Development of the Endosomal Escape Vehicle (EEV™) Platform to Enhance the Intracellular Delivery of Oligonucleotides

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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**).
  - The medicinal chemistry of cell-penetrating peptides is integral to their ability to efficiently deliver therapeutic cargo. As such, EEV peptides have been optimized for the efficient delivery of antisense oligonucleotides to target cells and tissue.[6]
- Here, we examined the EEV-PMO approach in multiple preclinical models of DMD.

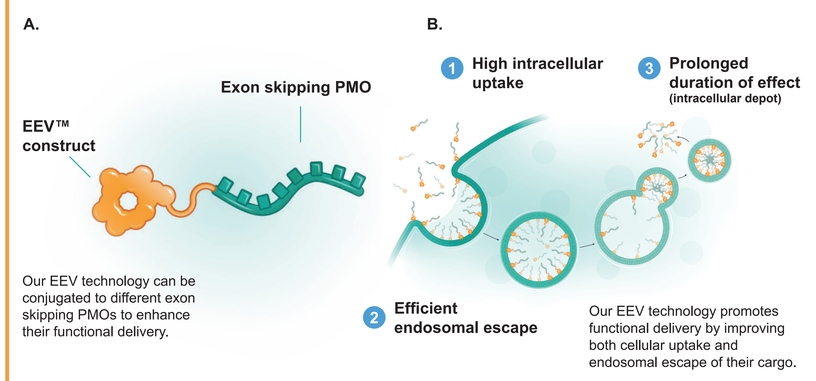
OBJECTIVE

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

MATERIALS AND METHODS

- *mdx* mice were evaluated for exon skipping 7 days following a single 20-mg/kg intravenous (IV) injection of saline, PMO-23, EEV1-PMO-23, or EEV2-PMO-23 (**Figure 2B**).
- EGFP-654 mice[7] were administered three once-weekly IV doses of 10 mg/kg PMO654 or EEV3-PMO654 and were evaluated for EGFP mRNA splice correction one week after the last dose (**Figure 2D**).
- ENTR-601-44, a DMD exon 44 skipping PMO conjugated to the EEV platform, was administered to human dystrophin (hDMD)–producing mice[8] and nonhuman primates (NHPs) to assess exon skipping in cardiac and skeletal muscles (**Figure 3**).
- Exon-skipping efficiency was analyzed by reverse-transcriptase polymerase chain reaction and LabChip (Perkin Elmer, Santa Clara, CA).

