Endosomal Escape Vehicles (EEV™) to Enhance the Functional Delivery of Oligonucleotides in Duchenne Muscular Dystrophy

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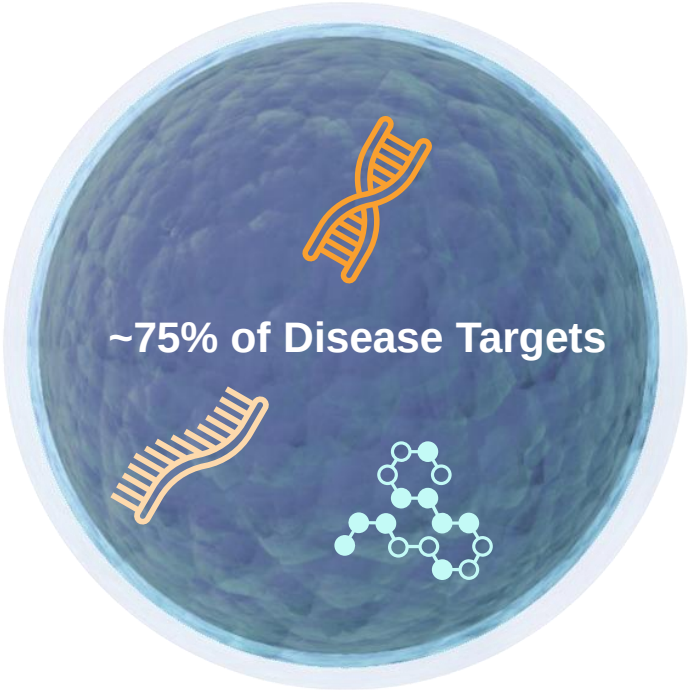
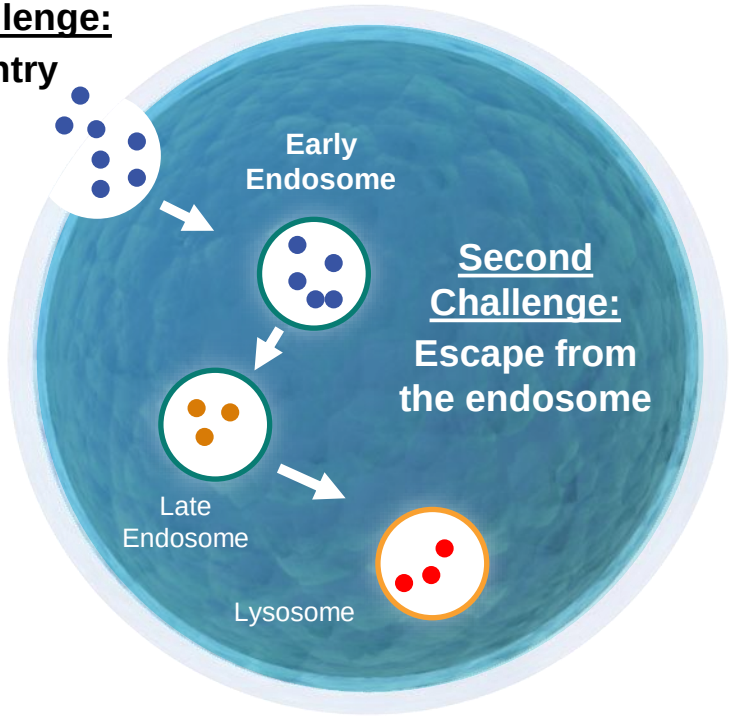
ENTRADA'S MISSION

Treating Devastating Diseases With Intracellular Therapeutics

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



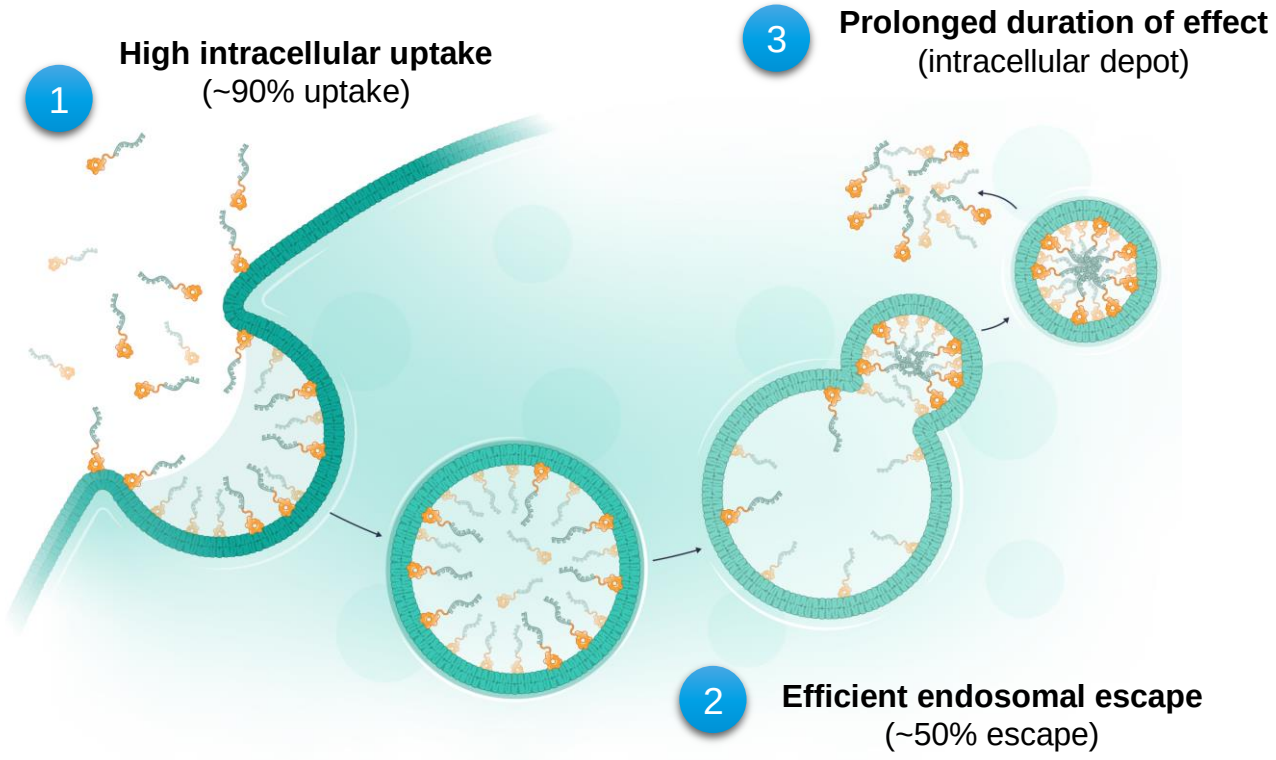


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

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

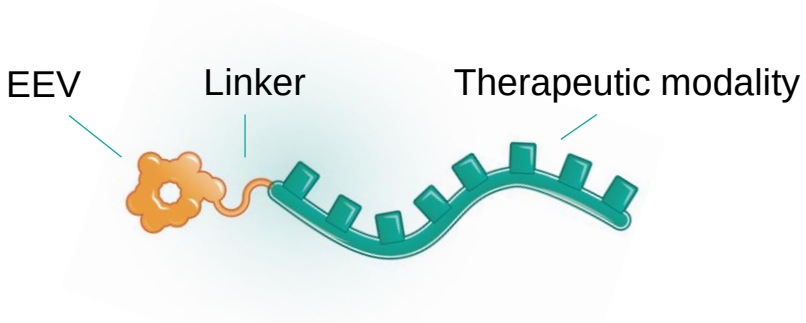
- Unique chemistry results in **improved uptake and endosomal escape**
- Cyclic structure designed to **extend half life and increase stability**
- Phospholipid binding potentially **enables broad biodistribution to all cells**
- Mechanism of **internalization conserved across species**



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.

