

## 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.<sup>1-2</sup>
- 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.<sup>3-4</sup>
- Limited exposure and poor endosomal escape in target tissue after systemic administration of antisense oligonucleotides remain significant hurdles for the treatment of patients with DMD and result in insufficient dystrophin protein restoration, especially in cardiac muscle.
- 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).<sup>5-6</sup>
- We previously demonstrated that D2-*mdx* mice treated with EEV-PMO-23 produced durable and robust exon 23 skipping and dystrophin production in both skeletal and cardiac muscle. Additionally, ENTR-601-44, a clinical candidate for exon 44 skip-amenable patients, produced robust exon 44 skipping in hDMD mice, and showed both robust exon skipping and dystrophin protein restoration in patient-derived skeletal muscle cells.<sup>7</sup>
- We developed ENTR-601-45 for the treatment of exon 45 skip-amenable mutations in DMD patients to further address unmet medical needs.

### **MATERIALS & METHODS**

- ENTR-601-45 is a DMD exon 45 skip-amenable PMO conjugated to the EEV platform.
- ENTR-601-45 efficacy was assessed in iPSC-derived skeletal and cardiac muscle cells from an exon 45 skip-amenable DMD patient harboring an exon 46-48 deletion mutation. Myotubes were treated for 24 hours and analyzed after 5 days of differentiation. Cardiomyocytes were treated for 24 hours and analyzed 48 hours later.
- Exon skipping efficiency was analyzed by RT-PCR and LabChip. Dystrophin protein restoration in cells was evaluated by Simple Western Jess and immunofluorescence.
- Humanized DMD (hDMD)<sup>8</sup> mice expressing the full-length human DMD gene were treated intravenously with ENTR-601-45 to evaluate exon 45 skipping efficacy in vivo.

### CONCLUSIONS

- ENTR-601-45 produced robust and dose-dependent exon skipping and dystrophin restoration in patient-derived skeletal and cardiac muscle cells.
- A single dose of ENTR-601-45 resulted in greater exon skipping in skeletal and cardiac muscles of hDMD mice compared with an EEV-casimersen sequence conjugate.
- Together, these findings indicate the potential for further study of ENTR-601-45 in patients with DMD amenable to exon 45 skipping.

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# Exon Skipping and Dystrophin Production With ENTR-601-45, a Novel EEV-Conjugated, Exon 45 Skip-Amenable PMO in Preclinical Models of DMD

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Concentrations provided are PMO equivalent. Casimersen is an exon 45 skipping PMO approved in the US.

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