EEV-Conjugated PMO Results in Nuclear Foci Reduction and Aberrant Splicing Correction in DM1 Cell and Animal Models

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Disclosure

- I am an employee of Entrada Therapeutics, the sponsor of this research

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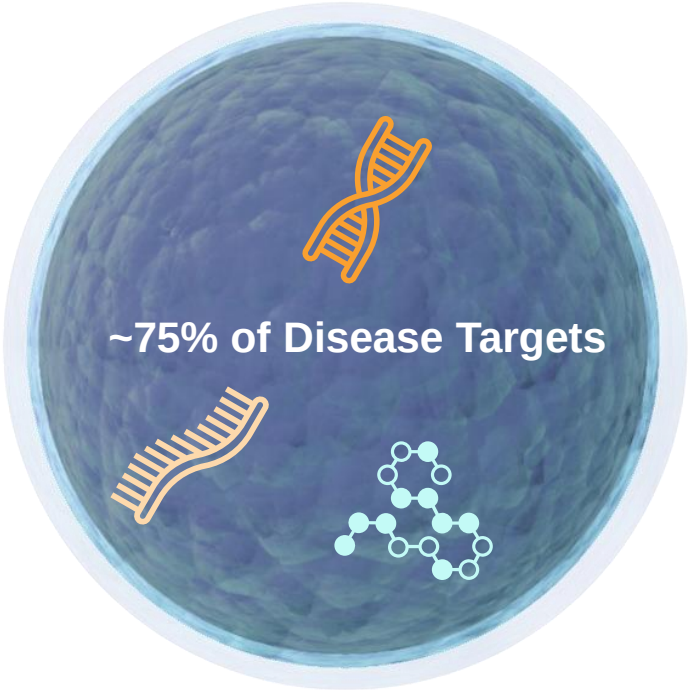
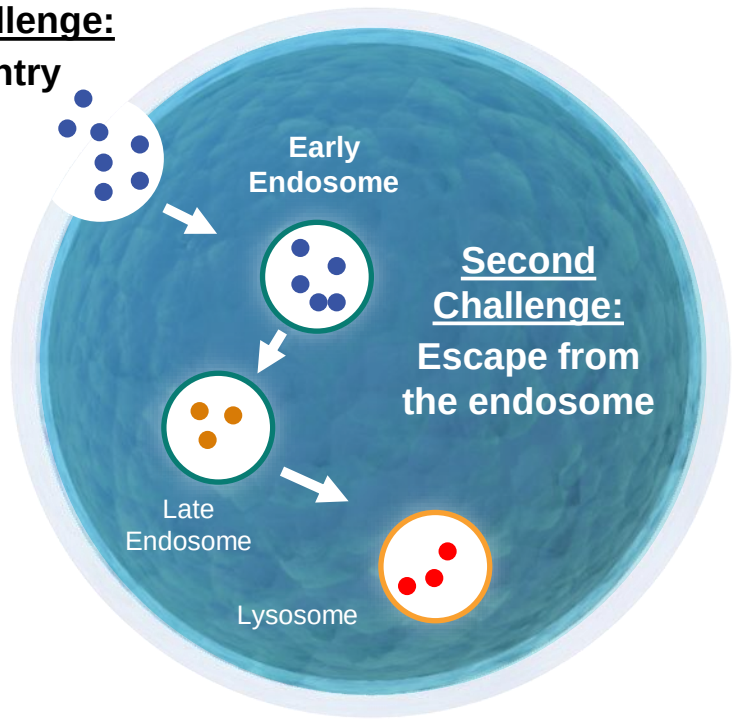
ENTRADA'S MISSION