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

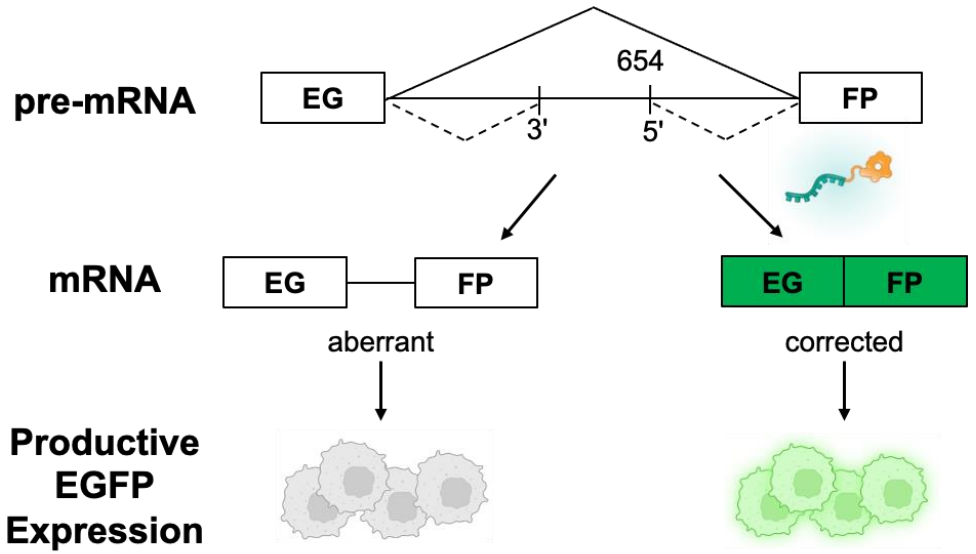
Fit-for-purpose EEV candidate for target indication

Identify EEV candidate with desired **therapeutic profile**

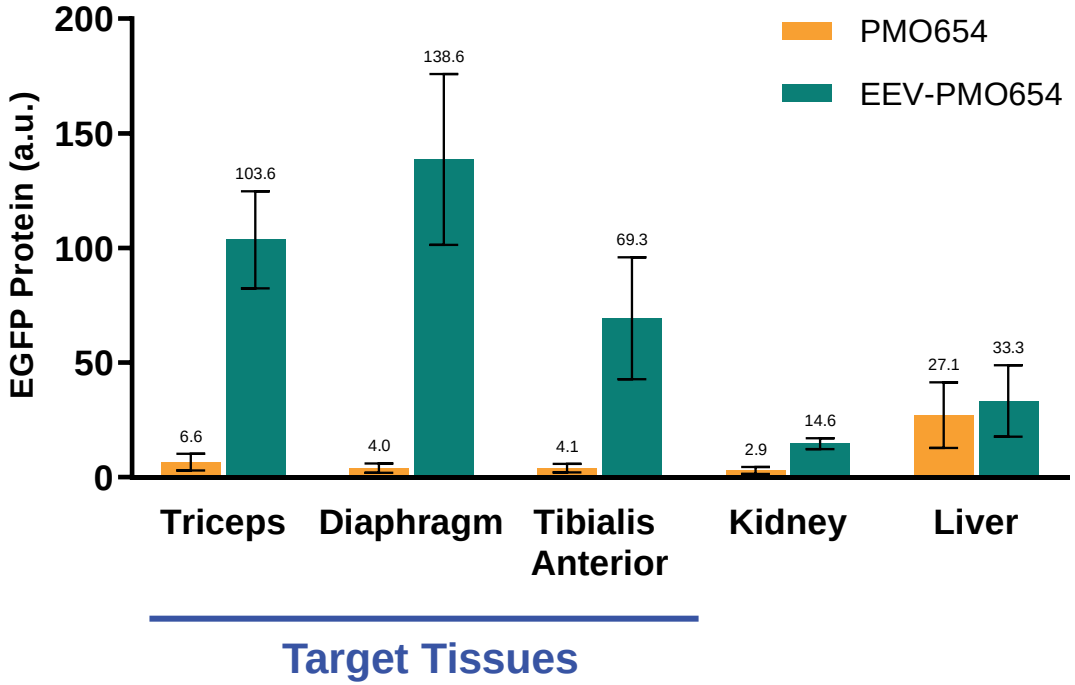
FUNCTIONAL DELIVERY FOR TARGET TISSUES

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

EGFP-654 Transgenic Mice



Functional Delivery to Target Tissues



PMO, phosphorodiamidate morpholino oligomer (Summerton, J. et al. *Antisense Nucleic Acid Drug Dev.* 1997); **EGFP-654** transgenic mouse model contains an EGFP gene interrupted by human beta-globin intron 2 with mutated nt654 (Sazani, P. et al. *Nature Biotech.* 2002); **PMO654**, splicing switching PMO targeting nt654; shown as mean ± standard deviation.

TRANSLATION FROM UPTAKE TO OUTCOMES

EEV-therapeutics possess favorable pharmacological properties: significant uptake in target tissues, efficient intracellular delivery and potent pharmacodynamic outcomes

Tissue Uptake in Muscle

- ✓ Skeletal muscle
- ✓ Cardiac muscle

+

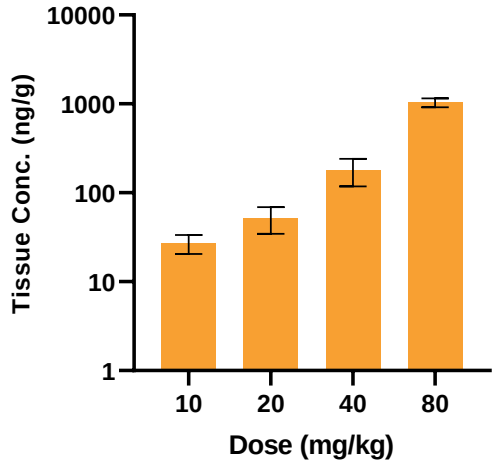
Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

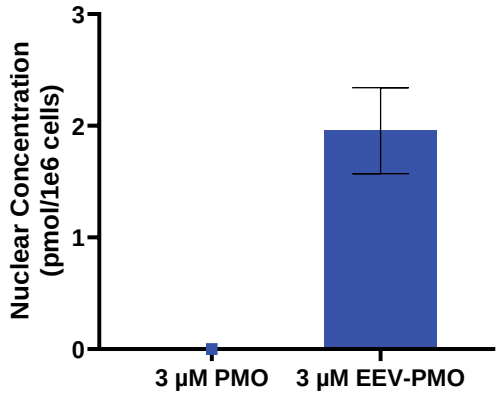
=

Pharmacodynamic Outcome

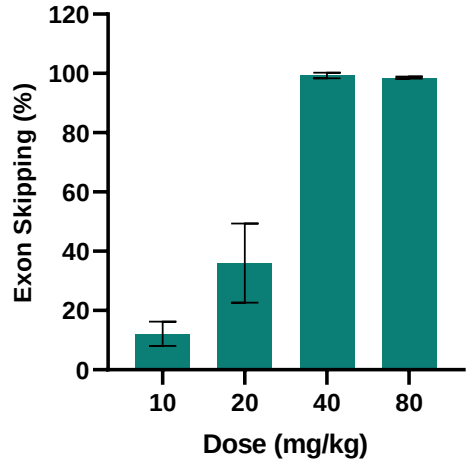
- ✓ Rapid, dose-dependent efficacy
- ✓ Duration of at least 12 weeks



IV, hDMD mice, 5-day p.i.



24-hour incubation



IV, hDMD mice, 5-day p.i.

hDMD mice express full-length human dystrophin gene. **p.i.** post injection; shown as mean ± standard deviation.

