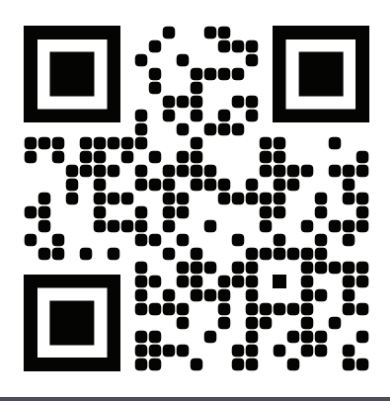


Preclinical Efficacy of ENTR-601-50, a Novel EEV™-Oligonucleotide Construct for the Treatment of Exon 50 Skip–Amenable Duchenne Muscular Dystrophy

Mahasweta Girgenrath, Nelsa L. Estrella, Amy N. Hicks, Holly McKee, Mary Lou Beermann, Jia Qi Cheng Zhang, Christopher M. Brennan, Pauline Tan, Yongchao Mou, Nick Long, Wenlong Lian, Terrance A. Stadheim, Mahboubeh Kheirabadi, Patrick G. Dougherty, Matthew Streeter, Ziqing Leo Qian

Entrada Therapeutics, Boston, MA

654P



BACKGROUND

- The Endosomal Escape Vehicle (EEV™) platform consists of a family of cyclic cell-penetrating peptides developed to enhance the delivery of therapeutics to target cells by overcoming barriers to cell entry and endosomal escape¹ (**Figure 1**).
- The EEV platform has been shown to improve antisense phosphorodiamidate morpholino oligomer (PMO) delivery to skeletal and cardiac muscle in preclinical models of Duchenne muscular dystrophy (DMD).²
- EEV-PMO therapeutics designed for exon 44 (ENTR-601-44) and exon 45 (ENTR-601-45) skip–amenable DMD, respectively, demonstrated robust exon skipping and dystrophin production in both cell and animal models, suggesting their therapeutic potential in patients with DMD.^{3,4}
- Furthermore, ENTR-601-44 was well tolerated and demonstrated significant exon 44 skipping compared with placebo in a phase 1 clinical trial of healthy human volunteers.⁵
- Here, we assessed the preclinical efficacy of ENTR-601-50, an exon 50 skipping EEV-PMO construct in development for the treatment of DMD amenable to exon 50 skipping.

