Development of Endosomal Escape Vehicles to Enhance the Intracellular Delivery of Oligonucleotides

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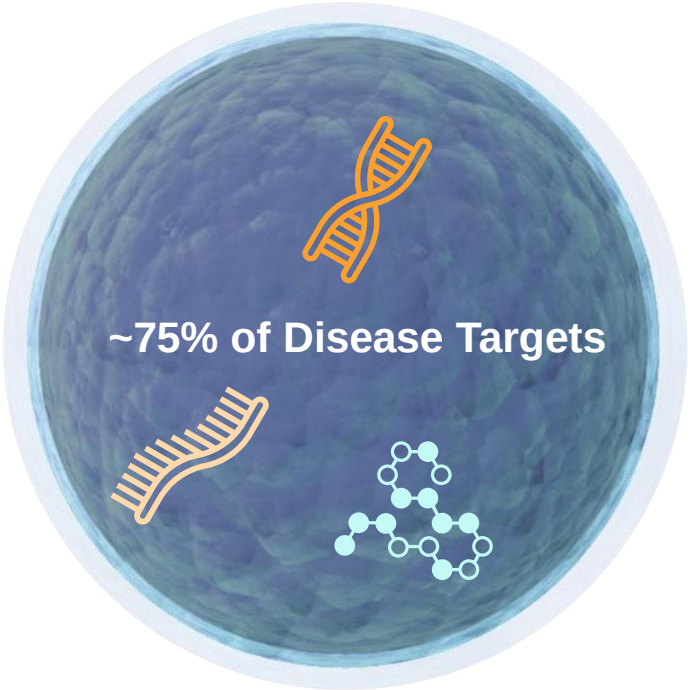
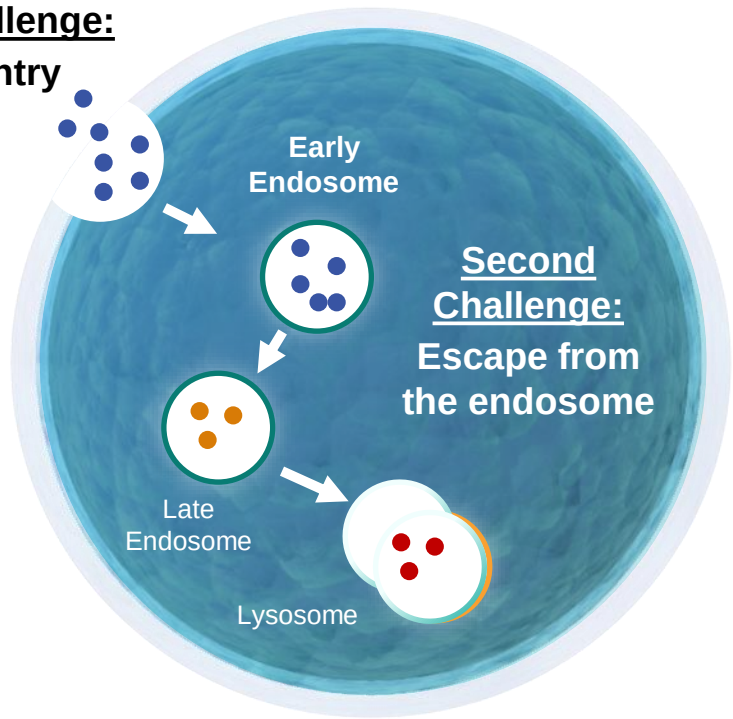
ENTRADA'S MISSION

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



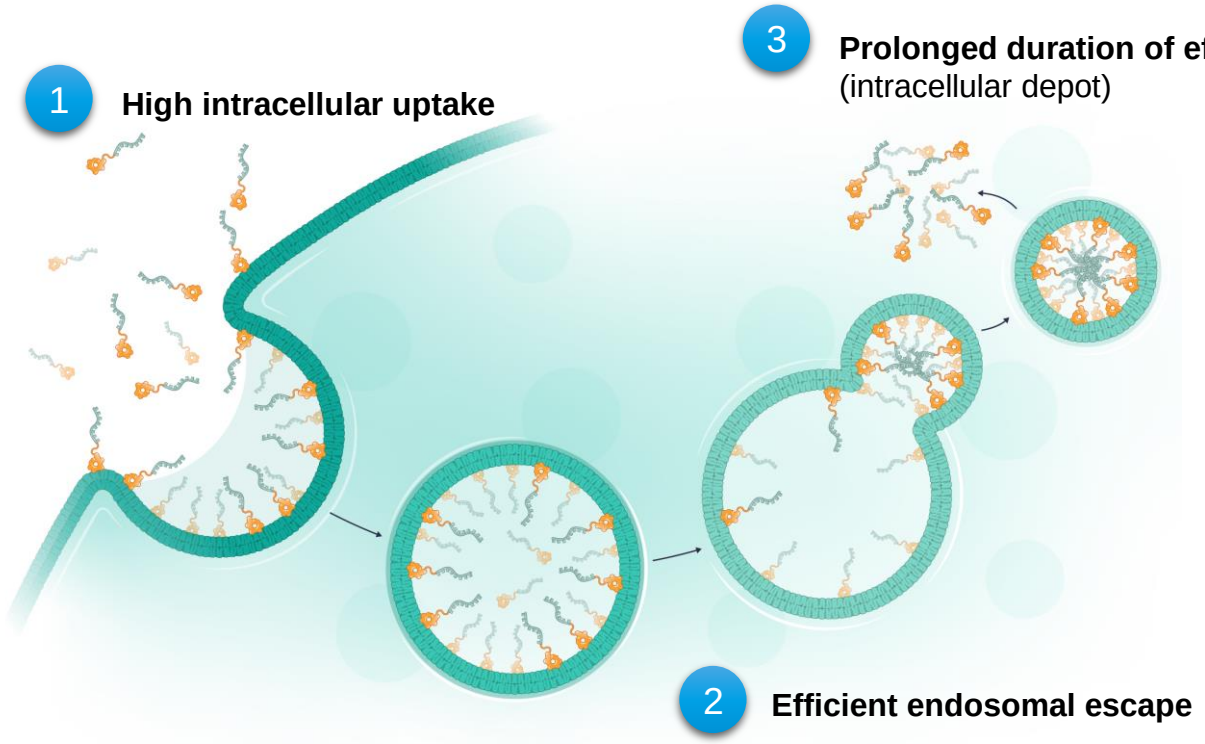


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

Entrada’s EEV platform consists of a library of proprietary cyclic peptides with unique chemistry that enable improved uptake and endosomal escape