DUCHENNE MUSCULAR DYSTROPHY (DMD)

SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

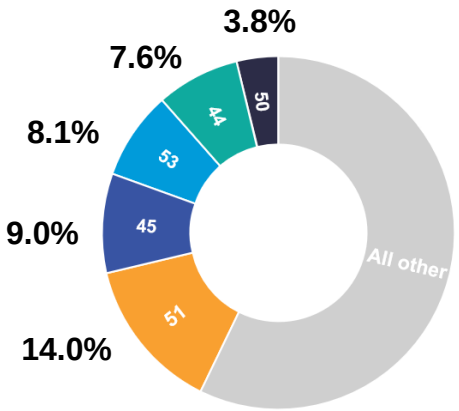
Duchenne is caused by **mutations in the DMD gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

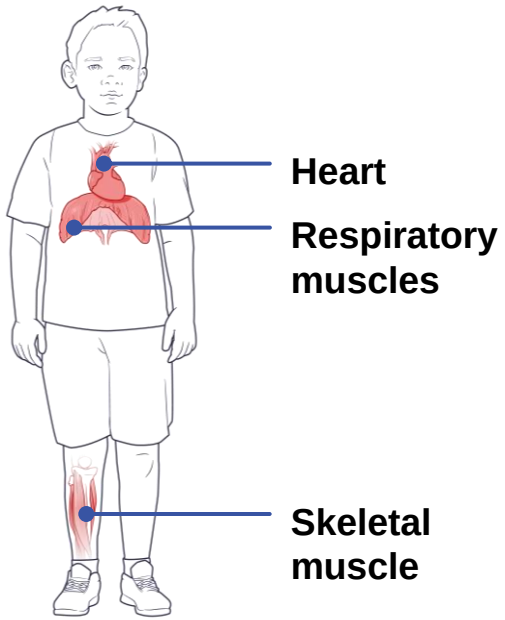
~30,000

people in the **U.S. and Europe** have Duchenne[1]

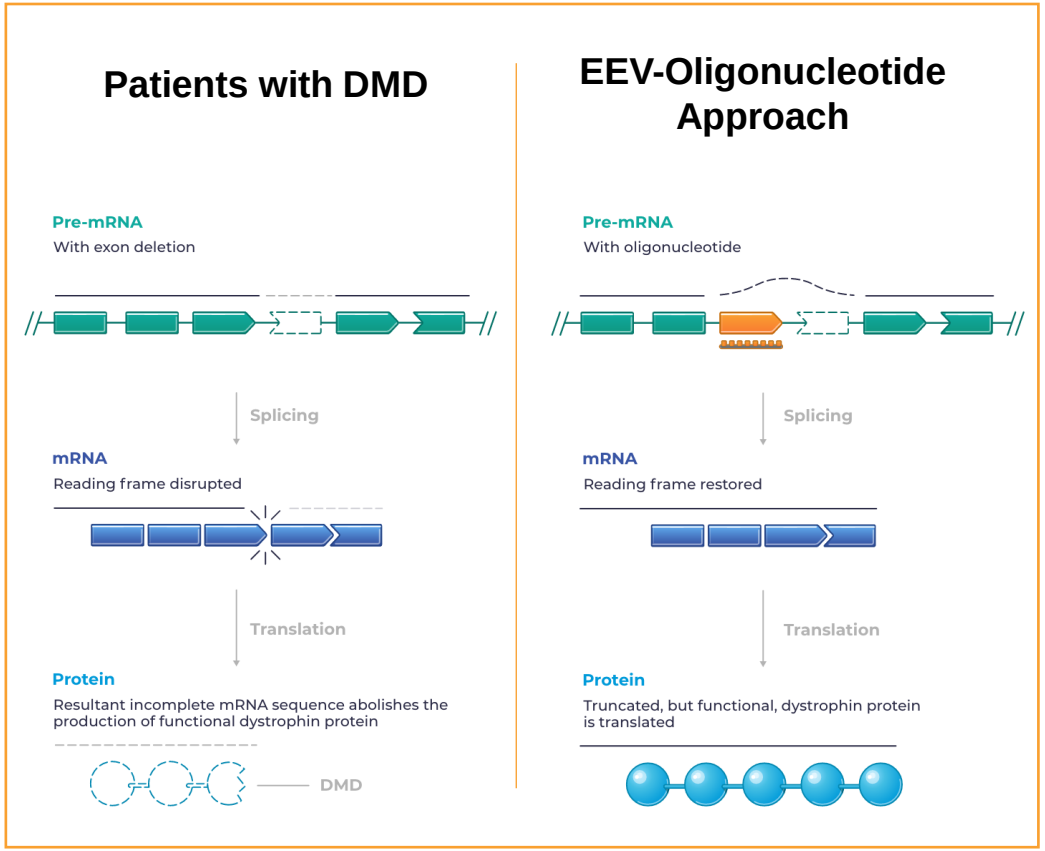
>40% of patients with Duchenne

have mutations amenable to exon skipping of exons 44, 45, 50, 51 and 53





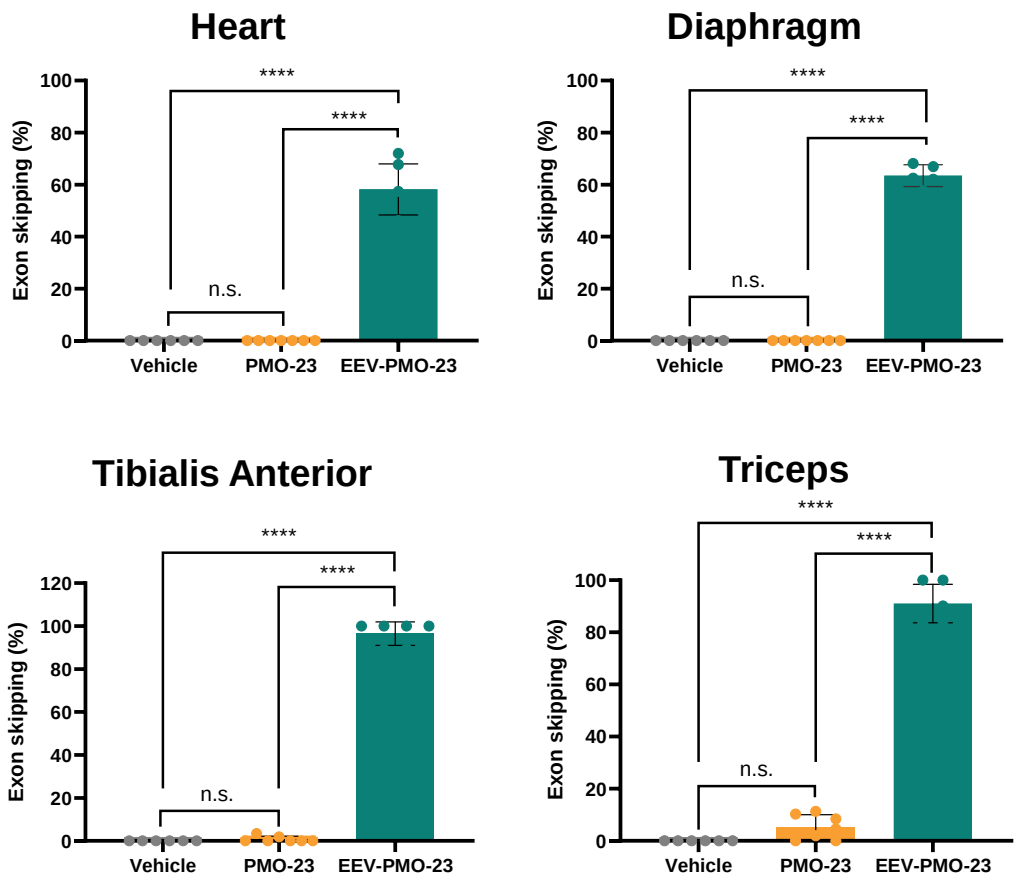
Exon skipping therapeutics have been approved based on **modest improvement in dystrophin levels ranging from ~1 to 6%**



