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

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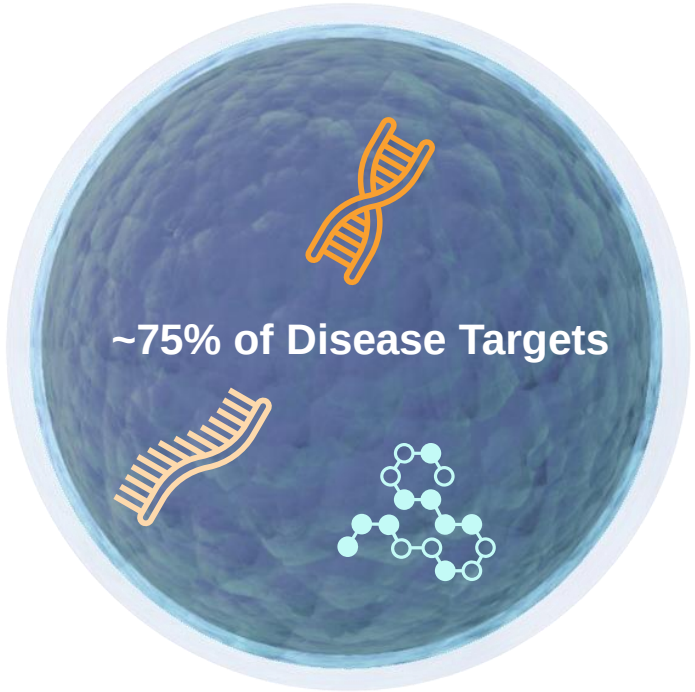
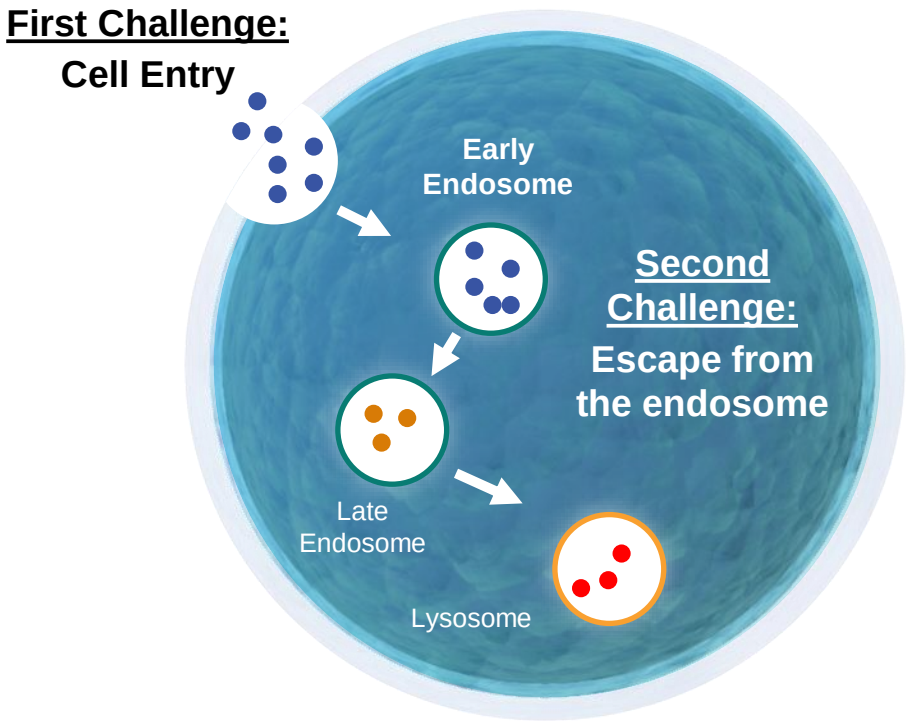
THE NEED FOR INTRACELLULAR THERAPEUTICS

Approximately 75% of all disease-causing targets are intracellular and difficult to reach, representing a significant issue when developing effective therapies



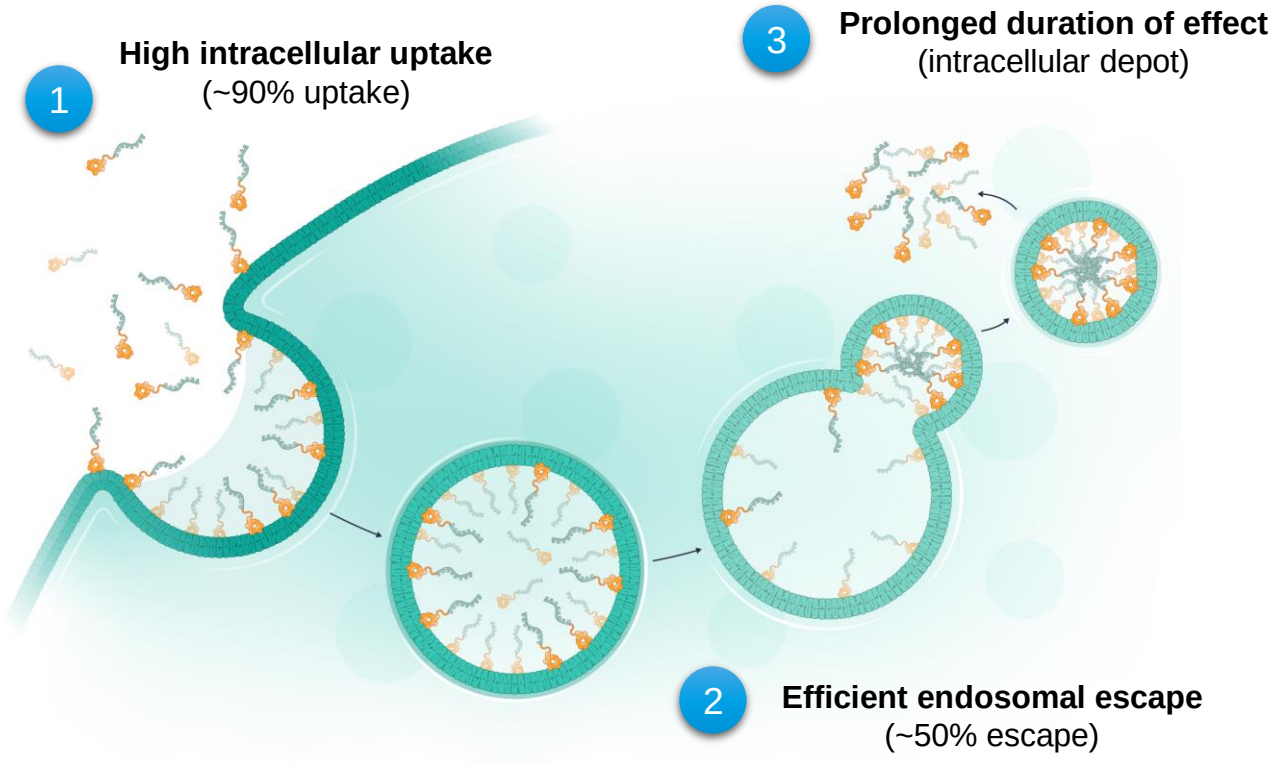


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

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

- 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**

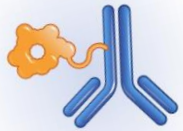


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

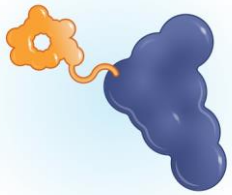
A BROADLY APPLICABLE PLATFORM

Entrada has demonstrated intracellular uptake of unique moieties ranging from 1 kDa to 600 kDa

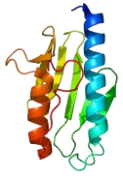
Antibodies



Enzymes

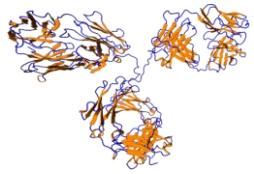


Oligonucleotides



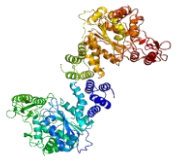
550-600 KDa

Hybrid frataxin



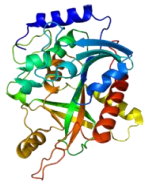
150 KDa

Antibody



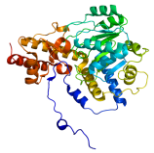
98 KDa

Thymidine phosphorylase



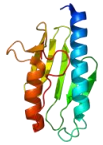
96 KDa

Purine nucleoside phosphorylase



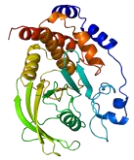
86 KDa

Alanine-glyoxylate aminotransferase



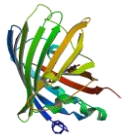
46 KDa

Human frataxin



37 KDa

PTP1B catalytic domain



32 KDa

EGFP

16 KDa

Nanobody

6 KDa

Oligonucleotide

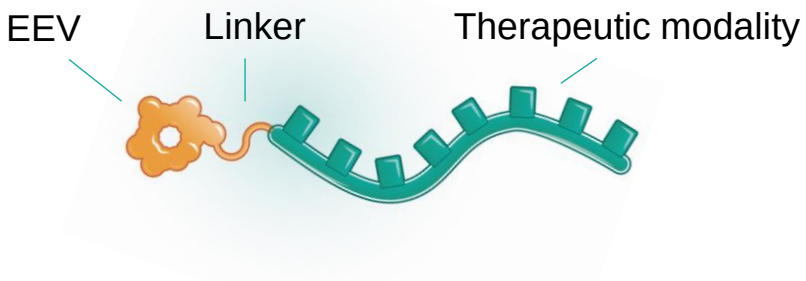
1-3 KDa

Various peptide cargos

EGFP, enhanced green fluorescent protein.

