Robust Exon Skipping and Dystrophin Production with Endosomal Escape Vehicle (EEV™)-Oligonucleotide Conjugates in Preclinical Models of Duchenne Muscular Dystrophy

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entrada THERAPEUTICS

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OUR MISSION

To Treat Devastating Diseases with Intracellular Therapeutics

entrada THERAPEUTICS

EEV™ PLATFORM

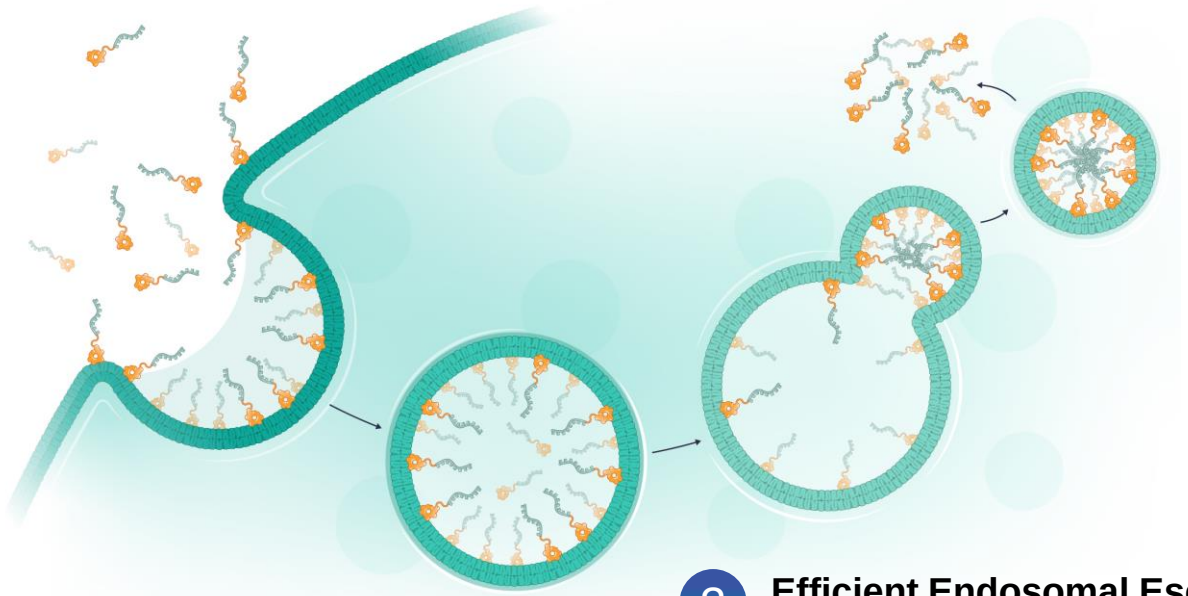
Endosomal Escape Vehicle (EEV™) Therapeutics

- Unique chemistry results in **improved uptake and endosomal escape**
- Cyclic structure designed to **extend half life and increase stability**
- Phospholipid binding potentially **enables broad biodistribution to all cells**
- Mechanism of **internalization conserved across species**

The EEV Platform seeks to solve a fundamental problem: a lack of efficient cellular uptake and escape from the endosome. Both are critical to intracellular target engagement and therapeutic benefit.

1 **High Intracellular Uptake**
(~90% Uptake)

3 **Prolonged Duration Of Effect**
(Intracellular Depot)

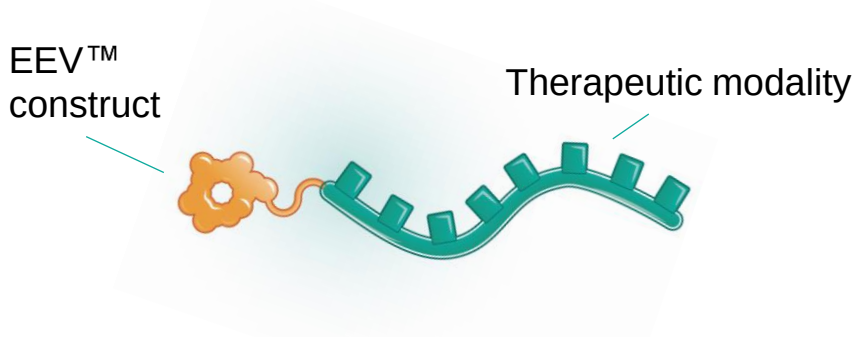


2 **Efficient Endosomal Escape**
(~50% Escape v. 2% Standard)

Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

EEV LIBRARY: SCREENING AND OPTIMIZATION

Discovery Engine for Intracellular Therapeutics



- Cyclic peptide library design and combinatorial synthesis to generate **EEV library**
- Delivery and counter-screening assays enabled for in vitro **high throughput screening**
- Functional screening of lead EEVs in vivo to select for **pharmacodynamic activity** in target tissues
- Optimize **conjugation chemistry** for desired therapeutic modality

Screening Cascade for EEV Candidates

Chemically-diverse >500 member EEV library

EEVs with robust target cell uptake and efficacy

In vitro **functional validation** in relevant cell types with therapeutic payload

Well-tolerated EEVs with desired tissue functional delivery

Assess in vivo **functional delivery** in wild-type and disease models

Fit-for-purpose EEV candidate for target indication

Identify EEV candidate with desired **therapeutic profile**

EEV, endosomal escape vehicle.