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

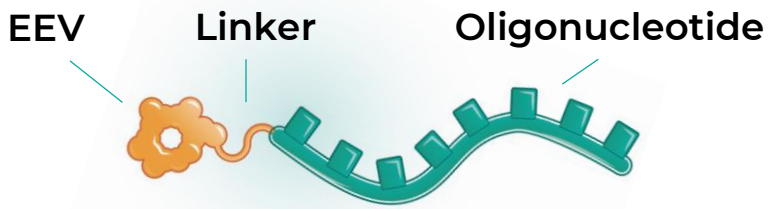




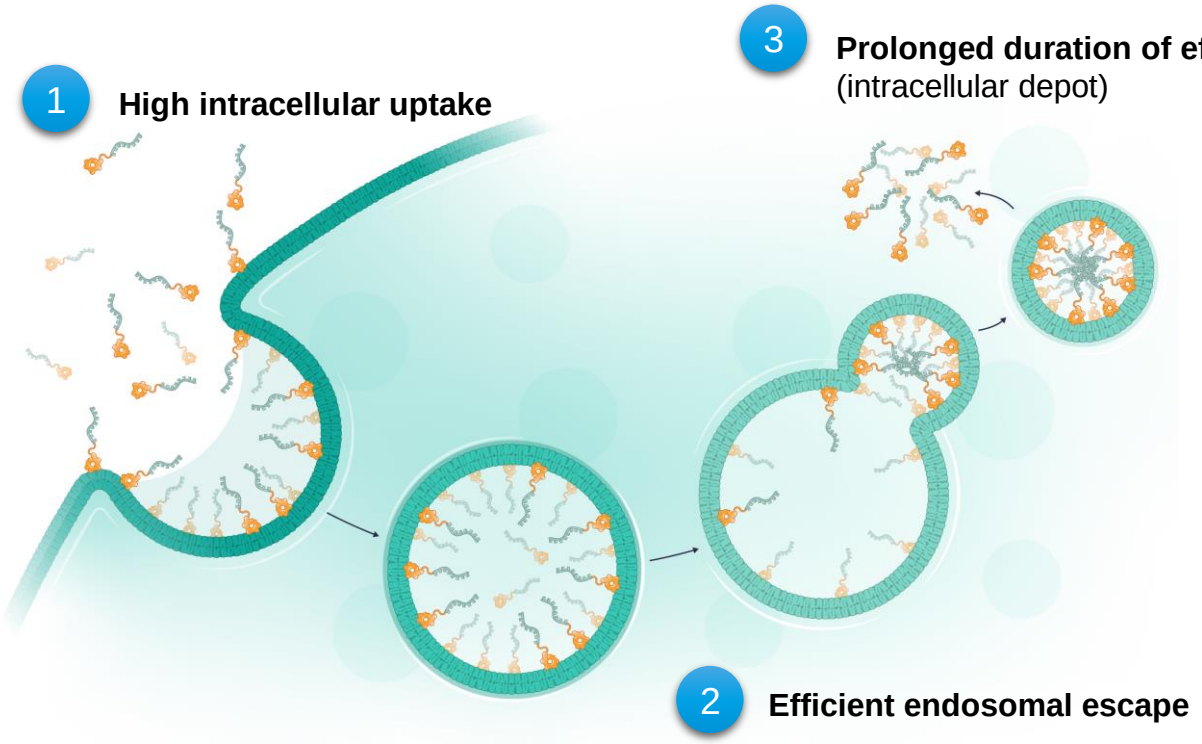
The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

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



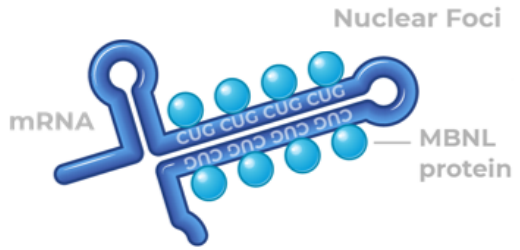
Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

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

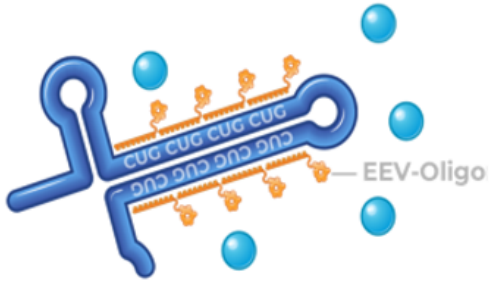
- **DM1 occurs in 1:8,000 people worldwide and affects ~40,000 patients in US and over 50,000 in Europe**
  - 75% of patients have adult-onset DM1
  - Multisystemic: including myotonia, muscle weakness and atrophy, cardiac conduction abnormalities, pulmonary complications, cataracts, and endocrine dysfunction[1]
  - **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