- Unique chemistry results in **improved uptake and endosomal escape**
- Cyclic structure enhances **proteolytic stability**
- Small and cyclic structure may **reduce immunogenicity risk**
- Mechanism of internalization **conserved across species**

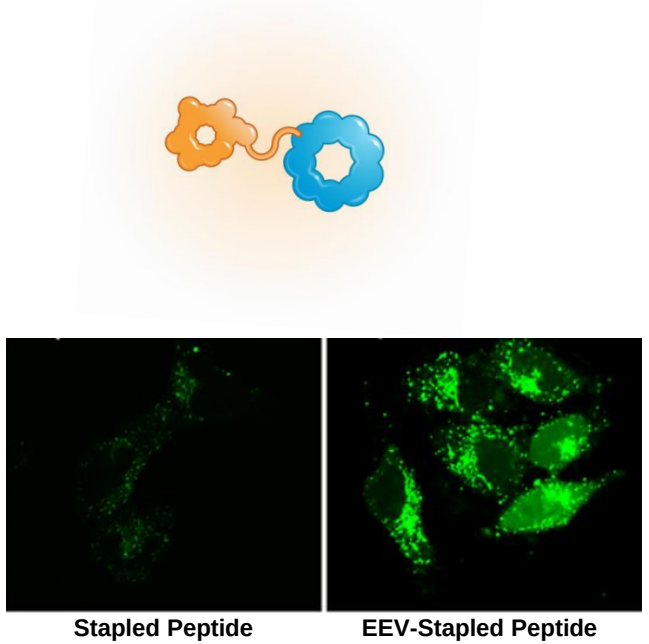


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

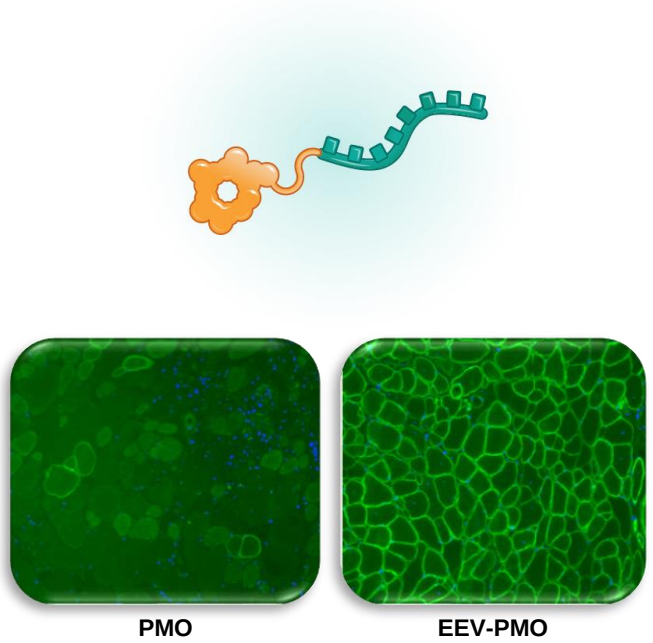
A BROADLY APPLICABLE PLATFORM

Entrada has demonstrated intracellular uptake of unique moieties ranging from 1 kDa to 600 kDa, including peptides, oligonucleotides, and proteins

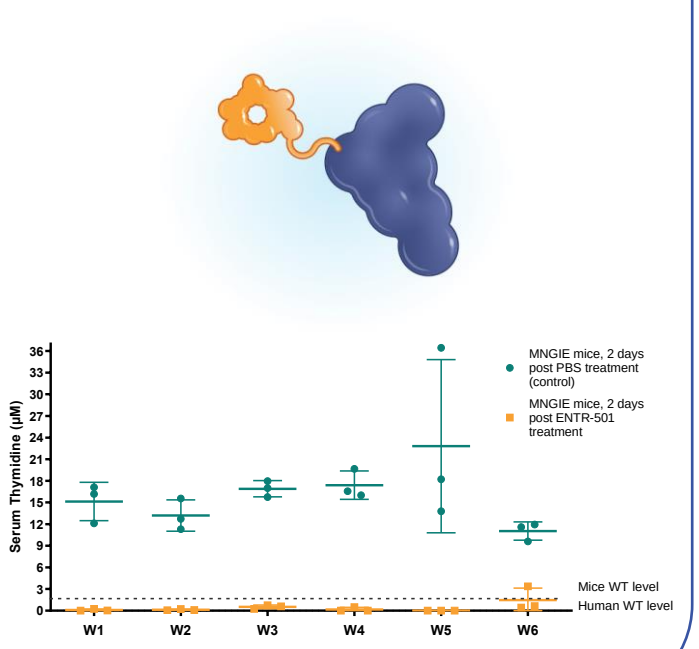
Enhanced Peptide Uptake



Functional Oligonucleotide Delivery



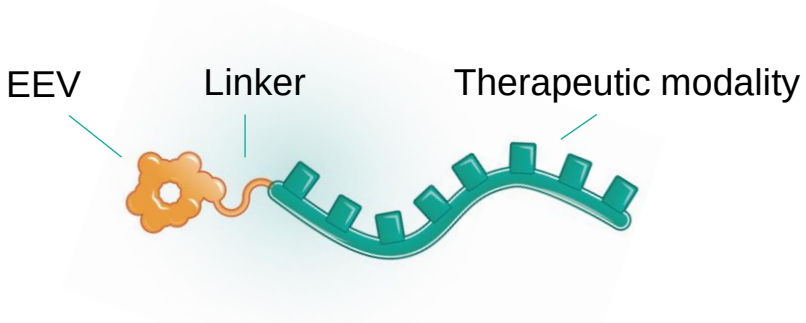
Functional Intracellular Protein Delivery