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 **linker & 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

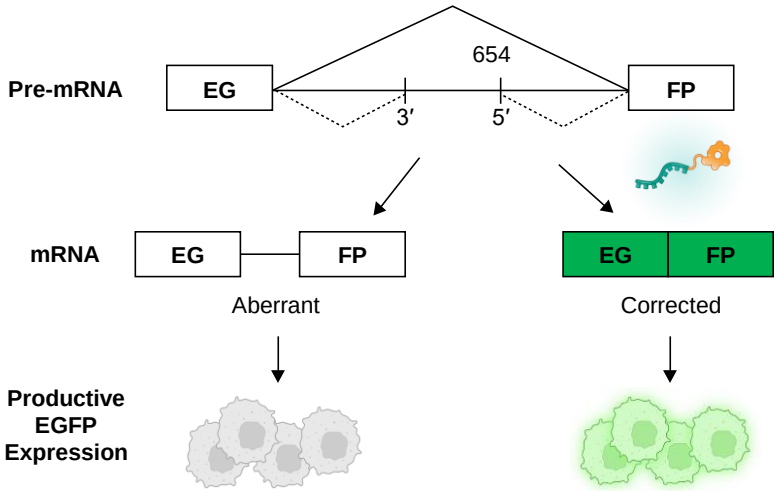
Identify EEV candidate with desired **therapeutic profile**

EEV, endosomal escape vehicle.

FUNCTIONAL DELIVERY FOR TARGET TISSUES

Entrada's EEV-therapeutics can be designed to enhance functional delivery to target tissues

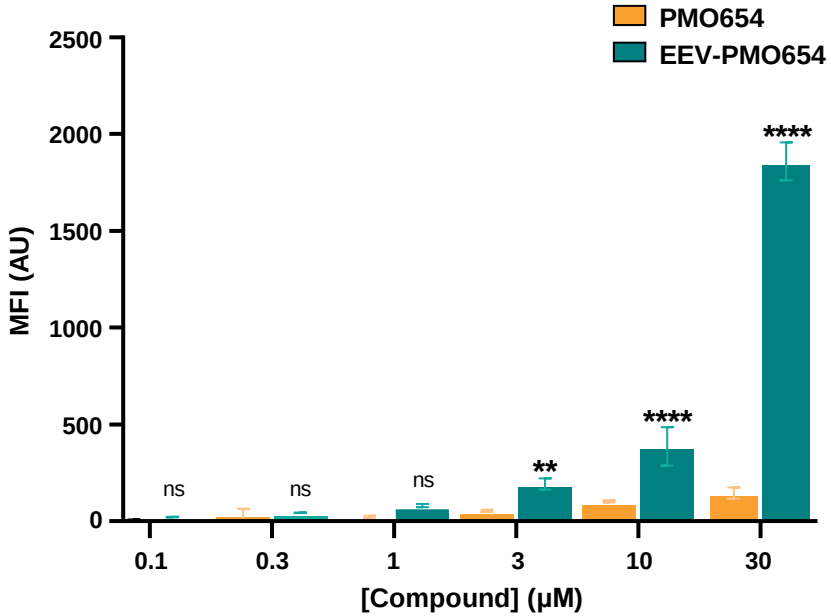
EGFP-654 Transgenic Model[1]



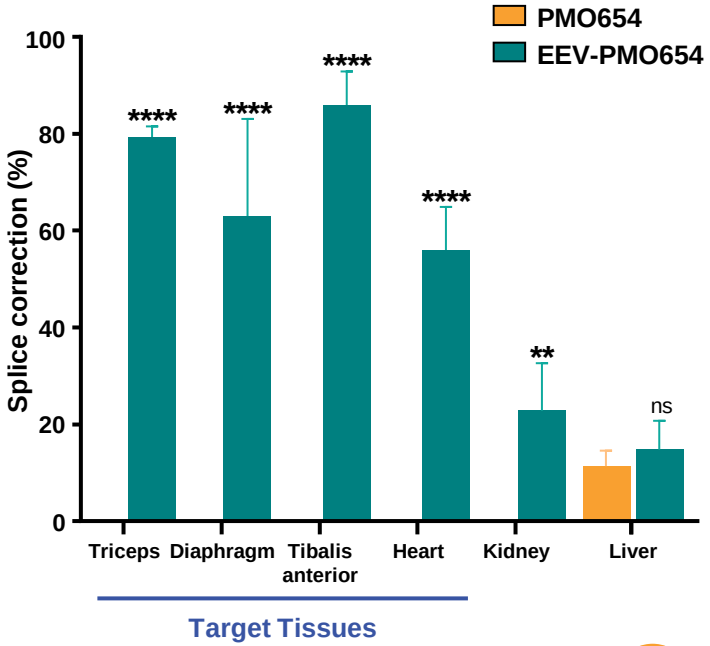
- The HeLa EGFP-654 cellular model was used to evaluate the suitability of the EEV platform for in vitro delivery of oligonucleotide cargo
- Successful treatment of HeLa EGFP-654 cells with antisense oligonucleotides (PMOs) would switch the splicing and restore expression of EGFP.

Enhanced Functional Delivery to Target Tissues with EEV Platform

Internalization Efficacy in HeLa-EGFP-654 Cells



Splicing Correction in EGFP-654 Mice



p<0.01, ****p<0.0001 vs. PMO654. These studies used EEV3-PMO654. Values are shown as mean ± standard deviation. **1. Li, X. et al. *Mol. Ther. Nucleic Acids* 2023. **EEV**, endosomal escape vehicle; **EGFP**, enhanced green fluorescent protein; **PMO**, phosphorodiamidate morpholino oligomer.

DUCHENNE MUSCULAR DYSTROPHY

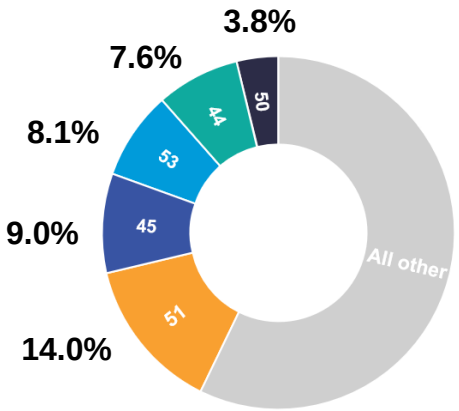
SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

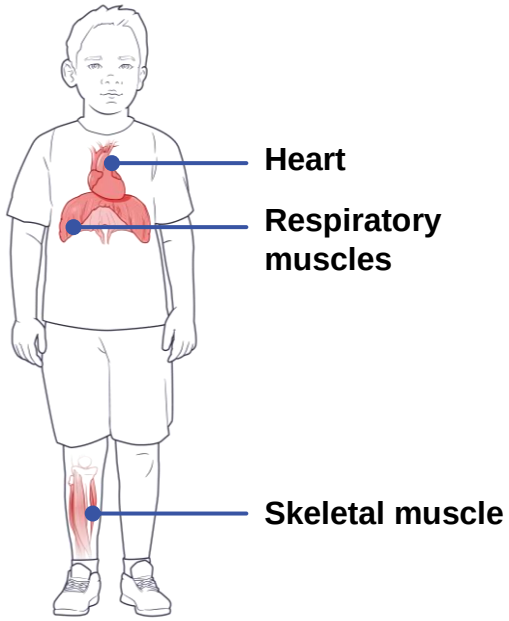
Duchenne is caused by **mutations in the DMD gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

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

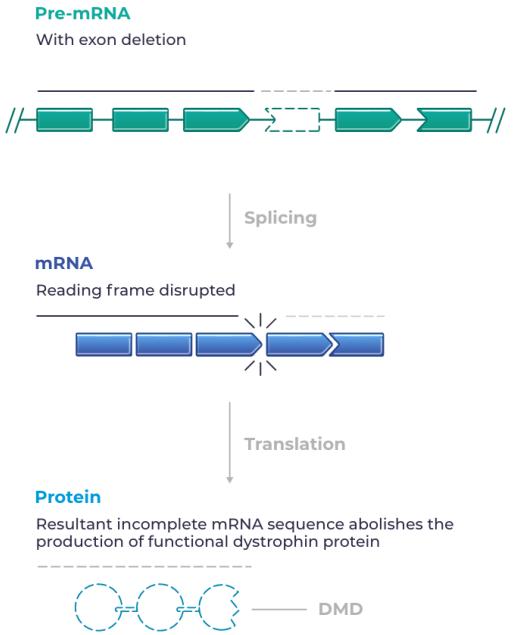
~15,000 people in the **U.S.**[1] **&** **~26,000** people in **Europe**[2] have Duchenne