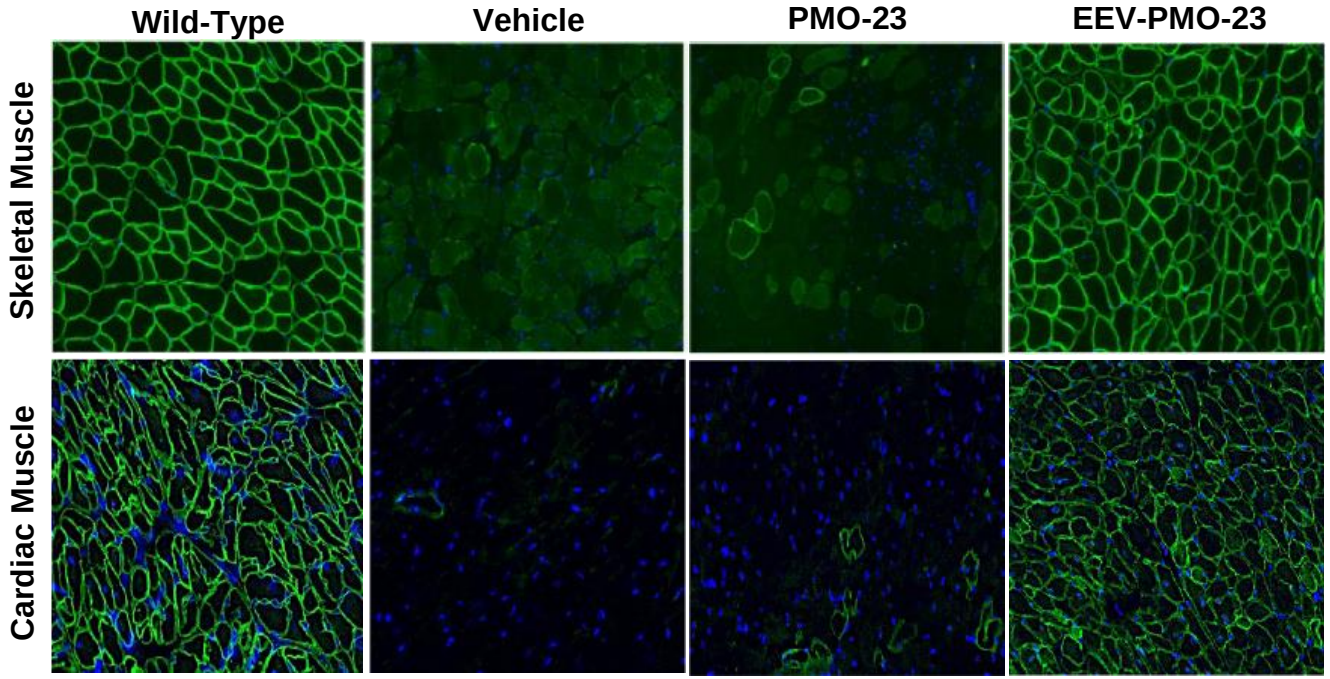
[1]Crispi V, Matsakas A. Duchenne muscular dystrophy: genome editing gives new hope for treatment. Postgraduate Medical Journal. 2018;94(1111):296-304. doi: 10.1136/postgradmedj-2017-135377.

REPEAT EEV-PMO TREATMENT IN 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



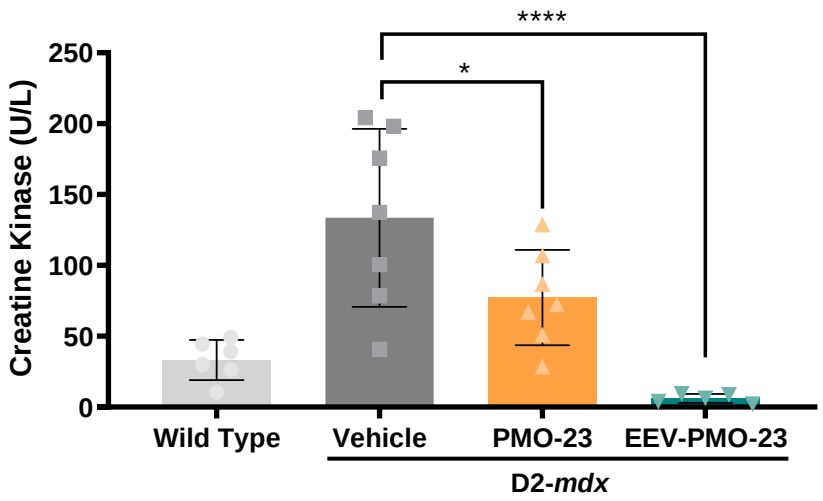
- D2-mdx mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg unconjugated PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose.

D2-*mdx* is a DMD mouse model with a nonsense mutation in *Dmd* exon 23 (Coley et al. Hum. Mol. Genet. 2016). **EEV**, Endosomal Escape Vehicle; **PMO-23**, mouse *Dmd* exon 23 skipping phosphorodiamidate morpholino oligomer. ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

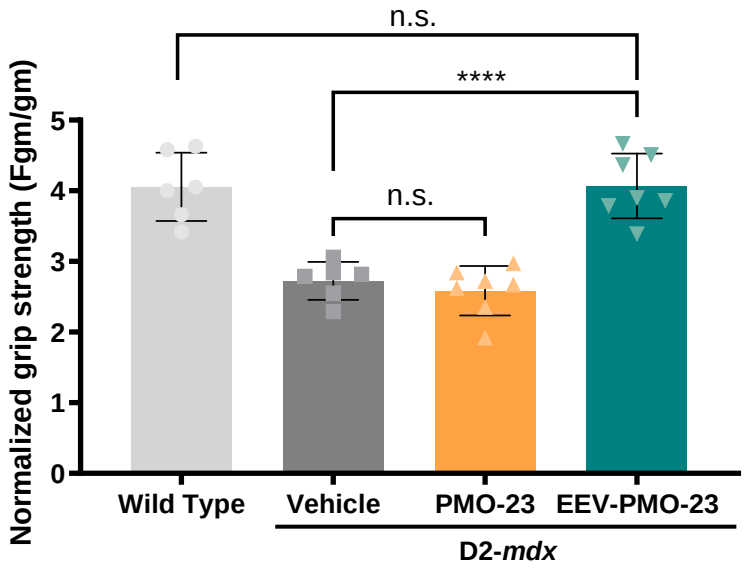
REPEAT EEV-PMO TREATMENT RESULTS IN CK CORRECTION AND FUNCTIONAL IMPROVEMENT

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

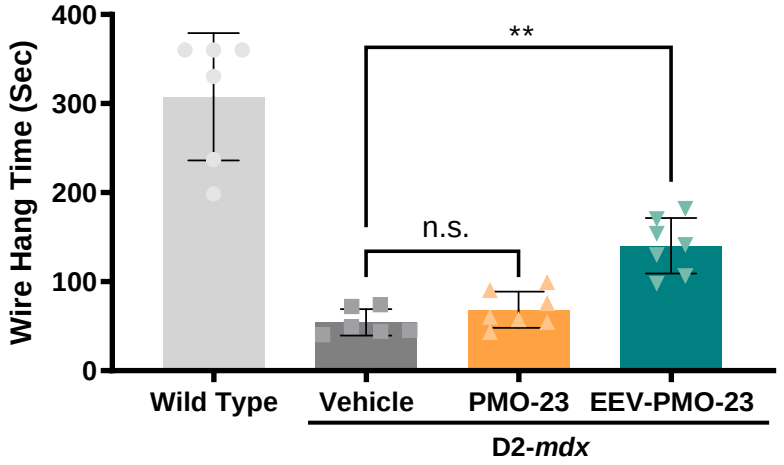
Serum CK Levels (Muscle Damage)



Grip Strength



Wire Hang Time



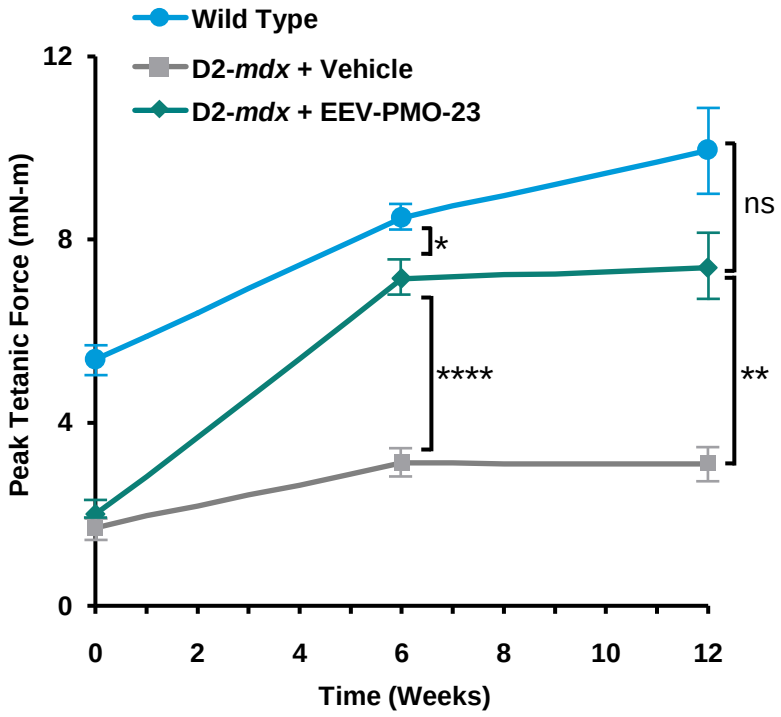
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~2 weeks after the last dose

D2-*mdx* is a DMD mouse model on DBA/2J background and better recapitulate disease pathology (Fukada et al. *Am. J. Path.* 2010). **CK**, creatine kinase; *p<0.05, **p<0.01, ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