Peptide Example: Enhanced cellular uptake in a cyclic CPP-dependent manner (Dougherty, P. et al. *J. Med. Chem.* 2020); **Oligonucleotide Example:** EEV-conjugated PMO delivery corrected dystrophin production in DMD mouse model; **Protein Example:** EEV-conjugated thymidine phosphorylase (ENTR-501) effectively reduces plasma Thd levels in MNGIE mice following weekly systemic administration via subcutaneous dosing.

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

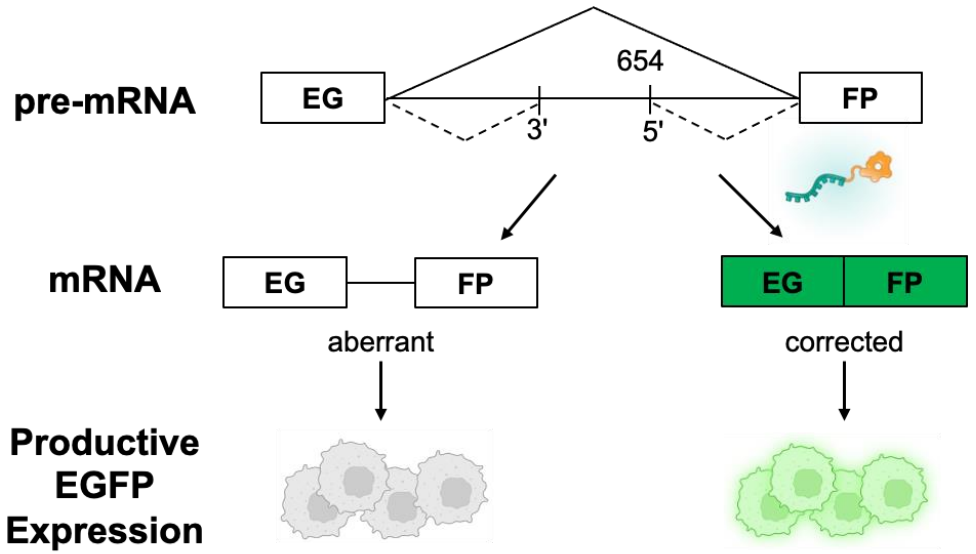
Fit-for-purpose EEV candidate for target indication

Identify EEV candidate with desired **therapeutic profile**

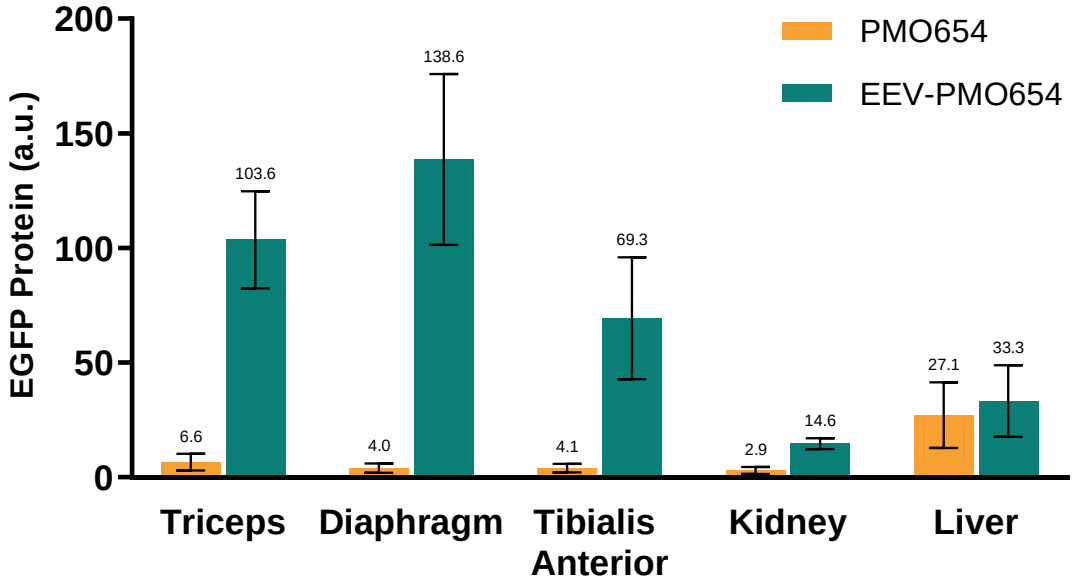
FUNCTIONAL DELIVERY FOR TARGET TISSUES

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

+

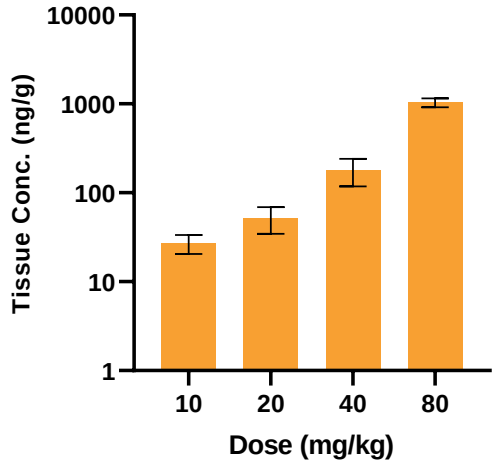
Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