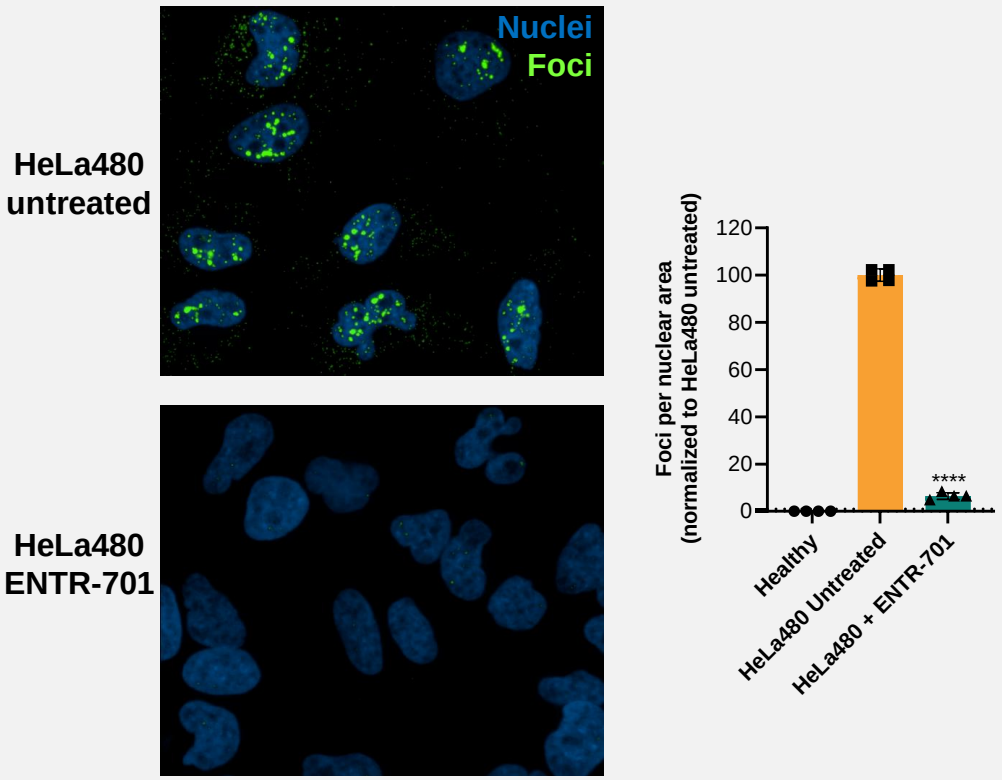
- Reduced nuclear foci
- Selective mRNA reduction
- Normal MBNL function
- Corrected spliceopathy

[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.

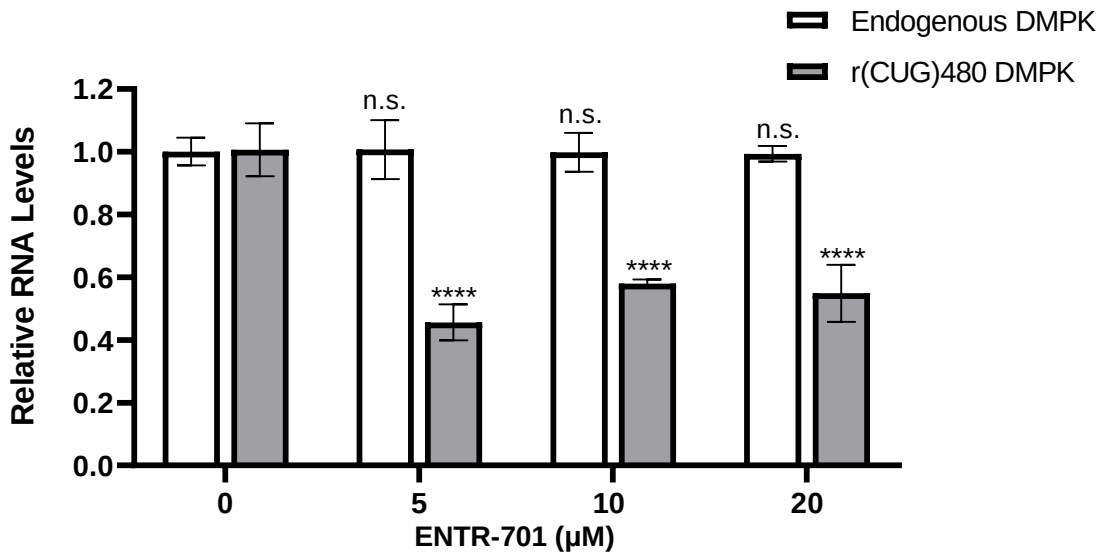
ENTR-701 IN DM1 CELL LINE WITH REPEAT EXPANSION

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 vs. untreated cells (left) or endogenous DMPK (right); shown as mean ± standard deviation.

