The Endosomal Escape Vehicle Platform Enhances the Delivery of Oligonucleotides to Skeletal and Cardiac Muscle

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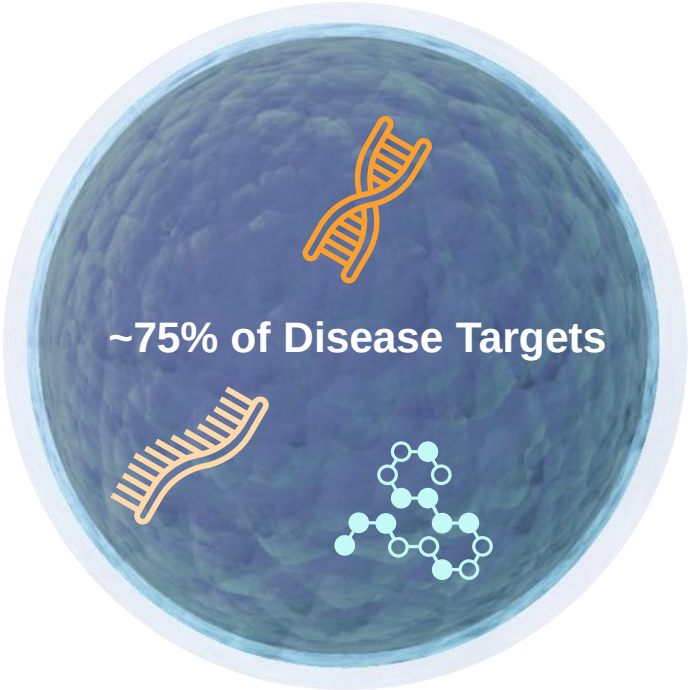
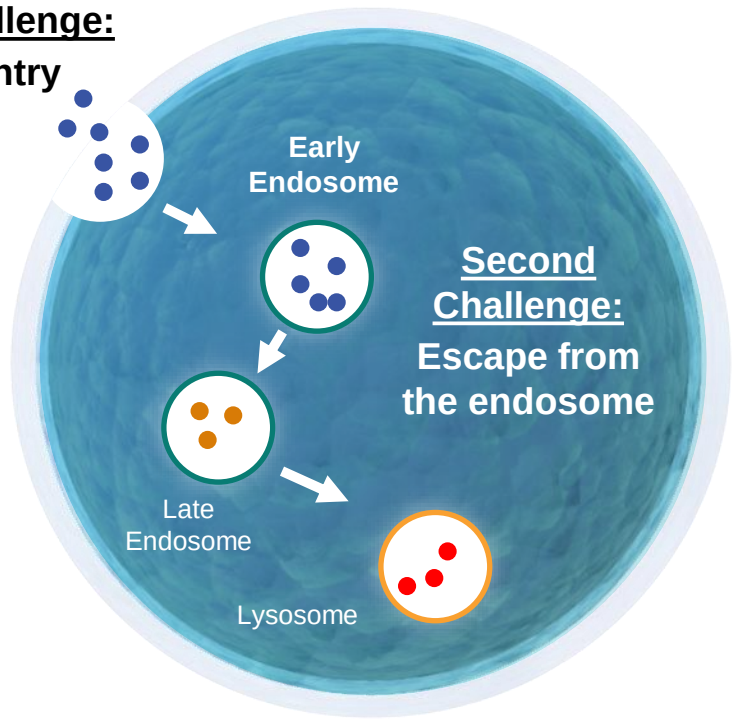
ENTRADA'S MISSION

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





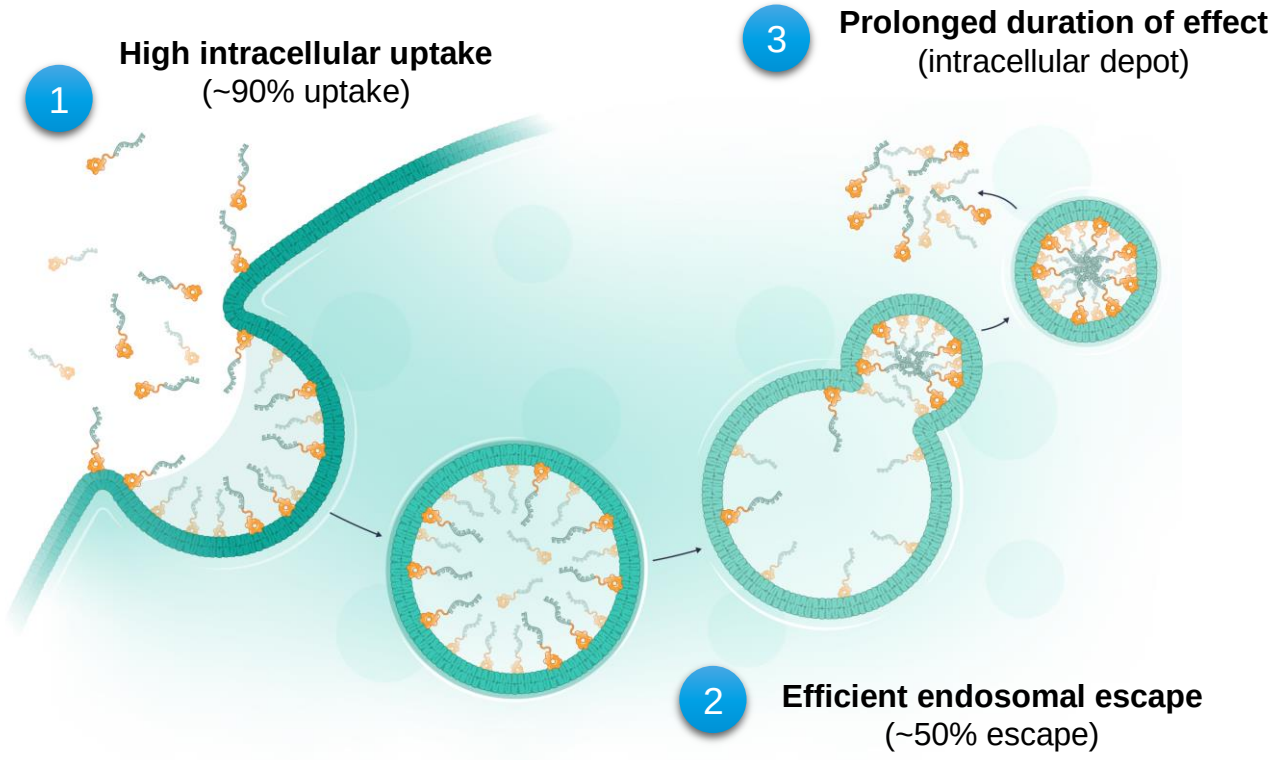
The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