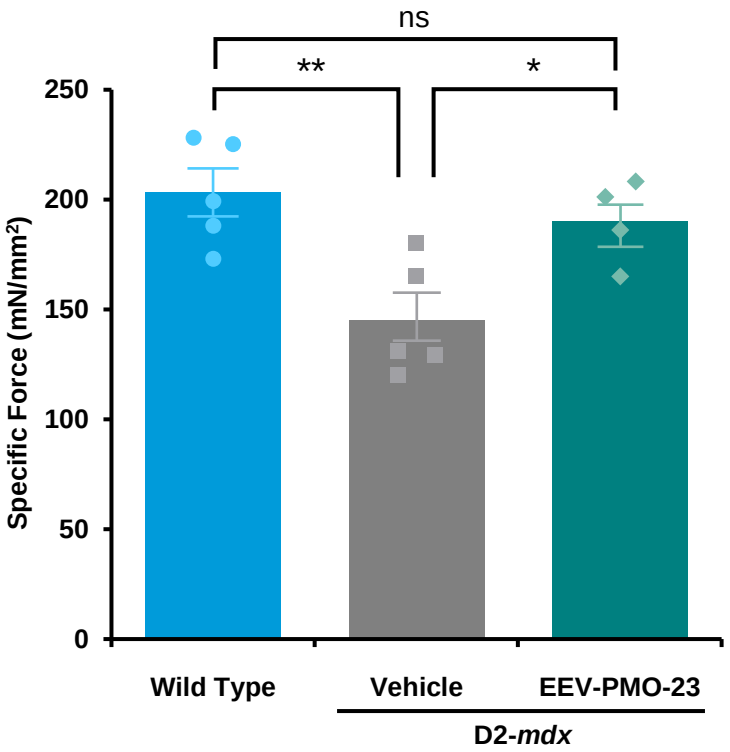
REPEAT EEV-PMO TREATMENT RESULTS IN IMPROVED MUSCLE CONTRACTILITY

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

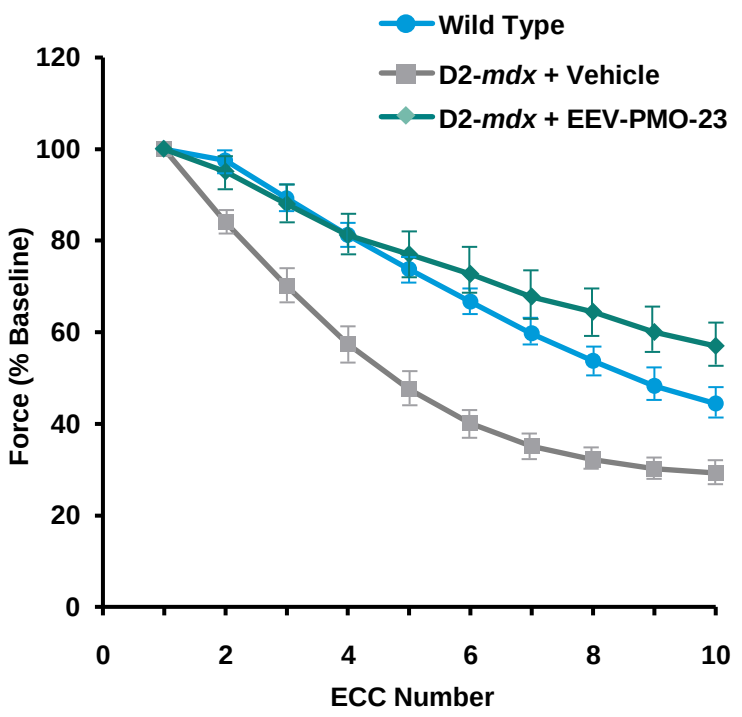
Longitudinal Tetanic Force



Week 12 Specific Force



Repeated Muscle Contraction

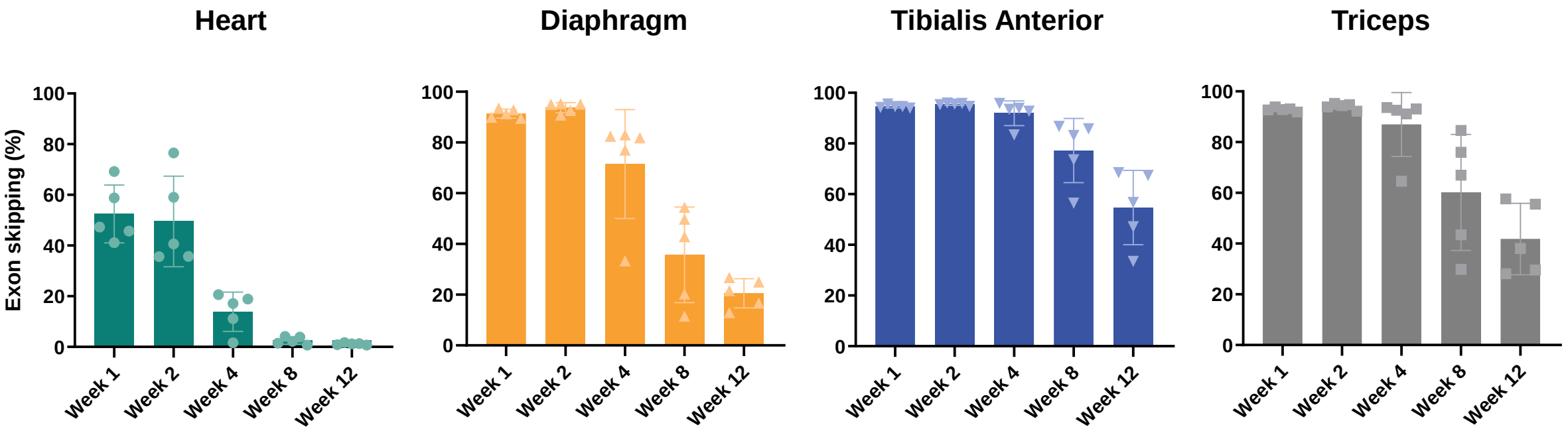


D2-*mdx* mice were treated with vehicle or 80 mg/kg (QW) EEV-PMO-23. Muscle contractility was assessed at Weeks 6 and 12 via isometric force generated by tetanic contraction of plantar flexor muscle group and eccentric force (ECC) generated by repeated tetanic contraction of TA muscle. *p<0.05, **p<0.01, ****p<0.0001; **n.s.**, not significant; shown as mean ± SEM.

ENTR-601-44

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



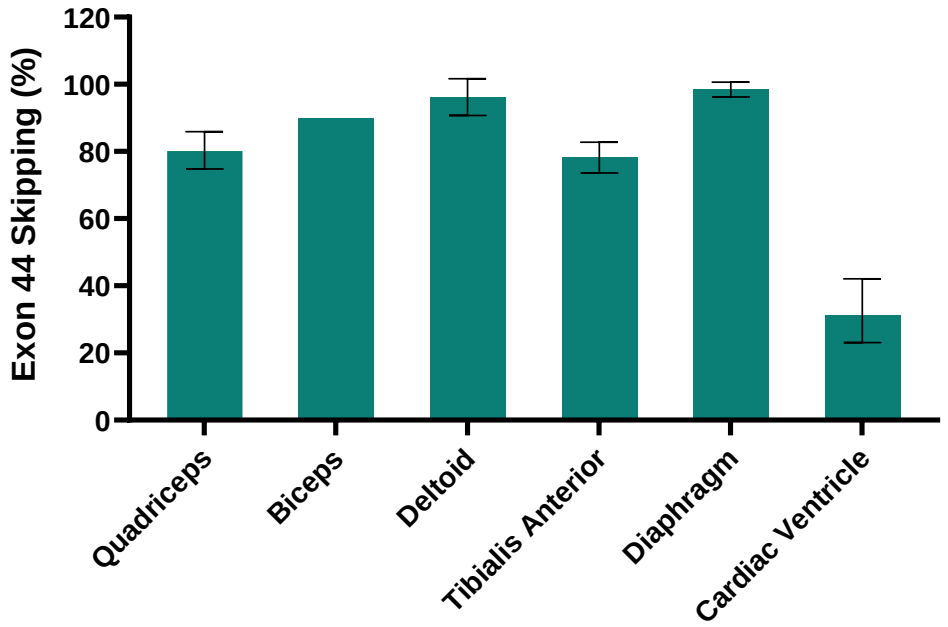
- Post single IV dosage of 60 mg/kg (PMO equivalent), robust exon skipping was observed in ENTR-601-44 treated hDMD mice

hDMD transgenic mice express full-length human dystrophin gene which allows for preclinical testing of human sequence-specific PMO for DMD transcript correction ('t Hoen et al. *J. Biol. Chem.* 2008); shown as mean ± standard deviation.