DUCHENNE MUSCULAR DYSTROPHY

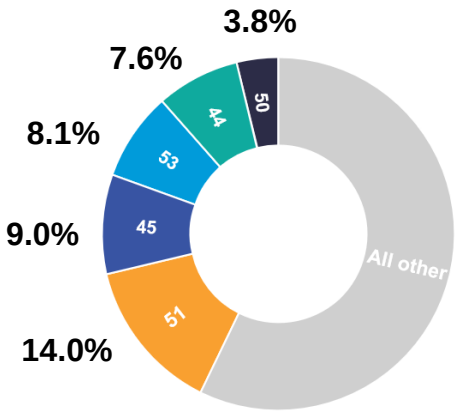
SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

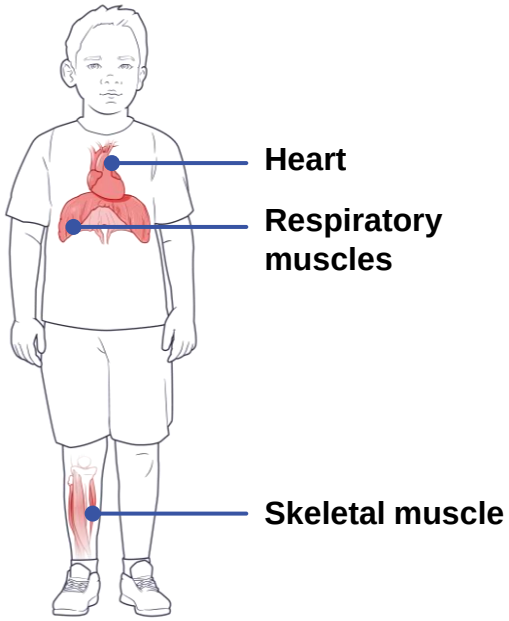
Duchenne is caused by **mutations in the *DMD* gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

Exon skipping therapeutics have been approved based on **modest improvement in dystrophin levels ranging from ~1% to 6%**[4-7]

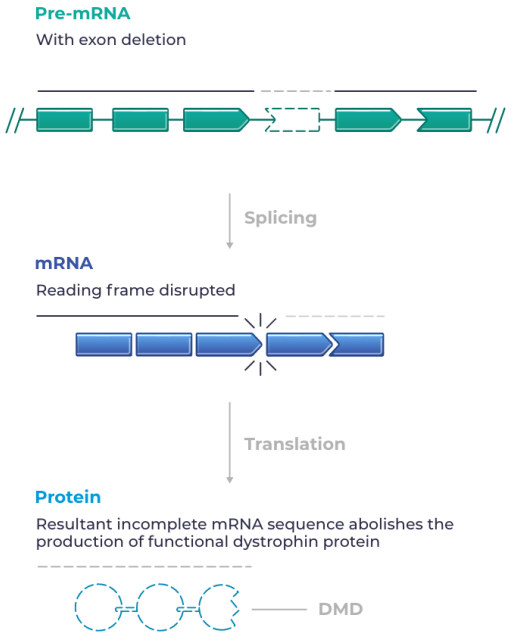
~15,000 people in the **U.S.**[1] **&** **~26,000** people in **Europe**[2] have Duchenne

>40% of patients with Duchenne[3] have mutations amenable to exon skipping of exons 44, 45, 50, 51 and 53

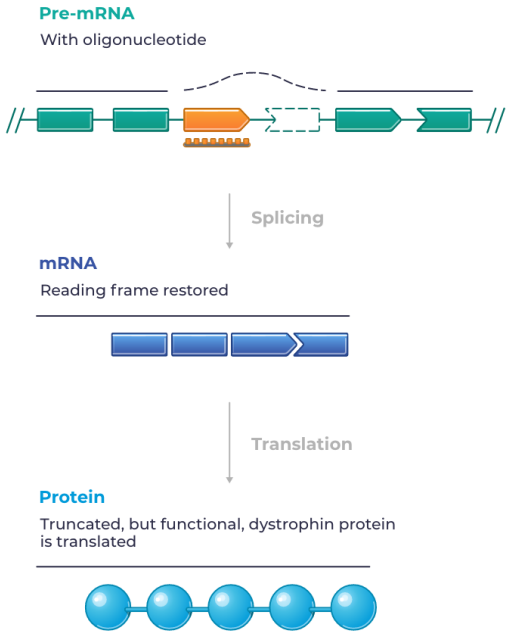




Patients with Duchenne



EEV-Oligonucleotide Approach



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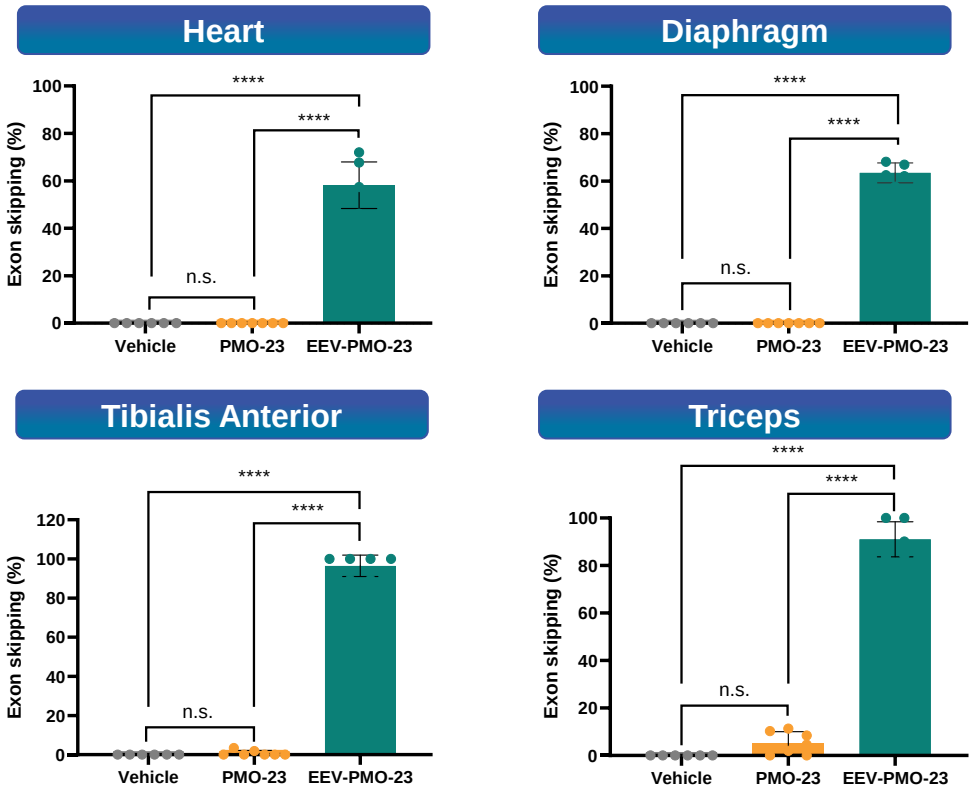
1. Parent Project Muscular Dystrophy. https://www.parentprojectmd.org/about-duchenne/. Accessed August 18, 2023. **2.** Europeans Medicines Agency. https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3202375. Accessed August 18, 2023. **3.** Bladen, C.L. et al. *Hum Mutat*. 2015. **4.** AMONDYS 45 PI. **5.** VILTEPSO PI. **6.** VYONDYS 53 PI. **7.** EXONDYS 51 PI. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle