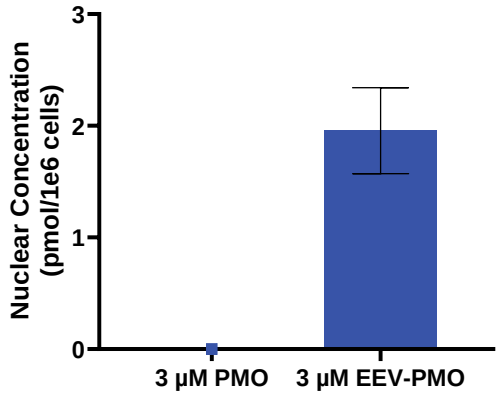
=

Pharmacodynamic Outcome

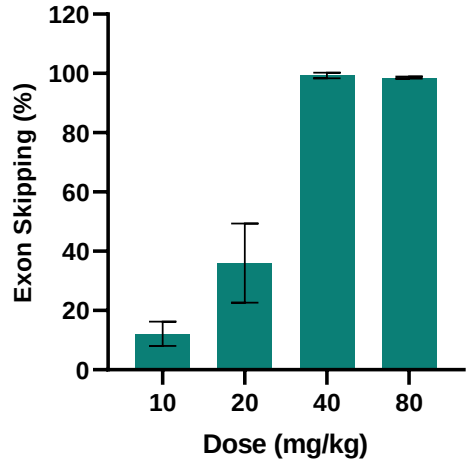
- ✓ Rapid, dose-dependent efficacy
- ✓ Duration of at least 12 weeks



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)

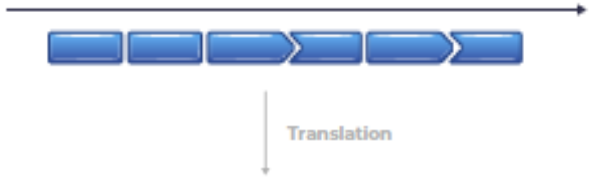
DMD OVERVIEW AND OUR THERAPEUTIC APPROACH

Duchenne muscular dystrophy (DMD) is a muscle wasting disease caused by a mutation in the *DMD* gene and lack of functional dystrophin protein in skeletal and cardiac muscle

Healthy Individual

All exons present in *DMD* pre-mRNA

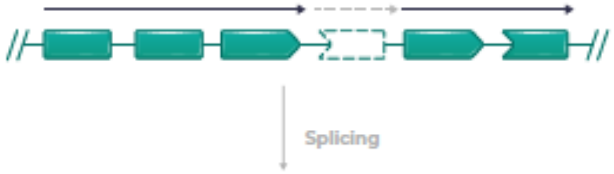
Normal reading frame



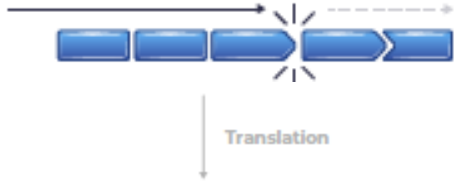
Fully translated and fully functional dystrophin protein