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

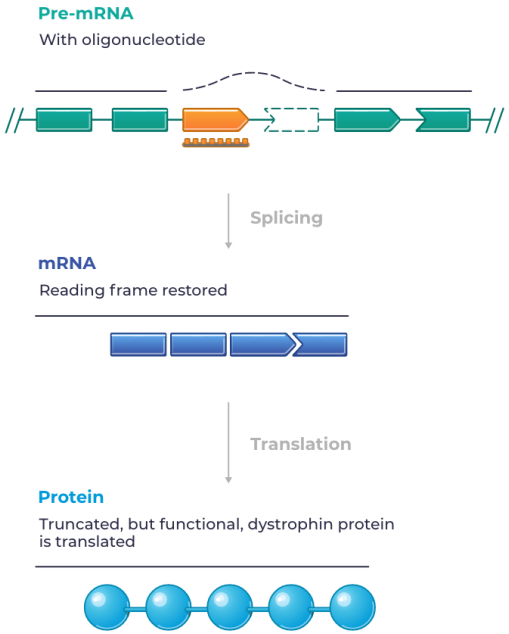




Patients with Duchenne



EEV-Oligonucleotide Approach

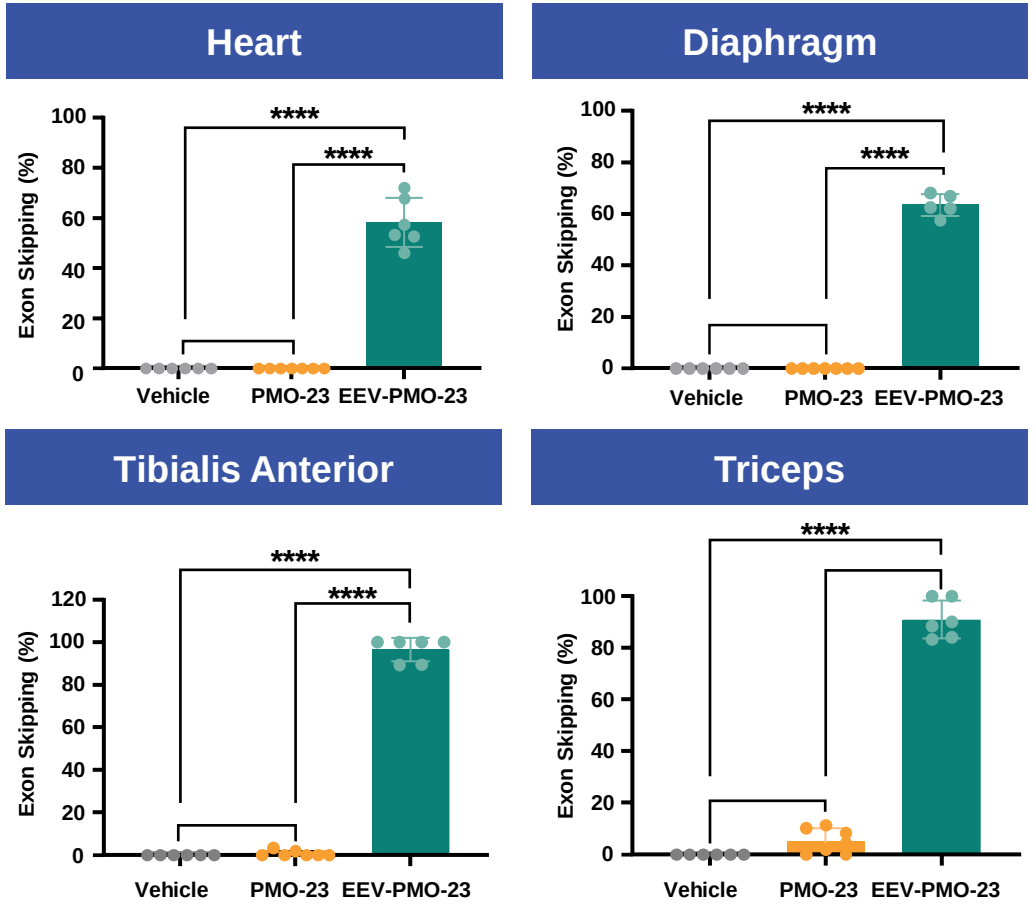


Frontiers in Myogenesis

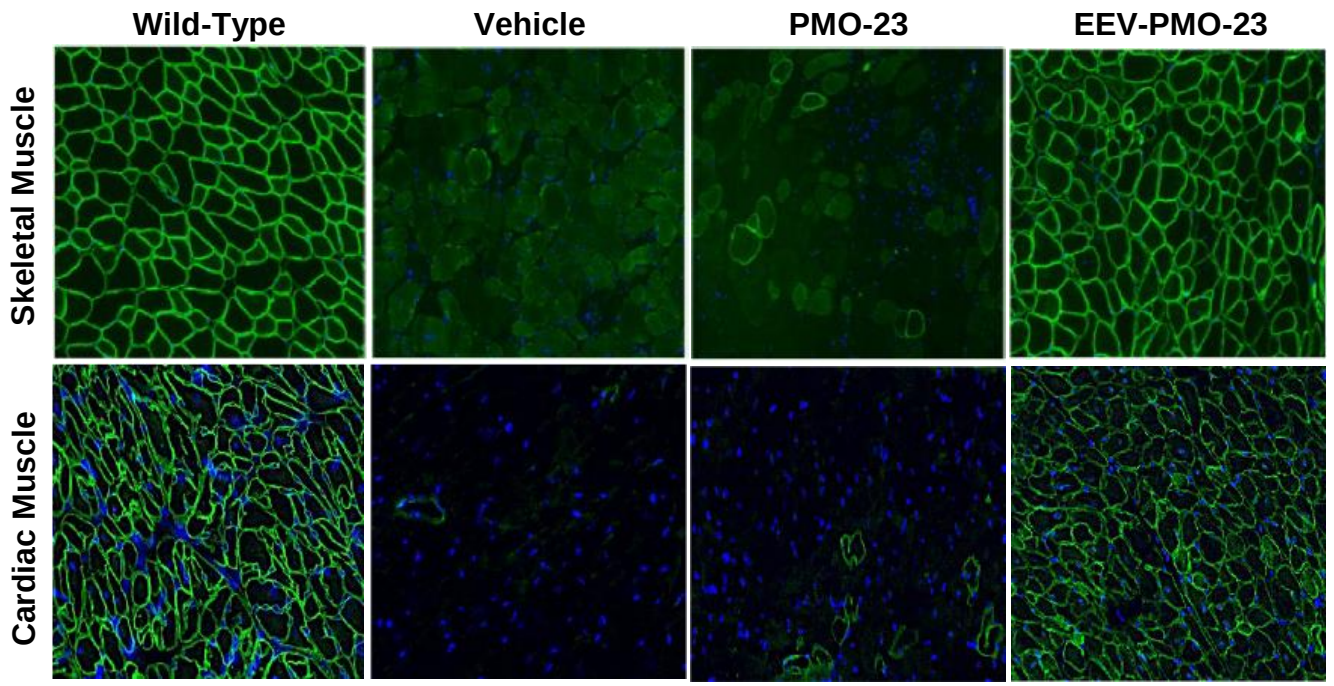
1. Parent Project Muscular Dystrophy. https://www.parentprojectmd.org/about-duchenne/. Accessed August 18, 2023. **2.** Europeans Medicines Agency. https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3202375. Accessed August 18, 2023. **3.** Bladen, C.L. et al. *Hum Mutat*. 2015. **4.**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

REPEAT EEV-PMO-23 TREATMENT IN D2-*mdx* MICE

Robust exon 23 skipping after 4 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



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

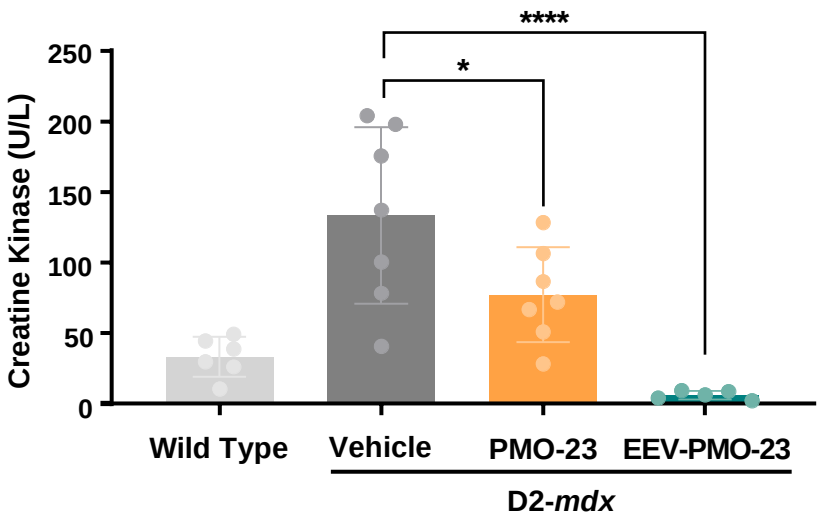
****p<0.0001. Values are shown as mean ± standard deviation. **D2-*mdx*** is a DMD mouse model with a nonsense mutation in *DMD* exon 23 (Coley, W.D. et al. *Hum. Mol. Genet*. 2016). Li, X. et al. *Mol. Ther. Nucleic Acids* 2023. **EEV**, endosomal escape vehicle; **DMD**, Duchenne muscular dystrophy; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer; **PMO-23**, mouse *DMD* exon 23 skipping phosphorodiamidate morpholino oligomer.