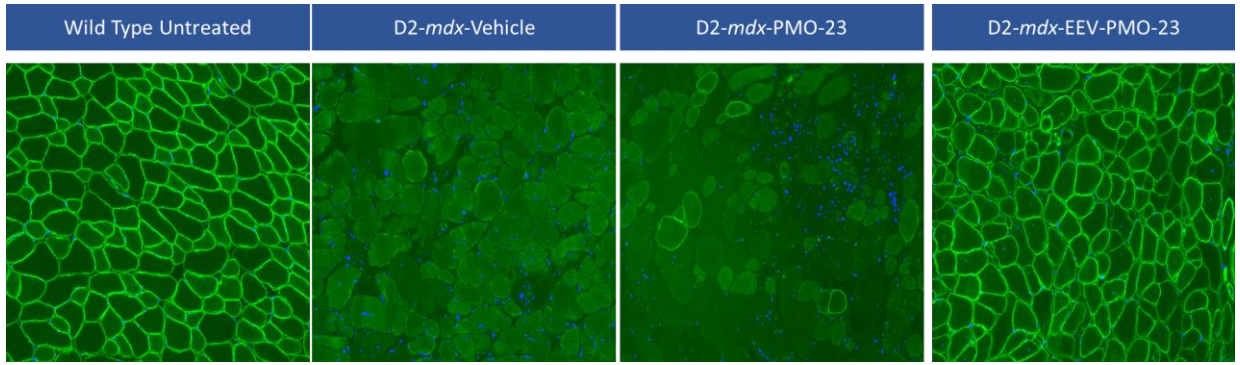
EEV-PMO RESTORES MUSCLE INTEGRITY
D2-*mdx* Mice

Robust exon 23 skipping after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

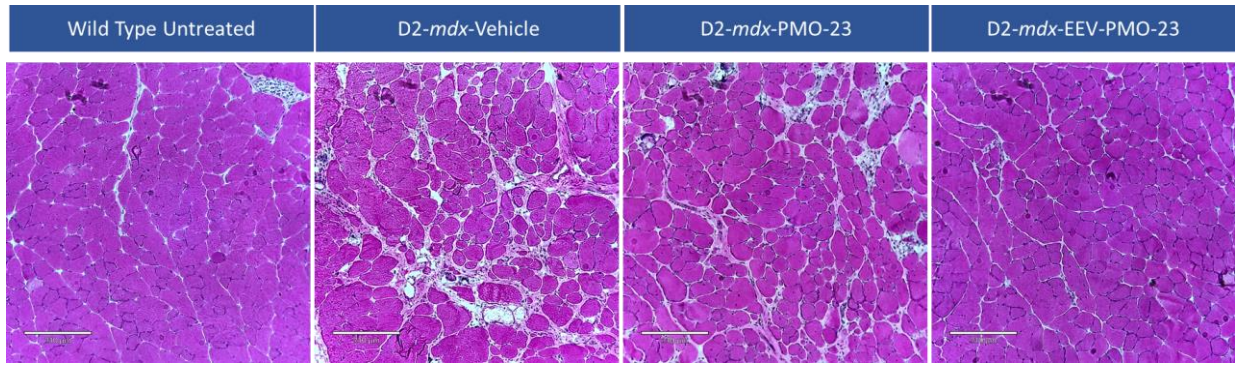


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



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

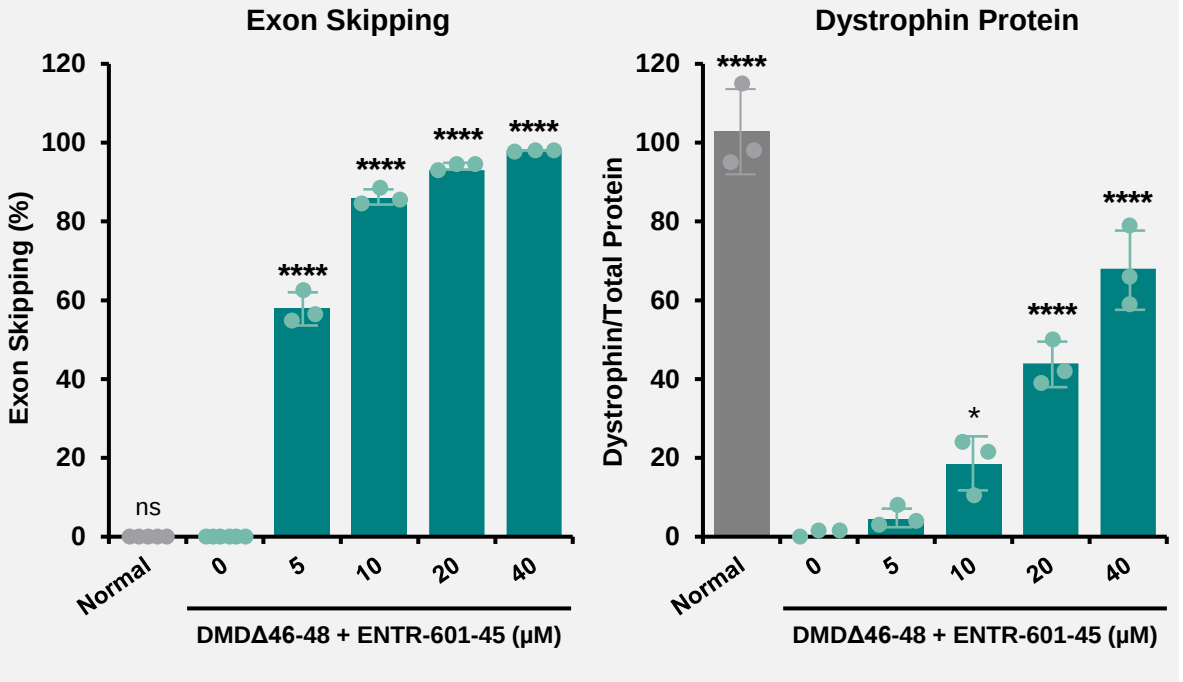
EEV, Endosomal Escape Vehicle; **PMO-23**, mouse *Dmd* exon 23 skipping phosphorodiamidate morpholino oligomer; **D2-*mdx*** is a DMD mouse model with a nonsense mutation in DMD exon 23 on a DBA/2J background and better recapitulates disease pathology (Fukada, S. et al. *Am. J. Path.* 2010, Coley, W .D. et al. *Hum. Mol. Genet.* 2016). ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