Individual With DMD

Mutated or missing exon



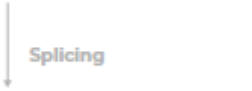
Disrupted reading frame



Functional dystrophin protein is not produced

EEV-Oligonucleotide Approach in DMD

EEV-oligonucleotide conjugates enable skipping of the mutated/missing exon

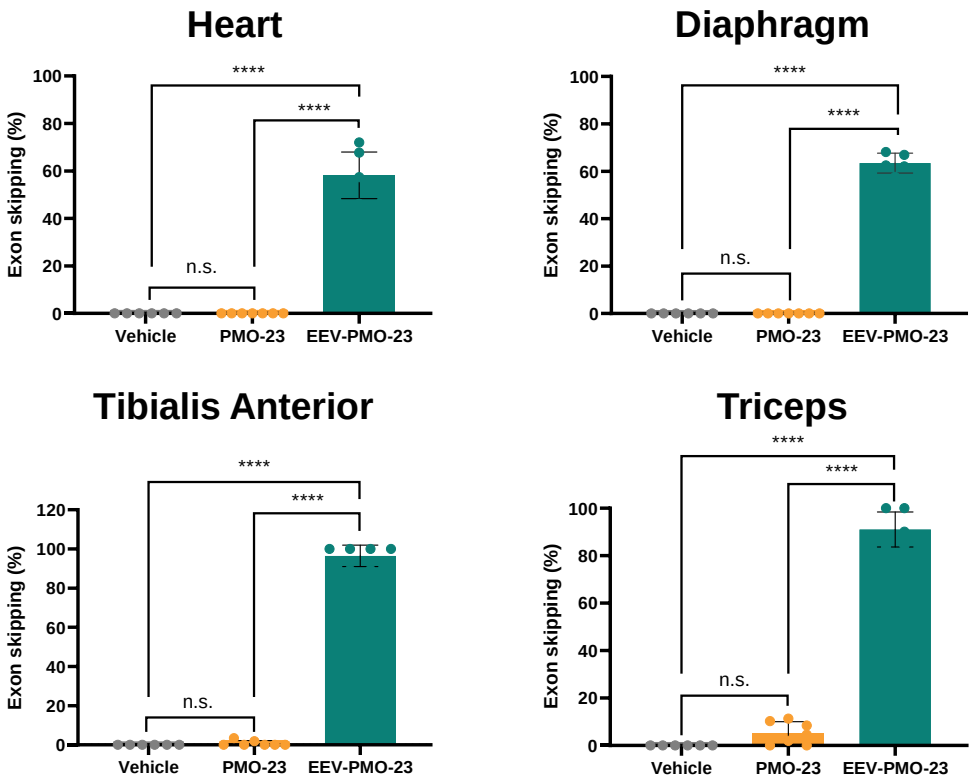


Normal reading frame

Truncated, but functional dystrophin protein

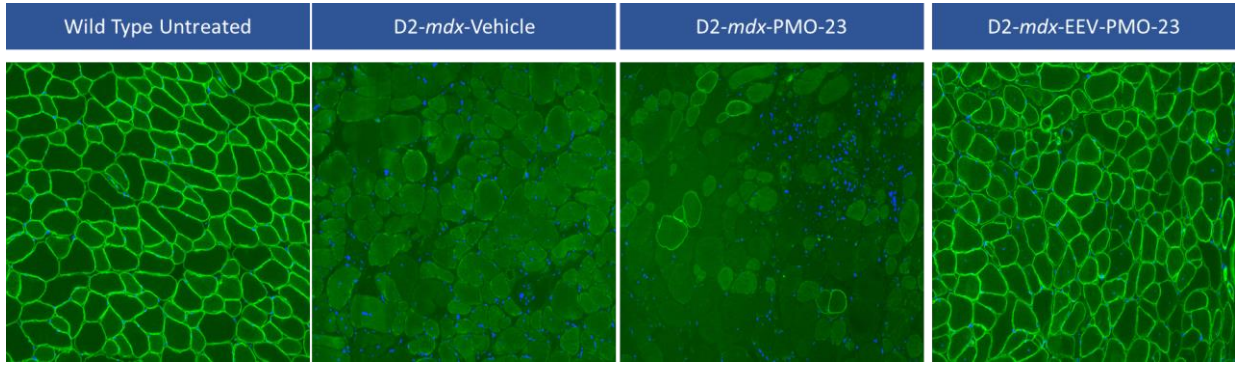
REPEAT EEV-PMO TREATMENT IN D2-*mdx* MICE

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

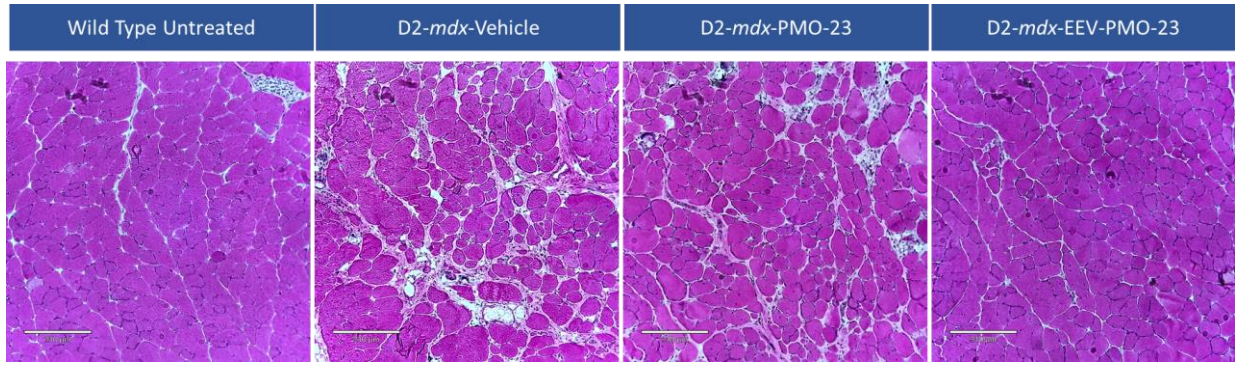


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



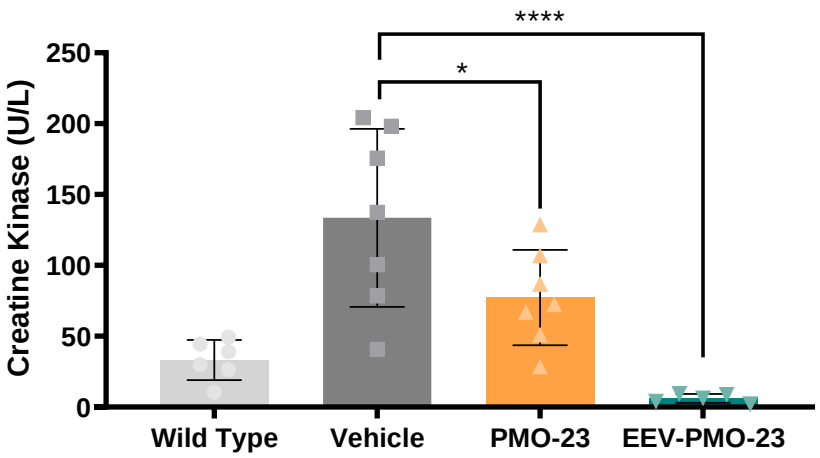
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose

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.

