Endosomal Escape Vehicles to Enhance the Functional Delivery of Oligonucleotides in Preclinical Models of Neuromuscular Diseases

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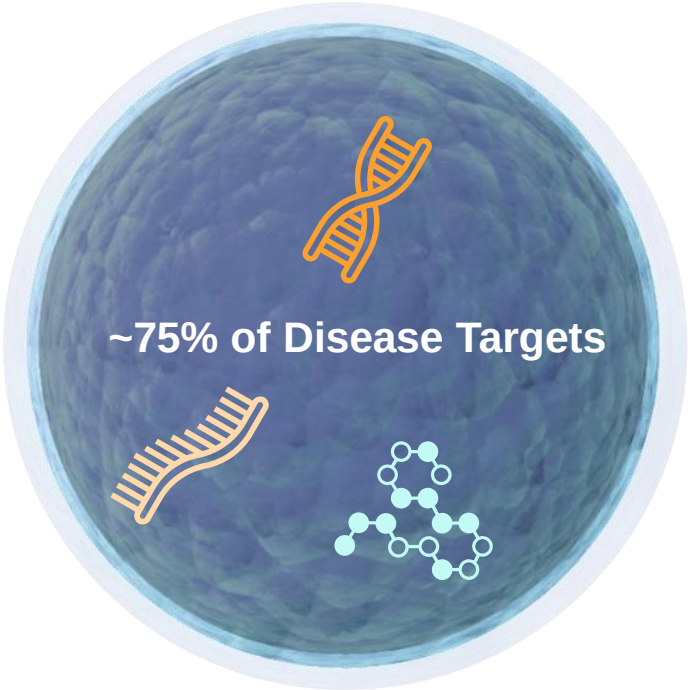
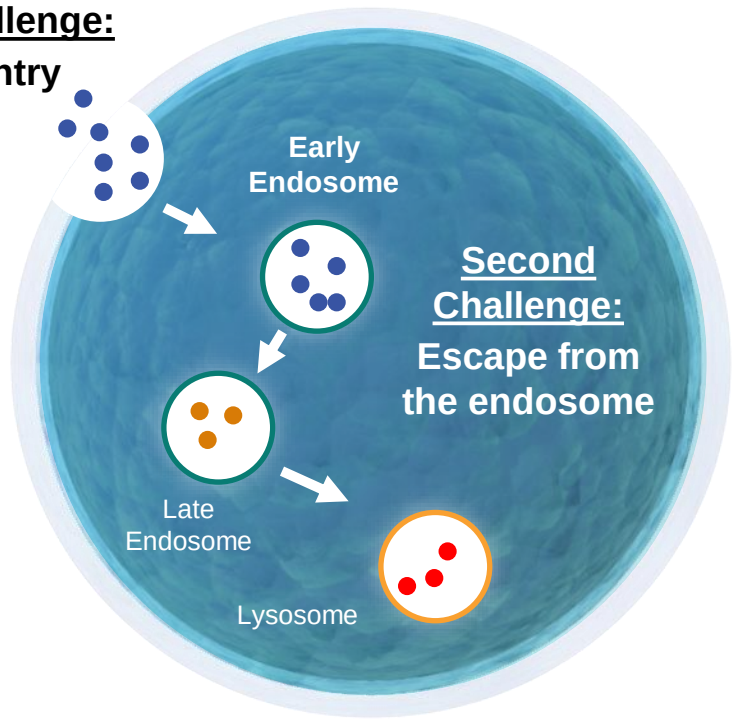
Certain information contained in this presentation and statements made orally during this presentation relate to or are based on studies, publications, surveys and other data obtained from third-party sources and our own internal estimates and research. While we believe these third-party studies, publications, surveys and other data to be reliable as of the date of this presentation, it has not independently verified, and makes no representations as to the adequacy, fairness, accuracy or completeness of, any information obtained from third-party sources. In addition, no independent source has evaluated the reasonableness or accuracy of our internal estimates or research and no reliance should be made on any information or statements made in this presentation relating to or based on such internal estimates and research.