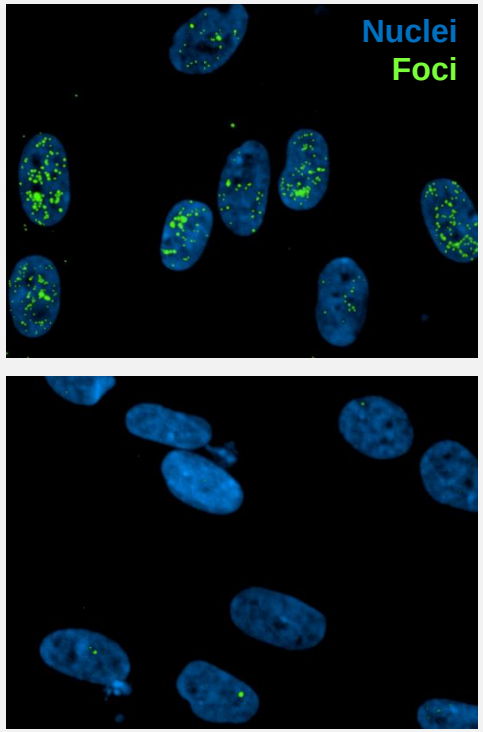
ENTR-701 IN DM1 PATIENT-DERIVED MYOTUBES

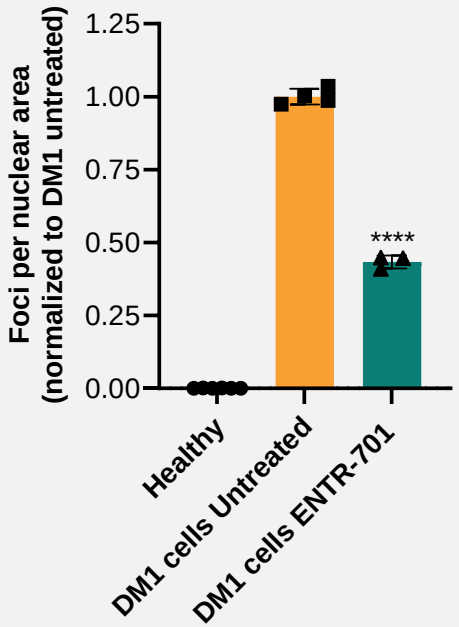
ENTR-701 treatment in DM1 patient-derived muscle cells resulted in significant nuclear foci reduction and correction of aberrant splicing

