entrada THERAPEUTICS

Endosomal Escape Vehicle (EEV™) Platform for Enhanced Functional Delivery of Oligonucleotides

Ziqing "Leo" Qian, PhD
Co-Founder & Vice President, Discovery Research

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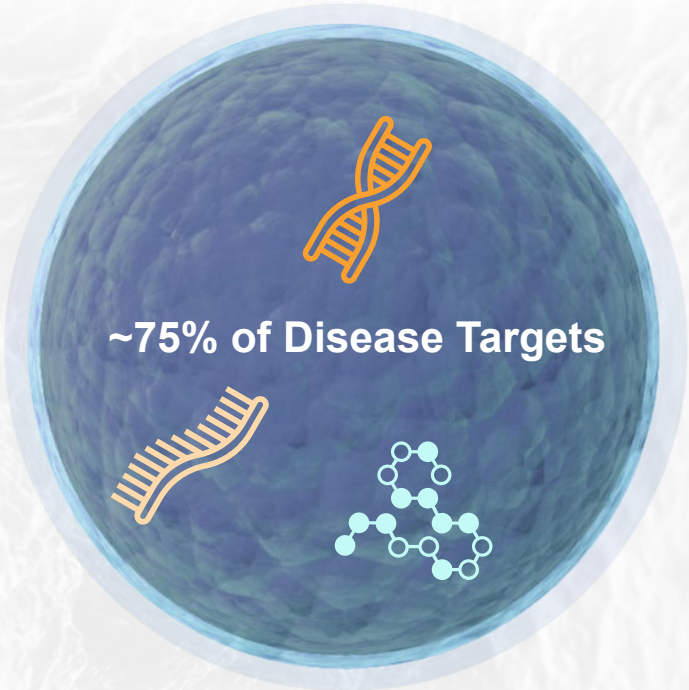
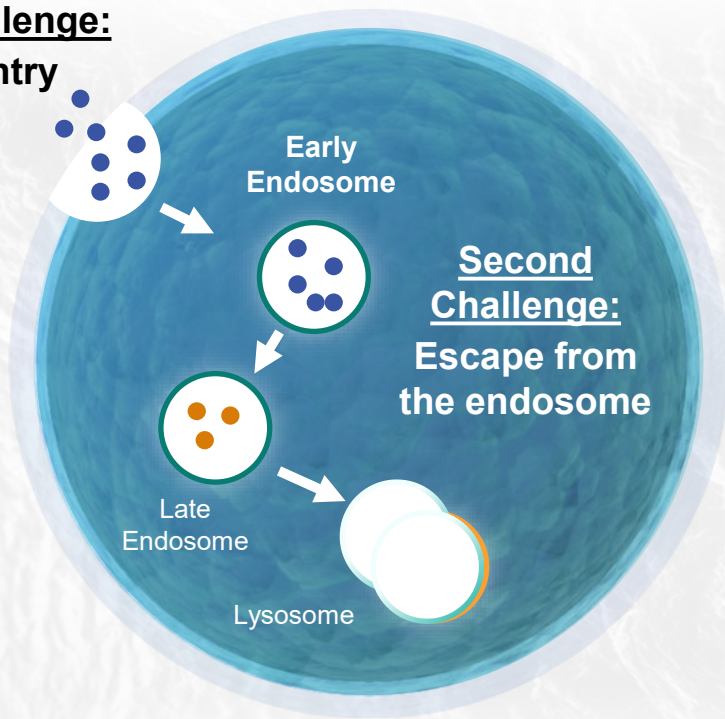
ENTRADA'S MISSION

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



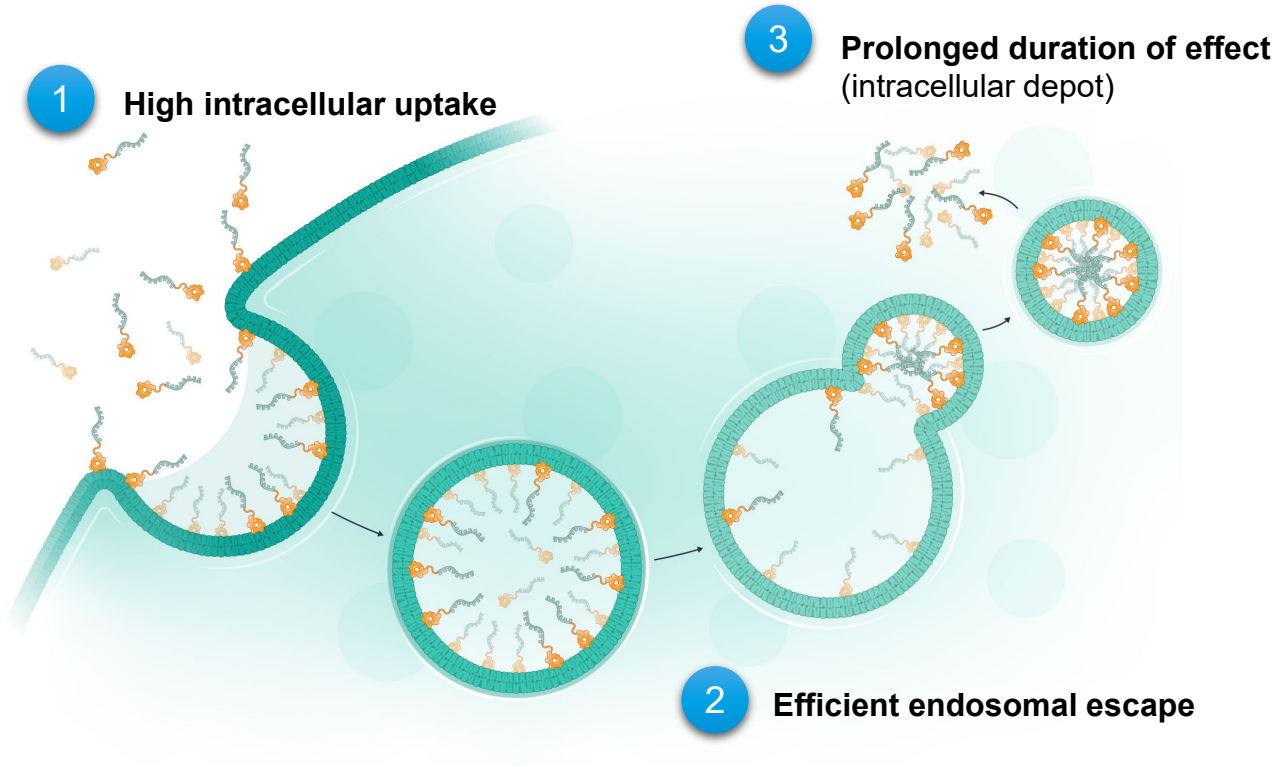


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

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

- Cyclic structure enhances proteolytic stability
- Small and cyclic structure may reduce immunogenicity risk
- Mechanism of internalization conserved across species
- Scalable and efficient peptide synthesis