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

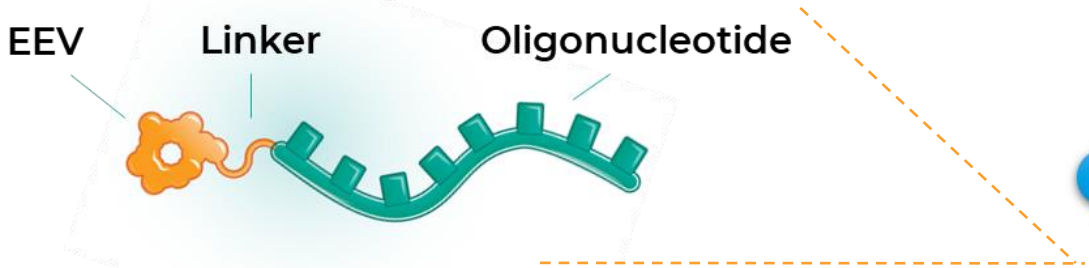


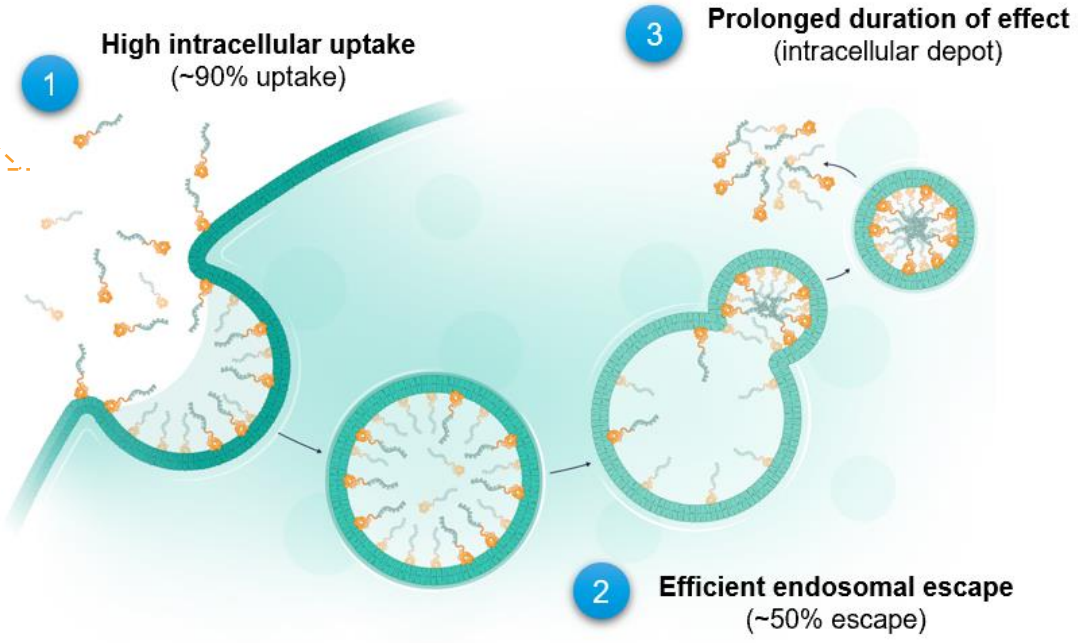


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

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





- Entrada's EEV™ platform consists of a library of proprietary cyclic peptides with unique chemistry that enable improved uptake into the cell and escape from the endosome
- Entrada's two lead clinical programs will evaluate our EEV-PMO approach in Duchenne muscular dystrophy (DMD) and myotonic dystrophy type 1 (DM1)

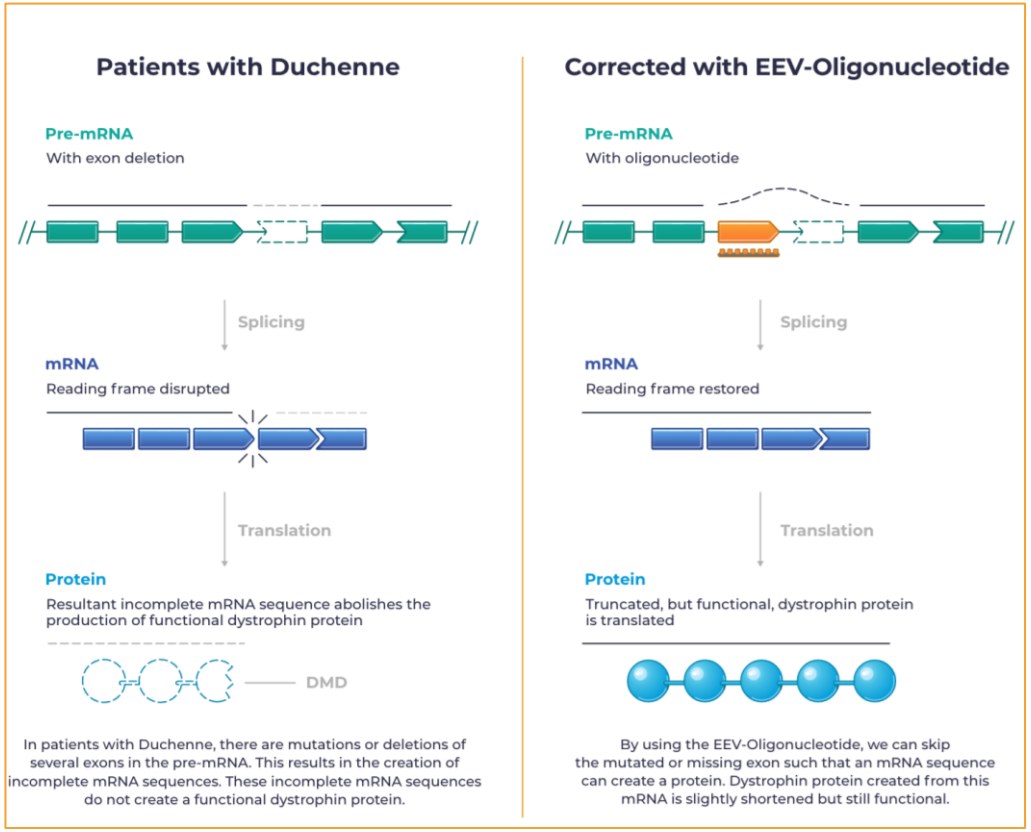
Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022. **PMO**, phosphorodiamidate morpholino oligomer

DUCHENNE MUSCULAR DYSTROPHY (DMD)

DMD OVERVIEW

Duchenne muscular dystrophy (DMD) is a progressive, devastating muscle wasting disease with significant unmet need. Entrada's first DMD program is for exon 44 skipping

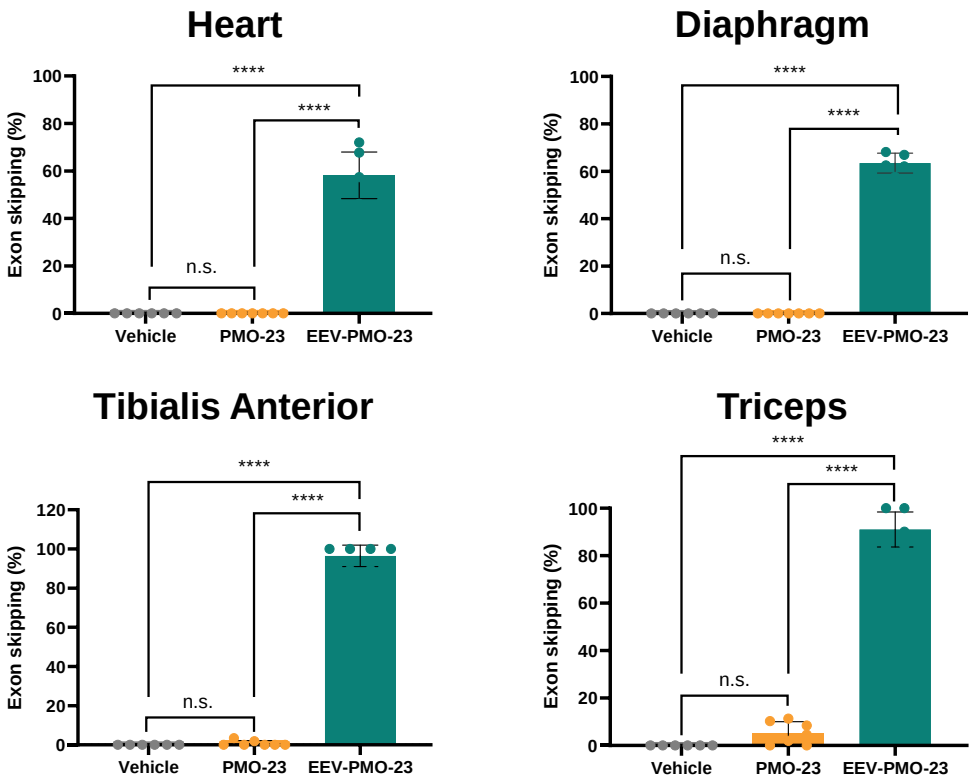
- DMD is caused by mutations in the *DMD* gene that encodes for dystrophin[1]
- Progressive muscle degeneration, wasting and paralysis generally lead to death via **respiratory and/or cardiac failure**[2]
- Exon skipping therapeutics (for exons 45, 51 and 53) using **PMO chemistry** were approved based on **a very modest improvement in dystrophin levels ranging from ~1 to 6%**
- 40% of patients with DMD have mutations amenable to exon skipping of exons 44, 45, 51 and 53.[3] **However, there is currently no approved therapy for patients with mutations amenable to exon 44 skipping**