ENTR-601-45

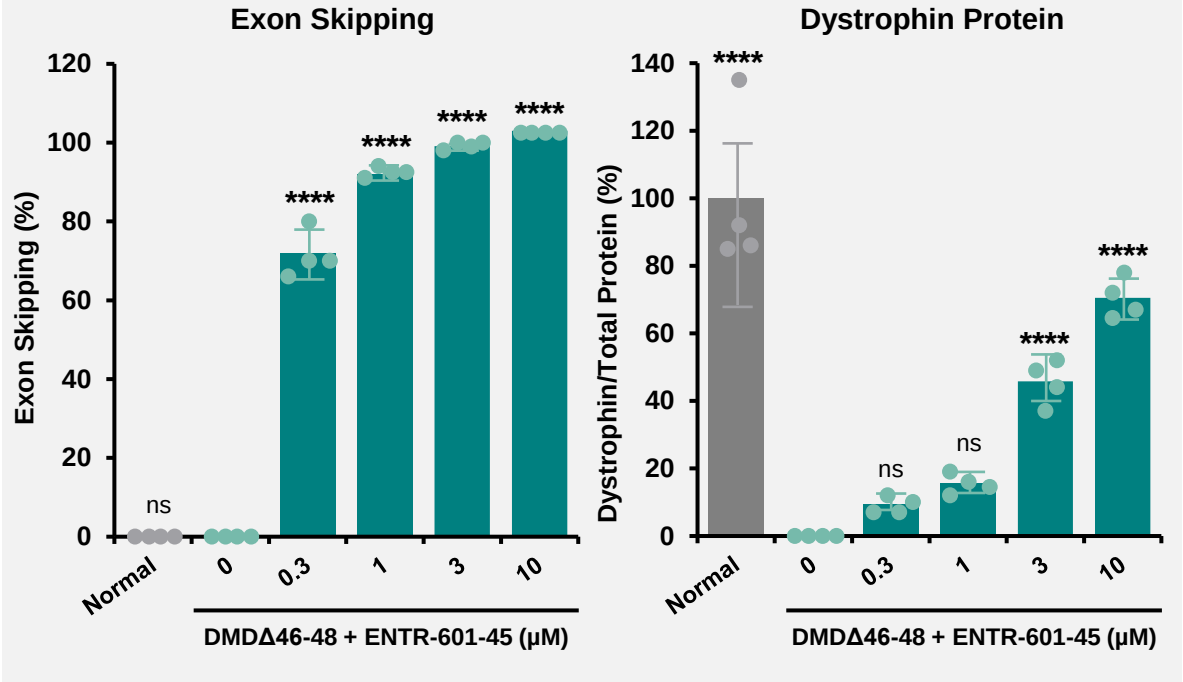
ENTR-601-45 IN VITRO EFFICACY

ENTR-601-45 showed robust exon skipping and dystrophin production in patient-derived skeletal and cardiac muscle cells