REPEAT EEV-PMO-23 TREATMENT RESULTS IN CK CORRECTION AND FUNCTIONAL IMPROVEMENT

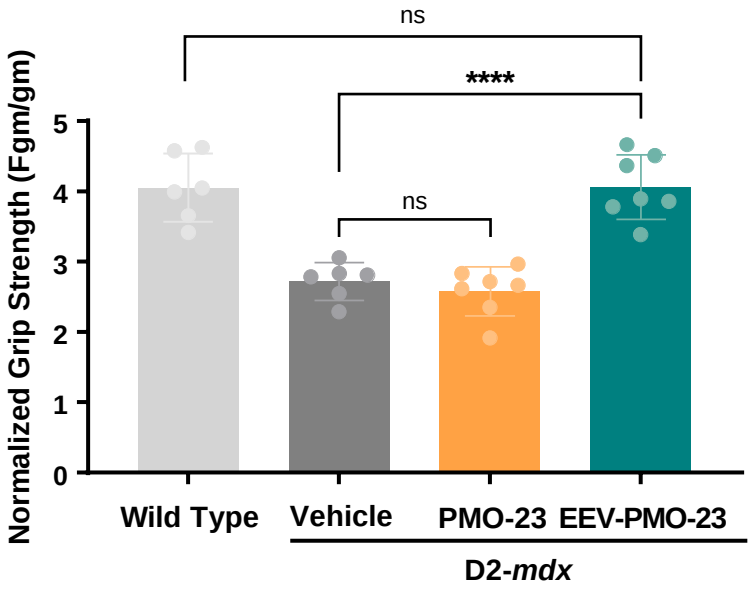
Repeat EEV-PMO-23 treatment normalized serum CK levels and showed significant improvements in muscle function when compared to PMO alone after four monthly IV doses in D2-*mdx* mice

*p<0.05, **p<0.01, ****p<0.0001

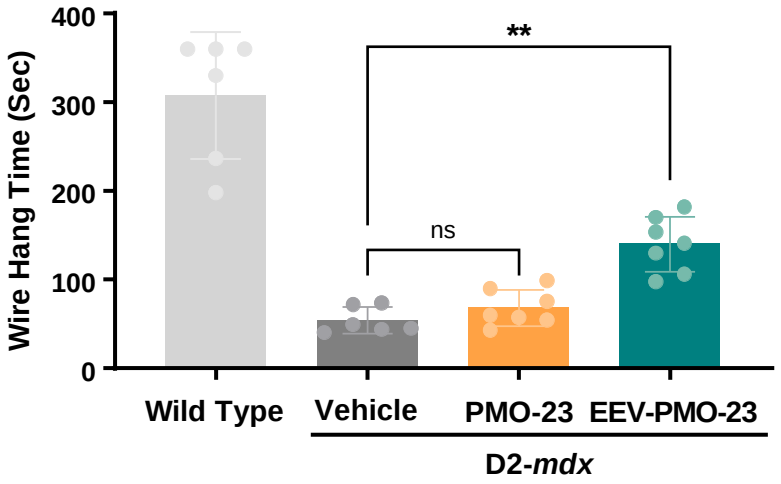
Serum CK Levels (Muscle Damage)



Grip Strength



Wire Hang Time



- 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 ~2 weeks after the last dose

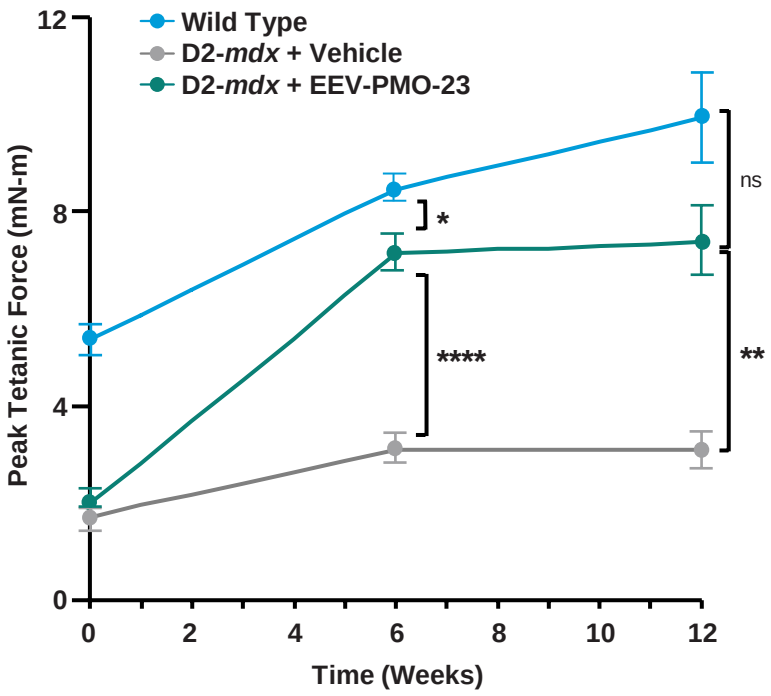
Values are shown as mean ± standard deviation. **D2-*mdx*** is a DMD mouse model with a nonsense mutation in *DMD* exon 23 (Coley, W.D. et al. *Hum. Mol. Genet*. 2016). The D2-*mdx* model is on a DBA/2J background and better recapitulates disease pathology (Fukada, S. et al. *Am. J. Path.* 2010). **CK**, creatine kinase; **EEV**, endosomal escape vehicle; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer; **PMO-23**, mouse *DMD* exon 23 skipping phosphorodiamidate morpholino oligomer.

REPEAT EEV-PMO-23 TREATMENT RESULTS IN IMPROVED MUSCLE CONTRACTILITY

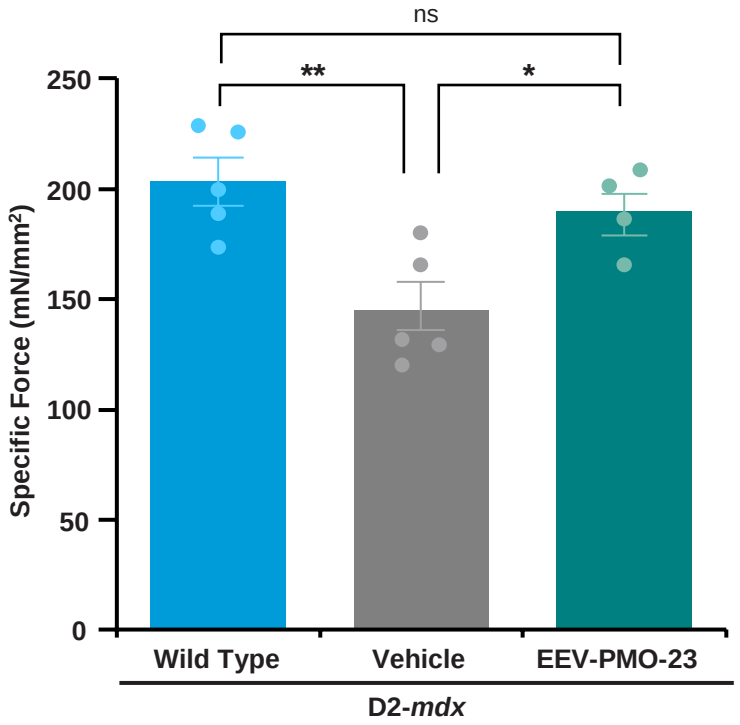
Bi-weekly treatment with EEV-PMO-23 improved skeletal muscle contractile force in D2-*mdx* mice and was not significantly different than wild-type mice at Week 12