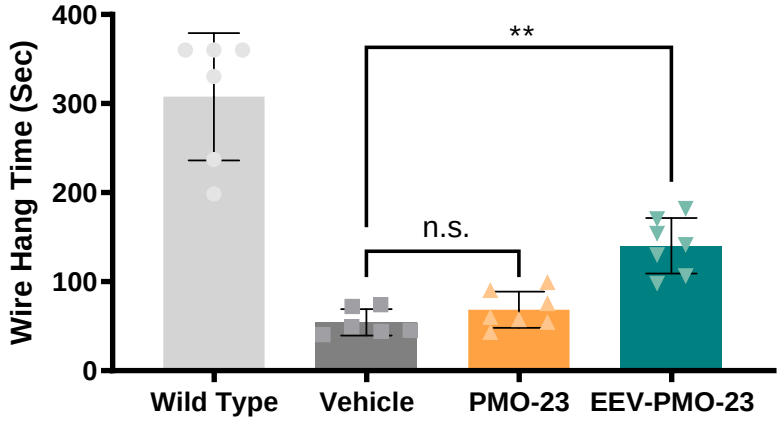
REPEAT EEV-PMO TREATMENT RESULTS IN CK CORRECTION AND FUNCTIONAL IMPROVEMENT

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

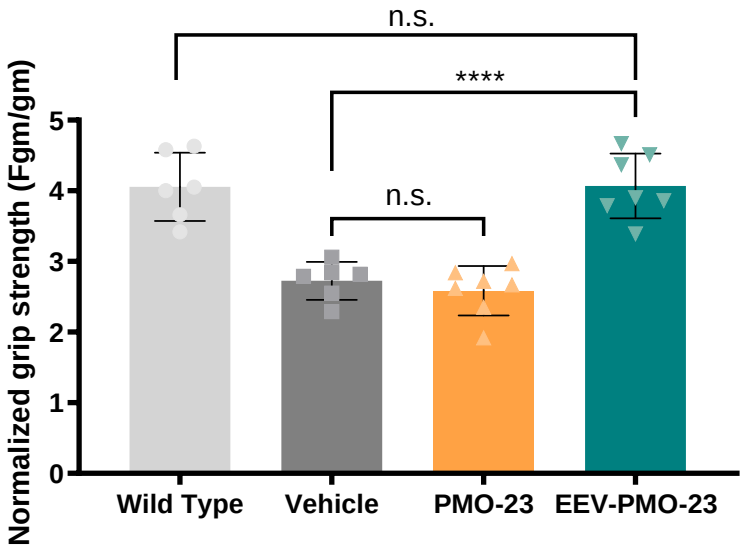
Serum CK Levels (Muscle Damage)



Wire Hang Time



Grip Strength



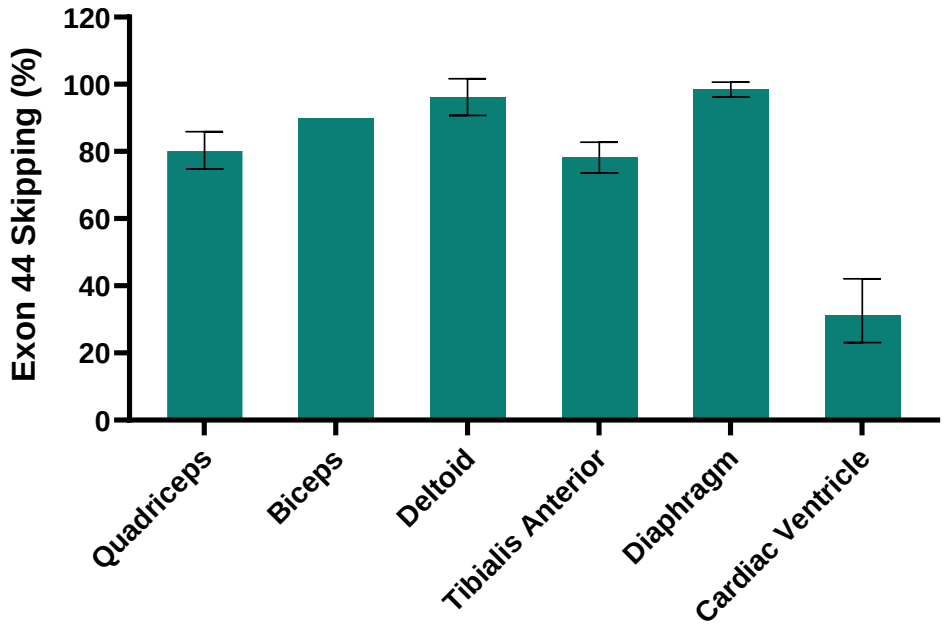
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~2 weeks after the last dose

CK, creatine kinase; *p<0.05, **p<0.01, ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

ENTR-601-44 IN NHP

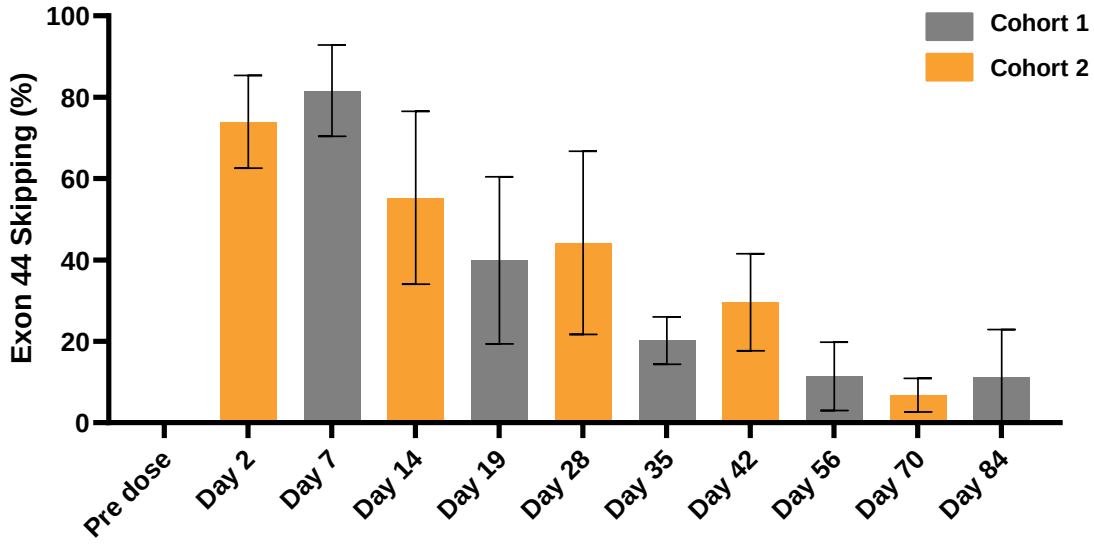
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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

ENTR-601-44 is the clinical candidate selected for DMD and is an exon 44 skipping EEV-oligonucleotide conjugate; **NHP**, non-human primates; shown as mean ± standard deviation.