[1]Hoffman, E.P. et al. *Cell* 1987; [2]Birnkrant, D.J. et al. *Lancet Neurol* 2018; [3]https://www.parentprojectmd.org/about-duchenne/what-is-duchenne/types-of-mutations/mutation-specific-therapies

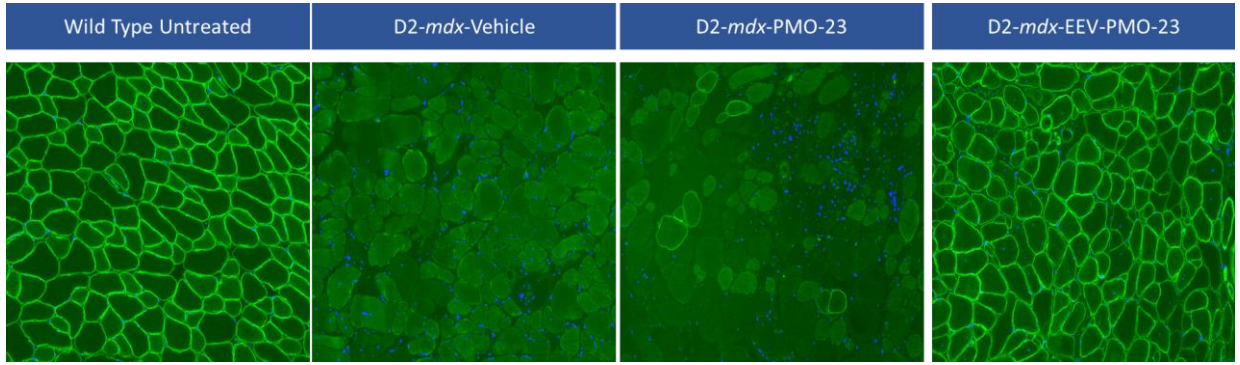
REPEAT EEV-PMO TREATMENT IN D2-*mdx* MICE

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

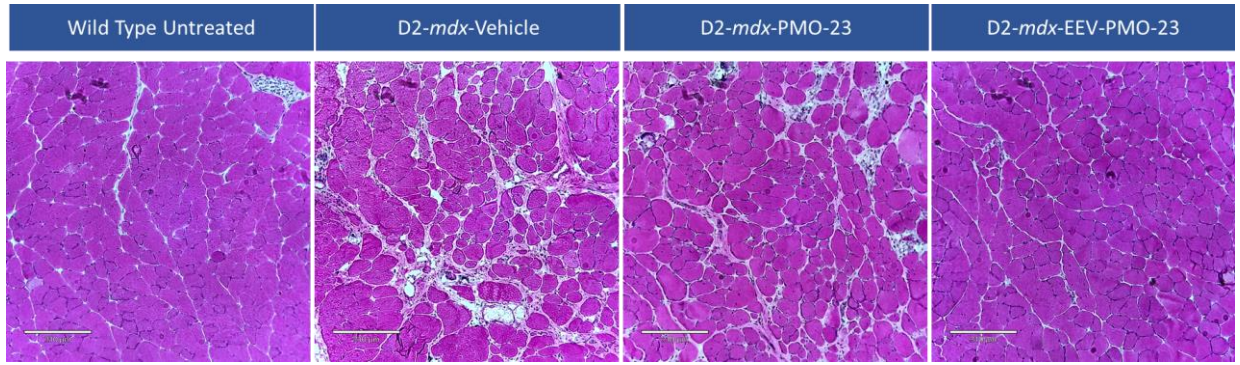


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



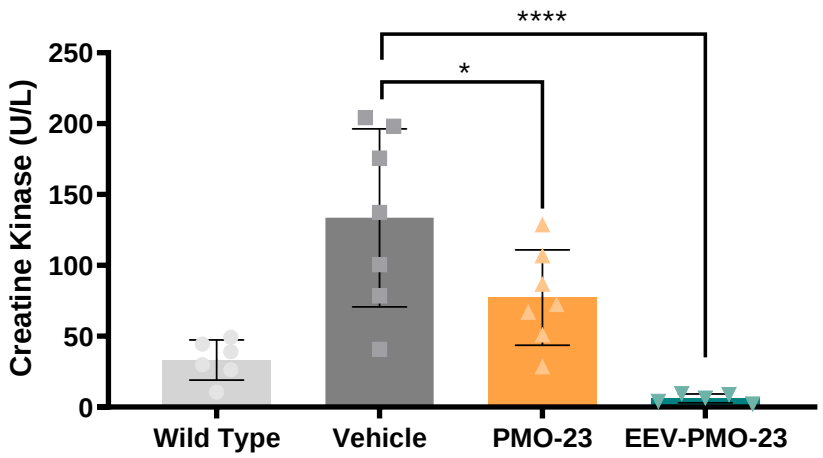
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose

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.