ENTR-601-45 in Skeletal Muscle Cells



ENTR-601-45 in Cardiac Muscle Cells

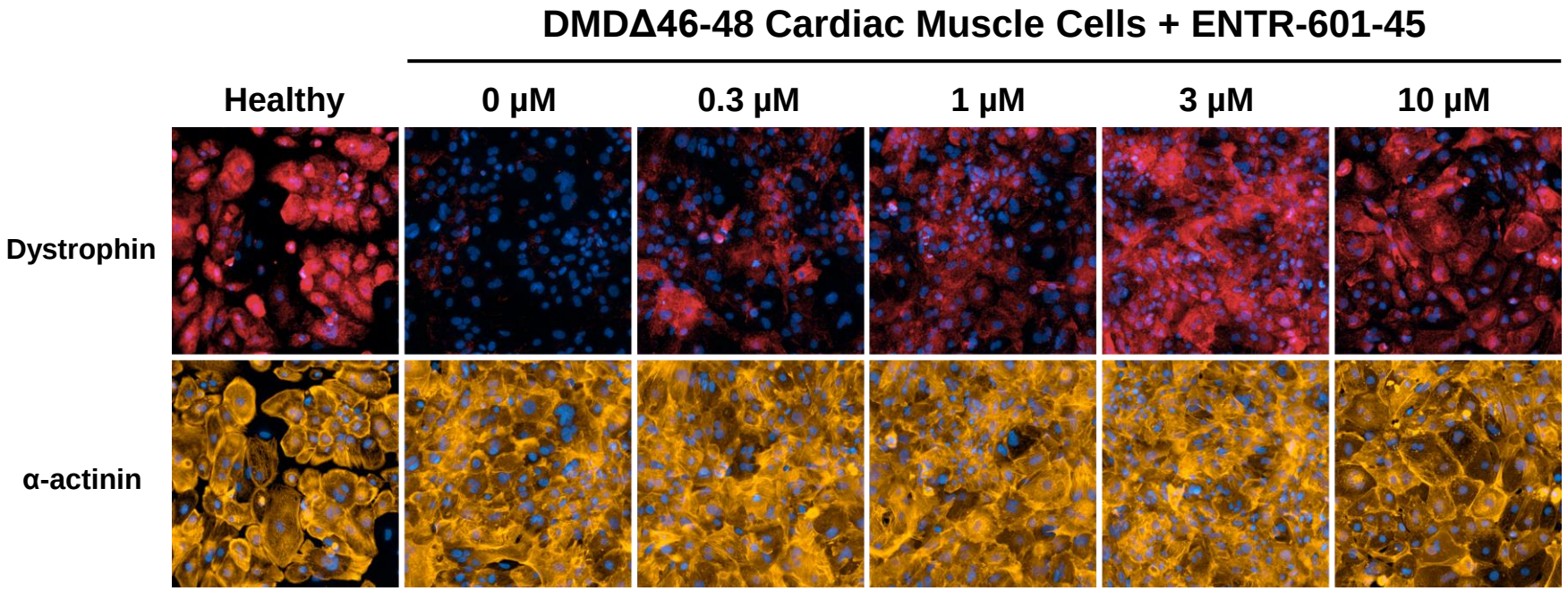


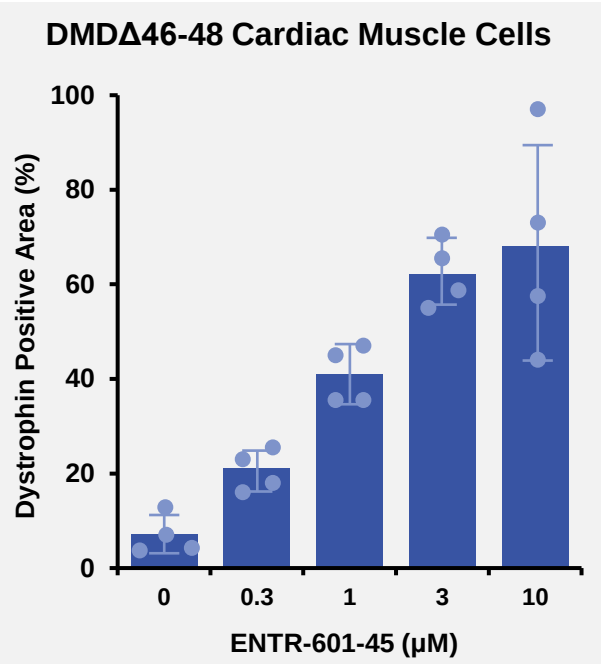
- DMD patient-derived skeletal (n=3) and cardiac (n=4) muscle cells (DMDΔ46-48) were treated with ENTR-601-45 for 24 hours

*p<0.05, ****p<0.0001 vs. untreated DMDΔ46-48 cells. Values are shown as mean ± standard deviation. **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide construct. **DMDΔ46-48** induced pluripotent stem cell-derived skeletal and cardiac muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601-45 IN CARDIAC MUSCLE CELLS

ENTR-601-45 produced dose-dependent dystrophin restoration in patient-derived cardiac muscle cells





- DMD patient-derived cardiac muscle cells (DMDΔ46-48, n=4) were treated with ENTR-601-45 for 24 hours and analyzed 48 hours later.

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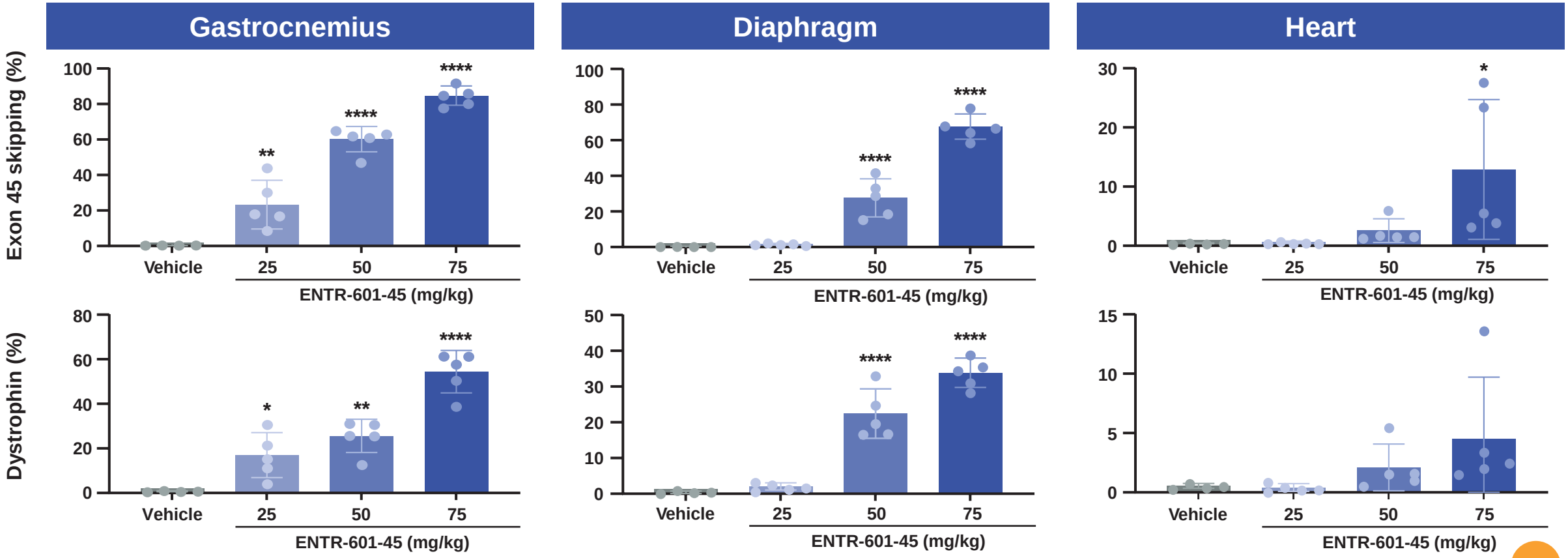
Values are shown as mean ± standard deviation. **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide construct. **DMDΔ46-48** induced pluripotent stem cell-derived cardiac muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation were treated for 24 hours and analyzed 48 hours later. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **iPSC**, induced pluripotent stem cells; **PMO**, phosphorodiamidate morpholino oligomer.