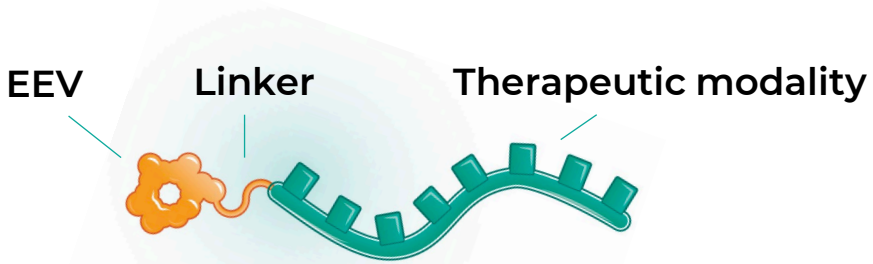


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

FUNCTIONAL DELIVERY FOR TARGET TISSUES

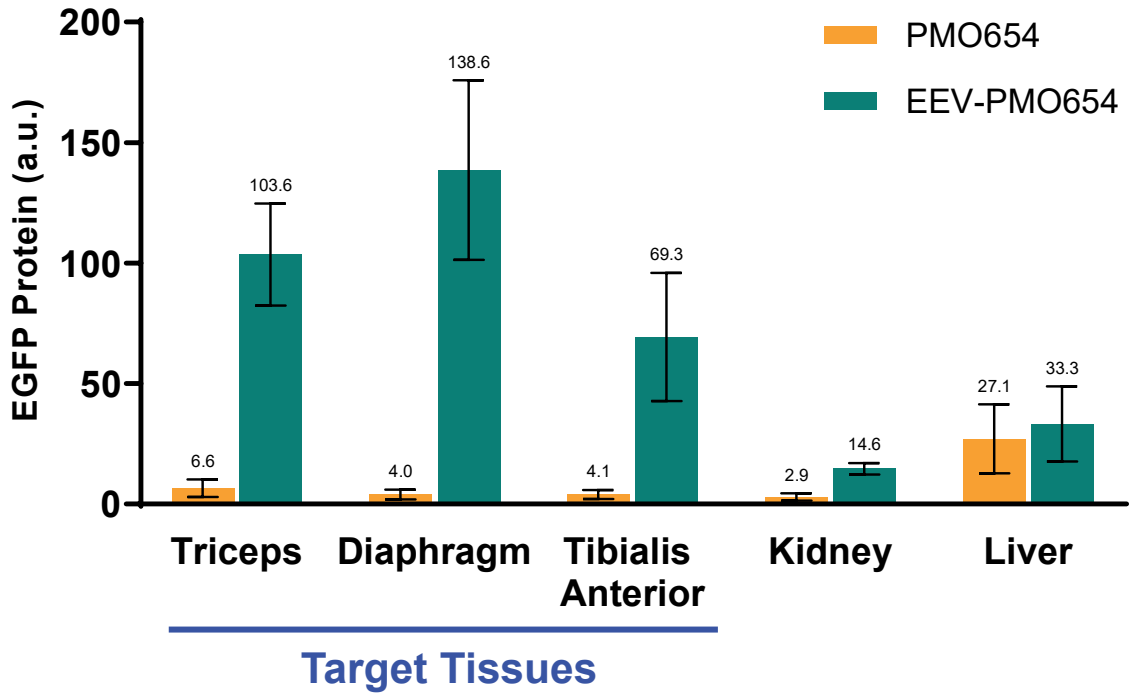
Entrada's EEV-therapeutics can be designed to enhance functional delivery to target tissues

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



PMO, phosphorodiamidate morpholino oligomer; **PMO654**, splicing switching PMO targeting nt654; **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.); 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

+

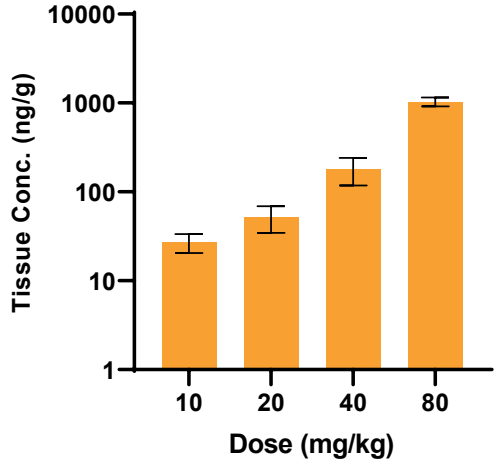
Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

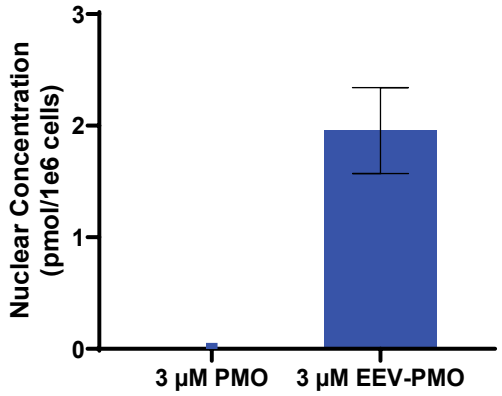
=

Pharmacodynamic Outcome

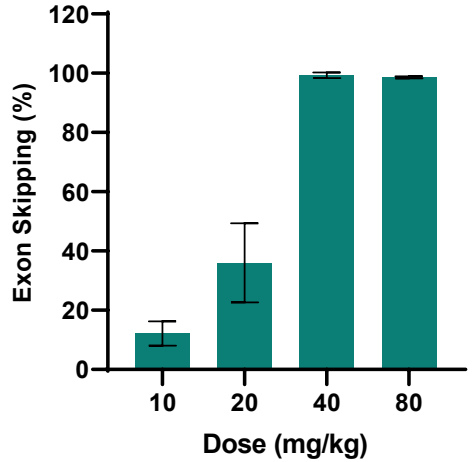
- ✓ Rapid, dose-dependent efficacy
- ✓ Duration of at least 12 weeks



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



24-hour incubation



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

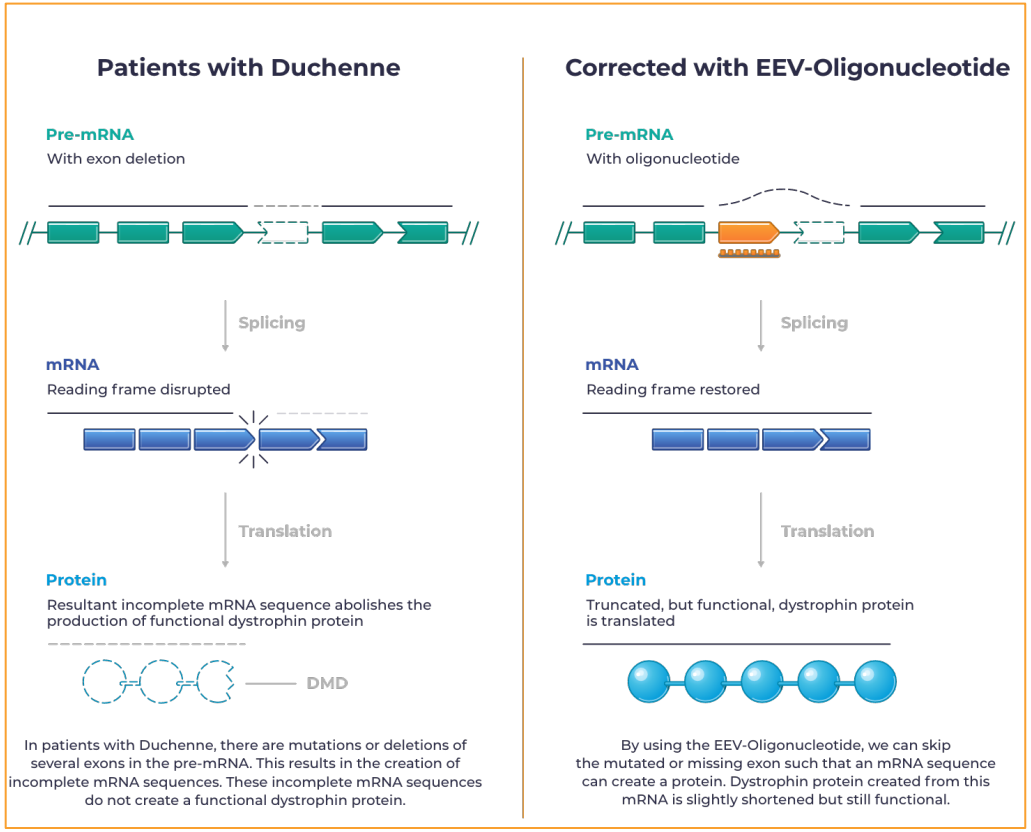
hDMD mice express full-length human dystrophin gene. **p.i.** post injection; shown as mean ± standard deviation.