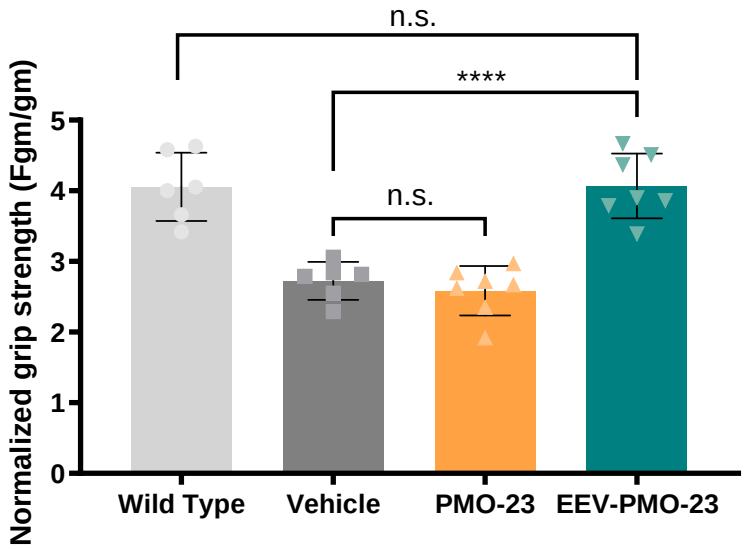
REPEAT EEV-PMO TREATMENT RESULTS IN CK CORRECTION AND FUNCTIONAL IMPROVEMENT

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

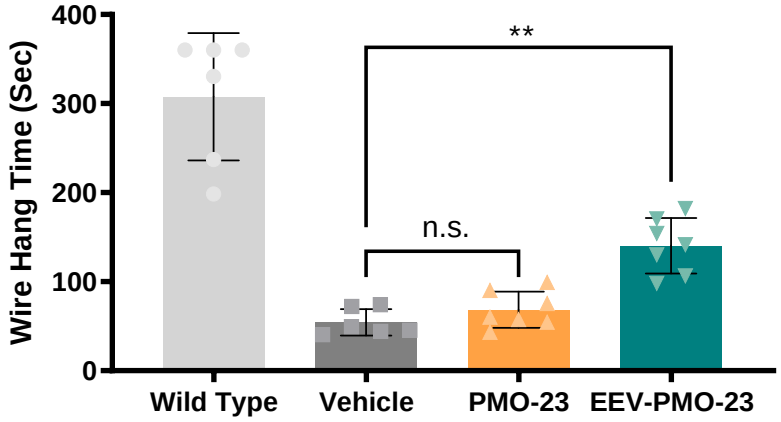
Serum CK Levels (Muscle Damage)



Grip Strength



Wire Hang Time



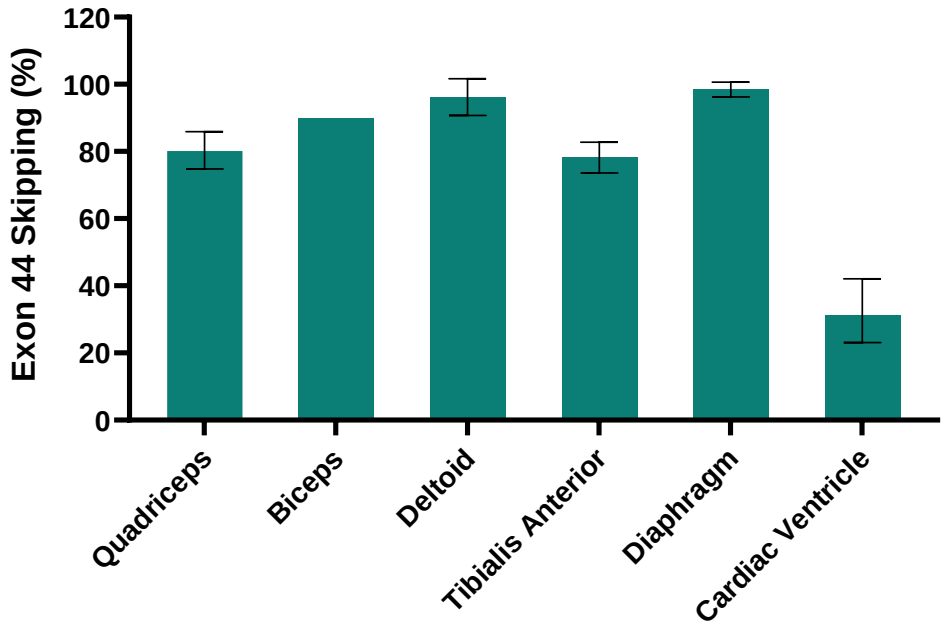
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~2 weeks after the last dose

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

ENTR-601-44: CLINICAL CANDIDATE FOR EXON 44 SKIP AMENABLE DMD

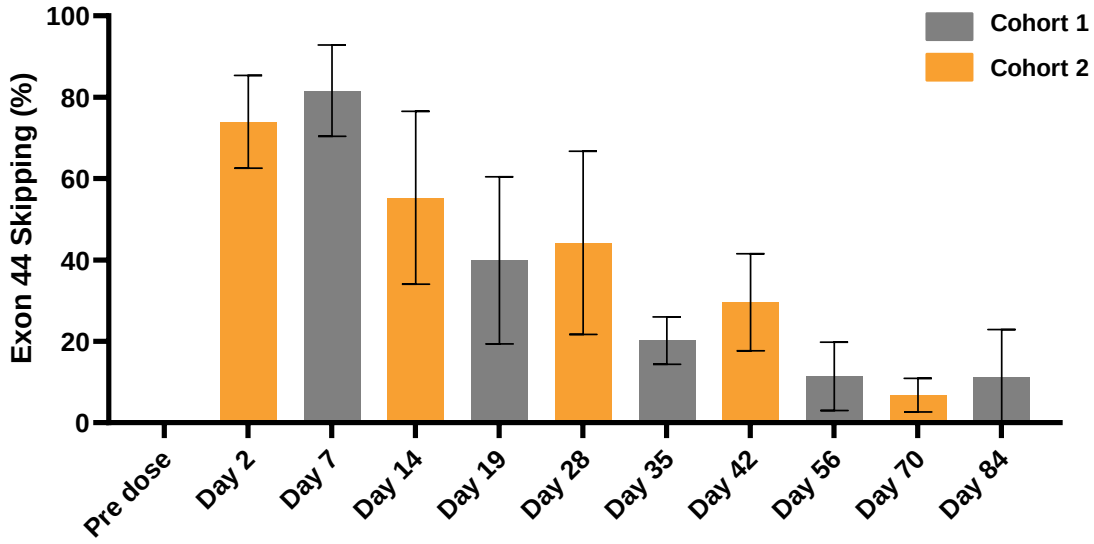
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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.