MYOTONIC DYSTROPHY TYPE 1 (DM1)

DM1 OVERVIEW AND OUR THERAPEUTIC APPROACH

Myotonic Dystrophy Type 1 (DM1) is a multisystemic disease caused by CUG trinucleotide repeats in *DMPK* mRNA that sequester MBNL proteins

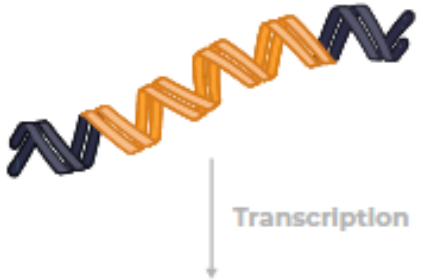
Healthy Individual

***DMPK* gene with few CTG repeats**

Normal transcription and proper downstream MBNL protein activity

Individual With DM1

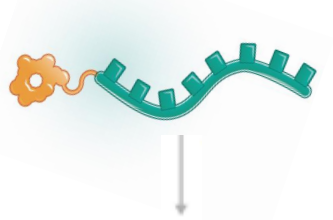
***DMPK* gene with 1000s of CTG repeats**



Mutant mRNA sequesters MBNL proteins and inhibit downstream splicing function

EEV-Oligonucleotide Approach in DM1

ENTR-701 is a CUG repeat blocking oligonucleotide conjugated to our EEV



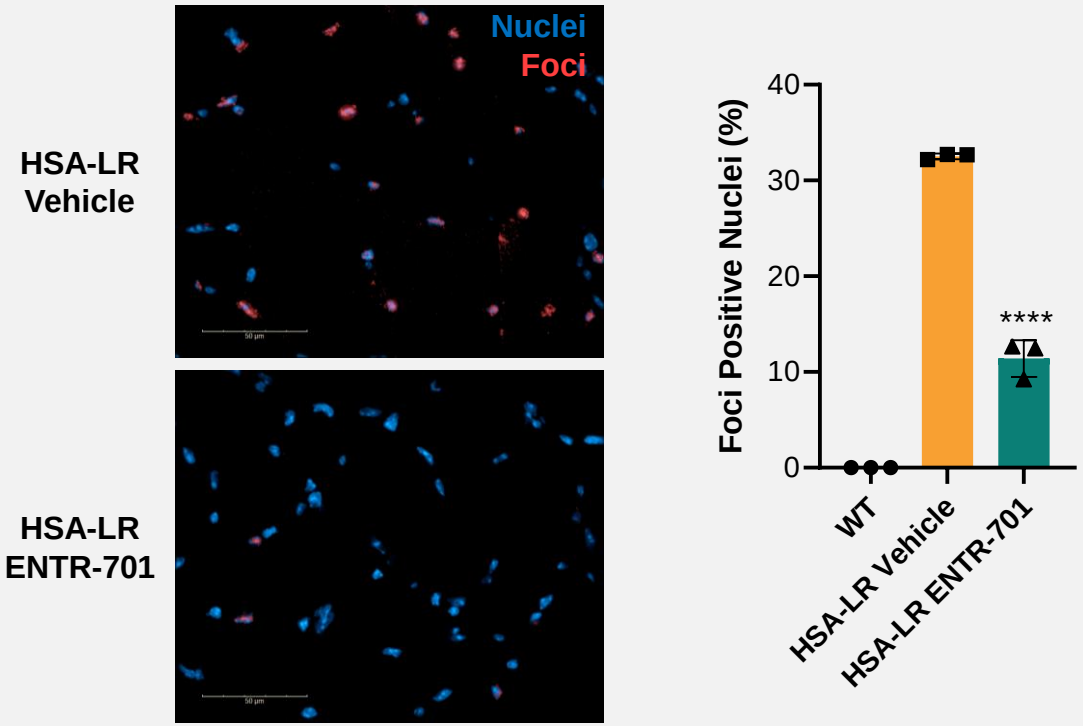
ENTR-701 blocks mutant DMPK mRNA and reduces nuclear foci, restores MBNL function and splicing defects

DMPK, DM1 protein kinase; **MBNL**, muscleblind like splicing regulator; **ENTR-701** is the clinical candidate selected for DM1.

EFFICACY OF ENTR-701 IN HSA-LR MICE

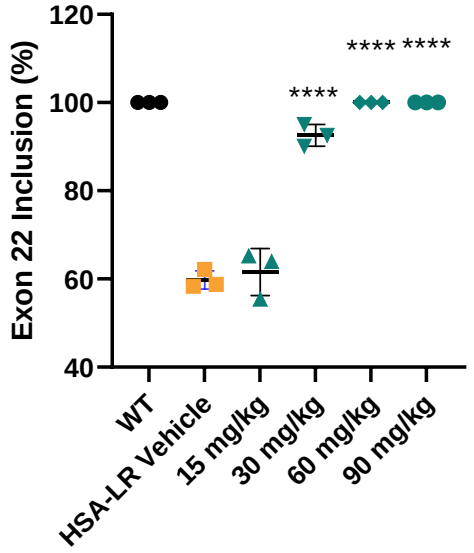
ENTR-701 treatment reduced nuclear foci and CUG-repeat expansion transcript level and corrected aberrant splicing in HSA-LR mice