*p<0.05, **p<0.01, ****p<0.0001

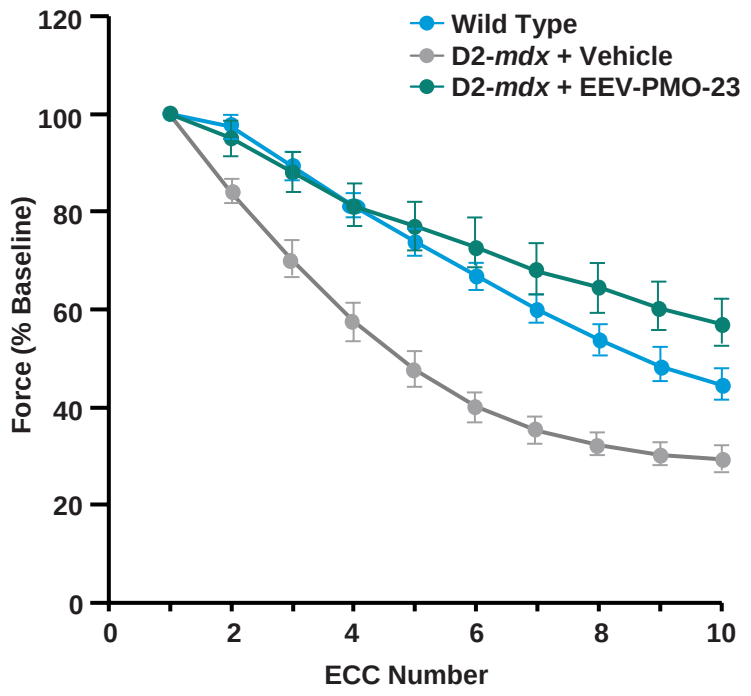
Longitudinal Tetanic Force



Week 12 Specific Force



Repeated Muscle Contraction



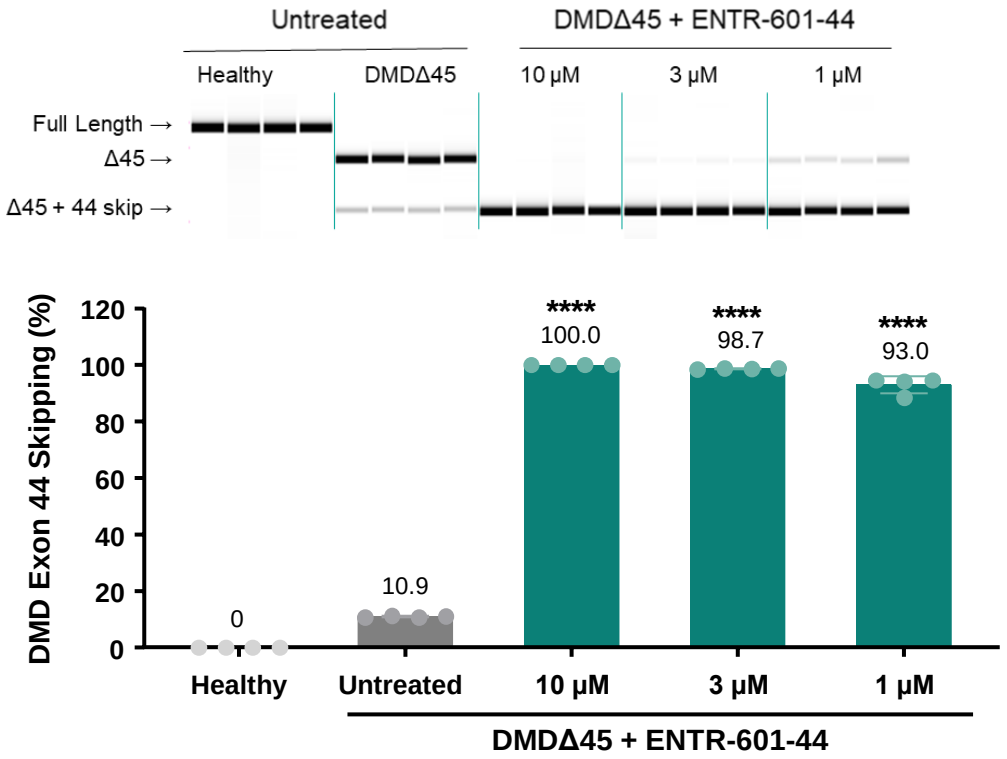
Data are shown as mean ± standard error of mean. D2-*mdx* mice were treated with vehicle or 80 mg/kg weekly EEV-PMO-23. Muscle contractility was assessed via isometric force generated by tetanic contraction of plantar flexor muscle group and ECC generated by repeated tetanic contraction of TA muscle. **ECC,** eccentric force; **EEV**, endosomal escape vehicle; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer; **PMO-23**, mouse *DMD* exon 23 skipping phosphorodiamidate morpholino oligomer.

ENTR-601-44

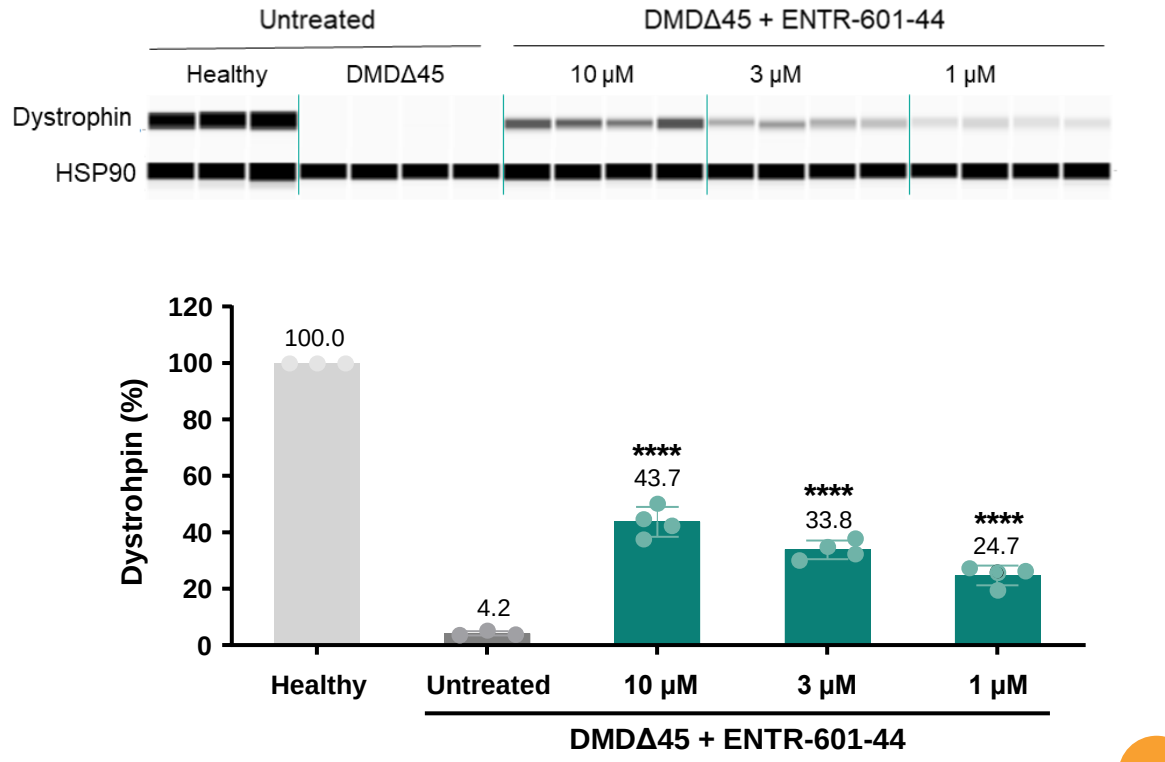
ENTR-601-44 IN PATIENT-DERIVED MYOTUBES

Robust dose-dependent exon 44 skipping and dystrophin protein production were observed in DMD patient-derived muscle cells treated with clinical candidate, ENTR-601-44

Exon Skipping



Dystrophin Protein Production



****p<0.0001 vs untreated DMDΔ45; Values shown as mean ± standard deviation. **DMDΔ45** are immortalized myoblasts from DMD patients harboring an out-of-frame exon 45 deletion and further differentiated into myotubes. **ENTR-601-44** is a DMD exon 44 skipping EEV-oligonucleotide conjugate. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **HSP90,** heat shock protein 90.

EXON 44 SKIPPING AND DYSTROPHIN RESTORATION WITH A HUMAN DMD CELL MODEL

EMT + EEV-PMO44

Proper dystrophin restoration with an exon 44 skipping EEV-PMO tool compound was observed using a DMD patient-derived, 3D organoid system