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

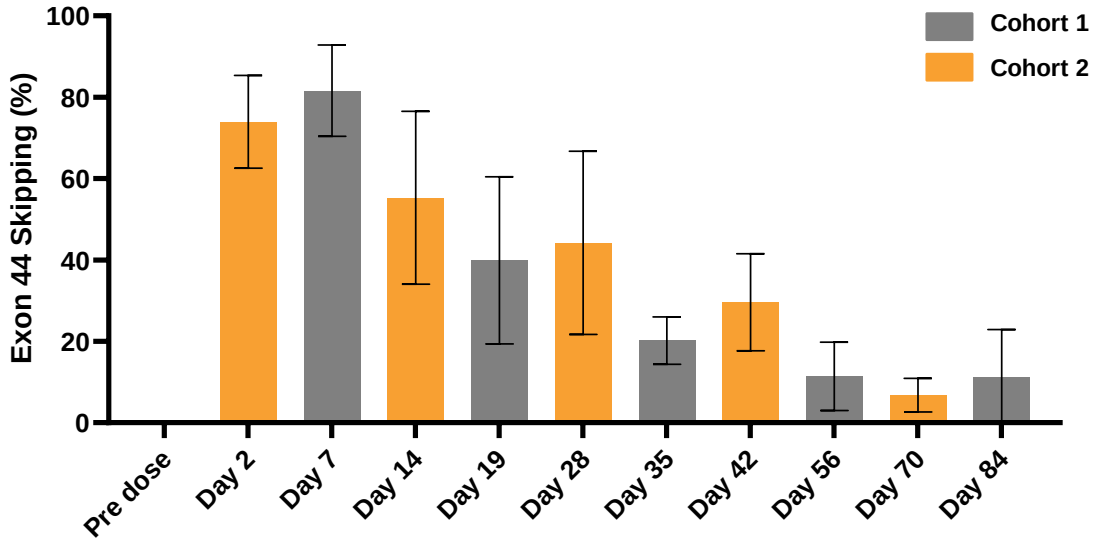
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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

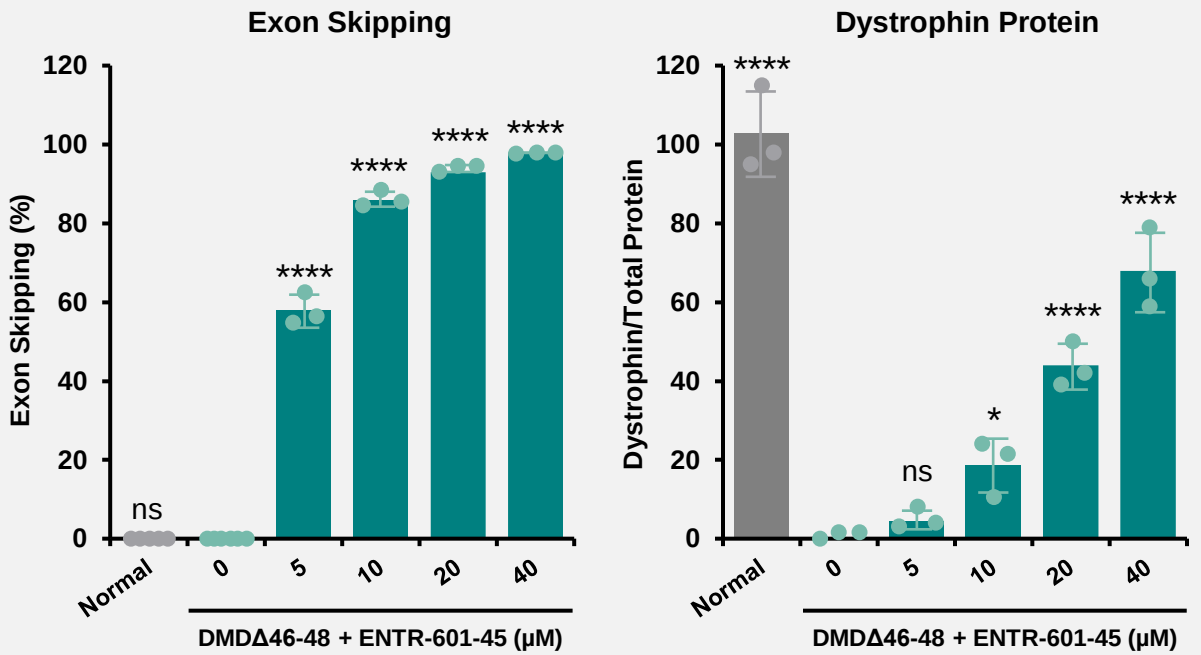
ENTR-601-44 is a DMD exon 44 skipping PMO conjugated to the EEV platform. **NHP**, non-human primates; shown as mean ± standard deviation.