EEV PLATFORM

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**

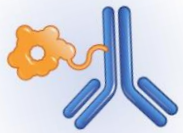


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

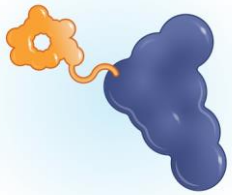
A BROADLY APPLICABLE PLATFORM

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

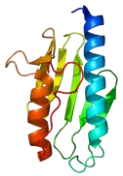
Antibodies



Enzymes

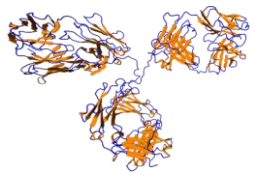


Oligonucleotides



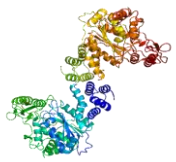
550-600 KDa

Hybrid frataxin



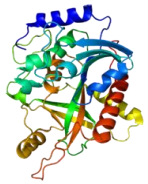
150 KDa

Antibody



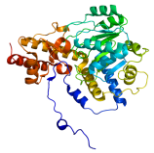
98 KDa

Thymidine phosphorylase



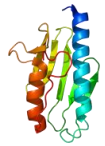
96 KDa

Purine nucleoside phosphorylase



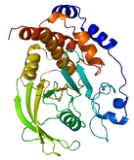
86 KDa

Alanine-glyoxylate aminotransferase



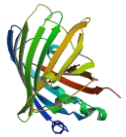
46 KDa

Human frataxin



37 KDa

PTP1B catalytic domain



32 KDa

EGFP

16 KDa

Nanobody

6 KDa

Oligonucleotide

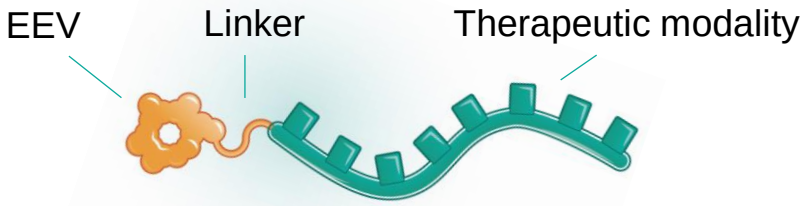
1-3 KDa

Various peptide cargos

FUNCTIONAL DELIVERY FOR TARGET TISSUES

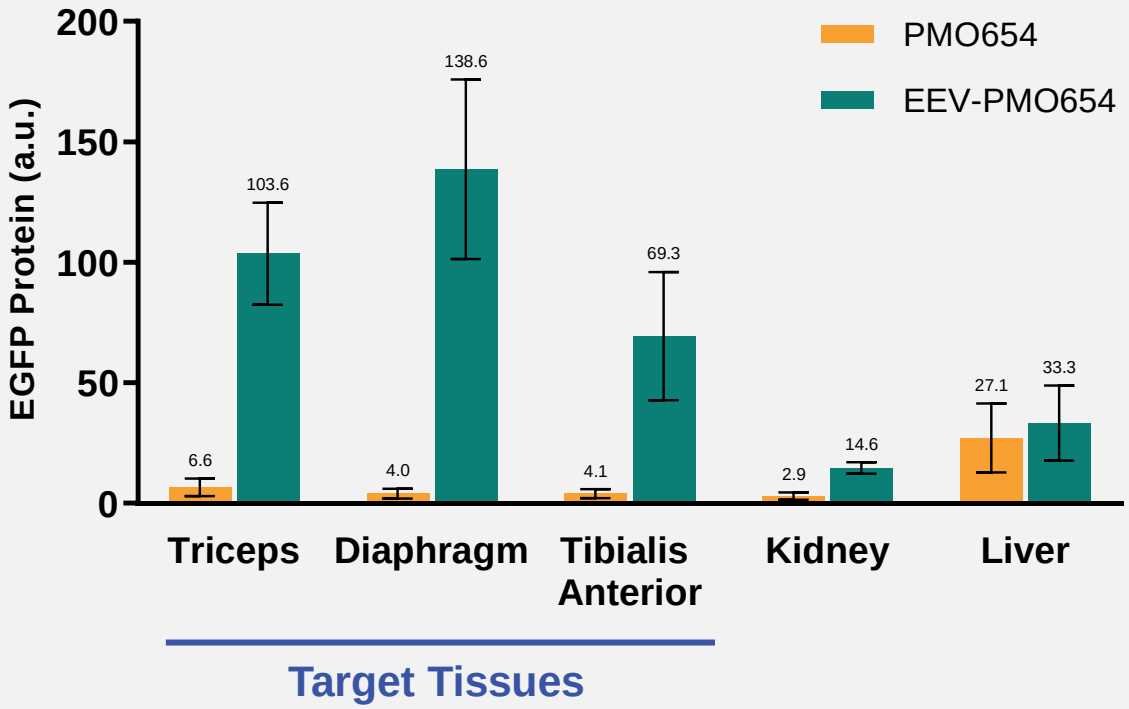
EEV-therapeutic candidates can be designed to enhance functional delivery to target tissues