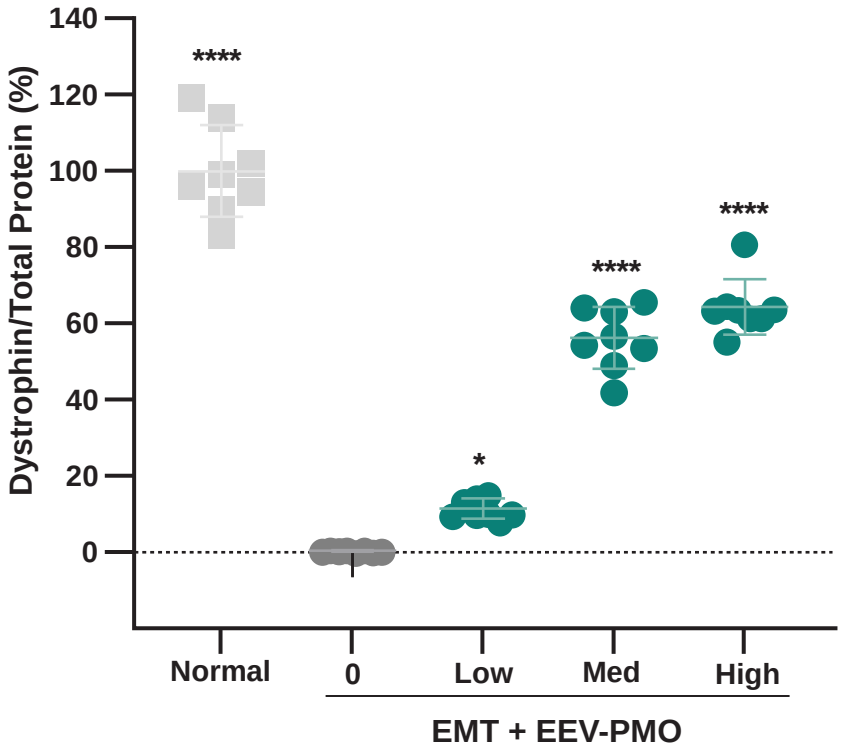
3D EMTs

DMD Patient-Derived Skeletal Muscle Cells

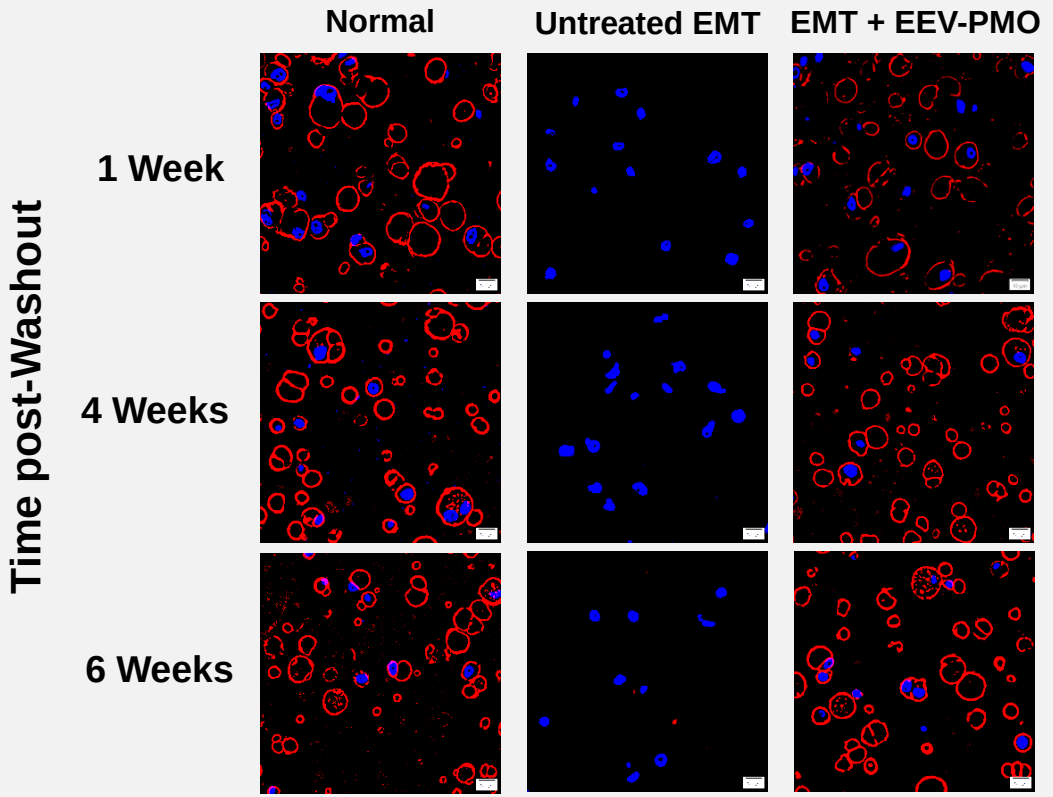
3D engineered skeletal muscle tissues (EMTs) are generated on the Mantarray™ System

Dystrophin Expression (6 weeks post-Washout)

*p<0.05, ****p<0.0001 vs. Untreated EMT



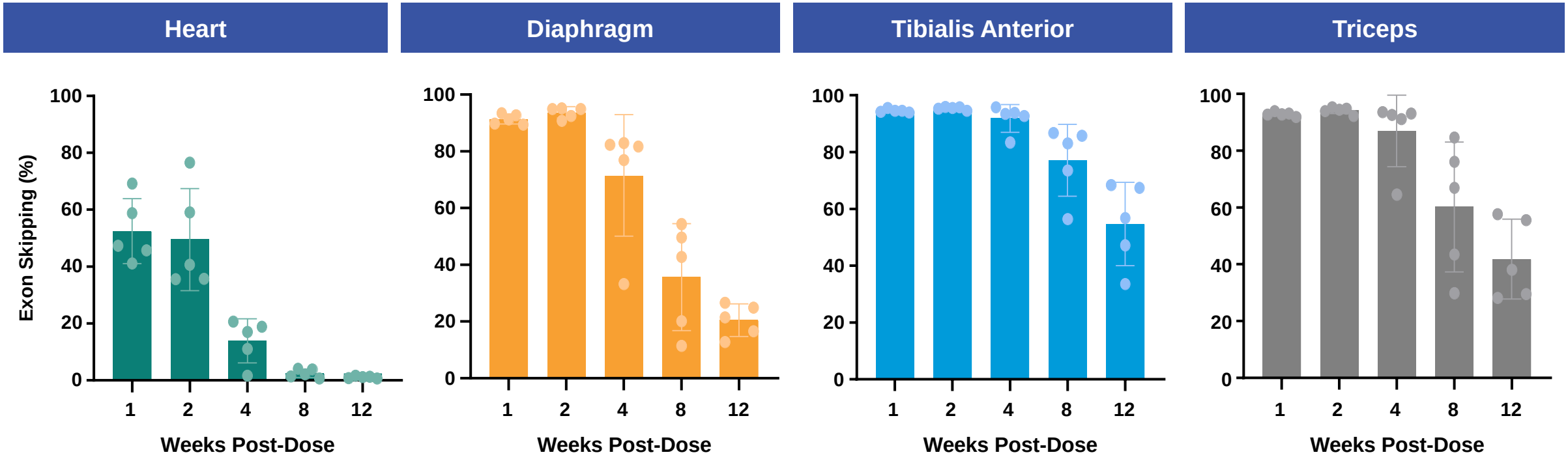
Dystrophin Restoration



Immortalized patient-derived skeletal myoblasts with exon 45 deletion in the *DMD* gene were engineered into EMTs (Mantarray™ system, Curi Bio, Seattle, WA). EMTs were treated with EEV-PMO for 24 hours and analyzed at 1-, 4-, and 6-weeks post-washout. Normal cells from non-DMD individuals were used as controls. Dystrophin quantified using RT-PCR (N=8). Data are shown as mean ± SD; Ordinary 1-way ANOVA and Dunnett's multiple comparison test. Cross-sectional images (40X cropped) of EMTs (N=4). Red, dystrophin; blue, DAPI. **EMT**, engineered muscle tissue; **Med**, medium.

ENTR-601-44 IN HUMAN DMD MICE (hDMD)

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



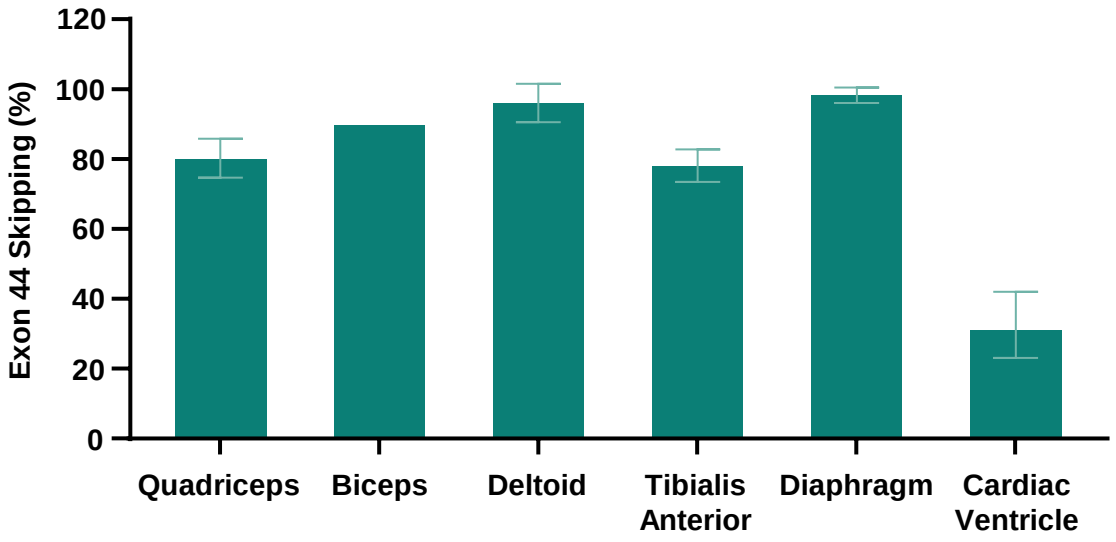
- Post single IV dosage of 60 mg/kg (PMO equivalent), robust exon skipping was observed in ENTR-601-44-treated hDMD mice

hDMD transgenic mice express full-length human dystrophin gene ('t Hoen, A.C. et al. *J. Biol. Chem.* 2008). Values are shown as mean ± standard deviation. **ENTR-601-44** is a DMD exon 44 skipping EEV-oligonucleotide conjugate. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **hDMD,** human Duchenne muscular dystrophy; **IV,** intravenous; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601-44 NON-HUMAN PRIMATES (NHP)

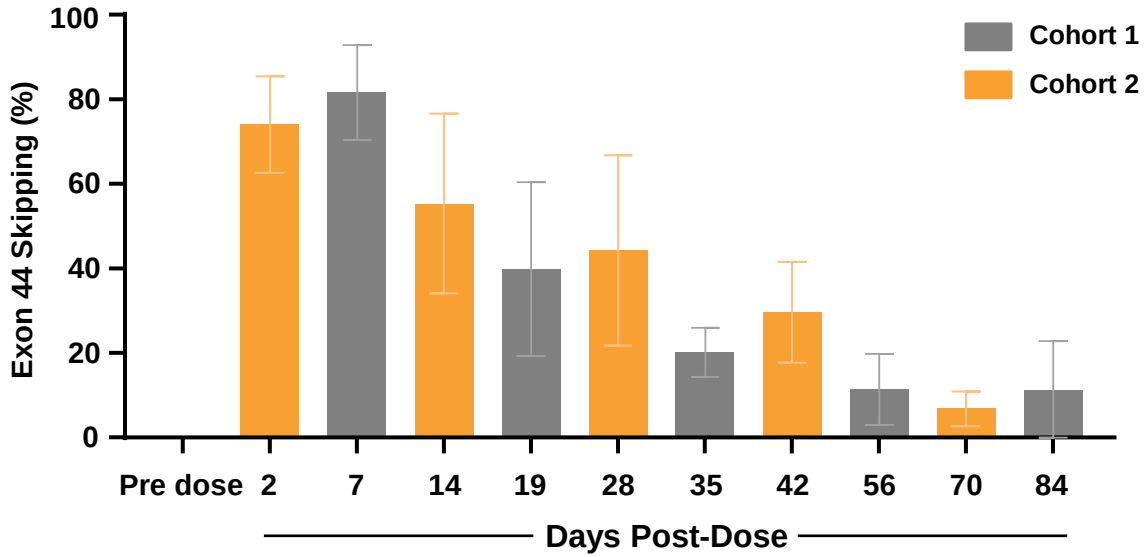
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