Nuclear Foci Reduction

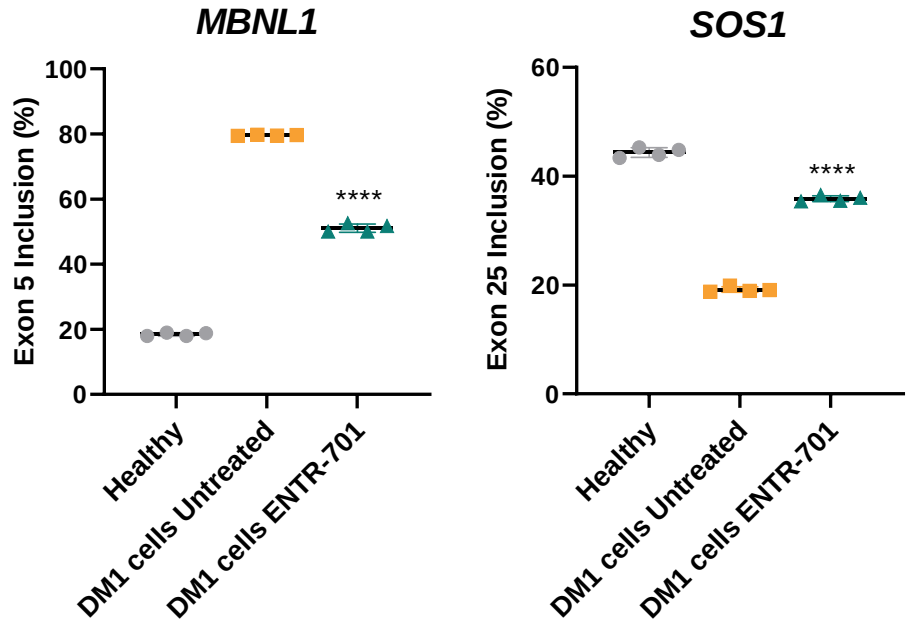
DM1 cells untreated

DM1 cells ENTR-701





Correction of Aberrant Splicing



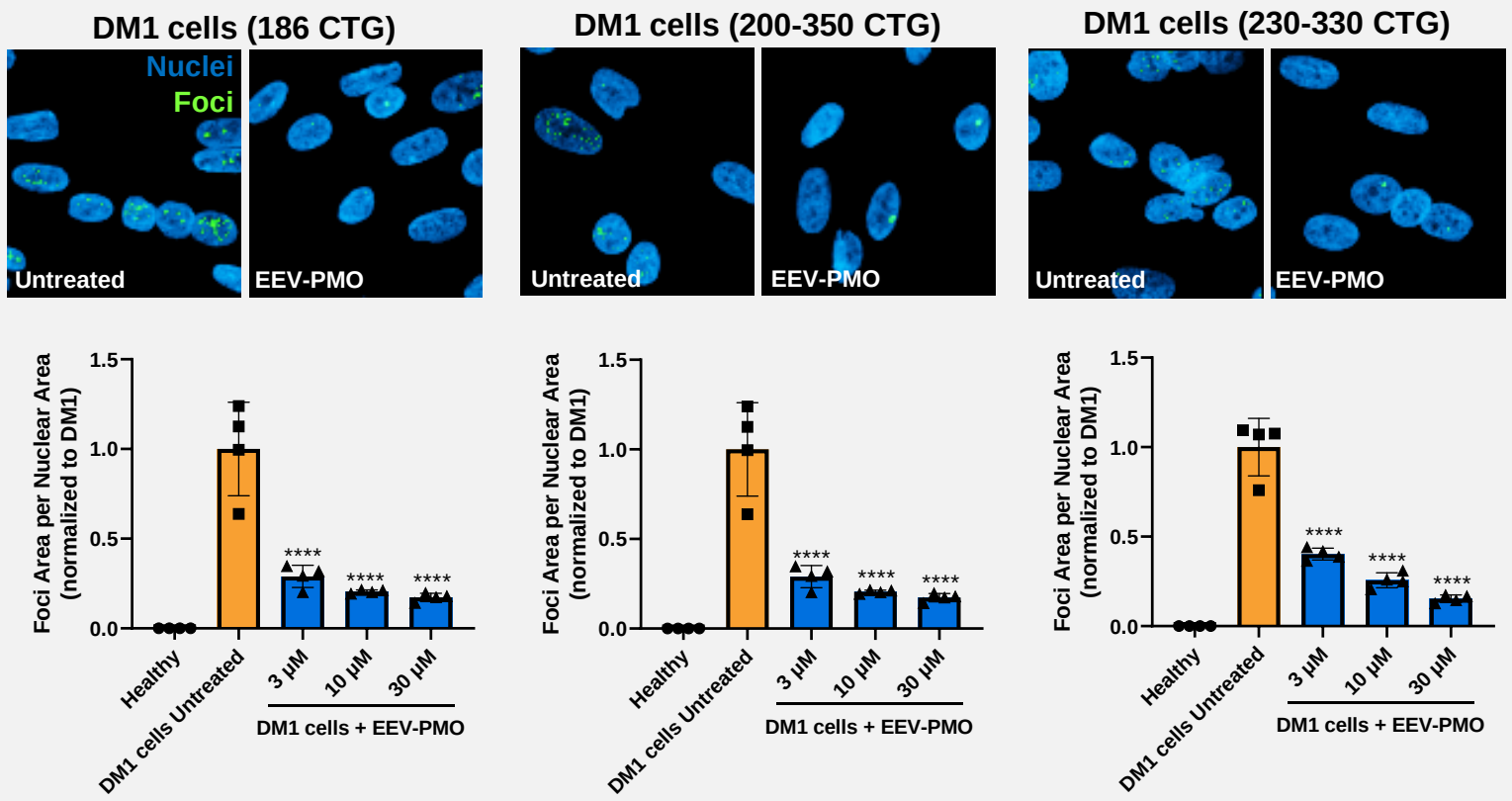
- Immortalized DM1 patient-derived (2,600 CUG repeats) muscle cells[1] were treated with ENTR-701 and analyzed for the reduction of nuclear foci and the correction of aberrant splicing