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



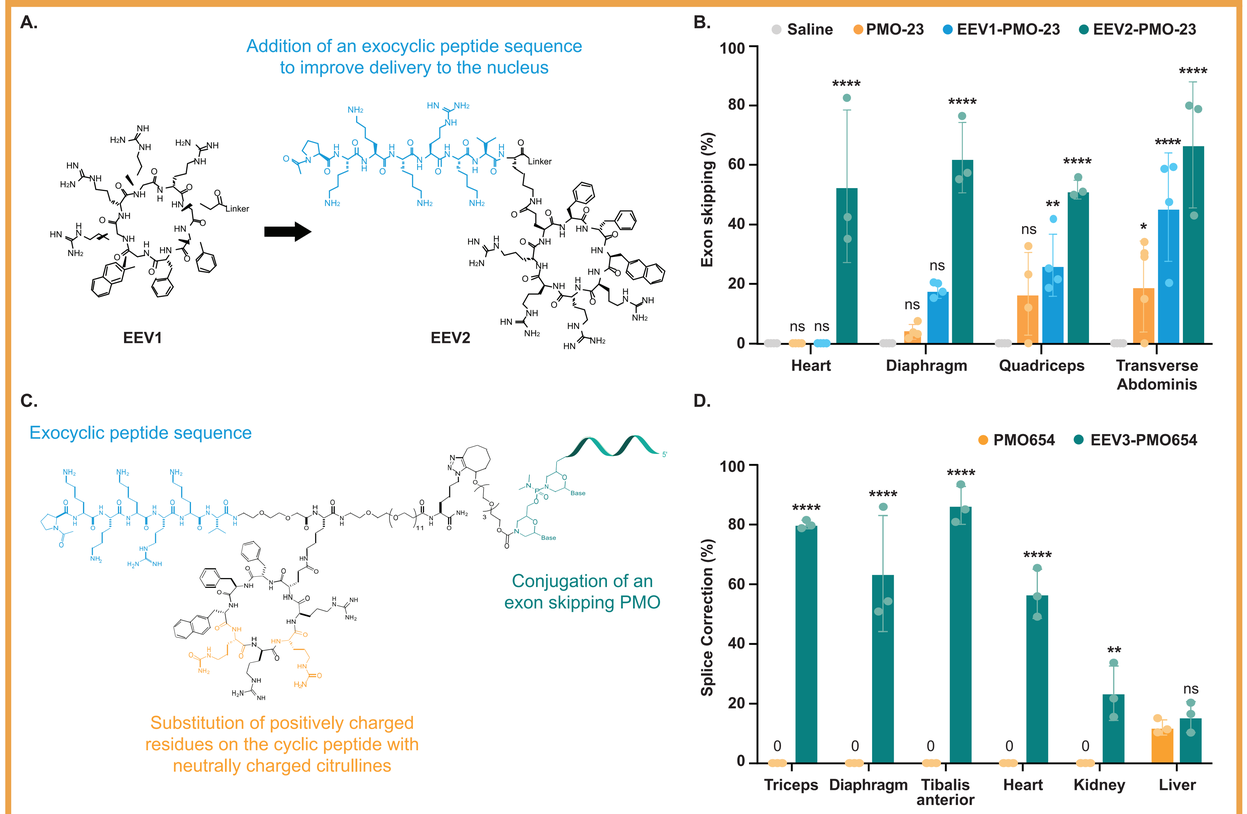
(A) Structure of EEV-PMO construct consisting of a cyclic cell-penetrating peptide-based EEV-construct and exon skipping PMO; **(B)** Mechanism of EEV construct-mediated delivery of PMOs.
EEV, endosomal escape vehicle construct; PMO, phosphorodiamidate morpholino oligomer.

RESULTS

EEV peptides to efficiently deliver PMOs to skeletal and cardiac muscle

- The addition of an exocyclic peptide sequence to a first-generation EEV (EEV1) (**Figure 2A**) increased exon skipping in cardiac and skeletal muscle of *mdx* mice (**Figure 2B**).
- The EEV platform was further optimized (EEV3) by replacing positively charged residues with neutrally charged residues (**Figure 2C**). These modifications resulted in enhanced splice correction in both skeletal and cardiac muscle (**Figure 2D**).

Figure 2. EEV Peptides to Efficiently Deliver PMOs to Skeletal and Cardiac Muscle.