Discovery Engine for Intracellular Therapeutics



- High-throughput **EEV library screening** *in vitro*
- Functional validation of lead EEVs with **PMO therapeutic modality** *in vitro* and *in vivo*
- **EEV** and **linker** optimized for the functional delivery to target tissues *in vivo*

Functional Delivery in the EGFP-654 Transgenic Mice[1]



PMO, phosphorodiamidate morpholino oligomer; 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. [1]Li X. et al. *Mol Therapy-Nucl Acids*. 2023.

TRANSLATION FROM UPTAKE TO OUTCOMES

Murine Example

EEV-therapeutic candidates have demonstrated favorable pharmacological properties: efficient intracellular delivery, significant uptake in target tissues and potent pharmacodynamic outcomes

Tissue Uptake in Muscle

- ✓ Skeletal muscle
- ✓ Cardiac muscle

+

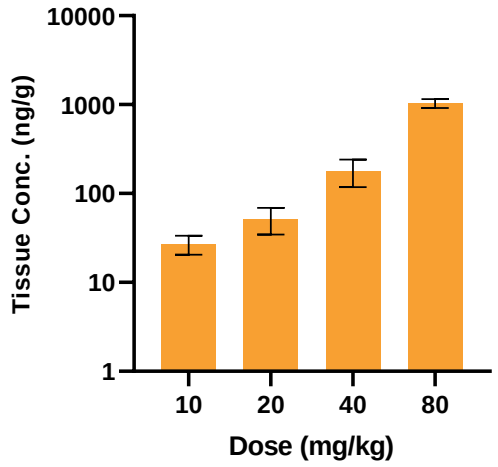
Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

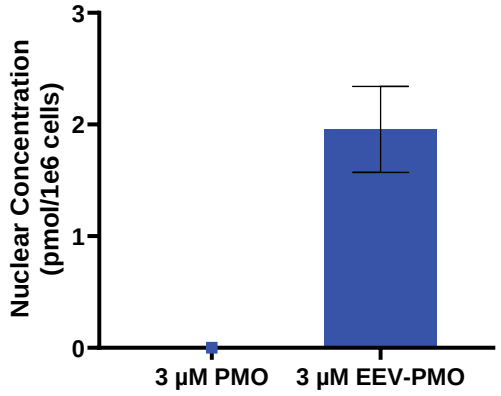
=

Pharmacodynamic Outcome

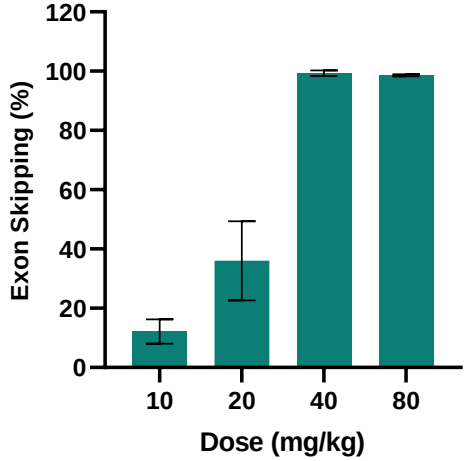
- ✓ Rapid, dose-dependent response
- ✓ Duration of at least 12 weeks