ENTR-601-45

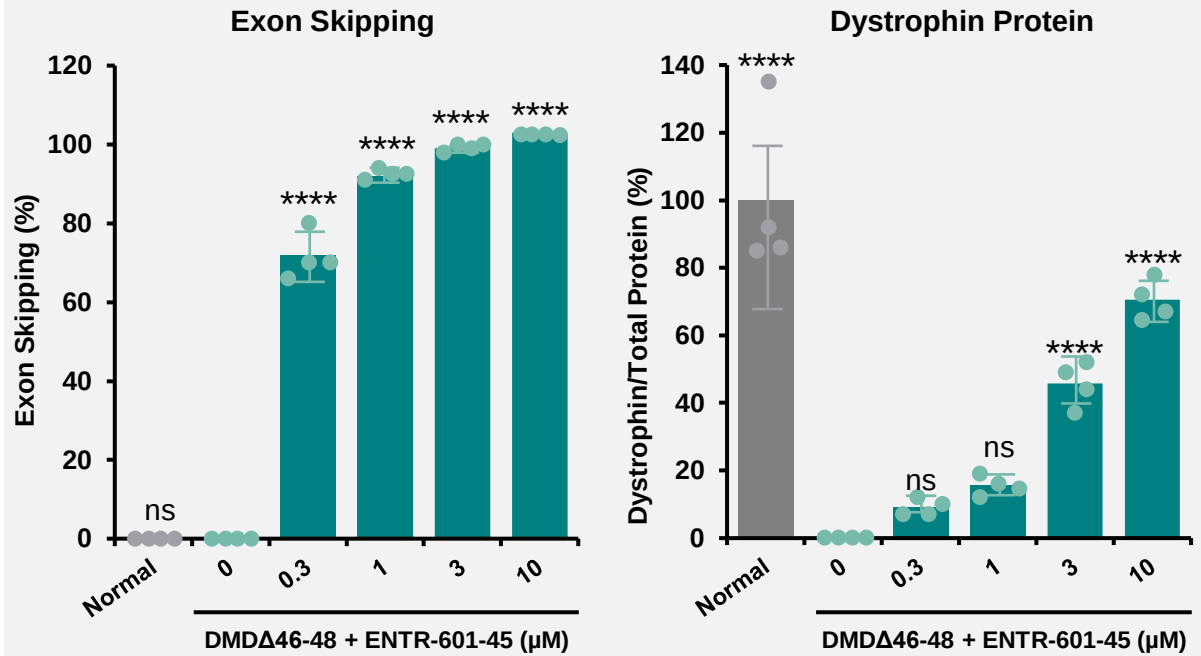
ENTR-601-45 IN VITRO EFFICACY

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

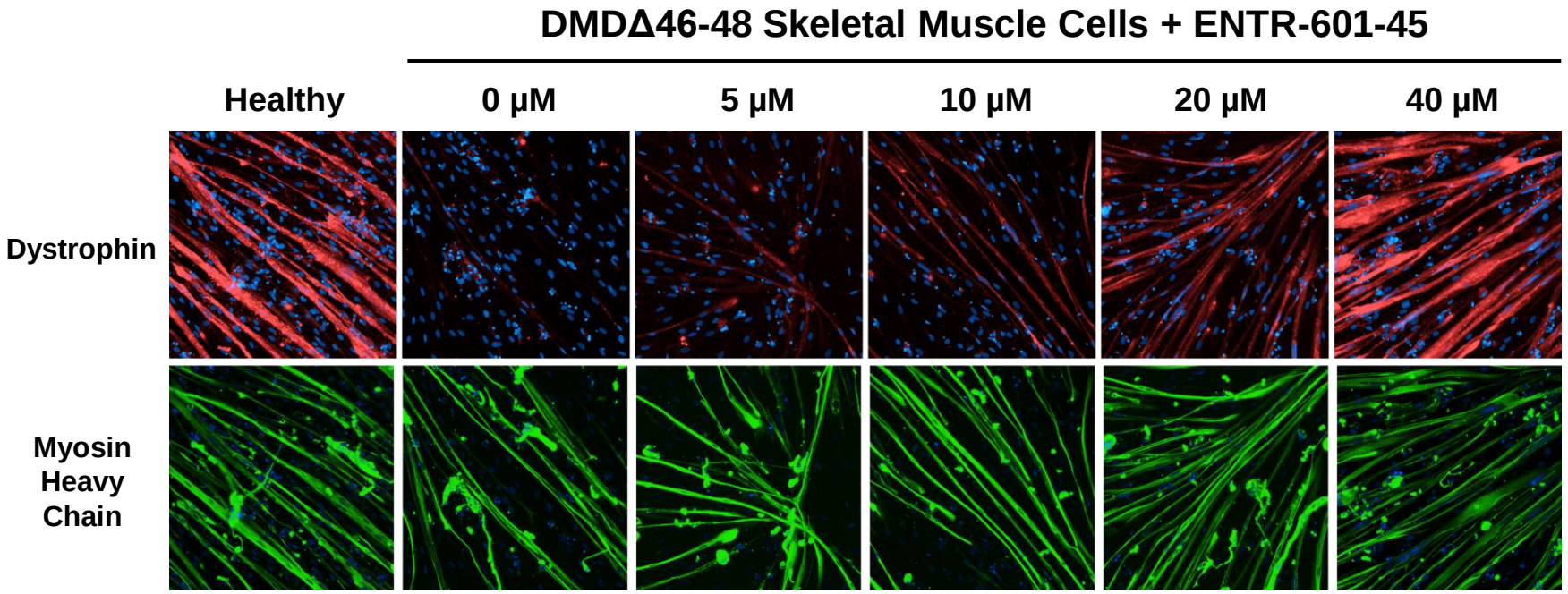


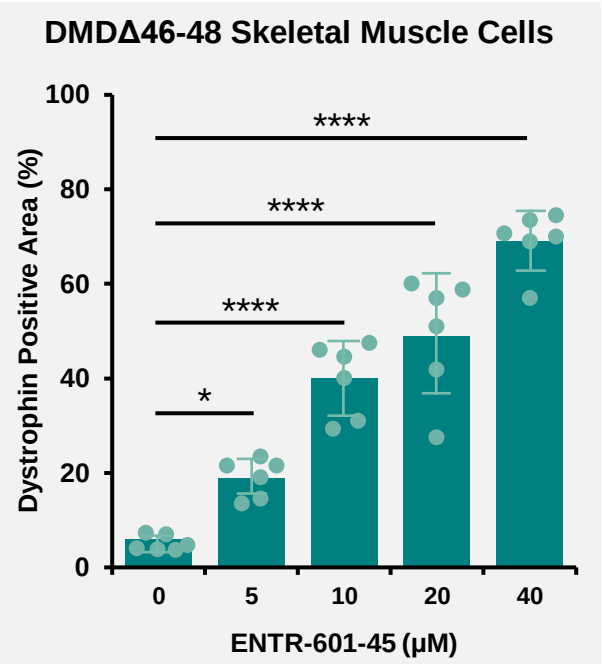
- DMD patient-derived skeletal and cardiac muscle cells (DMDΔ46-48) were treated with ENTR-601-45 for 24 hours

DMDΔ46-48 iPSC-derived skeletal and cardiac muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation. Data are shown as mean ± SD (n = 3 skeletal muscle; n = 4 cardiac muscle); one-way ANOVA; *p<0.05, ****p<0.0001; relative to untreated DMDΔ46-48 cells.

ENTR-601-45 IN SKELETAL MUSCLE CELLS

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



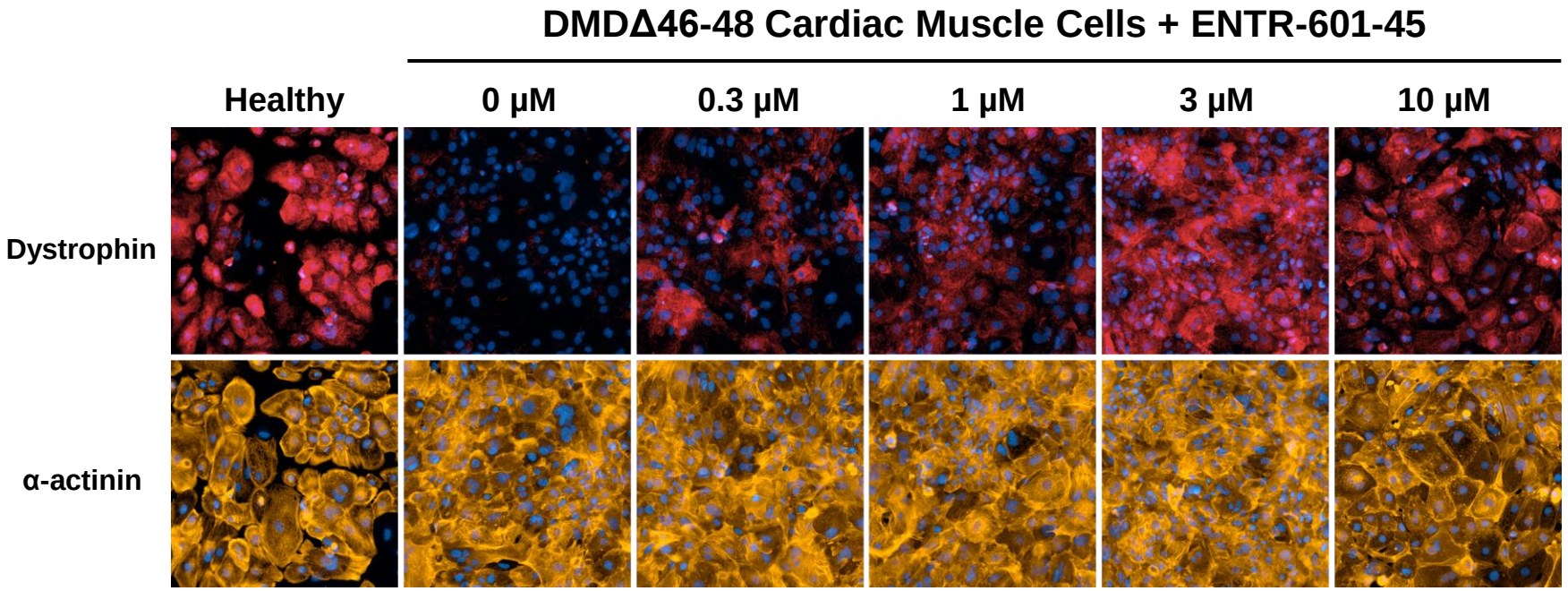


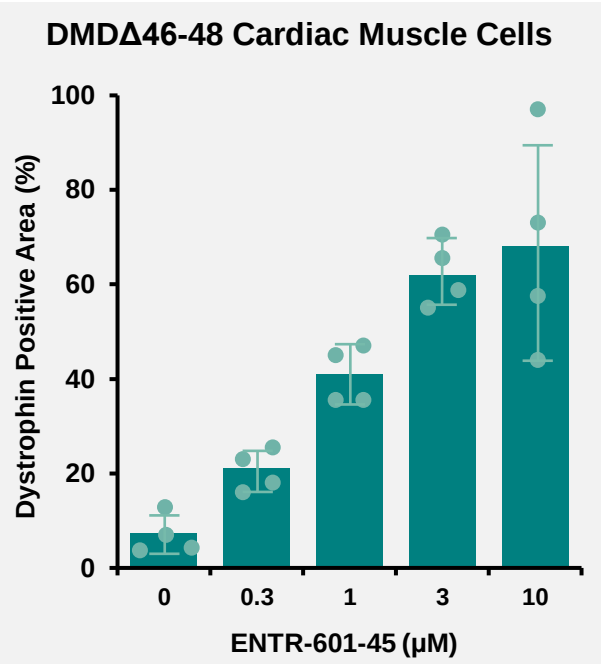
- DMD patient-derived skeletal muscle cells (DMDΔ46-48, n=6) were treated with ENTR-601-45 for 24 hours and analyzed 5 days later.