DUCHENNE MUSCULAR DYSTROPHY (DMD)

DMD OVERVIEW

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

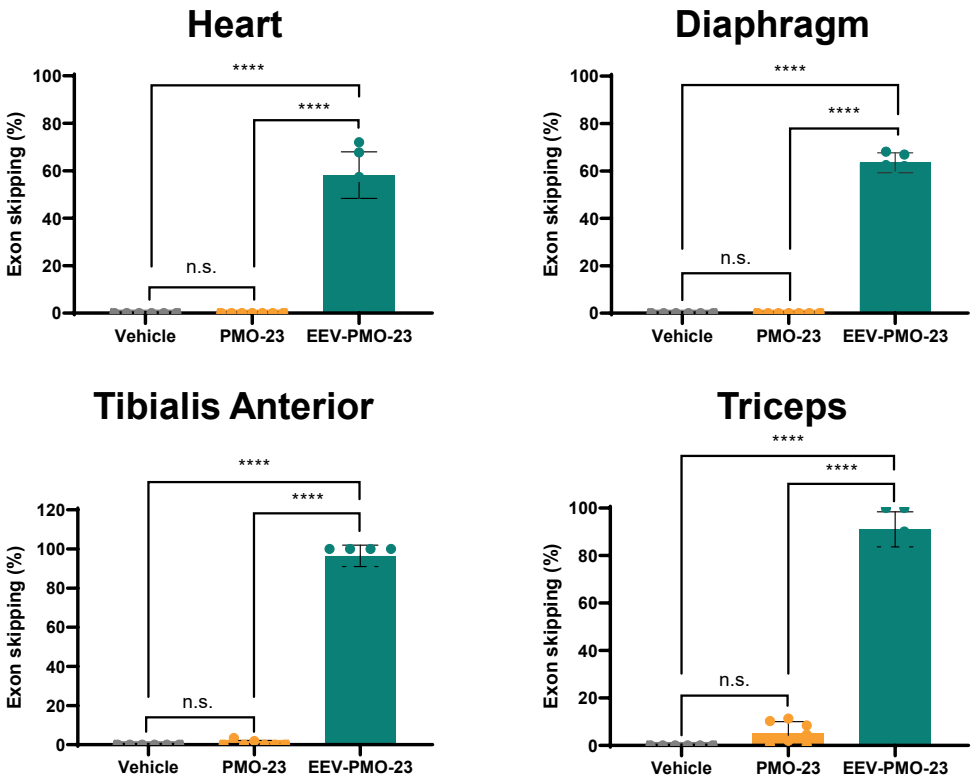
- DMD is caused by mutations in the *DMD* gene that encodes for dystrophin[1]
- Progressive muscle degeneration, wasting and paralysis generally lead to death via **respiratory and/or cardiac failure**[2]
- Exon skipping therapeutics (for exons 45, 51 and 53) using **PMO chemistry** were approved based 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

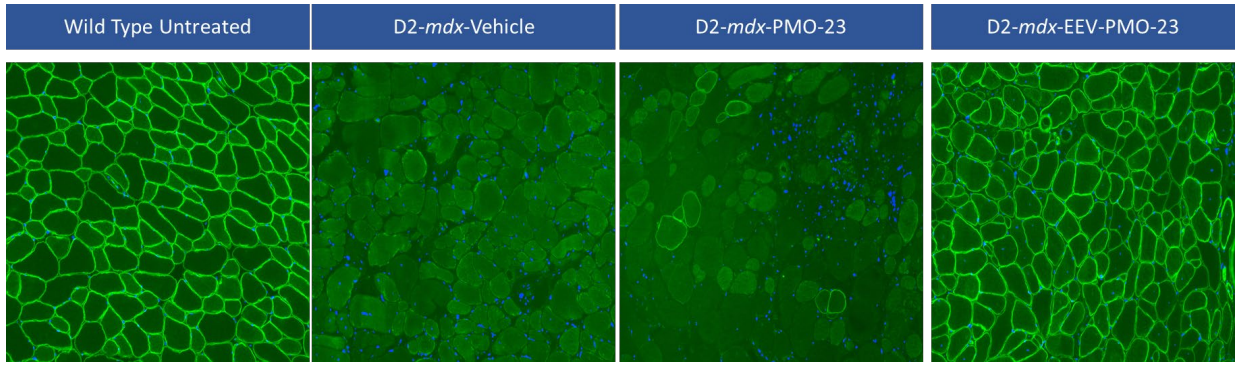
REPEAT EEV-PMO TREATMENT IN D2-*mdx* MICE

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

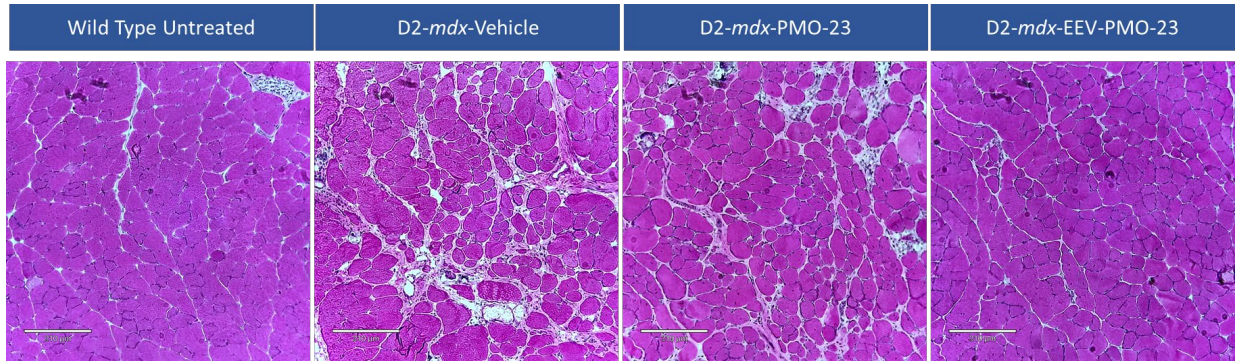


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



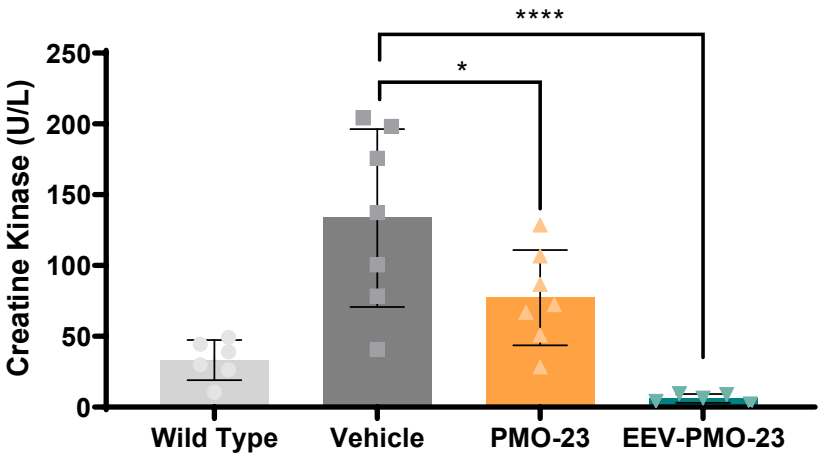
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose

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.