EEV-PMO-45 EFFICACY IN del44hDMD.*mdx* MICE

Three Q6W doses of ENTR-601-45 produced robust human *DMD* exon 45 skipping and dystrophin production 6 weeks after the third dose

*$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$ vs. Vehicle



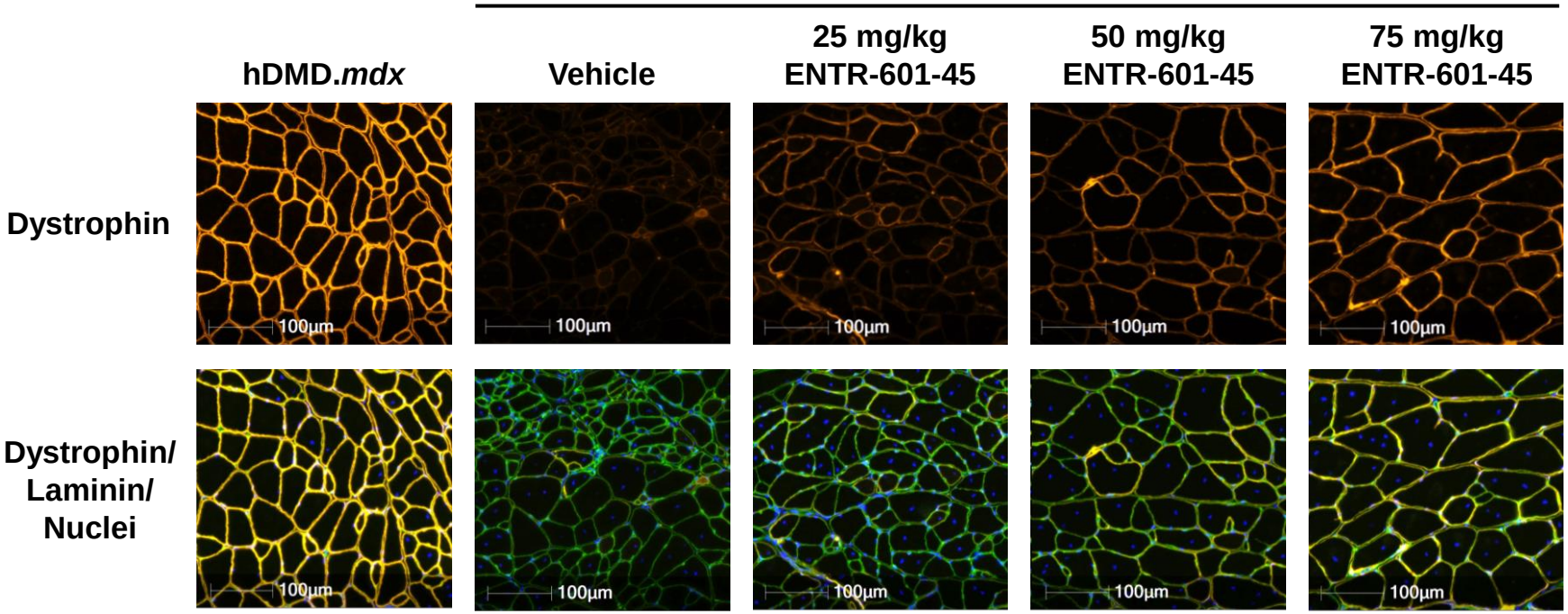
del44hDMD.*mdx* mice were treated with three Q6W IV injections of ENTR-601-45 or vehicle. Human *DMD* exon 45 skipping (top) and dystrophin protein expression (bottom) were analyzed in the gastrocnemius, diaphragm, and heart 6 weeks after the final dose. Percent dystrophin protein restoration is normalized to total protein and normalized to hDMD.*mdx* controls. Data shown as mean ± standard deviation. One-way ANOVA was used for statistical comparison; **ANOVA**, analysis of variance; **hDMD**, human dystrophin transgene; **IV**, intravenous; **Q6W**, every 6 weeks.

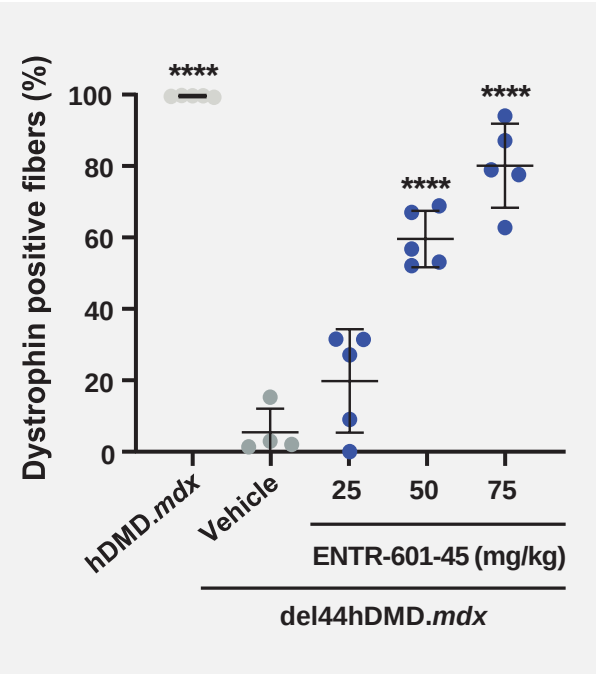
DYSTROPHIN LOCALIZATION WITH ENTR-601-45 IN del44hDMD.*mdx* Mice

ENTR-601-45 produced dose-dependent increases in dystrophin-positive muscle fibers localized to the sarcolemma of del44hDMD.*mdx* mice 6 weeks following the third Q6W dose

****$p \leq 0.0001$ vs. Vehicle

del44hDMD.*mdx* Skeletal Muscle





- del44hDMD.*mdx* mice were treated with three Q6W IV injections of ENTR-601-45 or vehicle. Dystrophin protein distribution and cellular localization was analyzed by immunofluorescence in the gastrocnemius 6 weeks after the final dose.