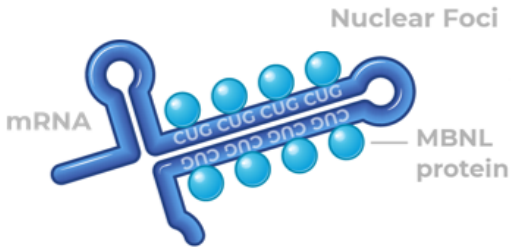
MYOTONIC DYSTROPHY TYPE 1 (DM1)

DM1 OVERVIEW

DM1 is a debilitating multi-systemic disease with no available treatments; CUG repeats in DMPK mRNA sequester MBNL proteins, resulting in nuclear foci, aberrant splicing, and disease

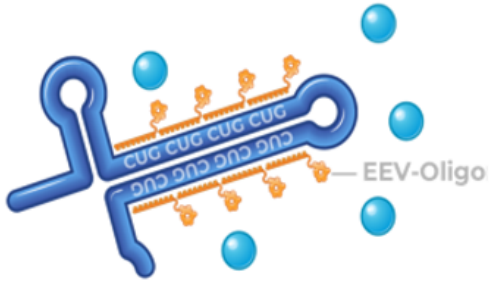
- **DM1 occurs in 1:8,000 people worldwide and affects ~40,000 patients in US and over 50,000 in Europe**
  - 75% of patients have adult-onset DM1
  - Multisystemic: including myotonia, muscle weakness and atrophy, cardiac conduction abnormalities, pulmonary complications, cataracts, and endocrine dysfunction[1]
  - **Currently there are no approved therapies**
- **DM1 is caused by CUG repeats in the mRNA that sequester MBNL proteins**[2]
  - Mutant *DMPK* mRNA and MBNL proteins form aggregates named nuclear foci[3]
- **MBNL activity is decreased as a result of sequestration leading to spliceopathy of downstream transcripts**[4,5]

Mutant *DMPK* mRNA



- Nuclear foci formation
- mRNA accumulation
- Reduced MBNL function
- Aberrant splicing

EEV-Oligonucleotide Approach



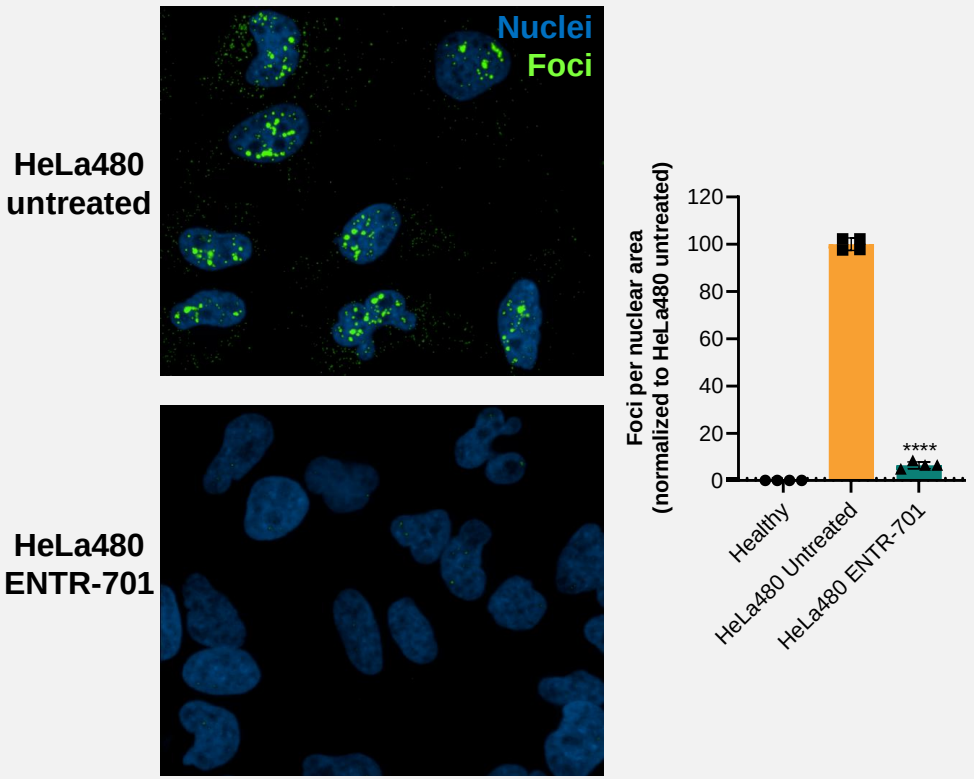
- Reduced nuclear foci
- Selective mRNA reduction
- Normal MBNL function
- Corrected spliceopathy

[1]Thornton C.A. *Neurol. Clin.* 2014, 705. [2]Philips, A.V. et al. *Science* 1998; [3]Pettersson, O.J. *NAR* 2015; [4]Wagner, S.D. et al. *PLoS Genet.* 2016; [5]Tanner, M. et al. *Nucleic Acids Res.* 2021.