MATERIALS AND METHODS

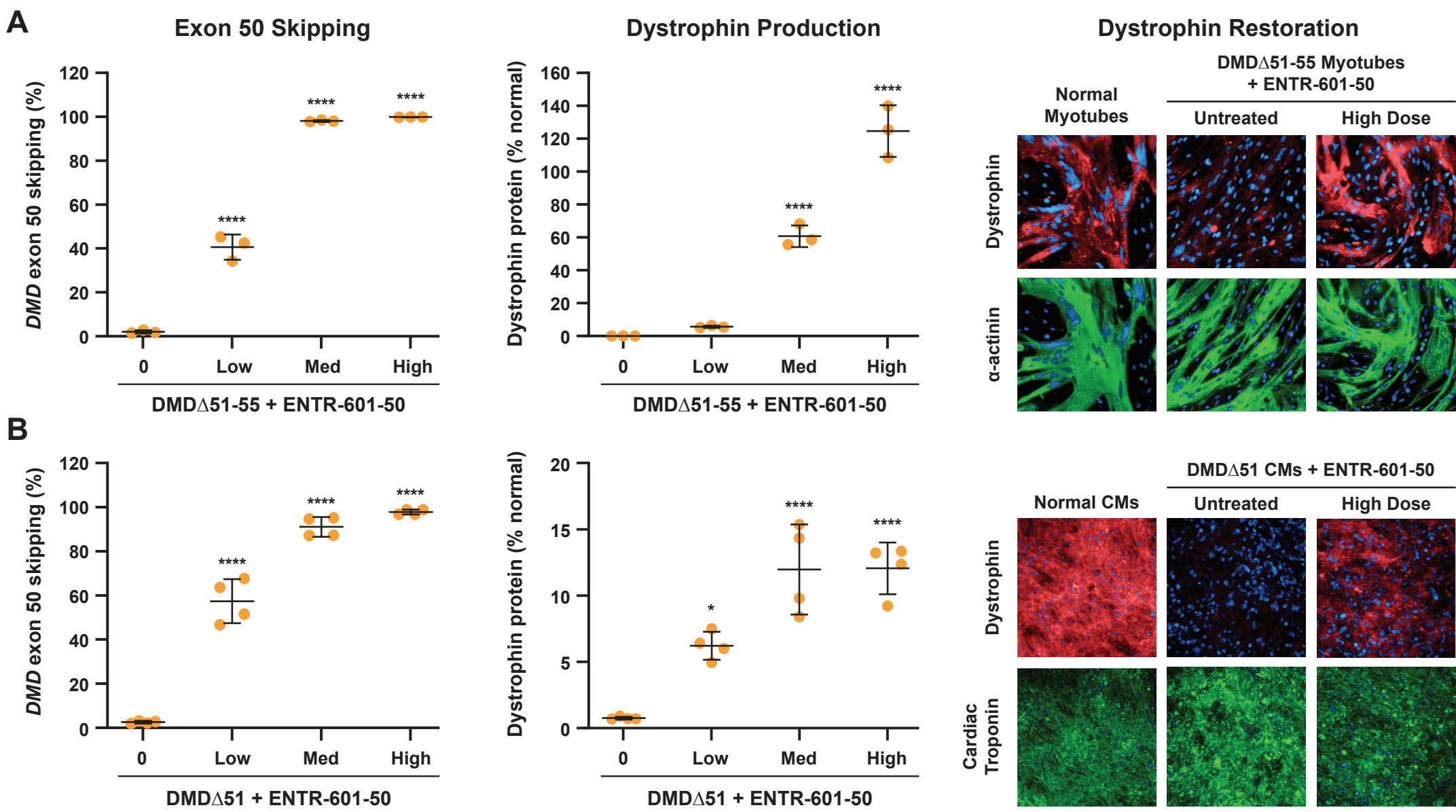
- ENTR-601-50 is a DMD exon 50 skipping PMO conjugated to the EEV platform, and is in development for the treatment of exon 50 skip–amenable DMD. EEV-PMO-50, an exon 50 skipping EEV-PMO tool compound, was used for the satellite cell study in Figure 6.
- Skeletal muscle cells were derived from a patient with exon 50 skip–amenable DMD harboring a deletion of DMD exons 51, 52, 53, 54, and 55 (DMDΔ51-55).
- Cardiomyocytes (CM) were derived from a patient with exon 50 skip–amenable DMD harboring a deletion of DMD exon 51 (DMDΔ51).
- del51hDMD.mdx mice (Taconic Biosciences, Germantown, NY) express human dystrophin (hDMD) and are engineered with a deletion in the hDMD exon 51 transgene on the mdx background, resulting in an exon 50 skip–amenable mouse line. hDMD.mdx mice were used as healthy controls for dystrophin quantification, as they contain a normal hDMD transgene on the mdx background.
- Exon-skipping efficiency was analyzed by digital droplet RT-PCR. Dystrophin restoration was evaluated by Simple Western Jess (Bio-Techne, Minneapolis, MN).

RESULTS

Exon Skipping and Dystrophin Restoration With ENTR-601-50 in DMD Patient-Derived Cells

- Treatment of patient-derived skeletal (**Figure 2A**) and cardiac (**Figure 2B**) muscle cells with ENTR-601-50 resulted in dose-dependent DMD exon 50 skipping and dystrophin protein restoration.

Figure 2. Efficacy of ENTR-601-50 in DMD Patient-Derived Skeletal and Cardiac Muscle Cells