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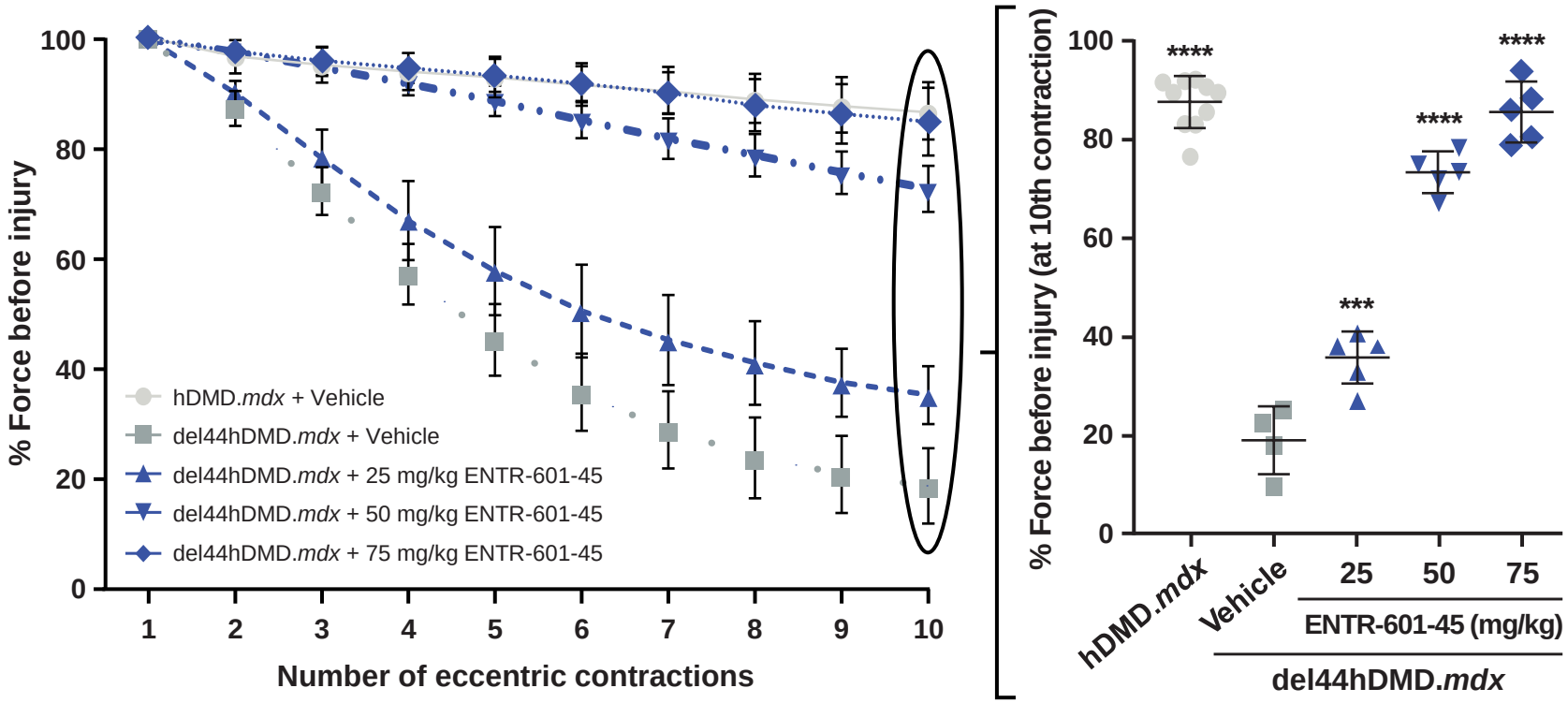
Quantification via Halo Image Analysis Software is shown as the percentage of dystrophin-positive muscle fibers relative to the total number of muscle fibers as determined by laminin staining (green) and dystrophin staining (red); co-localization to the sarcolemma appears yellow and nuclei appear blue. Data shown as mean ± standard deviation. One-way ANOVA was used for statistical comparison; **ANOVA**, analysis of variance; **hDMD**, human dystrophin transgene; **IV**, intravenous; **Q6W**, every 6 weeks.

ENTR-601-45 IMPROVES MUSCLE FUNCTION IN del44hDMD.*mdx* Mice

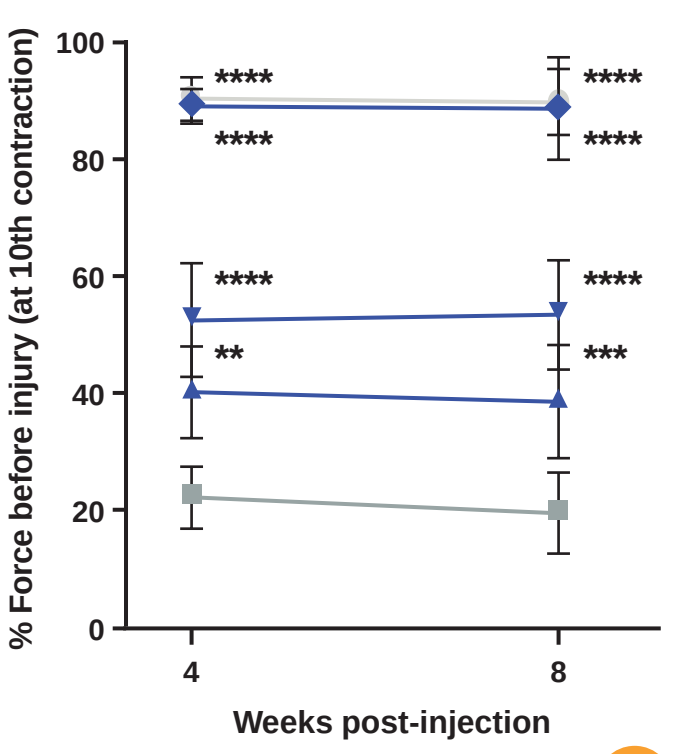
A dose-dependent increase in resistance to membrane damage was observed following the tenth contraction, which was maintained until at least 8 weeks after the third Q6W dose of ENTR-601-45

***p*<0.01, ****p*<0.001, *****p*<0.0001 vs. Vehicle

Skeletal Muscle Membrane Stability



Stability After Washout



del44hDMD.*mdx* mice were treated with three Q6W IV injections of ENTR-601-45 or vehicle. ECC-induced muscle force loss generated by repeated ECC contraction of the gastrocnemius muscle was assessed 5 weeks (left/center) or 4 and 8 weeks (right) after the third dose. Data (mean ± standard deviation) shown across 10 ECC contractions normalized into a percentage of the initial force before any ECC contractions and as the percentage of force retained after the 10th contraction. 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 del44hDMD.*mdx* mice. **ECC**, eccentric force; **hDMD**, human dystrophin transgene; **IV**, intravenous; **Q6W**, every 6 weeks.