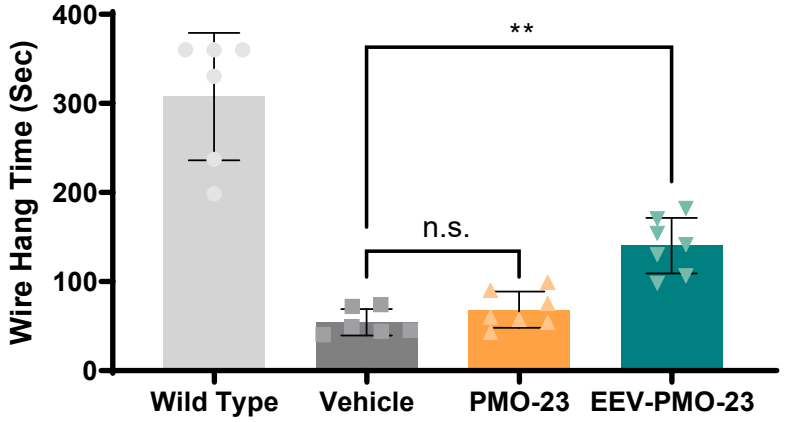
REPEAT EEV-PMO TREATMENT RESULTS IN CK CORRECTION AND FUNCTIONAL IMPROVEMENT

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

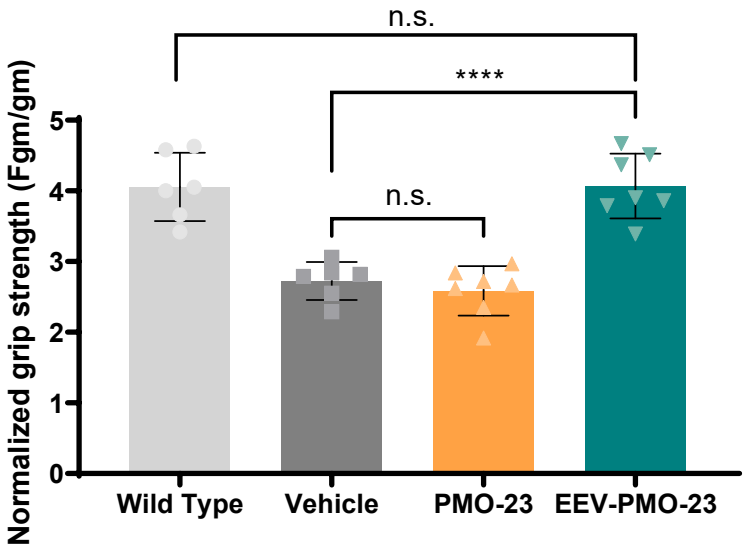
Serum CK Levels



Wire Hang Time



Grip Strength



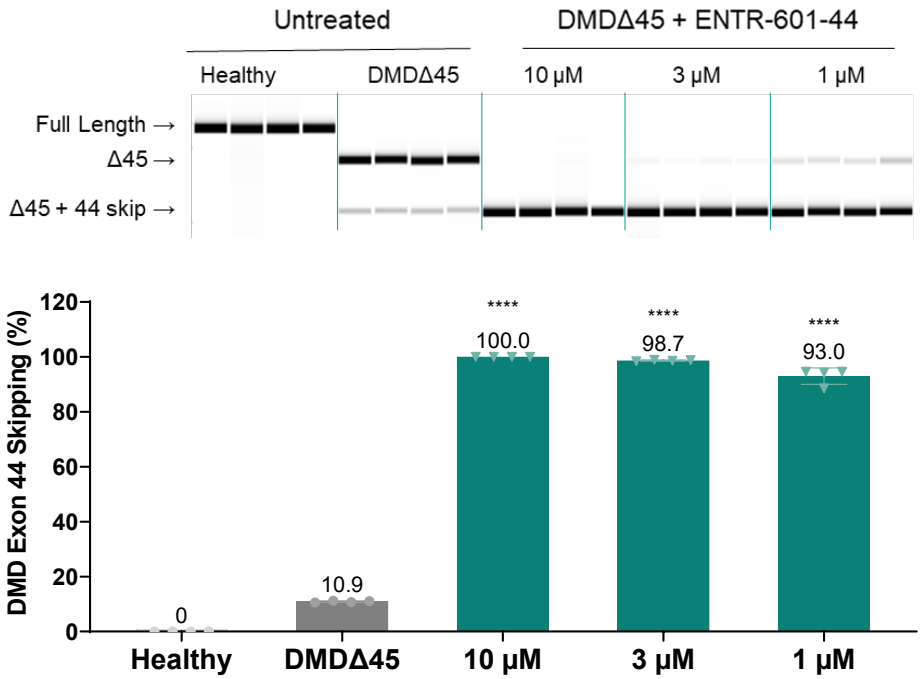
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~2 weeks after the last dose