DMDΔ46-48 iPSC-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. Data are shown as mean ± SD; one-way ANOVA and Dunnett's multiple comparison test *p<0.05, ****p<0.0001; relative to untreated DMDΔ46-48 cells.

ENTR-601-45 IN CARDIAC MUSCLE CELLS

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



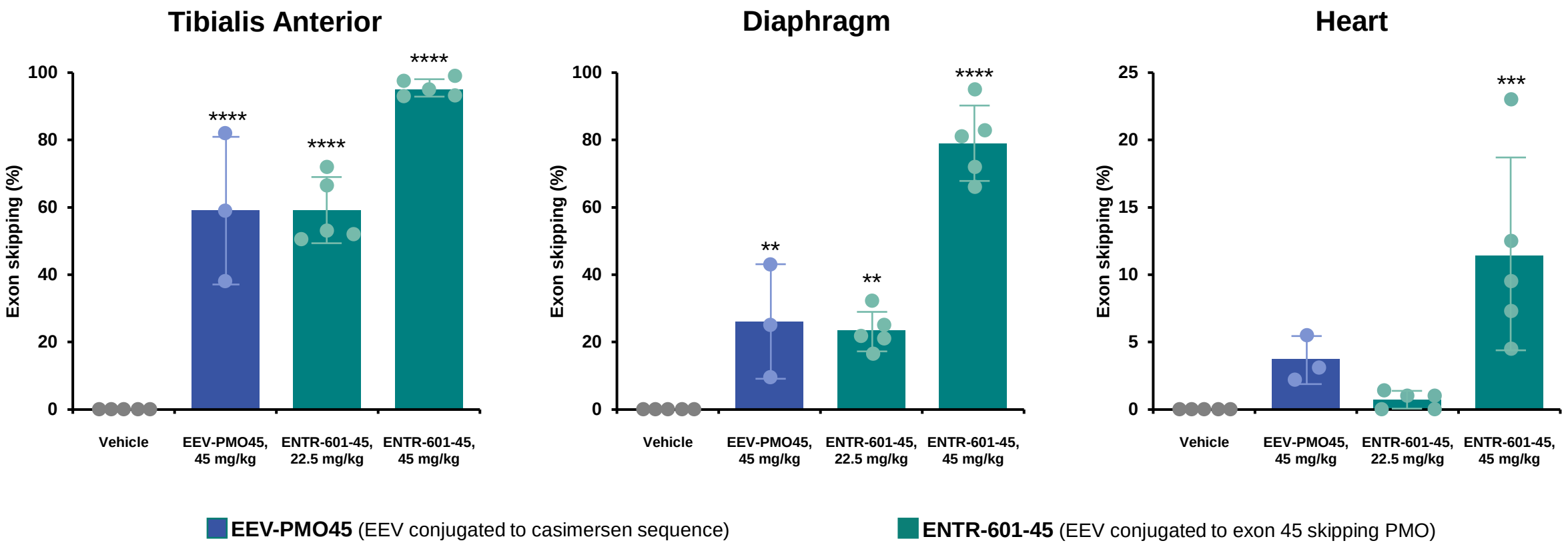


- DMD patient-derived cardiac muscle cells (DMDΔ46-48, n=4) were treated with ENTR-601-45 for 24 hours and analyzed 48 hours later.

DMDΔ46-48 iPSC-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. Data are shown as mean ± SD.

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



hDMD transgenic mice express full-length human dystrophin gene which allows for preclinical testing of human sequence-specific PMO for DMD transcript correction ('t Hoen et al. *J. Biol. Chem.* 2008). Casimersen is an exon 45 skipping PMO approved in the US. Data are shown as mean ± SD (n = 3-5); one-way ANOVA; **p<0.01, ***p<0.001, ****p<0.0001; relative to vehicle; Concentrations provided are PMO equivalent.

ENTRADA'S DMD PIPELINE

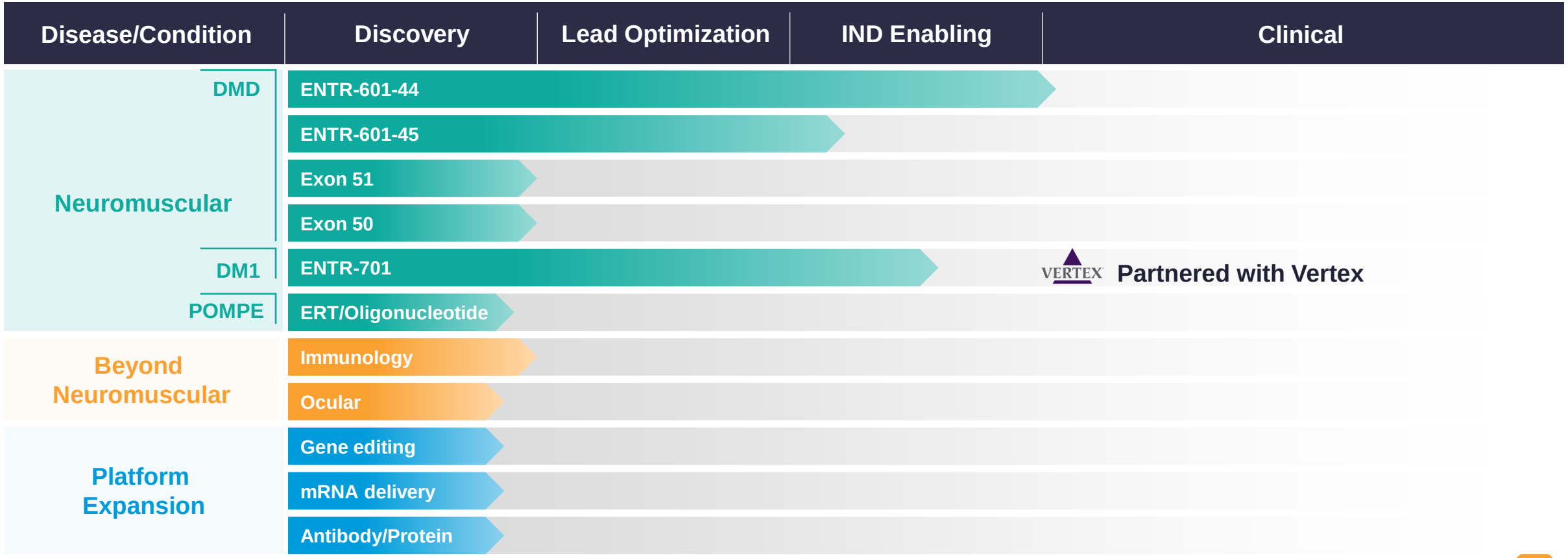
Entrada is advancing new therapeutic options for people living with exon 44 and exon 45 skip amenable DMD

- **ENTR-601-44 produced robust exon skipping and dystrophin production in several preclinical models of DMD**
  - Durable dystrophin production over 12+ weeks from a single injection of ENTR-601-44 was observed in the NHP
  - 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 Agency.
- **ENTR-601-45 IND filing is planned for H2 2024**
  - ENTR-601-45 showed 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

PIPELINE & DISCOVERY PROGRAMS

OUR DIFFERENTIATED AND EXPANDING PIPELINE

Entrada’s pipeline includes a diverse array of high potential and high value assets



ADDITIONAL PLATFORM OPPORTUNITIES

Entrada continues to invest in and build upon our EEV Platform to extend our efforts in developing novel EEV-therapeutic candidates

Target	Platform Approach	Goal
DNA	Gene editing	Deliver CRISPR enzyme and repair gene function with guide RNA
RNA	RNA editing	Deliver oligonucleotide therapeutics for RNA editing
	RNA splicing	Modify RNA via exon/intron splicing to activate protein expression
	RNA blocking	Block trinucleotide repeats in RNA to inhibit adverse binding
	RNA silencing	Silence or knockdown RNA to prevent protein expression
Protein	Protein replacement	Replace proteins and enzymes
	Protein inhibition	Inhibit protein signaling pathways
	Protein degradation	Degrade disease-causing proteins

ACKNOWLEDGEMENTS

Thank you!

WANT TO LEARN MORE?

Scan the QR code for access to our recent publications and conference presentations.