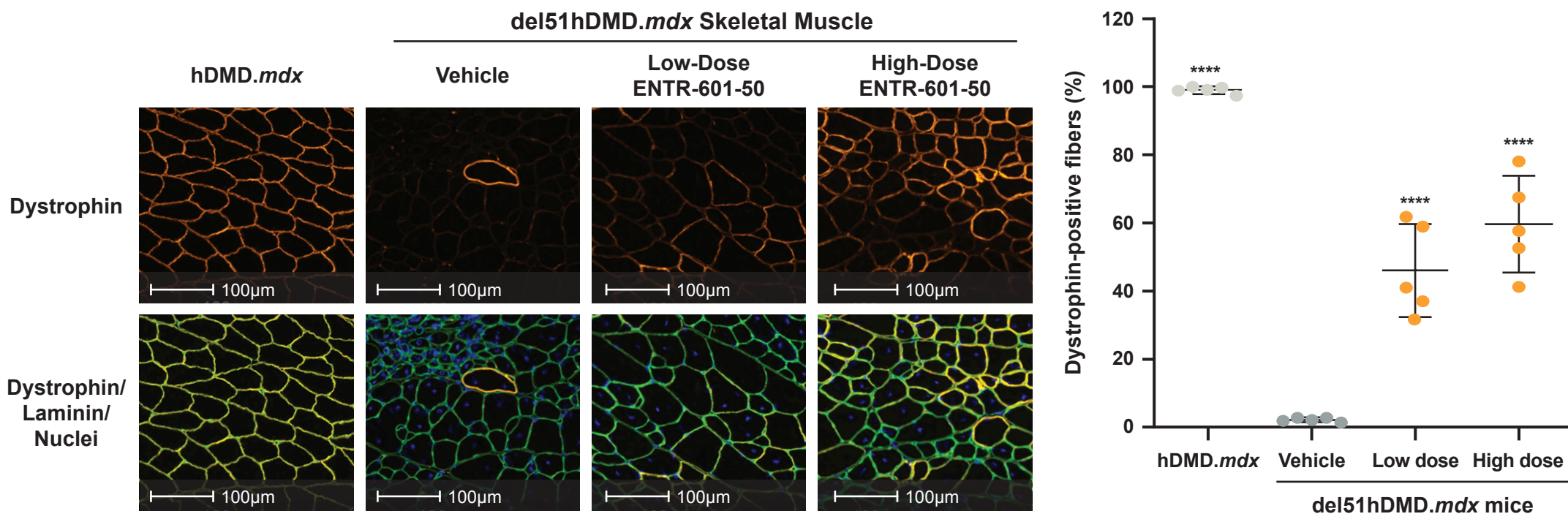


DMDΔ51-55 patient-derived skeletal (A) and DMDΔ51 patient-derived cardiac (B) muscle cells were incubated with ENTR-601-50. Skeletal myotubes (A) were treated for 24 hours and analyzed after 5 days of differentiation. Cardiomyocytes (B) were treated for 24 hours and analyzed 48 hours later. Dystrophin protein is shown in red, and nuclei appear blue. Data are shown as mean ± standard deviation; one-way ANOVA was used for exon skipping and dystrophin/total protein data (n=4 per cohort); *p<0.05, ****p<0.0001 compared with untreated DMDΔ51-55 cells in Panel A and untreated DMDΔ51 cells in Panel B. ANOVA, analysis of variance; CM, cardiomyocyte.

ENTR-601-50 Increased Dystrophin-Positive Fibers in Skeletal Muscle of Mice With an Exon 50 Skip–Amenable Mutation

- A single IV dose of ENTR-601-50 produced dose-dependent increases in dystrophin-positive muscle fibers, localizing in the gastrocnemius sarcolemma of del51hDMD.mdx mice 2 weeks post-dose (**Figure 4**). Significant increases in dystrophin-positive muscle fibers, evenly distributed across the tissue, were observed with both doses of ENTR-601-50 compared with vehicle.

Figure 4. Dystrophin Distribution and Localization in Skeletal Muscle of del51hDMD.mdx Mice With ENTR-601-50



del51hDMD.mdx mice were treated with a single IV injection of ENTR-601-50 or vehicle. Dystrophin protein distribution and cellular localization (red) were analyzed by immunofluorescence in the gastrocnemius 2 weeks after dosing. Quantification via Halo Image Analysis Software is shown as the percentage of dystrophin-positive muscle fibers relative to the total number of muscle fibers, as determined by laminin staining (green) and dystrophin staining (red); co-localization to the sarcolemma appears yellow, and nuclei appear blue. Data shown as mean ± standard deviation. One-way ANOVA was used for statistical comparison; ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; hDMD, human dystrophin transgene; IV, intravenous.