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

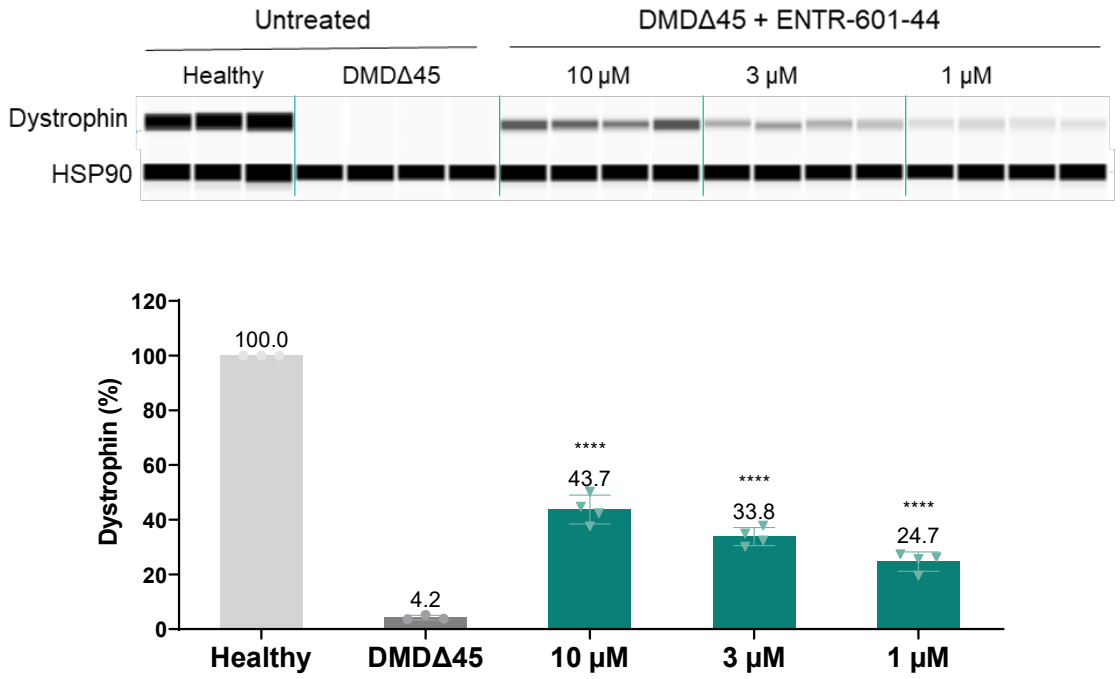
ENTR-601-44 IN PATIENT-DERIVED MYOTUBES

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



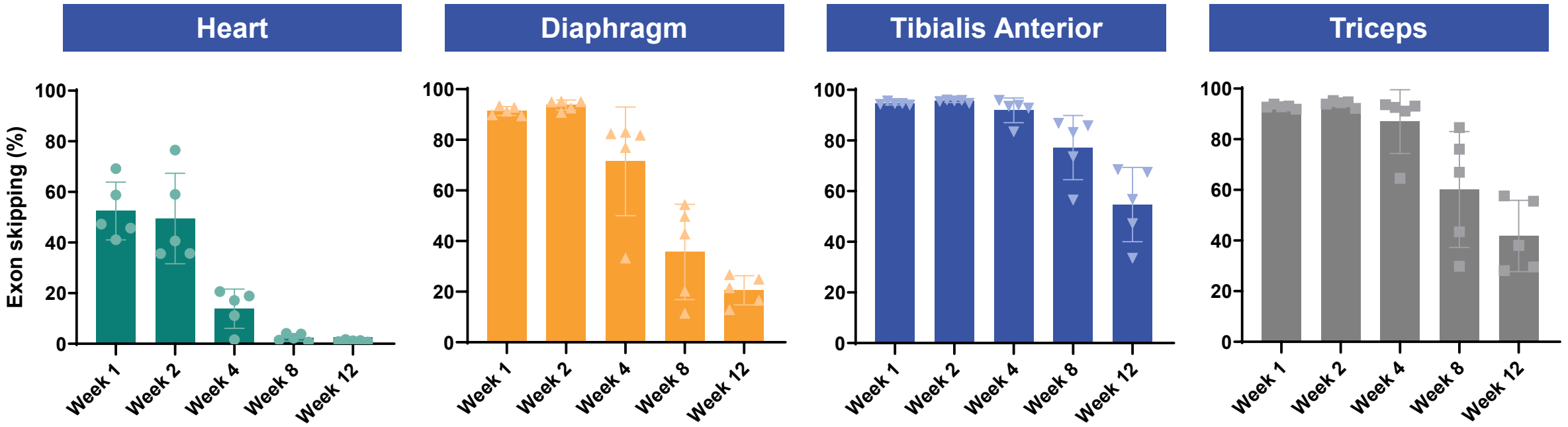
Dystrophin Protein Production



DMDΔ45, immortalized myoblasts from DMD patients harboring an out-of-frame exon 45 deletion and further differentiated into myotubes**; ENTR-601-44** is the clinical candidate selected for DMD exon 44 skipping EEV-oligonucleotide conjugate; ****p<0.0001; shown as mean ± standard deviation.

ENTR-601-44 IN hDMD MICE

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



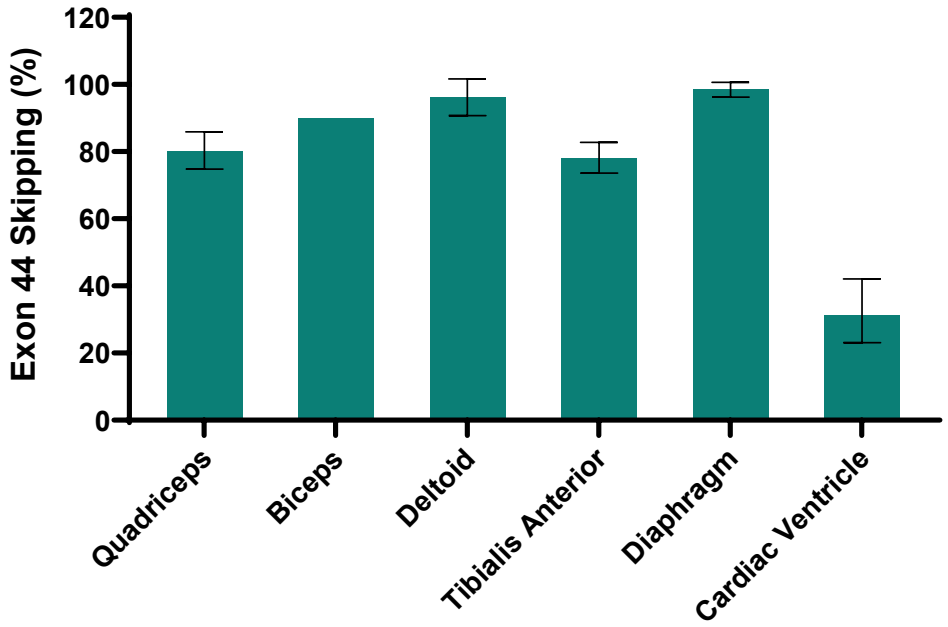
- Post single IV dosage of 60 mg/kg (PMO equivalent), robust exon skipping was observed in ENTR-601-44 treated hDMD mice

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 IN NHP

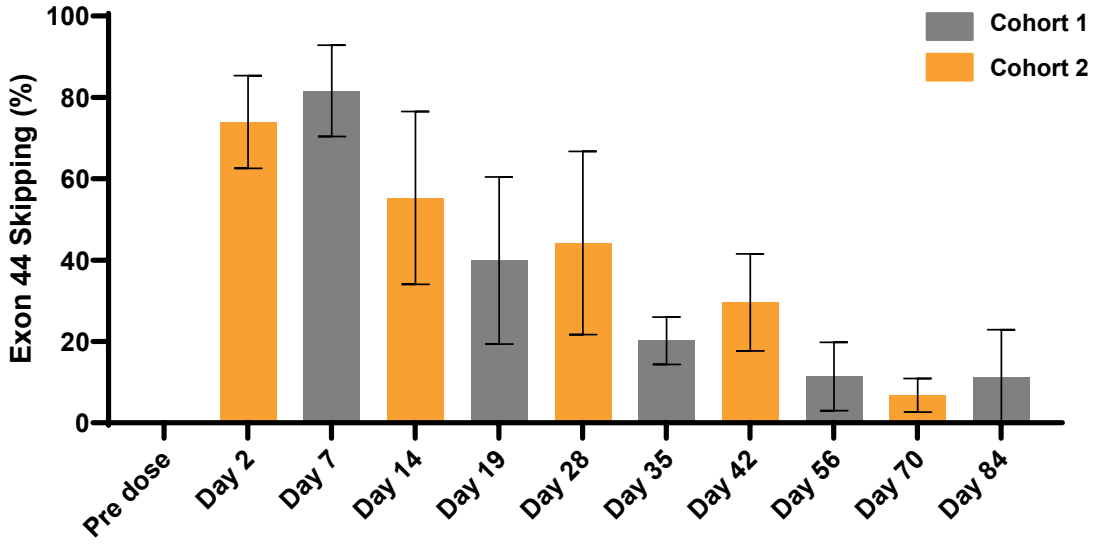
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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.

SUMMARY AND PATH FORWARD FOR DMD PROGRAMS

Entrada's DMD preclinical data are consistent across different model systems and represent a robust set of translational data

- Studies in the D2-*mdx* model of DMD demonstrate that our EEV-PMO approach produces **durable exon skipping and dystrophin production in both cardiac and skeletal muscle**
  - Restoration of cardiac and skeletal **muscle integrity**, as well as **functional improvements** are also observed further validating our EEV-PMO approach in DMD
- **ENTR-601-44** produces robust **exon skipping in both cardiac and skeletal muscle** of mice and NHP
  - A single dose of ENTR-601-44 produces **durable exon skipping for at least 12 weeks post-dose**

Path Forward for DMD Clinical Programs

- **ENTR-601-44** candidate selected with IND submission planned in **Q4 2022**
- DMD exon 45 candidate selection is ongoing

D2-*mdx* is a DMD mouse model with a nonsense mutation in *Dmd* exon 23; **NHP**, non-human primates; **ENTR-601-44** is the clinical candidate selected for DMD and is an exon 44 skipping EEV-oligonucleotide conjugate