Exon Skipping in NHP Muscles at Day 7



- At 7 days post IV infusion of 30 mg/kg (PMO equivalent), robust exon 44 skipping across different muscle groups isolated from the ENTR-601-44-treated NHP

Duration of Effect in NHP Biceps for at Least 12 Weeks



- Post IV infusion of 35 mg/kg (PMO equivalent), robust exon 44 skipping observed in biceps in the ENTR-601-44-treated NHP (n=3 per cohort) for at least 12 weeks

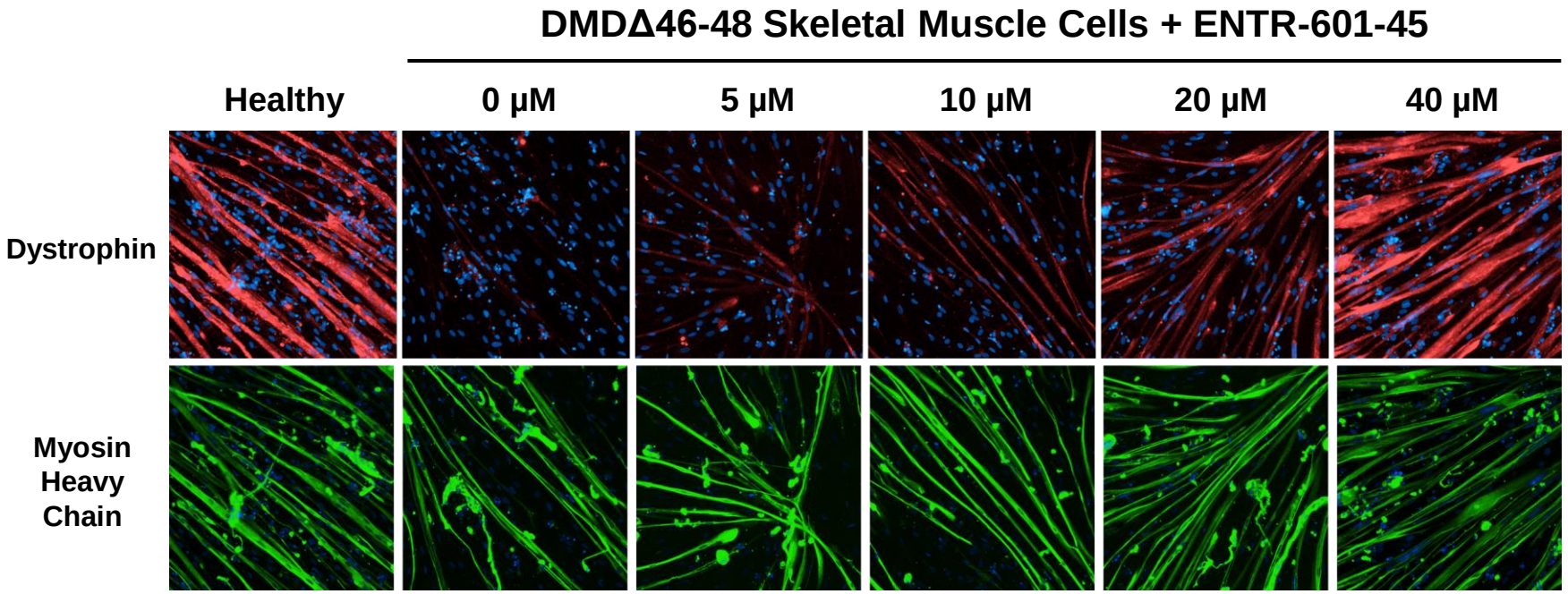
Values are shown as mean ± standard deviation. **ENTR-601-44** is a DMD exon 44 skipping EEV-oligonucleotide conjugate. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **IV,** intravenous; **NHP**, non-human primate; **PMO**, phosphorodiamidate morpholino oligomer.