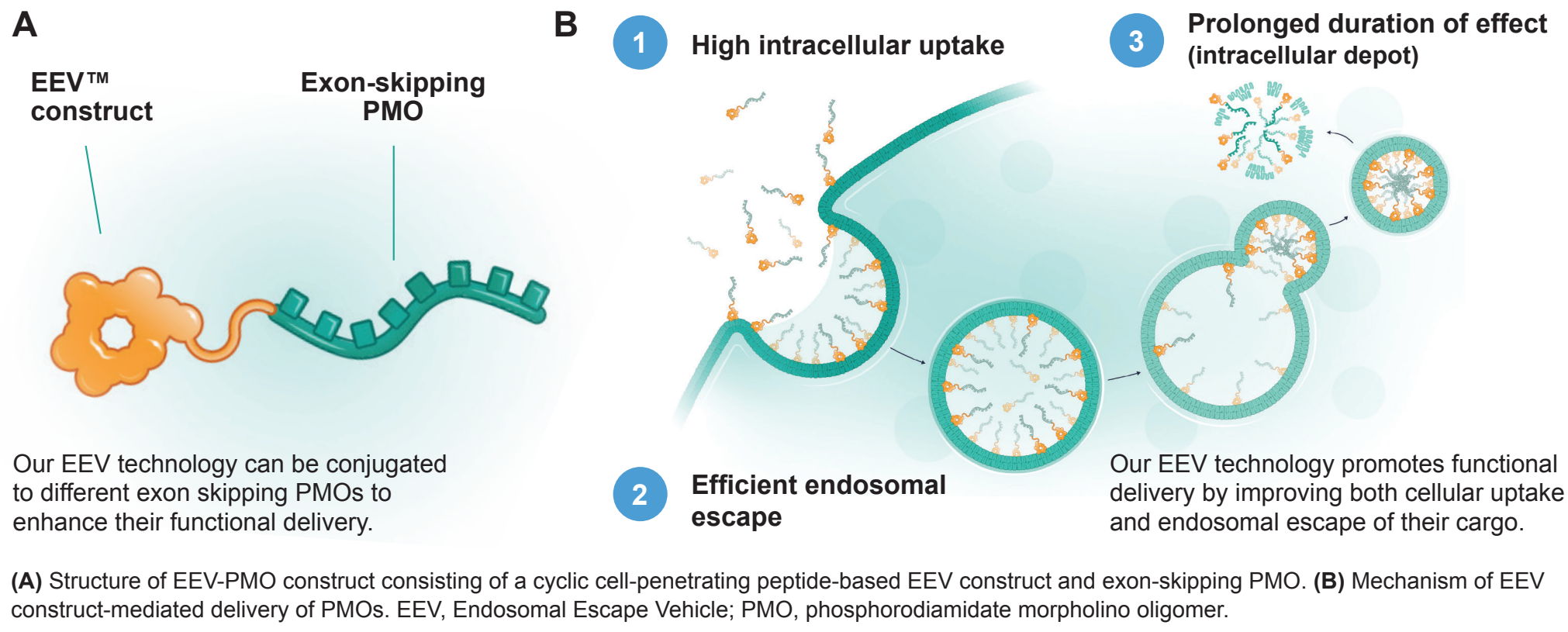
CONCLUSIONS

- ENTR-601-50 demonstrated significant dose-dependent exon skipping and dystrophin production in both cell and animal models of DMD amenable to exon 50 skipping.
- In animals treated with ENTR-601-50, dystrophin was correctly localized at the sarcolemma of muscle fibers.
- Treatment with ENTR-601-50 resulted in the expression of a functional protein, as evidenced by enhanced skeletal muscle function in a mouse model amenable to exon 50 skipping.
- Additionally, EEV-PMO-50 effectively penetrated the satellite cell compartment in mouse skeletal muscle, potentially enhancing dystrophin expression in functionally impaired satellite cells and promoting regeneration.
- These findings demonstrate the therapeutic potential of ENTR-601-50 and support further studies in patients with DMD amenable to exon 50 skipping.

ACKNOWLEDGMENTS

This research was funded by Entrada Therapeutics, Inc (Boston, MA). The authors would like to thank Aji Nair for assistance with poster development (Entrada Therapeutics, Inc). Editorial and studio support for this poster was provided by Ashfield MedComms (US), an Inizio company, and was funded by Entrada Therapeutics, Inc. References: 1. Qian Z, et al. ACS Chem. 2013. 2. Li, X, et al. Mol Ther Nucleic Acids. 2023. 3. Kumar A, et al. MDA 2022. Poster 126. 4. Girgenrath M, et al. WMS 2024. Poster. 5. Sethuraman N, et al. WMS 2025. Poster.

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



Our EEV technology can be conjugated to different exon skipping PMOs to enhance their functional delivery.

(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.