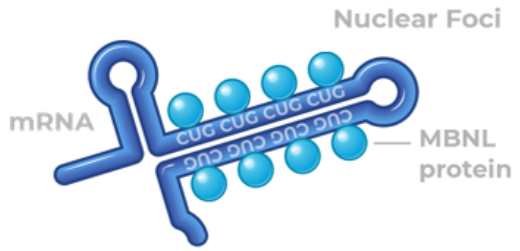
MYOTONIC DYSTROPHY TYPE 1 (DM1)

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

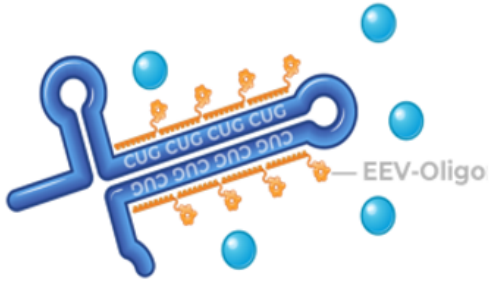
- **DM1 occurs in 1:8,000 people worldwide and affects ~40,000 patients in US and over 50,000 in Europe**
  - 75% of patients have adult-onset DM1
  - Multisystemic: including myotonia, muscle weakness and atrophy, cardiac and pulmonary complications, cataracts and endocrine dysfunction
  - **Currently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

Mutant *DMPK* mRNA



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

EEV-Oligonucleotide Approach



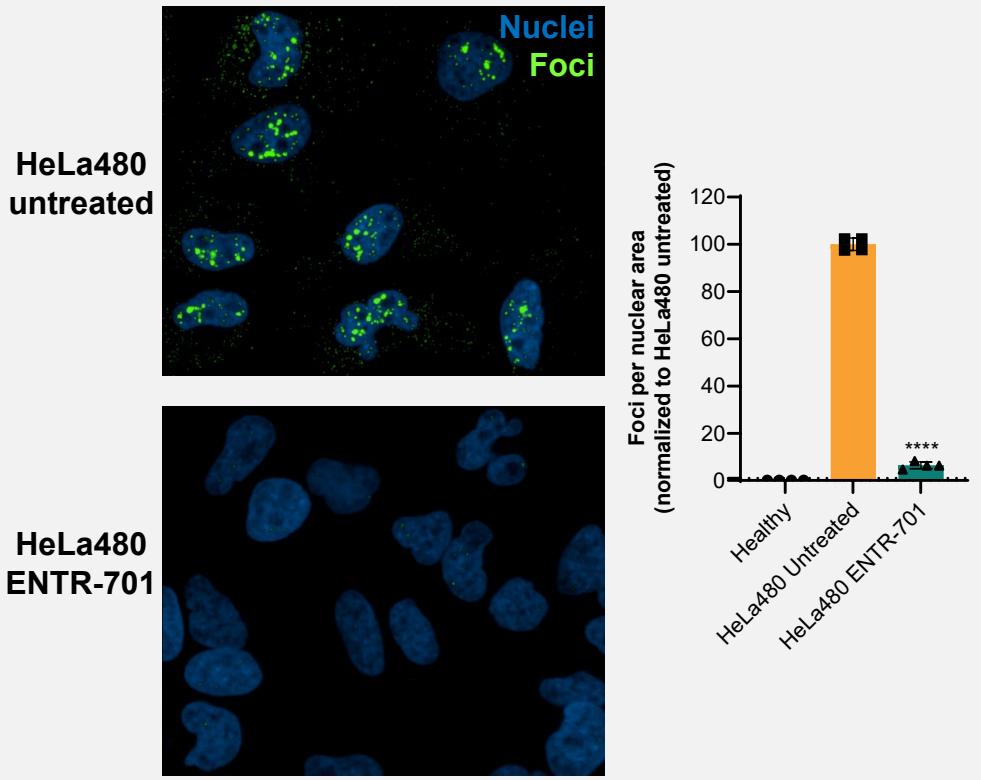
- Reduced nuclear foci
- Selective mRNA reduction
- Normal MBNL function
- Corrected spliceopathy

Thornton C.A. *Neurol. Clin.* 2014, 705. Philips, A.V. et al. *Science* 1998; Pettersson, O.J. *NAR* 2015; Wagner, S.D. et al. *PLoS Genet.* 2016; Tanner, M. et al. *Nucleic Acids Res.* 2021; Mulders, S.A.M. et al. *Proc. Natl. Acad. Sci.* 2009.

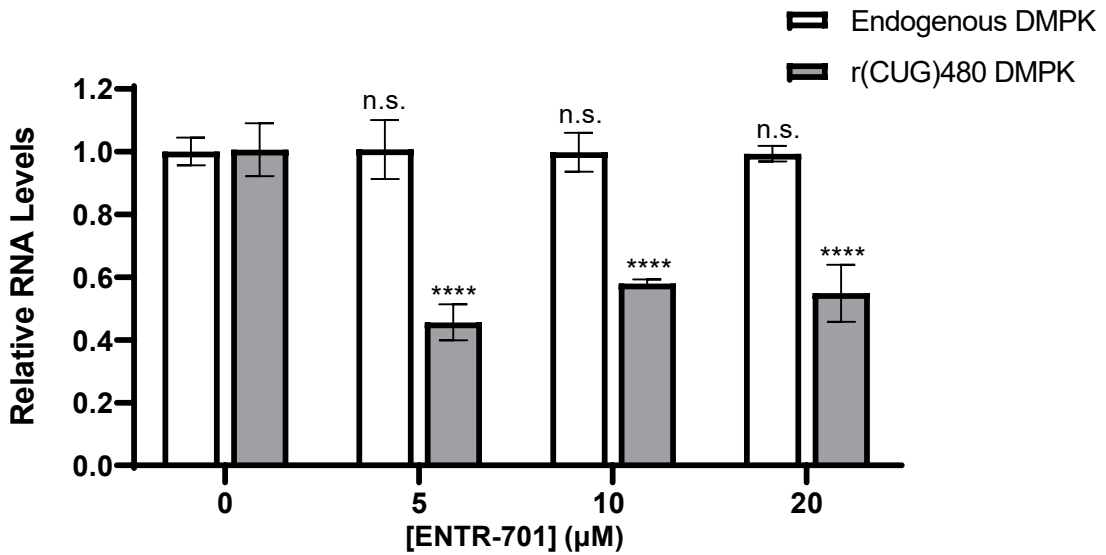
ENTR-701 IN DM1 MODEL CELL LINE WITH REPEAT EXPANSION

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.

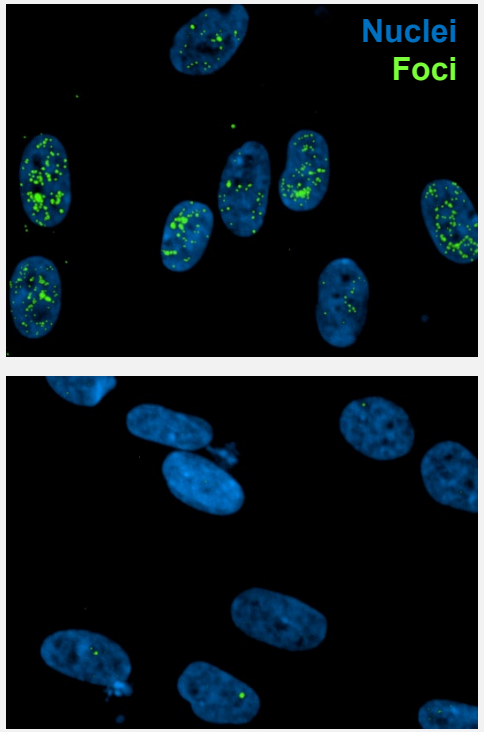
ENTR-701 IN DM1 PATIENT-DERIVED MYOTUBES