Nuclear Foci Reduction

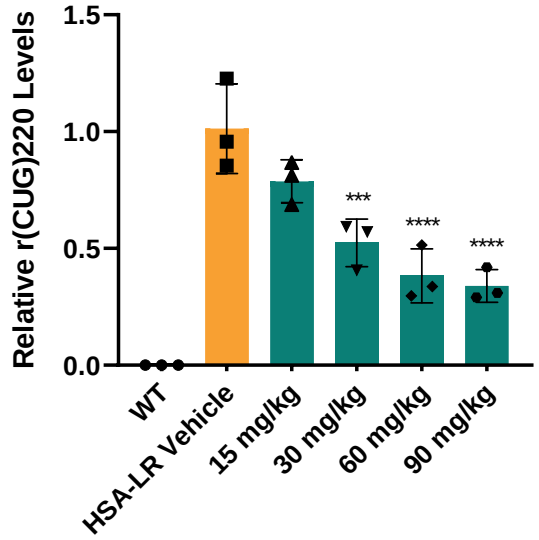


Tibialis anterior section

Atp2a1 Splicing Correction



HSA-r(CUG)220 Reduction

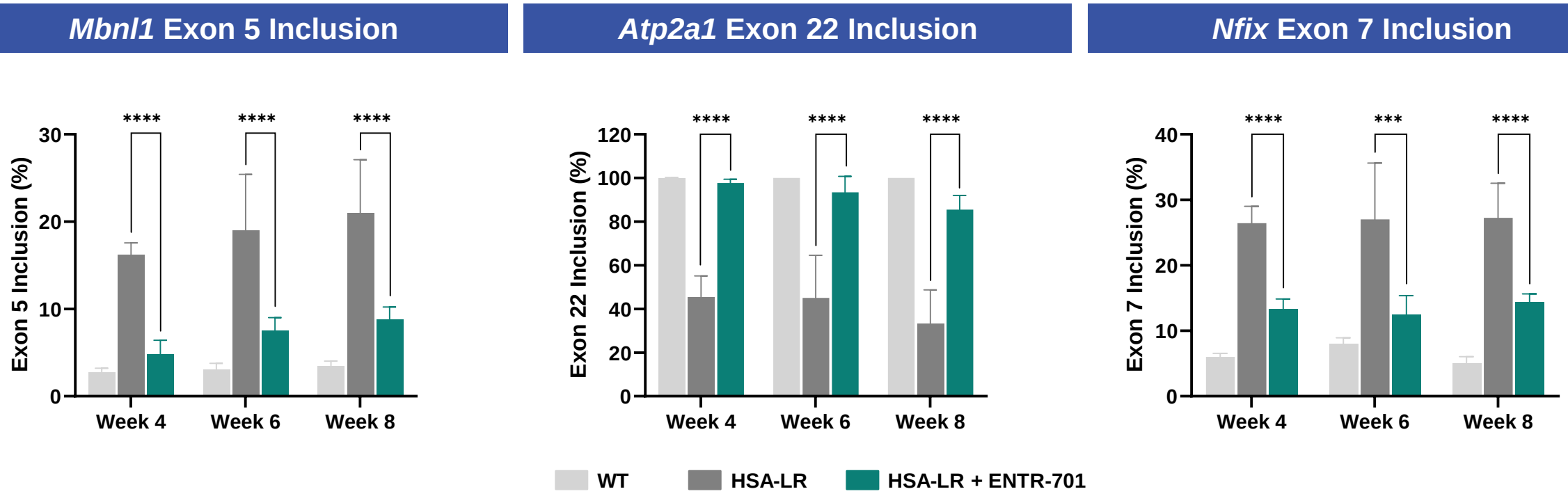


- HSA-LR mice were dosed with vehicle or ENTR-701 and taken down 1-week post injection; tibialis anterior samples analyzed as a representative skeletal group

HSA-LR model, carrying a transgene resulting in CUG repeat expansion and recapitulates DM1 phenotype and molecular pathology (Mankodi, A. et al. *Science* 2000); ***Atp2a1***, sarcoplasmic/endoplasmic reticulum calcium ATPase; ***p<0.001, ****p<0.0001, shown as mean ± standard deviation.

DURABILITY OF ENTR-701 IN HSA-LR MICE

A single dose of ENTR-701 resulted in splicing correction in HSA-LR mice for at least 8 weeks



- Post single IV dosage of 60 mg/kg (PMO equivalent), robust splicing correction in the ENTR-701 treated HSA-LR mice 4, 6, or 8 weeks post injection

Mbnl1, muscleblind like splicing regulator 1; ***Atp2a1***, sarcoplasmic/endoplasmic reticulum calcium ATPase; ***Nfix***, nuclear factor I X; ***p<0.001, ****p<0.0001, shown as mean ± standard deviation.

MYOTONIA CORRECTION IN HSA-LR MICE

A single dose of ENTR-701 ameliorated pinch-induced myotonia for at least 8 weeks

HSA-LR Mouse: **Non-treated**

HSA-LR Mouse: **ENTR-701 Treated**

SUMMARY AND PATH FORWARD TO THE CLINIC

Established that the **Endosomal Escape Vehicle (EEV™) platform** consists of a library of proprietary cyclic peptides with unique chemistry that enable improved cellular uptake, endosomal escape and consistent translation across species

ENTR-601-44, our clinical candidate for DMD exon 44 skipping amenable patients, produces durable and robust exon skipping in both cardiac and skeletal muscles of mice and NHP

- IND submission is planned in **Q4 2022**

ENTR-701, our clinical candidate for DM1, corrects splicing deficits and ameliorates myotonia in mice. A durable effect was observed 8 weeks following a single dose

- IND submission is planned in **2023**

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

entrada THERAPEUTICS

Thank you!

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LABORATORY

UF UNIVERSITY of FLORIDA