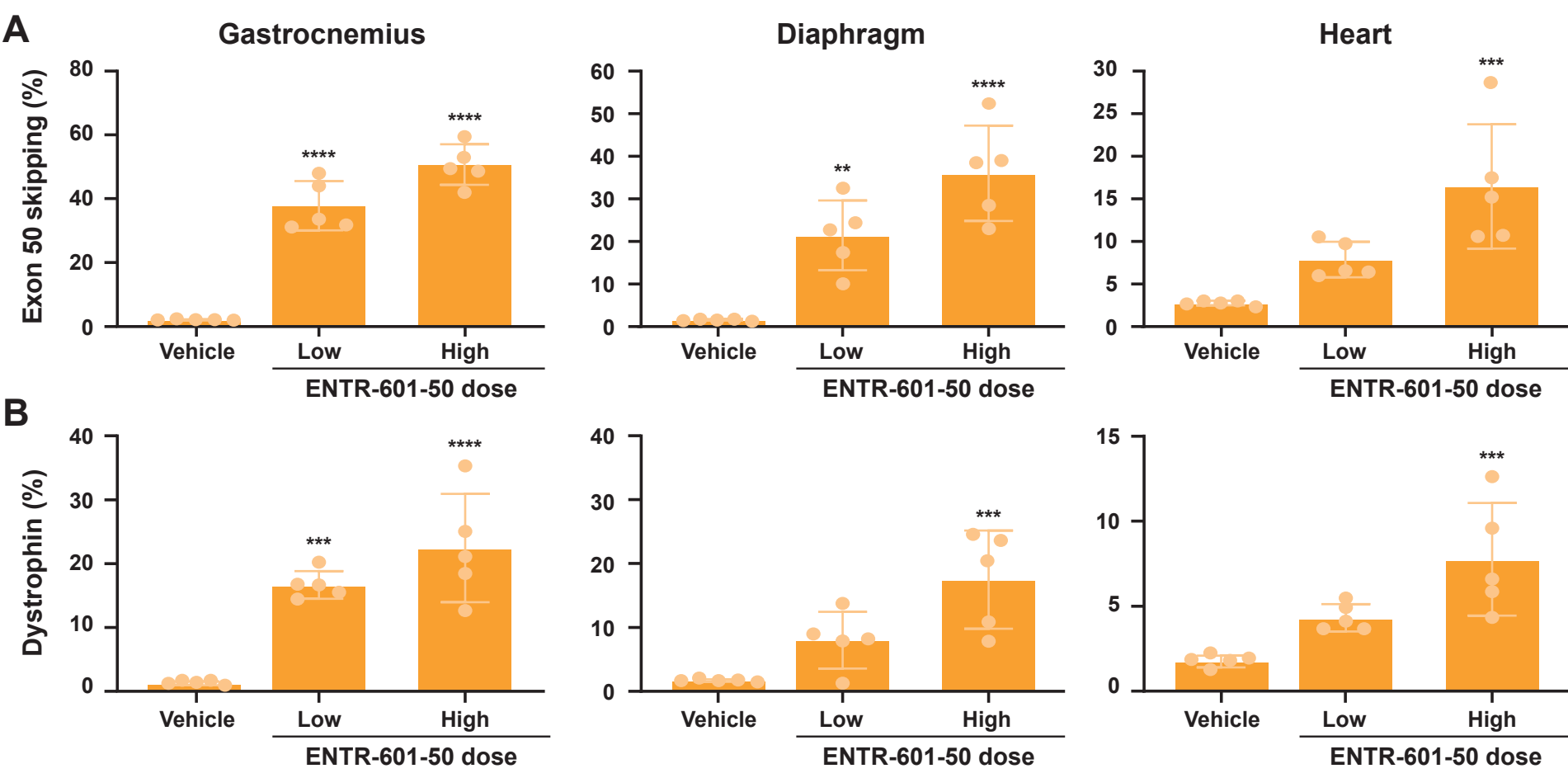
OBJECTIVE

- To assess the efficacy and therapeutic potential of ENTR-601-50 in cell and animal models of DMD amenable to exon 50 skipping.

Exon Skipping and Dystrophin Production With ENTR-601-50 in Mice With an Exon 50 Skip–Amenable Mutation

- A single intravenous (IV) dose of ENTR-601-50 led to robust human DMD exon 50 skipping (**Figure 3A**) and dystrophin production (**Figure 3B**) in del51hDMD.mdx mice harboring an exon 50 skip–amenable mutation 2 weeks after dosing.