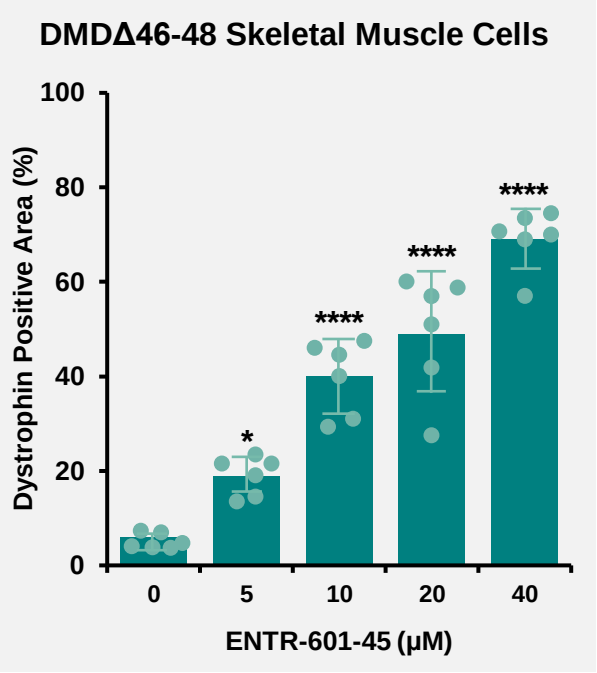
ENTR-601-45

ENTR-601-45 IN SKELETAL MUSCLE CELLS

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

*p<0.05, ****p<0.0001 vs. Vehicle



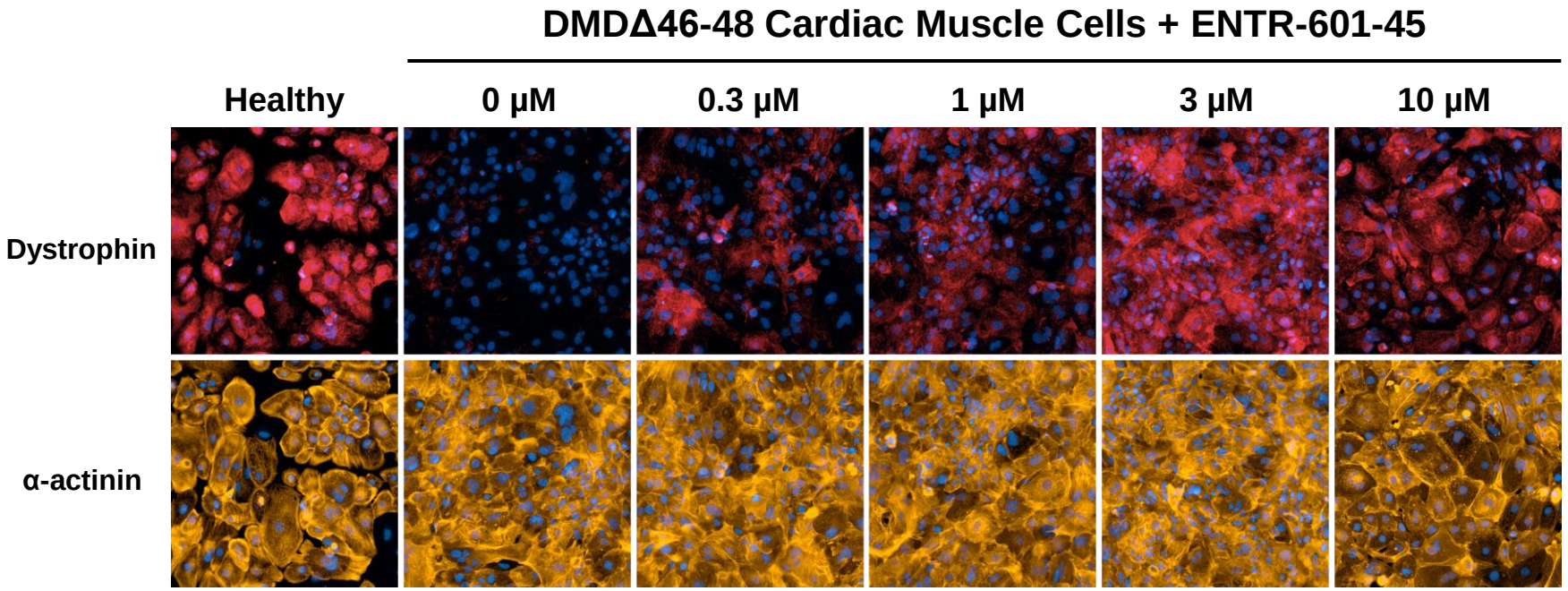


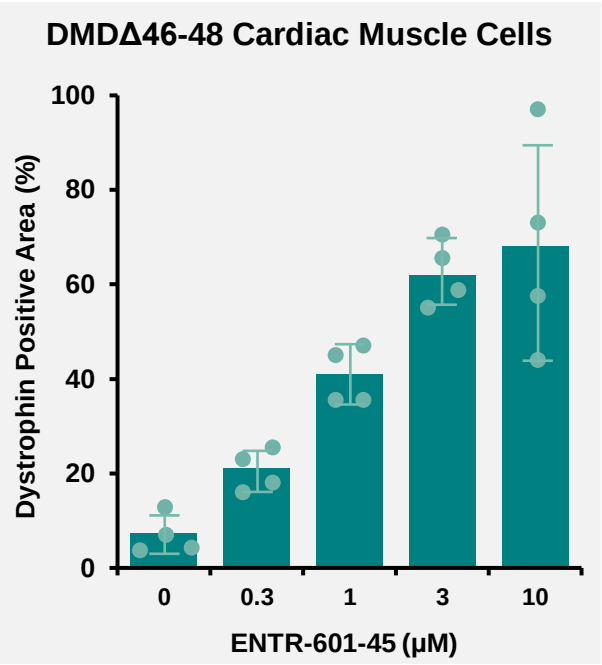
- DMD patient-derived skeletal muscle cells (DMDΔ46-48, n=6) were treated with ENTR-601-45 for 24 hours and analyzed 5 days later.

Values are shown as mean ± standard deviation. **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide conjugate. **DMDΔ46-48** induced pluripotent stem cell-derived skeletal muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation were treated for 24 hours and analyzed after 5 days of differentiation. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **iPSC**, induced pluripotent stem cells; **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





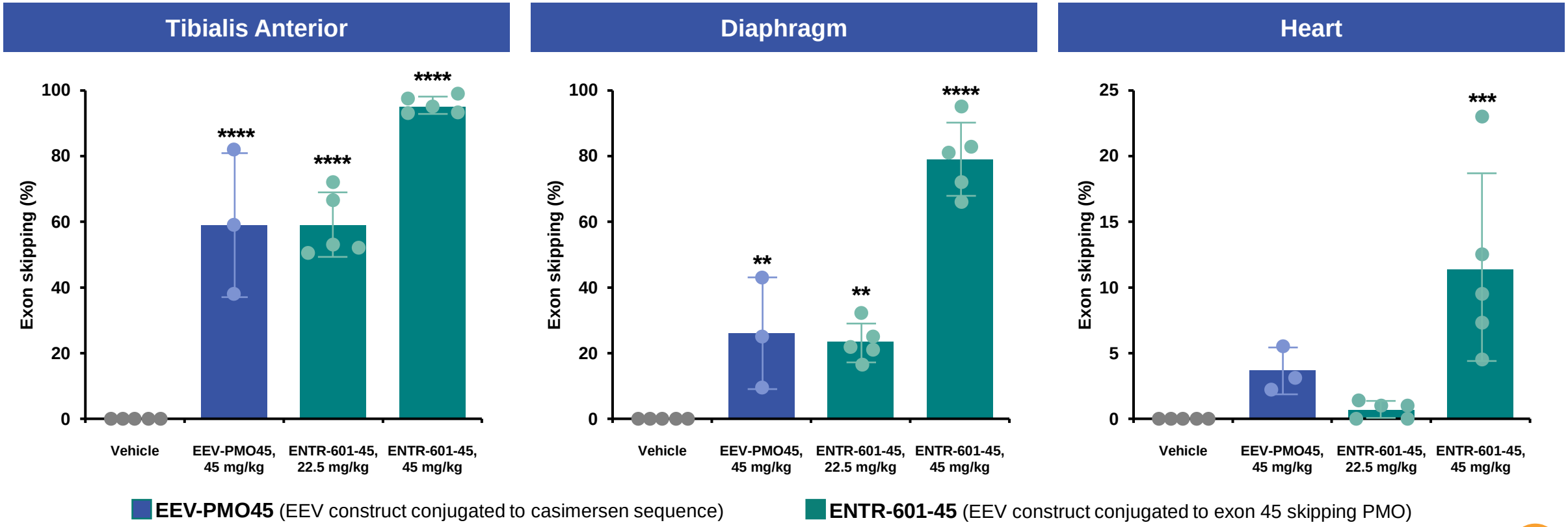
- DMD patient-derived cardiac muscle cells (DMDΔ46-48, n=4) were treated with ENTR-601-45 for 24 hours and analyzed 48 hours later.

Values are shown as mean ± standard deviation. **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide conjugate. **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.

ENTR-601-45 TARGET ENGAGEMENT IN hDMD MICE

A single dose of ENTR-601-45 delivered up to a two-fold improvement in exon skipping when compared to an equivalent dose of the same EEV conjugated to a casimersen sequence

*p<0.05, **p<0.01, ****p<0.0001 vs. Vehicle



Values are shown as mean ± standard deviation (n=3-5). **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide conjugate. **hDMD** transgenic mice express full-length human dystrophin gene ('t Hoen, P.A. et al. *J. Biol. Chem.* 2008). Casimersen is an exon 45 skipping PMO approved in the US. Concentrations provided are PMO equivalent.
DMD, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **hDMD,** human Duchenne muscular dystrophy; **PMO**, phosphorodiamidate morpholino oligomer.

EEV-PMO IN DMD

These results demonstrate that the EEV Platform efficiently delivers oligonucleotides to skeletal and cardiac muscles in preclinical models of Duchenne muscular dystrophy

ENTR-601-44*

- High levels of exon skipping across mdx, D2-*mdx*, human dystrophin (hDMD) mouse and NHP studies
- Exon skipping translates to promising dystrophin production in heart and skeletal muscles
- Dystrophin production observed to result in functional improvement

ENTR-601-45

- Robust exon skipping and dystrophin protein production in patient-derived cardiac and skeletal muscle cells
- High levels of exon skipping were measured in hDMD mouse heart and skeletal muscle tissue

Entrada received authorization in the U.K. to initiate a Phase 1 clinical trial in healthy volunteers

- First participant was dosed in September 2023 with data anticipated in 2H 2024

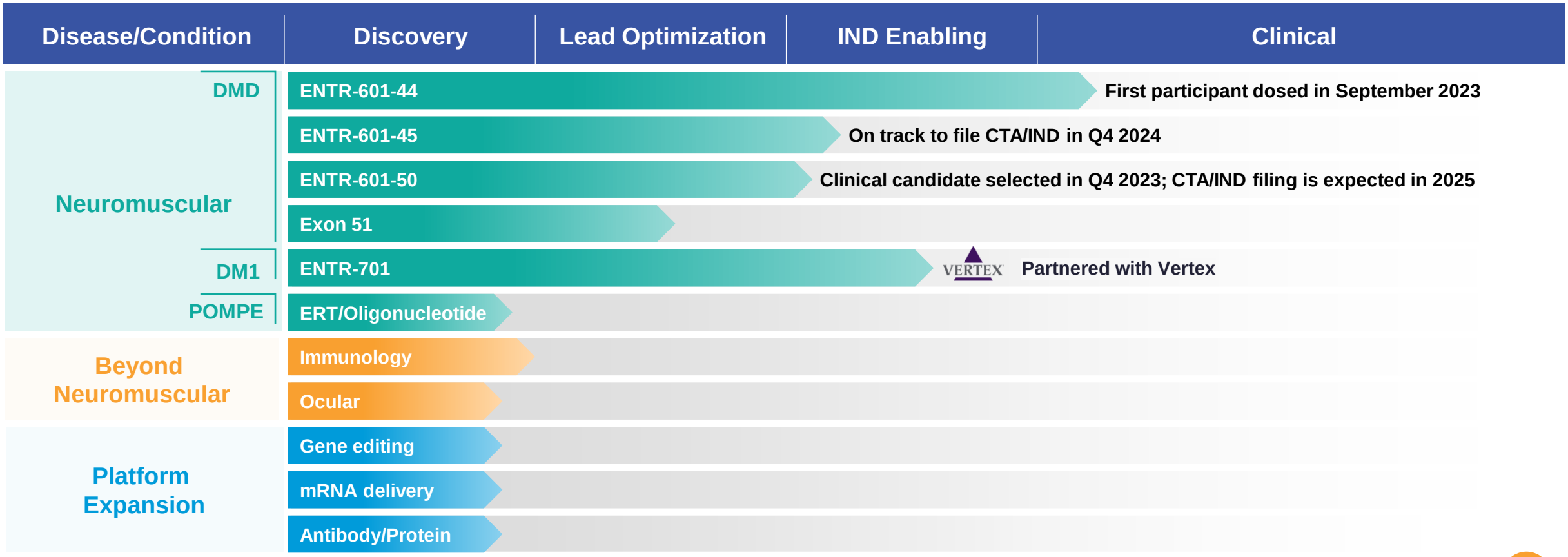
Entrada is planning for regulatory submission in Q4 2024

- Planning for a direct to MAD trial in Duchenne patients for ENTR-601-45

*Entrada received a clinical hold notice from the FDA regarding the IND for ENTR-601-44 in December 2022. Entrada plans to share additional updates pending further communications with the FDA.

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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