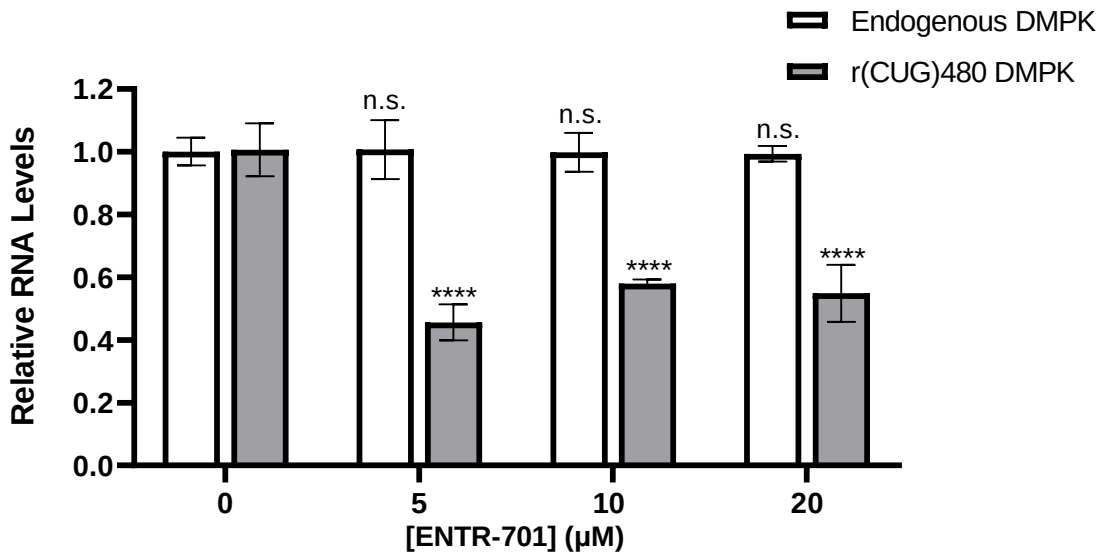
ENTR-701 IN DM1 MODEL CELL LINE WITH REPEAT EXPANSION

DM1 clinical candidate, ENTR-701, showed reduction of nuclear foci and selective reduction of repeat expansion-containing DMPK transcript in the HeLa480 cell line

Nuclear Foci Reduction



Selective Reduction of Mutant DMPK mRNA



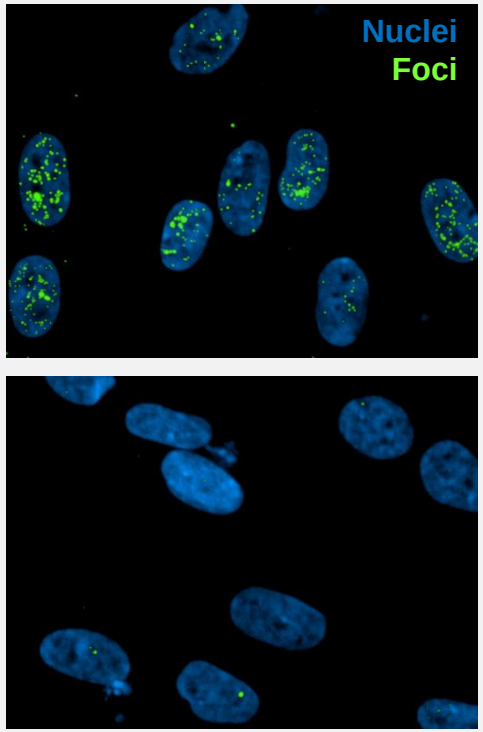
- Free uptake of ENTR-701 reduced nuclear foci and selectively reduced (CUG)480 containing DMPK mRNA

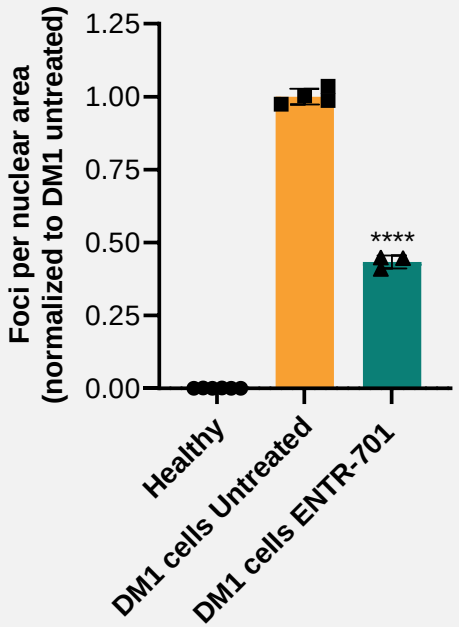
ENTR-701 is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV; **HeLa480** cell line was constructed by integrating (CTG)480 and (CTG)0 containing DMPK transgenes, which showed MBNL1-dependent aberrant splicing (Reddy, K. et al. *Proc. Natl. Acad. Sci.* 2019); ****p<0.0001, **n.s.**, not significant; shown as mean ± standard deviation.

ENTR-701 IN DM1 PATIENT-DERIVED MYOTUBES

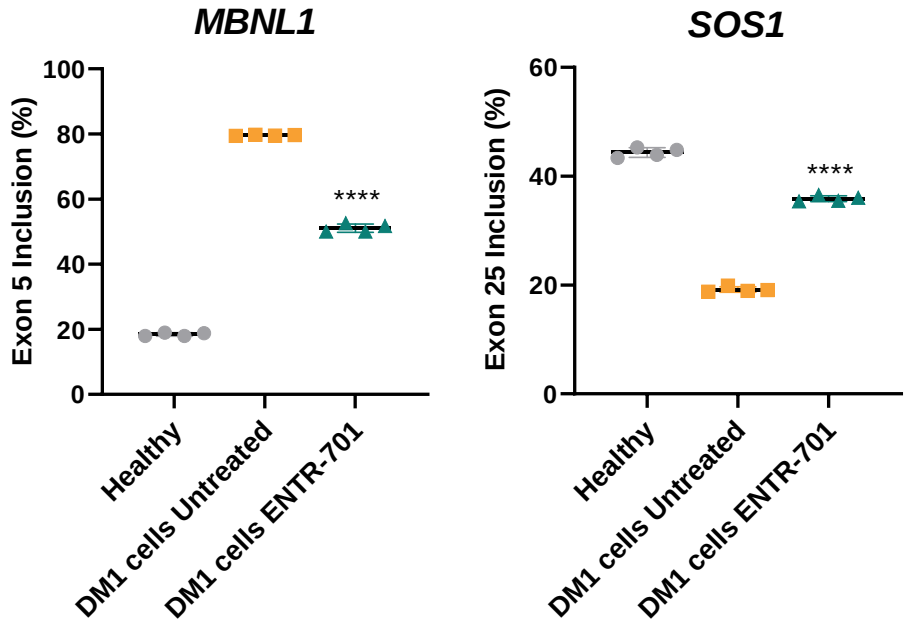
ENTR-701 treatment in DM1 patient-derived muscle cells resulted in significant nuclear foci reduction and correction of aberrant splicing