Figure 3. Efficacy of ENTR-601-50 in del51hDMD.mdx Mice

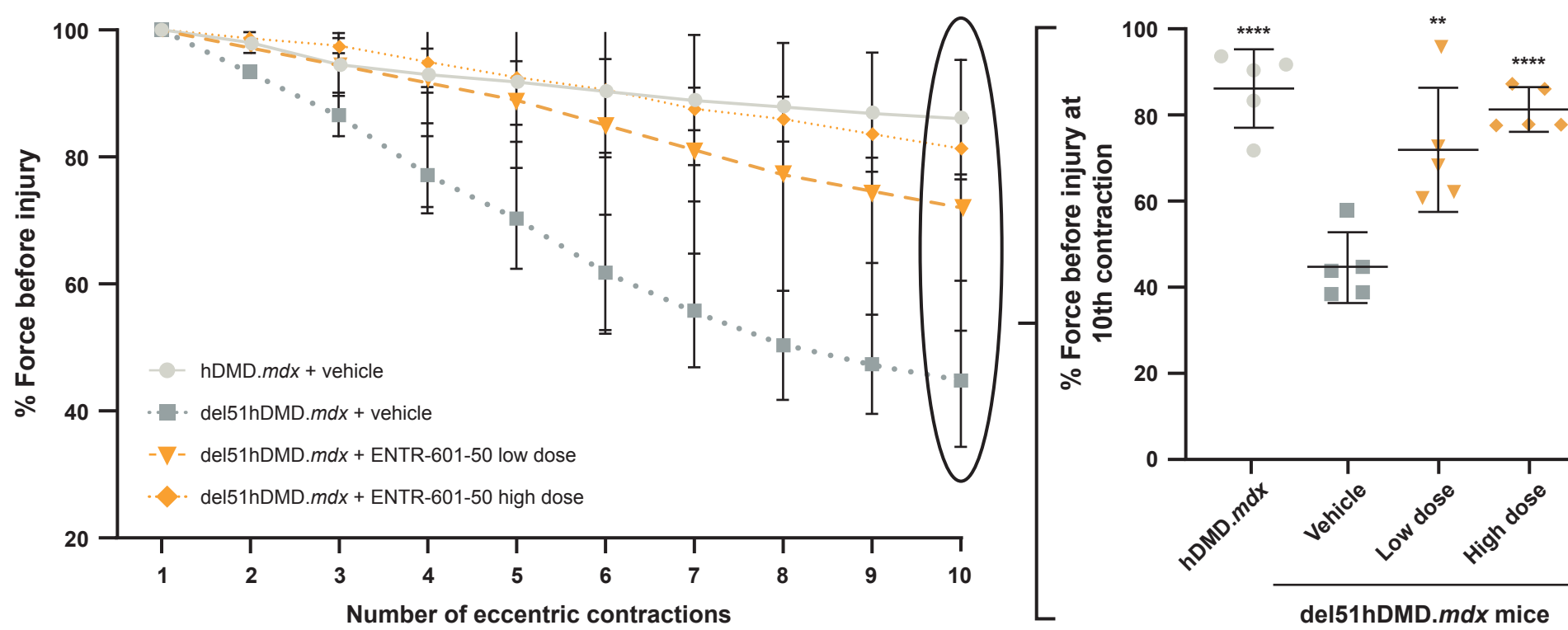


del51hDMD.mdx mice were treated with a single IV injection of ENTR-601-50 or vehicle. Human DMD exon 50 skipping (A) and dystrophin protein expression (B) were analyzed in the gastrocnemius, diaphragm, and heart 2 weeks post-dose. Percent dystrophin protein restoration is normalized to total protein and normalized to hDMD.mdx controls. Data shown as mean ± standard deviation. One-way ANOVA was used for statistical comparison; **p<0.01, ***p<0.001, ****p<0.0001 vs. vehicle. ANOVA, analysis of variance; hDMD, human dystrophin transgene; IV, intravenous.

ENTR-601-50 Improved Skeletal Muscle Function in Mice With an Exon 50 Skip–Amenable Mutation

- A significant and dose-dependent increase in resistance to membrane damage was observed following the tenth contraction with both doses of ENTR-601-50 two weeks after dosing (**Figure 5**).