[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.

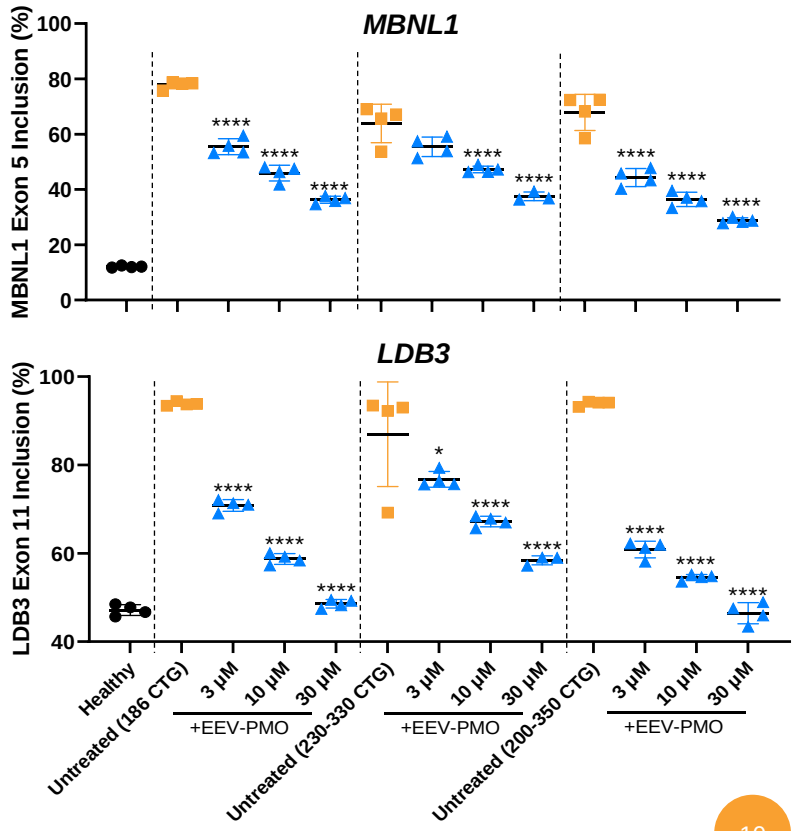
EEV-PMO EFFICACY IN PRIMARY DM1 PATIENT CELLS

Treatment of primary DM1 patient-derived cell lines with a CUG-blocking EEV-PMO conjugate confirmed foci reduction and correction of aberrant splicing regardless of CTG repeat number