IV, hDMD mice, 5-day post injection



24-hour incubation

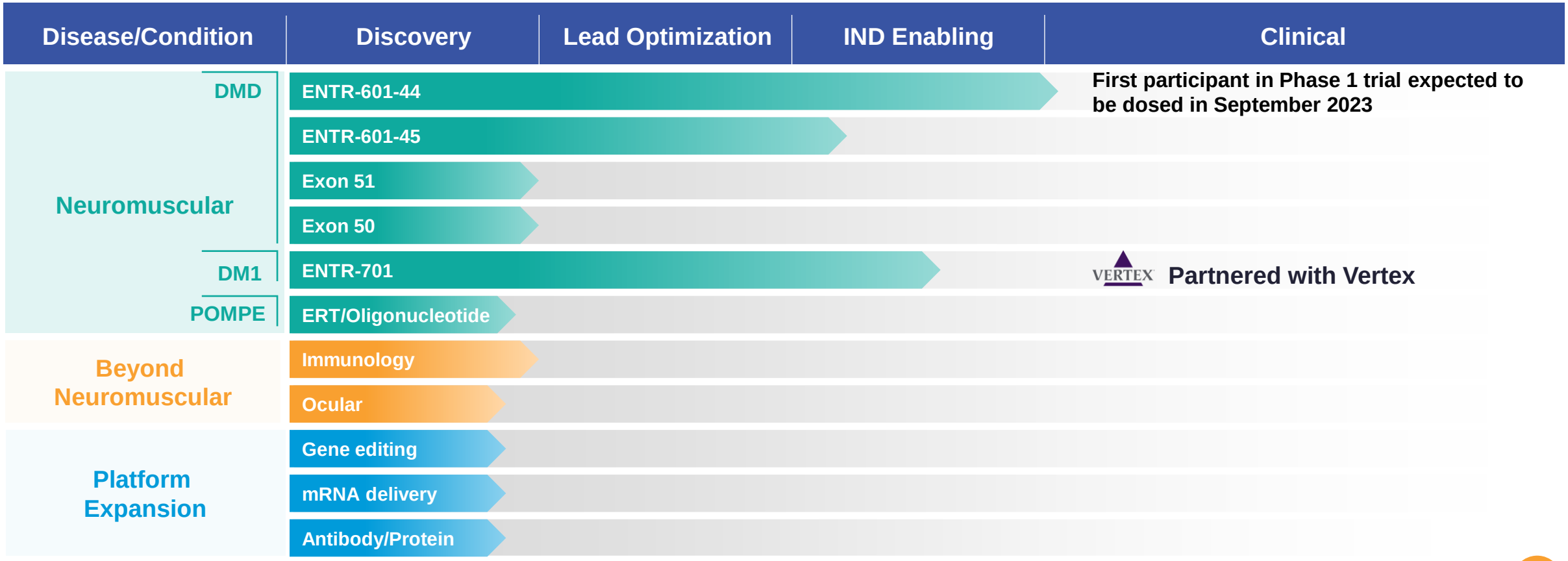


IV, hDMD mice, 5-day post injection

hDMD mice express full-length human dystrophin gene.

OUR DIFFERENTIATED AND EXPANDING PIPELINE

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



DUCHENNE MUSCULAR DYSTROPHY

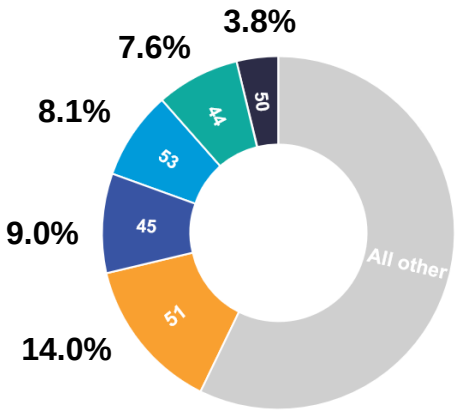
SIGNIFICANT THERAPEUTIC NEED EXISTS FOR THE TREATMENT OF DMD

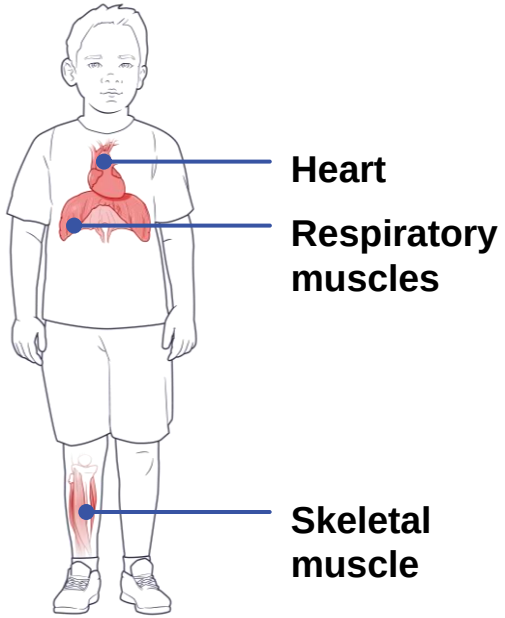
Duchenne is caused by **mutations in the *DMD* gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

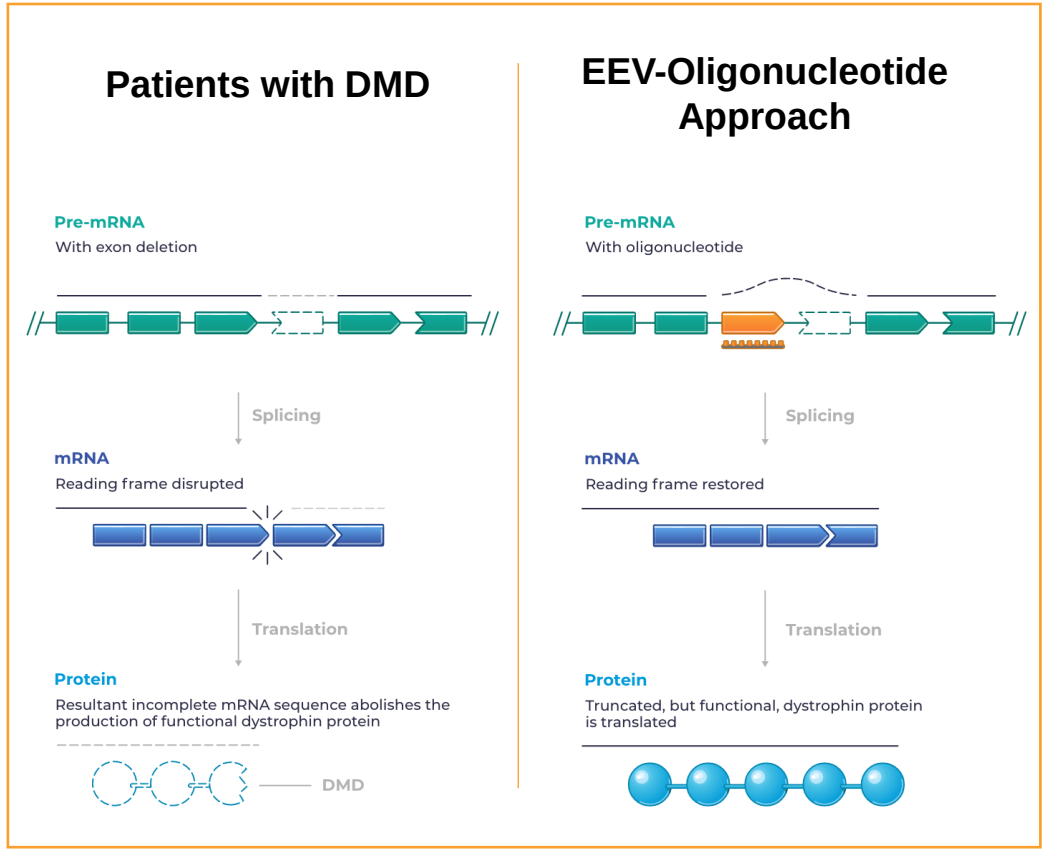
Exon skipping therapeutics have been approved based on **modest improvement in dystrophin levels ranging from ~1 to 6%**