ENTR-601-45 Data Summary

ENTR-601-45 consistently demonstrated robust *in vitro* and *in vivo* data;
Regulatory submissions planned in Q4 2024

- **Patient-derived cells**
  - ENTR-601-45 showed robust exon skipping and dystrophin protein production in patient-derived cardiac and skeletal muscle cells
- **DMD mouse models amenable to exon 45 skipping**
  - ENTR-601-45 produced robust dose-dependent exon skipping and dystrophin restoration in both in vitro and in vivo models of exon 45 skip–amenable DMD.
  - Improved skeletal muscle function in an exon 45 skip–amenable DMD mouse model suggests that ENTR-601-45 is capable of producing functional dystrophin protein in vivo.
  - At the highest dose of ENTR-601-45 examined, dystrophin production and muscle function were similar to healthy control mice.

Next Steps

- Planning for a global MAD trial in Duchenne patients
- Regulatory submissions expected in Q4 2024

ENTR-601-44

ENTR-601-44 CLINICAL PROGRAM

First-in-Human Trial

Completed

Single Ascending Dose (SAD) Study
in Healthy Volunteers (ENTR-601-44-101)

- **Study met all study objectives in healthy male volunteers with no AEs related to ENTR-601-44 administration**
- **No adverse findings or clinically relevant changes to any renal markers measured during the study**
- **Dose-dependent concentration and significant exon 44 skipping in skeletal muscle with 6 mg/kg ENTR-601-44**

Planned Multiple Ascending Dose/Phase 2b (Global)

Regulatory filings expected in Q4 2024

Multiple Ascending Dose (MAD) Study*
in Exon 44 Skipping Amenable Patients

- **Juvenile patients**
- **3 MAD cohorts (final design TBD)**
- **Dosing initiation target of 6 mg/kg**
- **Dosing interval ≥ every 6 weeks**

Phase 2b Study*
in Exon 44 Skipping Amenable Patients

Target Product Profile:

- **Double digit dystrophin improvement from baseline**
- **Dosing interval ≥ every 6 weeks**

File for Accelerated Approval

Outcome Measures

- Safety and tolerability
- Evaluation of PK and PD
- Evaluation of exon skipping and dystrophin production (skeletal muscle)

Primary Efficacy Measures

- Change in dystrophin level (skeletal muscle)

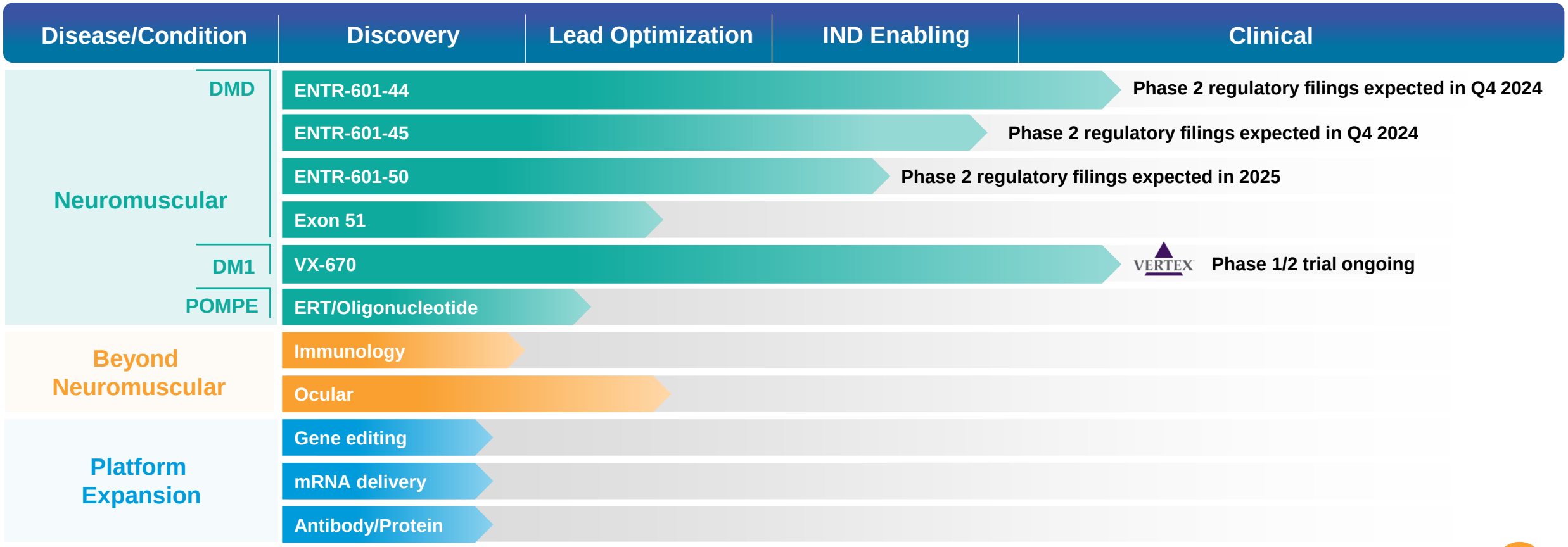
Secondary/Exploratory Efficacy Measures

- Change from baseline in the 10-meter walk/run
- Change from baseline in the timed rise from floor
- Other parameters may include NSAA, FVC, QoL

*MAD/Phase 2b study is subject to regulatory feedback. **ENTR-601-44** is a DMD exon 44 skipping EEV-oligonucleotide construct. **AE,** adverse event; **FVC**, forced vital capacity; **NSAA**, North Star Ambulatory Assessment: **PD**, pharmacodynamics; **PK**, pharmacokinetics; **Q,** quarter; **QoL**, quality of life; **TBD**, to be determined.

A DIFFERENTIATED AND EXPANDING PIPELINE

Entrada’s pipeline includes a diverse array of high potential and high value assets;
Each disease has a substantial patient population with a significant unmet medical need



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