Nuclear Foci Reduction





Correction of Aberrant Splicing



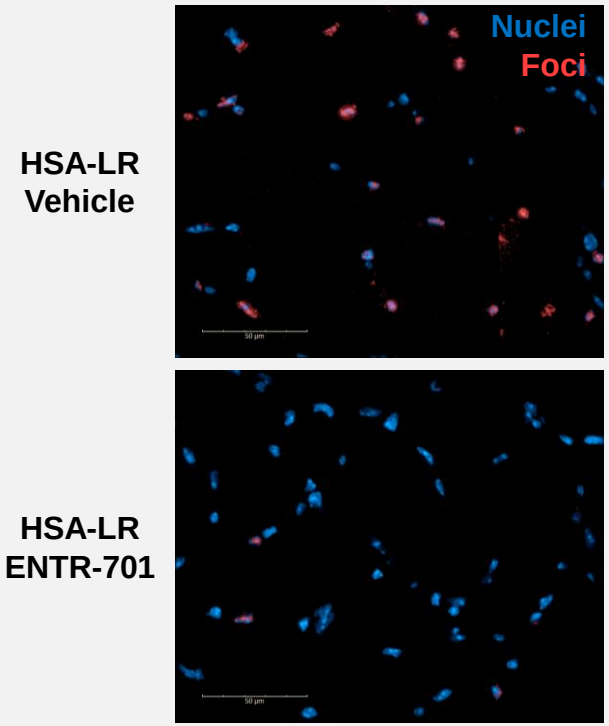
- Immortalized DM1 patient-derived (2,600 CUG repeats) muscle cells[1] were treated with ENTR-701 and analyzed for the reduction of nuclear foci and the correction of aberrant splicing

[1]Arandel, L. et al. *Dis. Model. Mech.* 2017. **ENTR-701** is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV; ****p<0.0001 for ENTR-701 compared to untreated; shown as mean ± standard deviation.

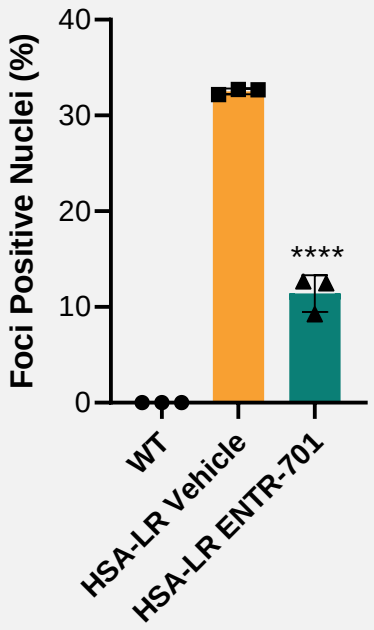
EFFICACY OF ENTR-701 IN HSA-LR MICE

ENTR-701 treatment reduced nuclear foci and CUG-repeat expansion transcript level and corrected aberrant splicing in HSA-LR mice 7 days post-dose