~15,000 people in the US and **~26,000** people in Europe **have Duchenne muscular dystrophy[1,2]**

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



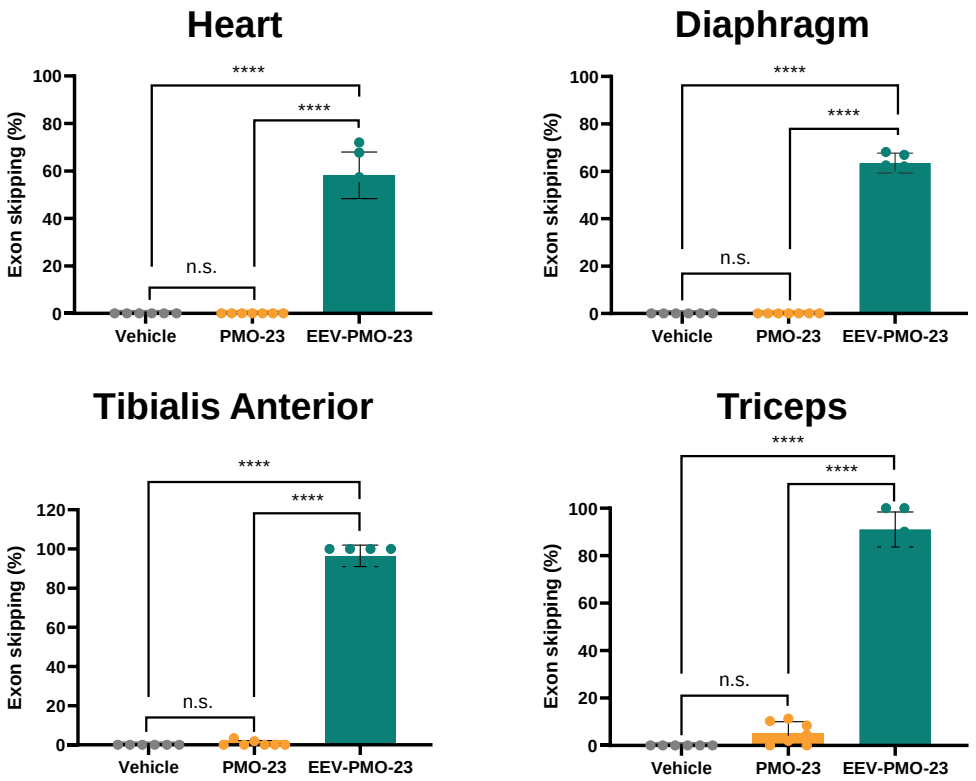




[1]Parent Project Muscular Dystrophy: About Duchenne. [2]Europeans Medicines Agency: Orphan designation for the treatment of Duchenne muscular dystrophy. [3]Bladen, C.L. et al *HUMAN MUTATION*, 2015.