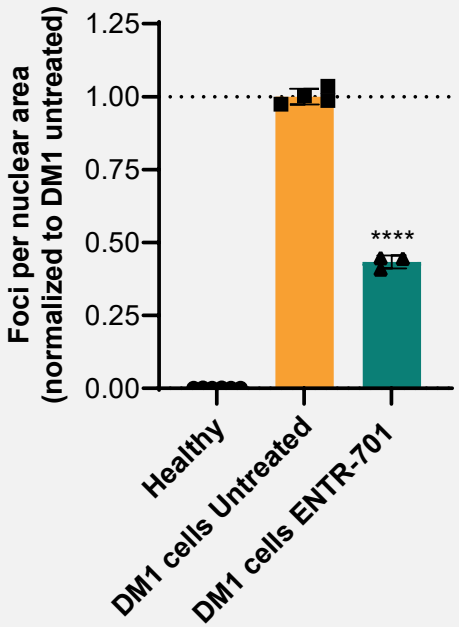
ENTR-701 treatment in DM1 patient-derived muscle cells resulted in significant nuclear foci reduction and correction of aberrant splicing

Nuclear Foci Reduction

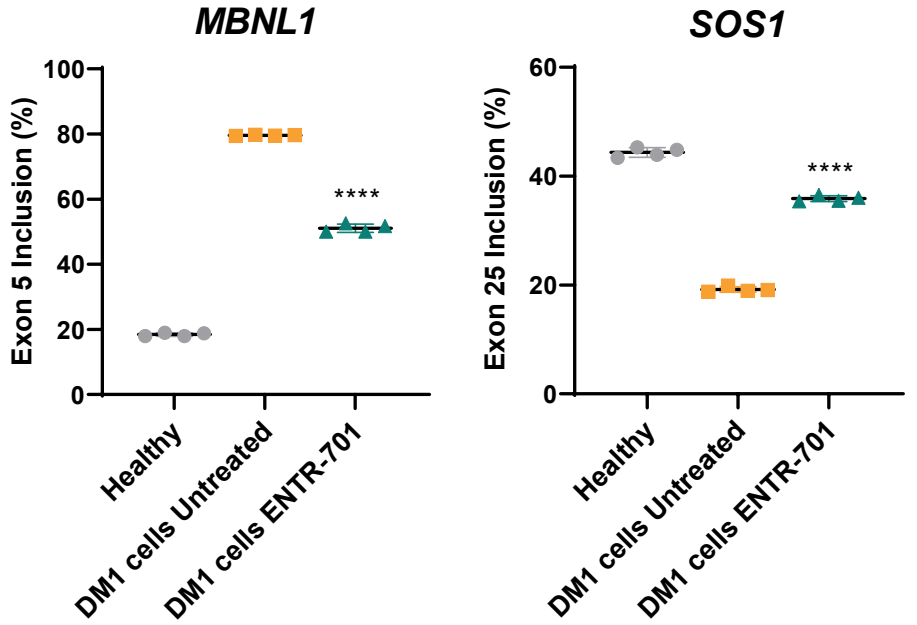
DM1 cells untreated

DM1 cells ENTR-701





Correction of Aberrant Splicing



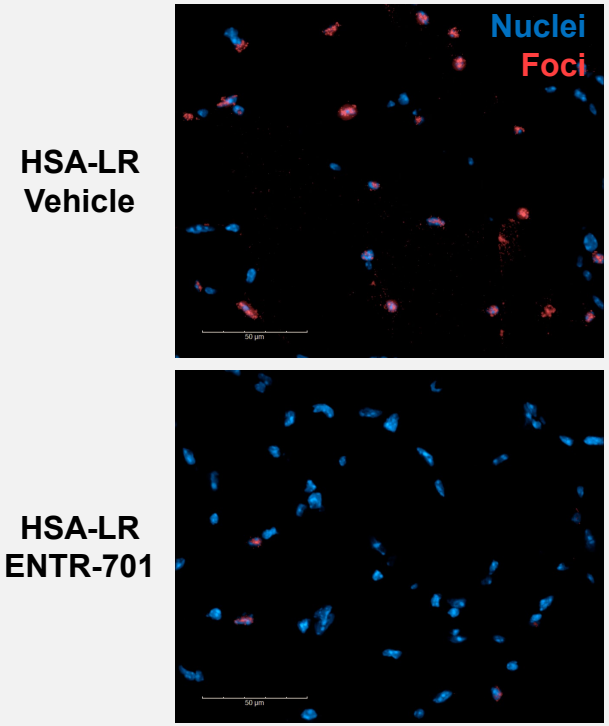
- Immortalized DM1 patient-derived (2,600 CUG repeats) muscle cells[1] were treated with ENTR-701 and analyzed for the reduction of nuclear foci and the correction of aberrant splicing

[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.