Figure 5. Improved Skeletal Muscle Function With ENTR-601-50 in del51hDMD.mdx Mice

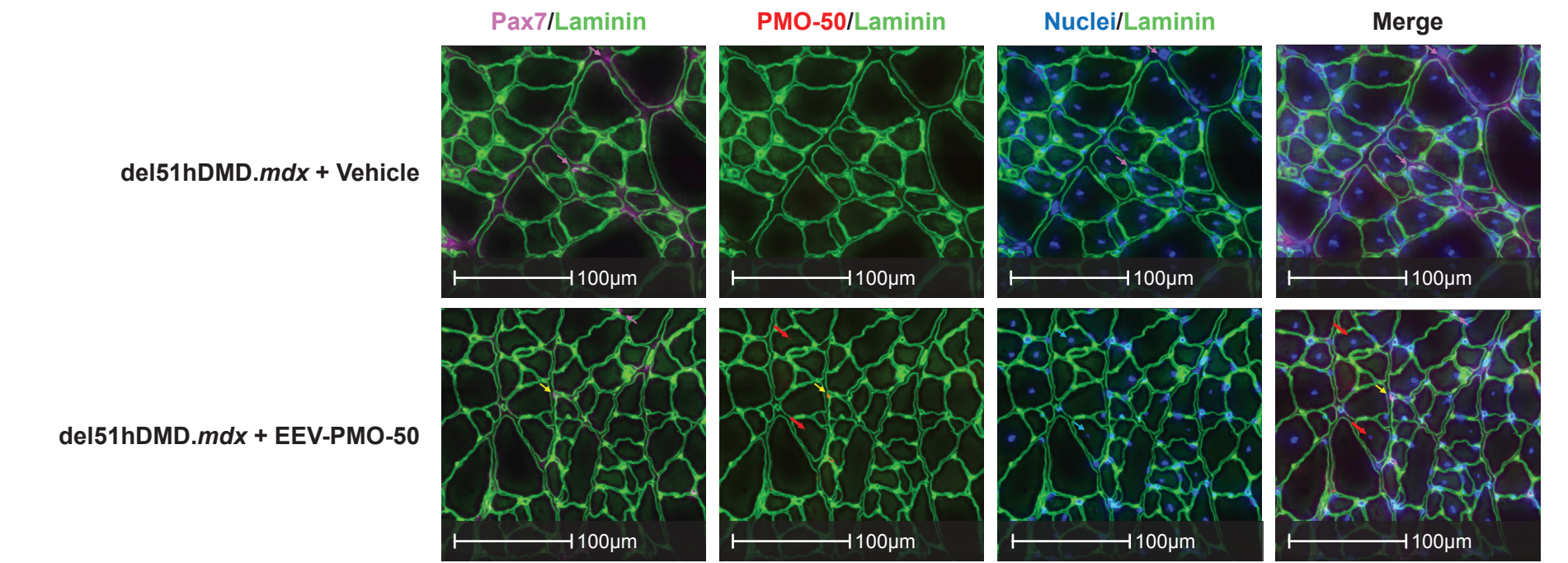


del51hDMD.mdx mice were treated with a single IV injection of ENTR-601-50 at low or high dose or with vehicle. ECC-induced muscle force loss generated by repeated ECC contraction of the gastrocnemius muscle was assessed 2 weeks after dosing. Data (mean ± standard deviation) are shown across 10 ECC contractions normalized as a percentage of the initial force before any ECC contractions (left) and as the percentage of force retained after the 10th contraction (right). Vehicle-treated hDMD.mdx mice were used as a control group for normal muscle function. One-way ANOVA was used for statistical comparison to vehicle-treated del51hDMD.mdx mice; **p<0.01, ****p<0.0001. ANOVA, analysis of variance; ECC, eccentric force; hDMD, human dystrophin transgene; IV, intravenous.

Localization of PMO-50 to Satellite Cells in Skeletal Muscle of del51hDMD.mdx Mice

- PMO-50 co-localized with satellite cells and newly generated centrally nucleated fibers in skeletal muscle 2 weeks post-dose.

Figure 6. PMO-50 Co-Localizes With Satellite Cells and Centrally Nucleated Fibers in Skeletal Muscle



del51hDMD.mdx mice were treated with a single IV dose of EEV-PMO-50 or vehicle. Gastrocnemius muscle was analyzed with immunohistochemistry for Pax7 (purple arrows, satellite cell marker), PMO-50 (red), and laminin (green) 2 weeks post-dose. Nuclei appear blue. Red arrows show PMO-50-positive centrally nucleated fibers. Yellow arrows show co-localization of PMO-50 with satellite cells. EEV, Endosomal Escape Vehicle; IV, intravenous; Pax7, paired box protein 7; PMO, phosphorodiamidate morpholino oligomer.