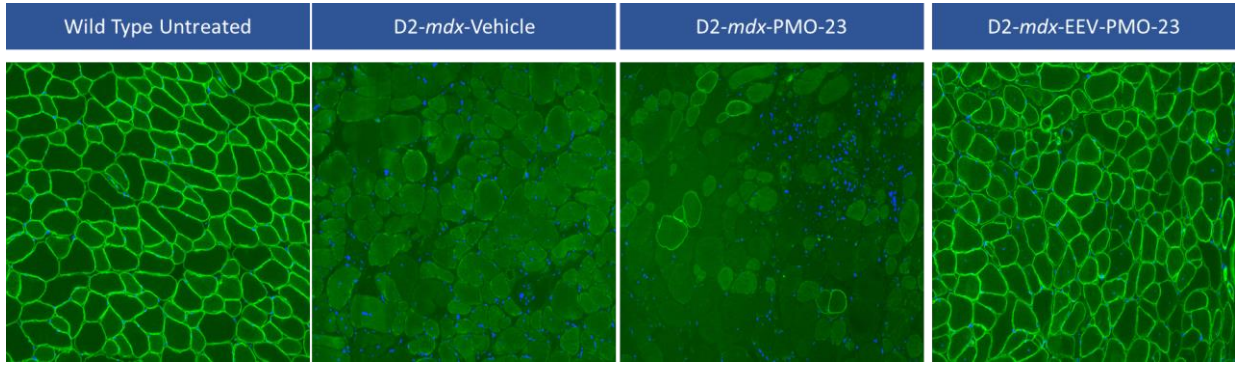
REPEAT EEV-PMO TREATMENT RESTORES MUSCLE INTEGRITY IN D2-*mdx* MICE

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

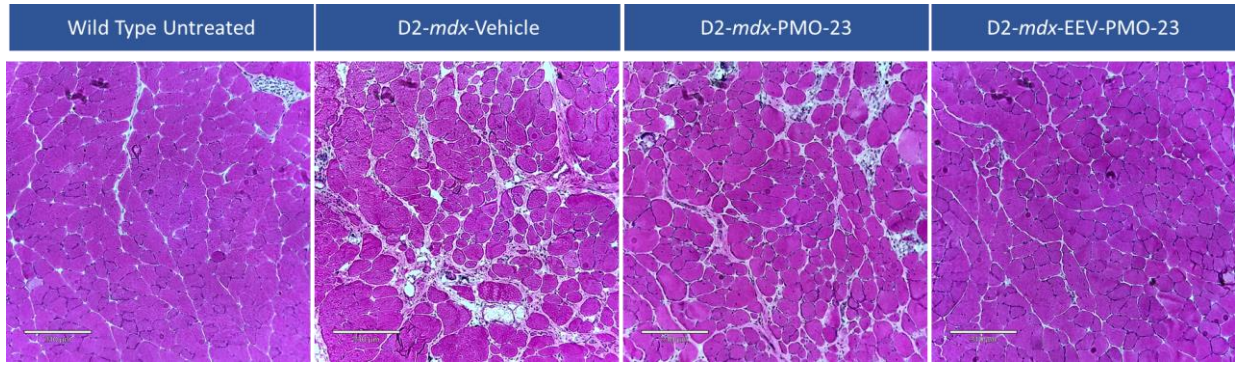


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)

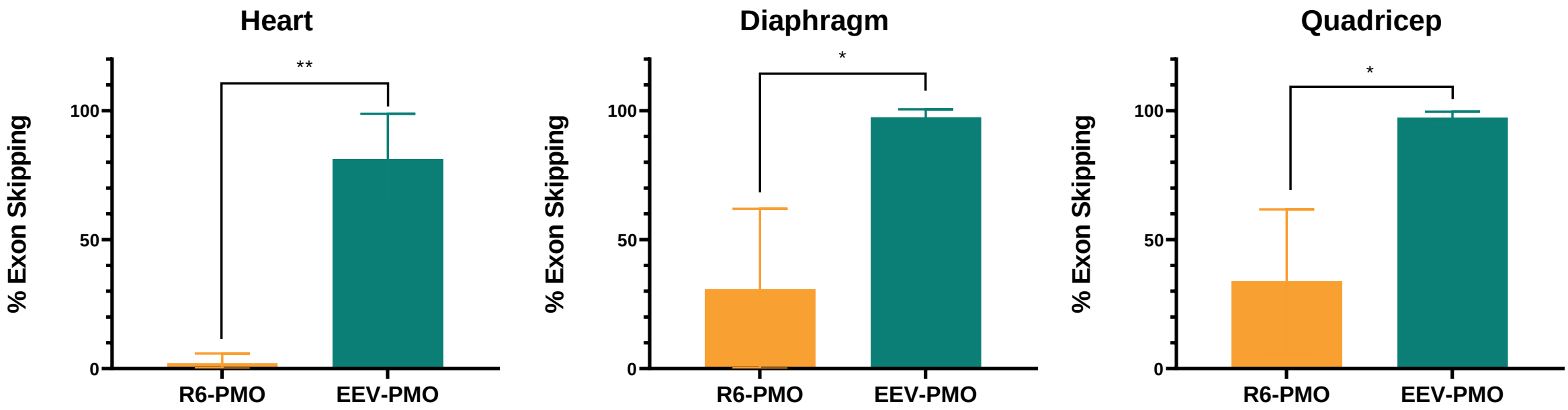


- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose

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 (Coley et al. *Hum. Mol. Genet.* 2016); ****p<0.0001; n.s., not significant; shown as mean ± standard deviation.

COMPARISON TO ALTERNATIVE R6-PMO

EEV-PMO significantly improved exon 23 skipping after 3 days in *mdx* mice as compared to competitive R6-PMO