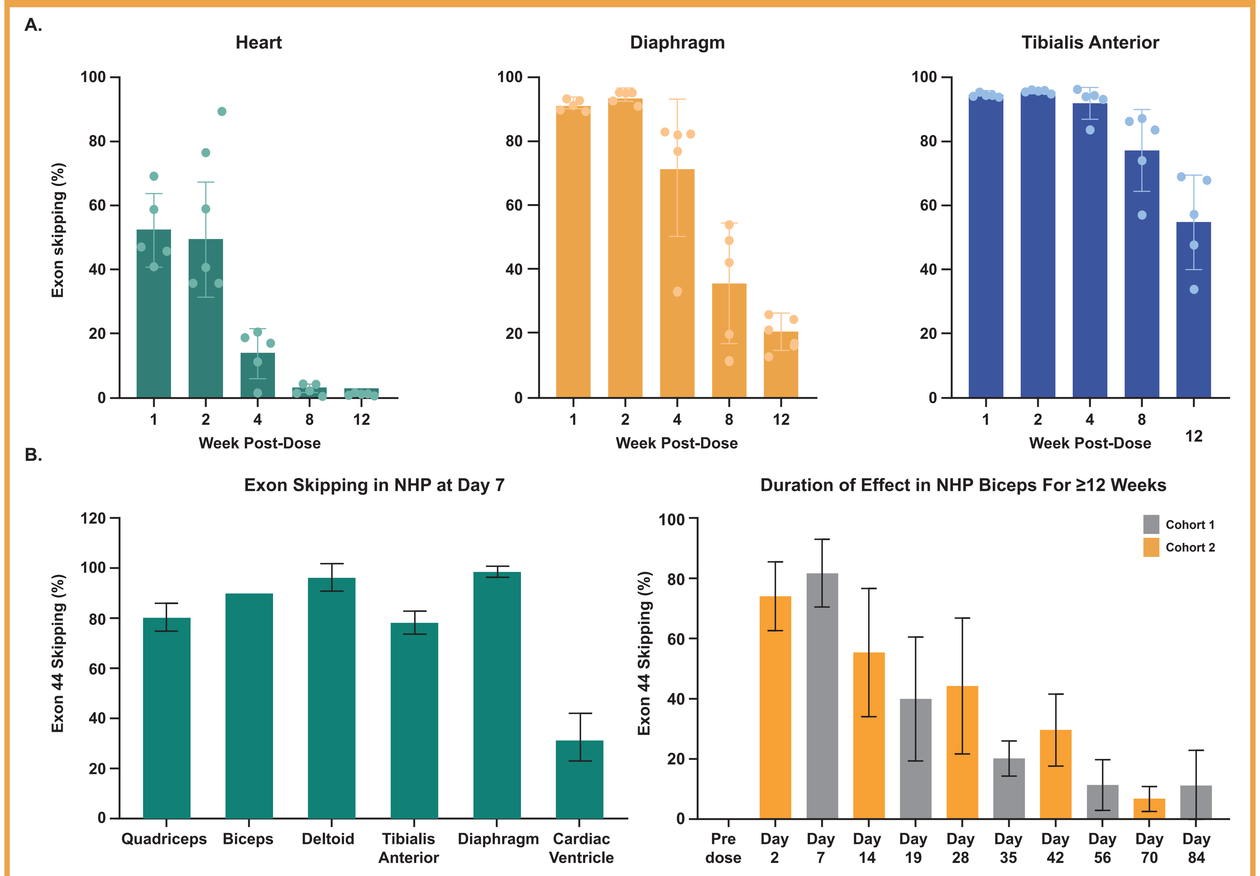


(A) Development of a second-generation EEV (EEV2); **(B)** EEV2 enhances PMO-23 exon skipping in *mdx* mice (**p<0.01, ****p<0.0001 vs. vehicle); **(C)** Development of a third-generation EEV (EEV3); **(D)** EEV3 efficiently delivers PMO654 to target tissues in EGFP-654 mice (**p<0.01, ****p<0.0001 vs. PMO654). EEV, Endosomal Escape Vehicle construct; PMO, phosphorodiamidate morpholino oligomer; ns, not significant.

Exon Skipping and Durable Efficacy of ENTR-601-44 in hDMD Mice 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 3A**) and NHPs (**Figure 3B**) for at least 12 weeks.

Figure 3. Exon 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). hDMD, human dystrophin; IV, intravenous; NHP, nonhuman primate.

CONCLUSIONS

- Development of the EEV platform led to efficient delivery of exon skipping PMOs to skeletal and cardiac muscle in preclinical models of DMD.
  - These results underscore the importance of the medicinal chemistry of cell penetrating peptides for successful delivery of PMOs to target tissues.
- ENTR-601-44 showed robust exon skipping efficacy in cell and 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 subjects is ongoing with expected completion in the second half of 2024.

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