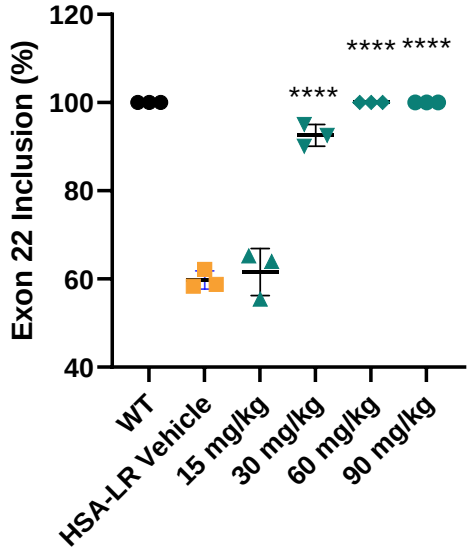
Nuclear Foci Reduction



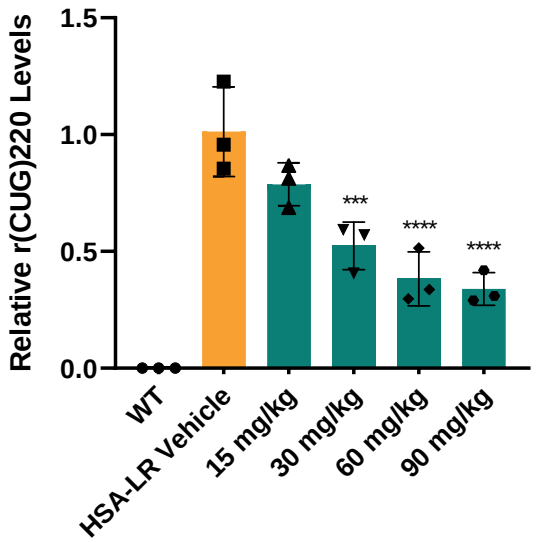
Tibialis anterior section



Atp2a1 Splicing Correction



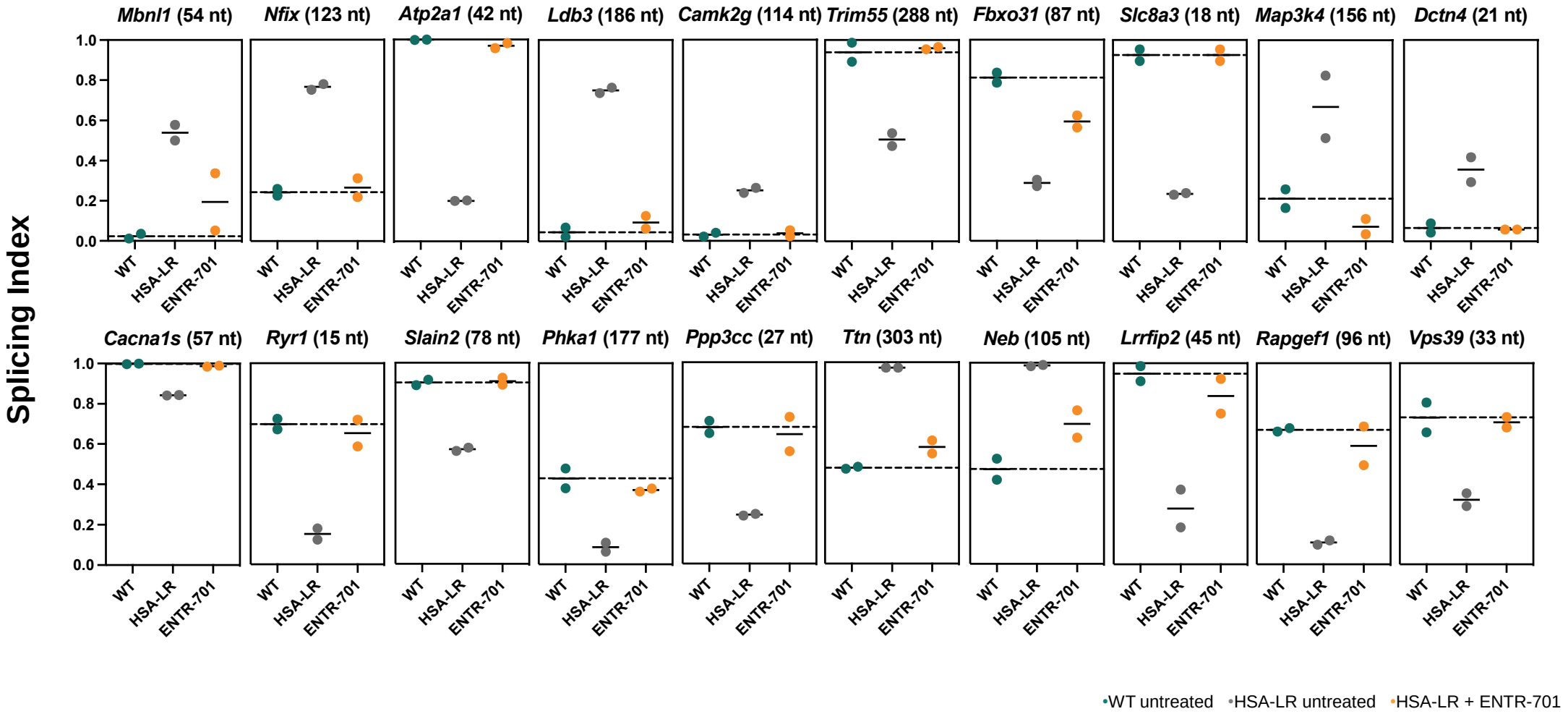
HSA-r(CUG)220 Reduction



- 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.

ENTR-701 CORRECTED SPLICEOPATHY IN HSA-LR MICE

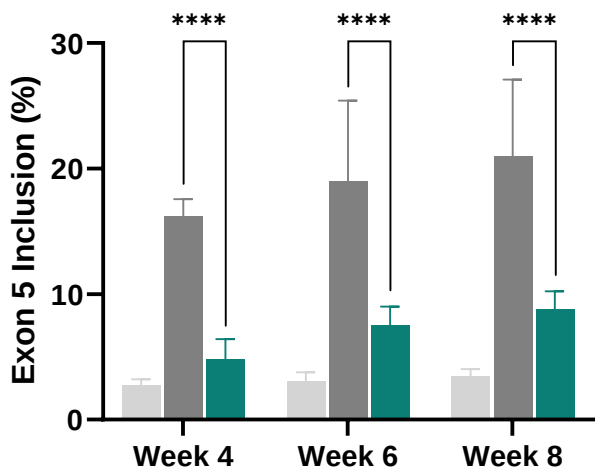


DM1-affected splicing events analyzed by RNA-seq; **ENTR-701** is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV

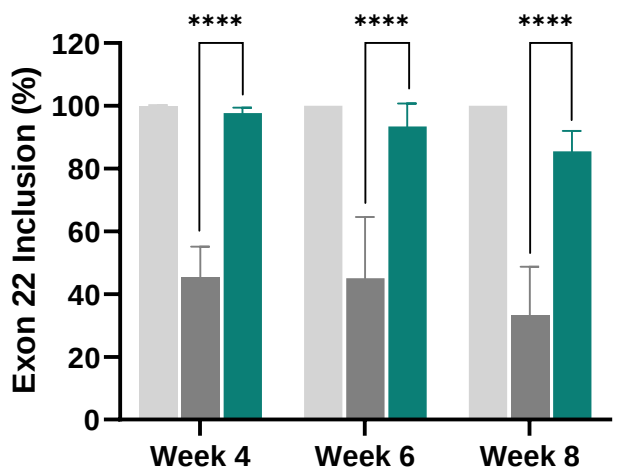
DURABILITY OF ENTR-701 IN HSA-LR MICE

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

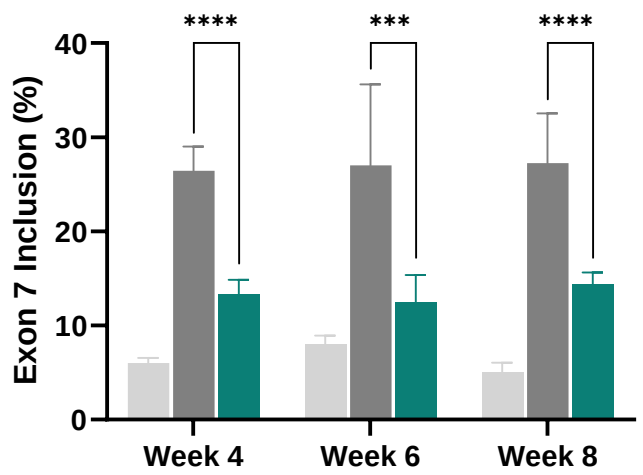
Mbnl1 Exon 5 Inclusion



Atp2a1 Exon 22 Inclusion



Nfix Exon 7 Inclusion



- 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**

ACKNOWLEDGEMENTS

entrada THERAPEUTICS

Thank you!

ERIC T. WANG
LABORATORY

UF UNIVERSITY of FLORIDA