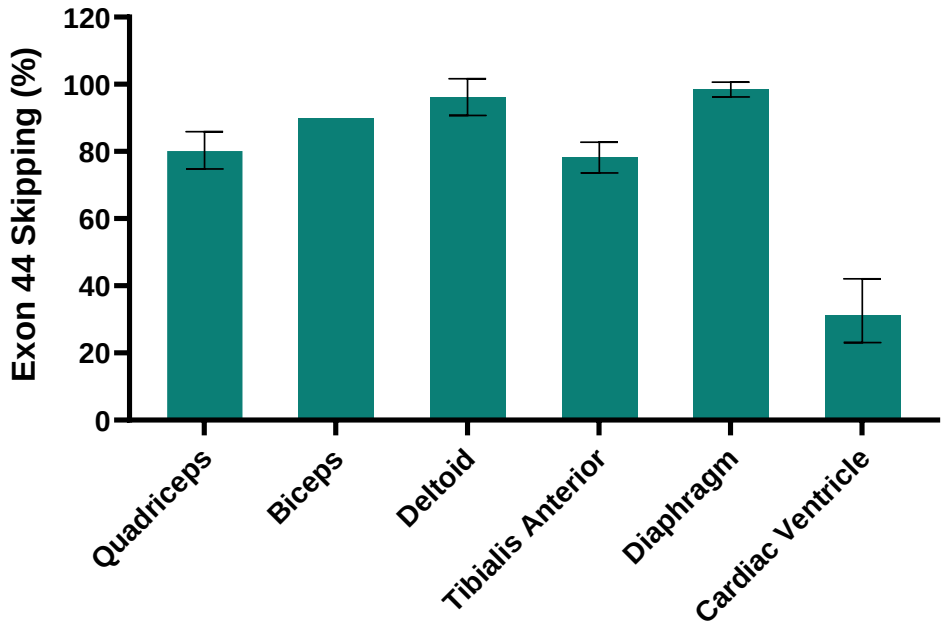
*p<0.05, **p<0.01

- EEV-PMO-23 demonstrates significantly improved PD effects after single 40 mg/kg IV dose in *mdx* mice

ENTR-601-44 IN NHP

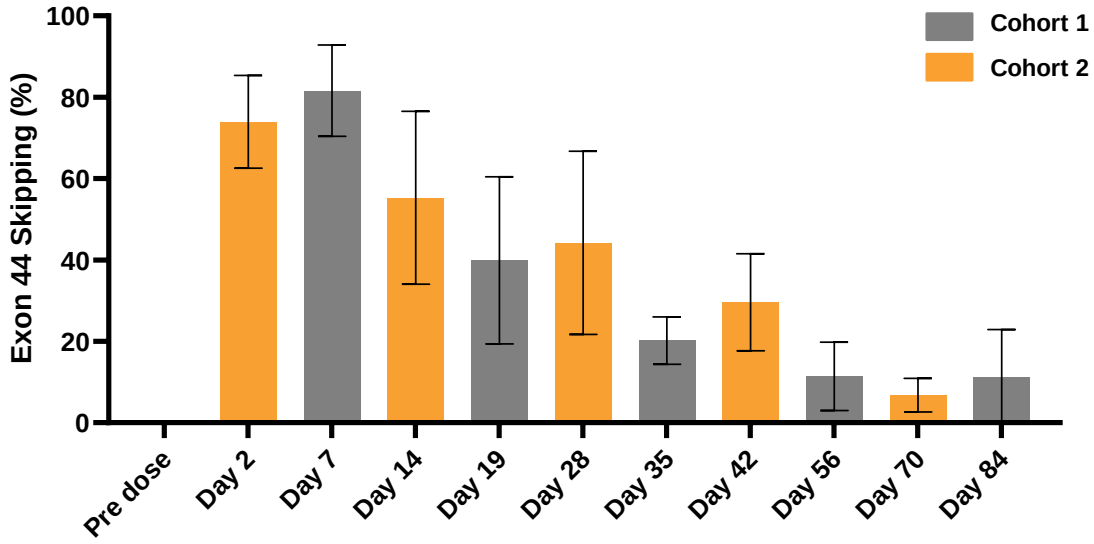
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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



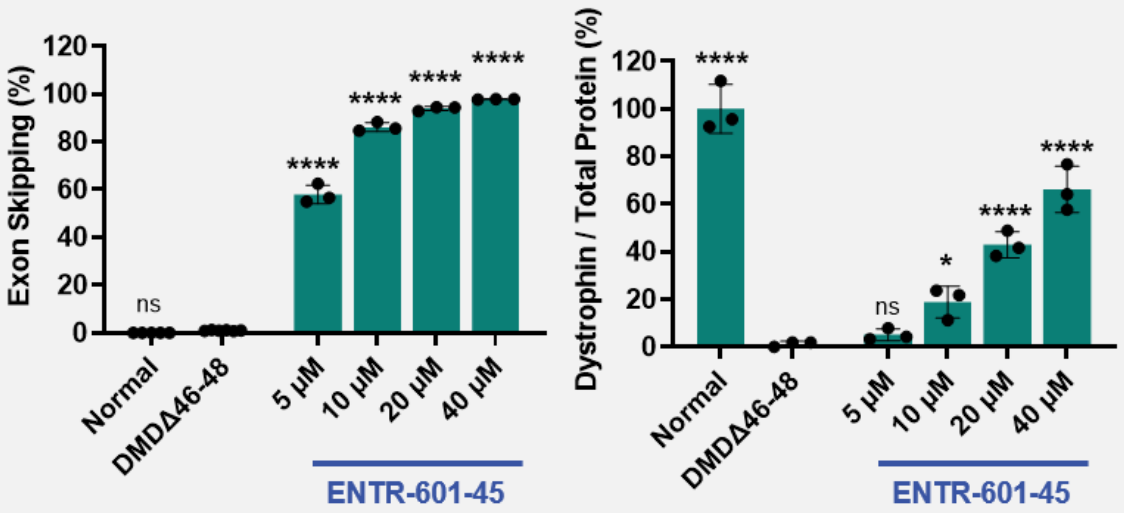
- Post IV infusion of 35 mg/kg (PMO equivalent), robust exon 44 skipping observed in biceps in the ENTR-601-44 treated NHP (n=3 per cohort) for at least 12 weeks

NHP, non-human primates; shown as mean ± standard deviation.