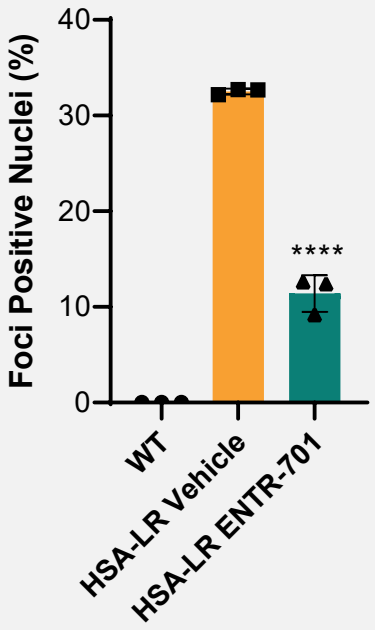
EFFICACY OF ENTR-701 IN HSA-LR MICE

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

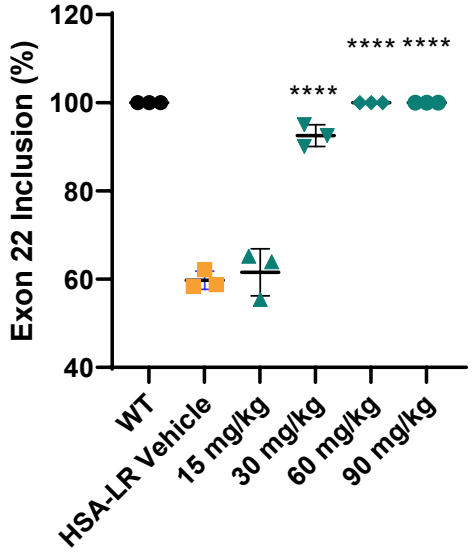
Nuclear Foci Reduction



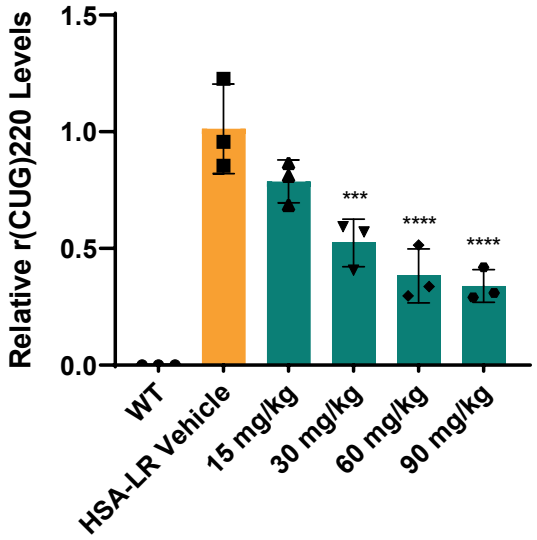
Tibialis anterior section



Atp2a1 Splicing Correction



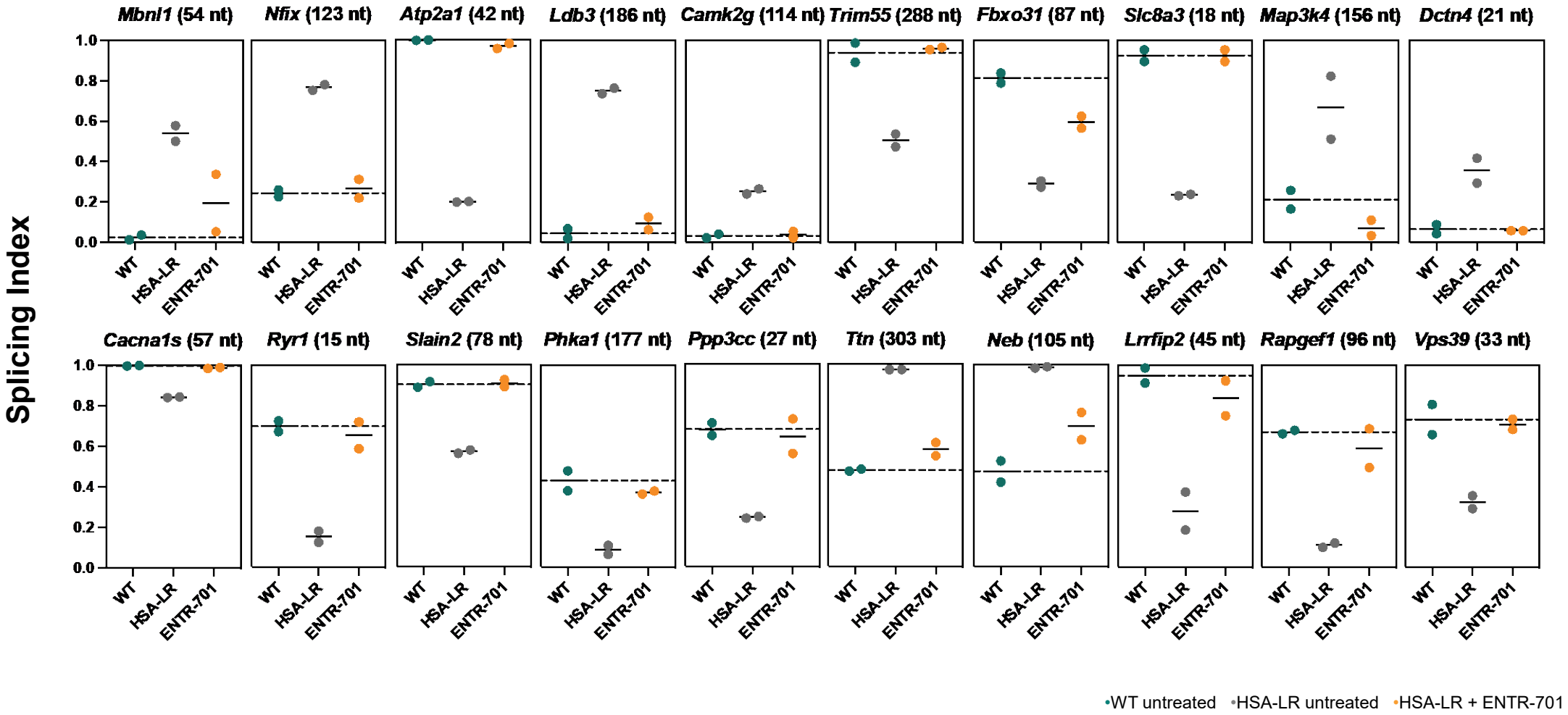
HSA-r(CUG)220 Reduction



- 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

[1]**HSA-LR** model, carrying a transgene resulting in CUG repeat expansion and recapitulates DM1 phenotype and molecular pathology (Mankodi, A. et al. *Science* 2000); **ENTR-701** is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV; ***p<0.001, ****p<0.0001, shown as mean ± standard deviation.

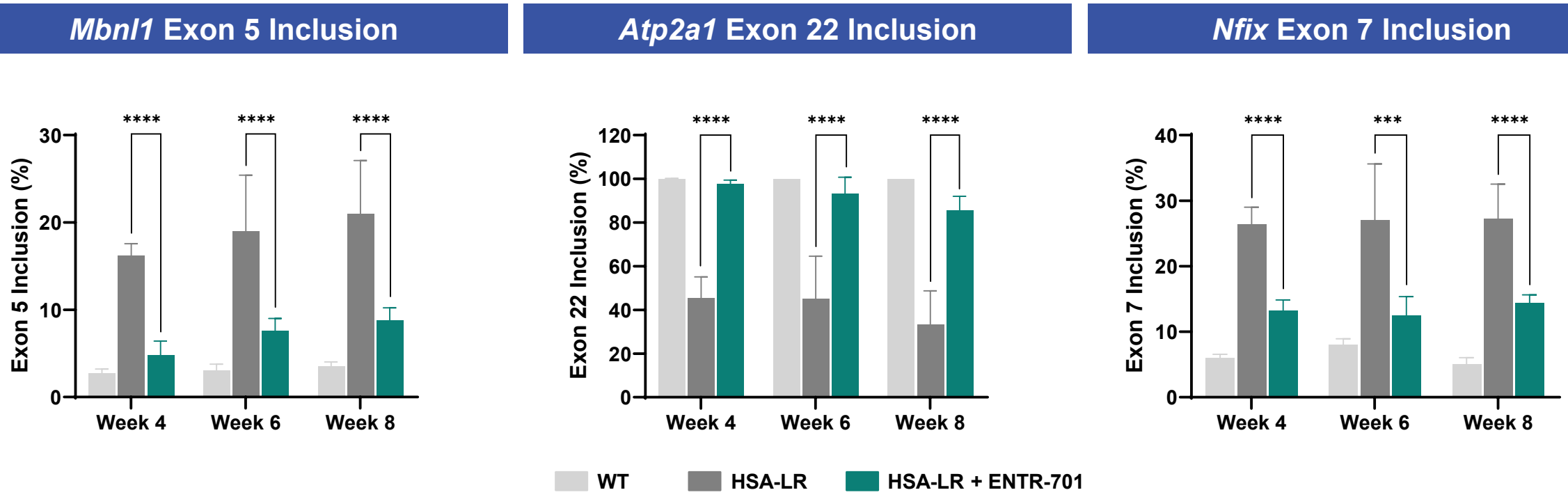
ENTR-701 CORRECTED SPLICEOPATHY IN HSA-LR MICE



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



- 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

ENTR-701 is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV; ***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**

NOTE: this was a video during the TIDES presentation

ENTR-701 is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV

SUMMARY AND PATH FORWARD FOR DM1 PROGRAM

Entrada's DM1 preclinical data are consistent across different model systems, leading to selection of ENTR-701 as the DM1 clinical candidate

- Our DM1 clinical candidate, **ENTR-701**, reduces nuclear foci and CUG-repeat expansion containing transcript levels, leading to **corrected aberrant splicing** in the HeLa480 cell model, DM1 patient derived cells, as well as HSA-LR mouse model of DM1
- A single dose of ENTR-701 demonstrates **durable splicing correction and amelioration of myotonia** for at least 8 weeks post-dose

Path Forward for DM1 Clinical Program

- **ENTR-701** candidate selected with IND submission planned in **2023**

HSA-LR model, carrying a transgene resulting in CUG repeat expansion; **ENTR-701** is a CUG-repeat blocking PMO conjugated to EEV

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

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entrada THERAPEUTICS

Thank you!

ERIC T. WANG
LABORATORY

UF UNIVERSITY of FLORIDA