Skipping with ENTR-601-44 in NHP.



Exon skipping in NHP skeletal and cardiac muscle 7 days post IV infusion of 30 mg/kg PMO equivalent (top panel). Duration of effect in biceps of NHP for up to 12 weeks post IV infusion of 35 mg/kg PMO equivalent (bottom panel). Data shown as mean ± standard deviation.

CONCLUSIONS

- Studies in the D2-*mdx* model of DMD demonstrate that our EEV-PMO approach produces durable exon skipping and dystrophin production in cardiac and skeletal muscle.
  - D2-*mdx* mice also showed restoration of cardiac and skeletal muscle integrity and functional improvements.
- Patient-derived muscle cells treated with ENTR-601-44 produced robust and dose-dependent exon skipping and dystrophin protein restoration.
- A single dose of ENTR-601-44 produces robust and durable exon skipping in muscles of hDMD mice and non-human primates for at least 12 weeks post-dose.
- Together, these findings indicate the potential for further study in patients with DMD amenable to exon 44 skipping.

ACKNOWLEDGEMENTS

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