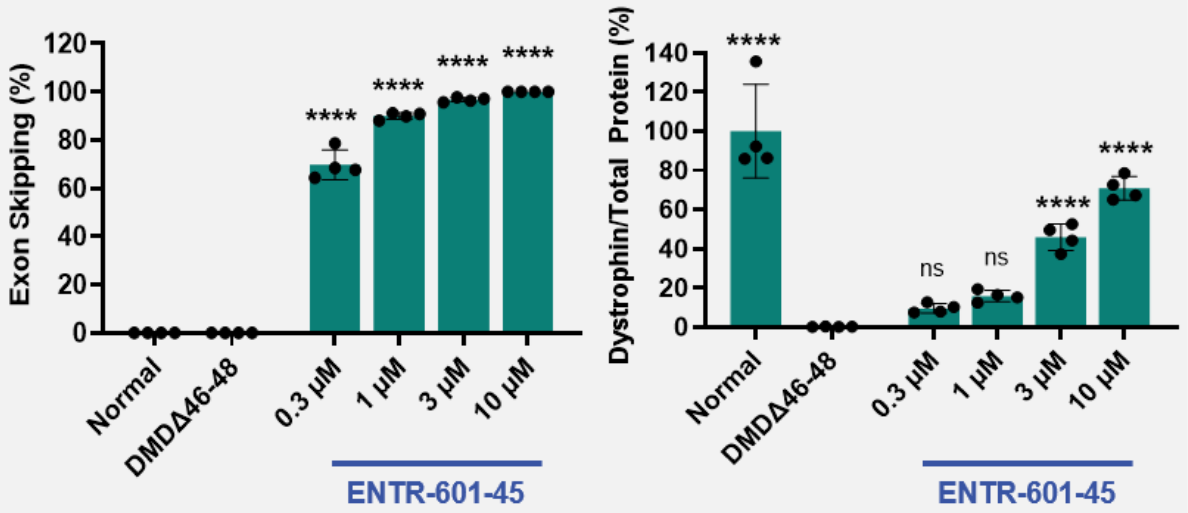
ENTR-601-45 *IN VITRO* EFFICACY

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

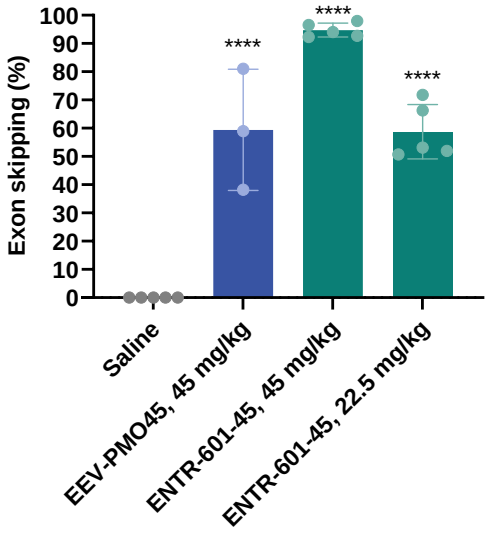


Data are shown as mean ± SD (n = 3 skeletal muscle; n = 4 cardiac muscle); one-way ANOVA; *p<0.05, ****p<0.0001; relative to untreated DMDΔ46-48.
Concentrations provided are PMO equivalent.

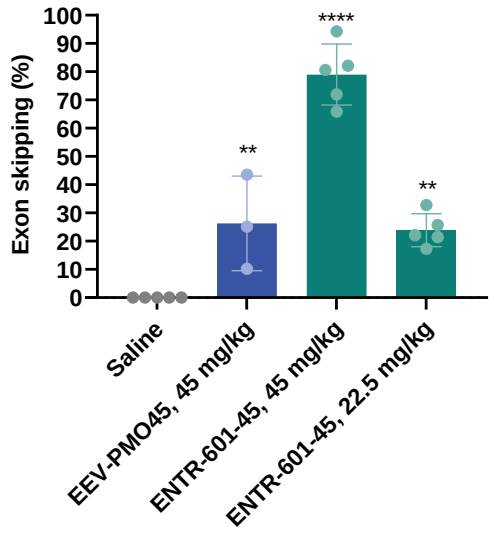
ENTR-601-45 TARGET ENGAGEMENT IN HUMAN DMD MICE

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

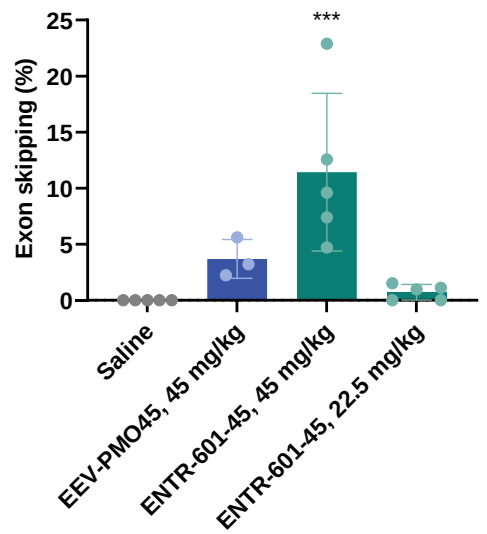
Tibialis Anterior



Diaphragm



Heart



- A single IV dose of ENTR-601-45 resulted in high levels of exon skipping in hDMD in the mouse skeletal muscle and heart after 1 week
- Both EEV-PMO45 (casimersen sequence) and ENTR-601-45 utilize the same EEV
- EEV-PMO45 uses the same oligonucleotide sequence as casimersen

Data are shown as mean ± SD (n = 3-5); one-way ANOVA; **p<0.01, ***p<0.001, ****p<0.0001; relative to saline; Concentrations provided are PMO equivalent.

DMD DATA SUMMARY

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[a]

- 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 is expected to be dosed in September 2023 with data anticipated in the 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

[a]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.

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

THANK YOU

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