Nuclear Foci Reduction



Correction of Aberrant Splicing

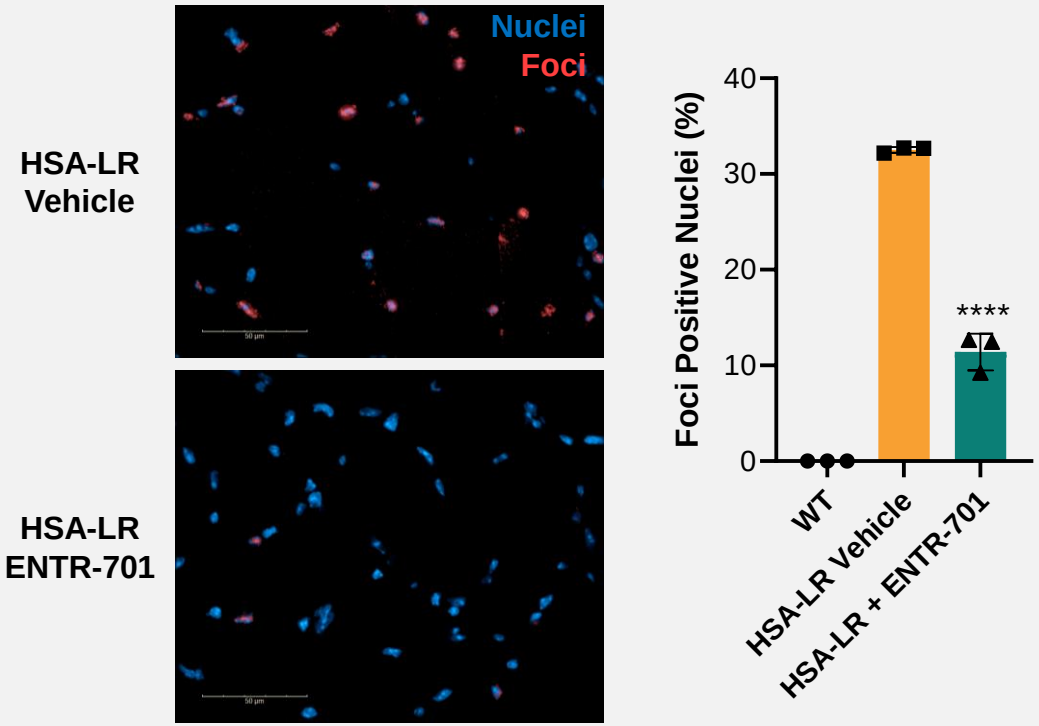


Primary DM1 patient-derived muscle cells were treated with an EEV-PMO conjugate and analyzed for the reduction of nuclear foci and the correction of aberrant splicing.
EEV-PMO is composed of CUG-repeat blocking PMO conjugated our EEV platform; ****p<0.0001, *p<0.05 for EEV-PMO compared to untreated; shown as mean ± standard deviation.

EFFICACY OF ENTR-701 IN HSA-LR MICE

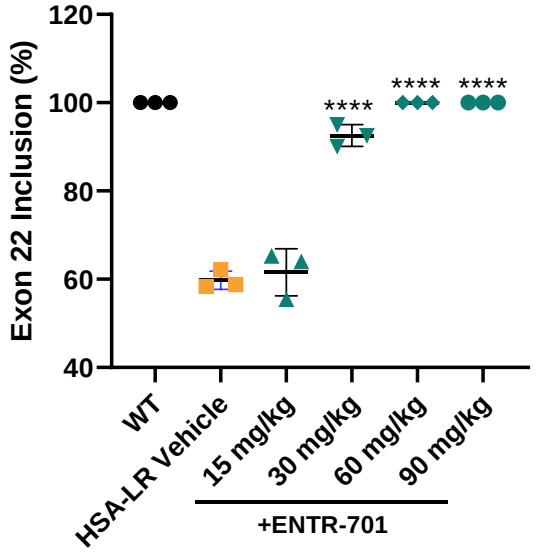
ENTR-701 treatment reduced nuclear foci and CUG-repeat expansion transcript level and corrected aberrant splicing in HSA-LR mice

Nuclear Foci Reduction

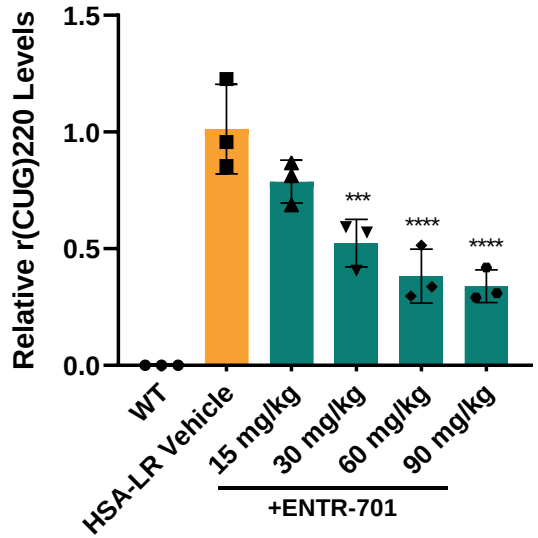


Tibialis anterior section

Atp2a1 Splicing Correction



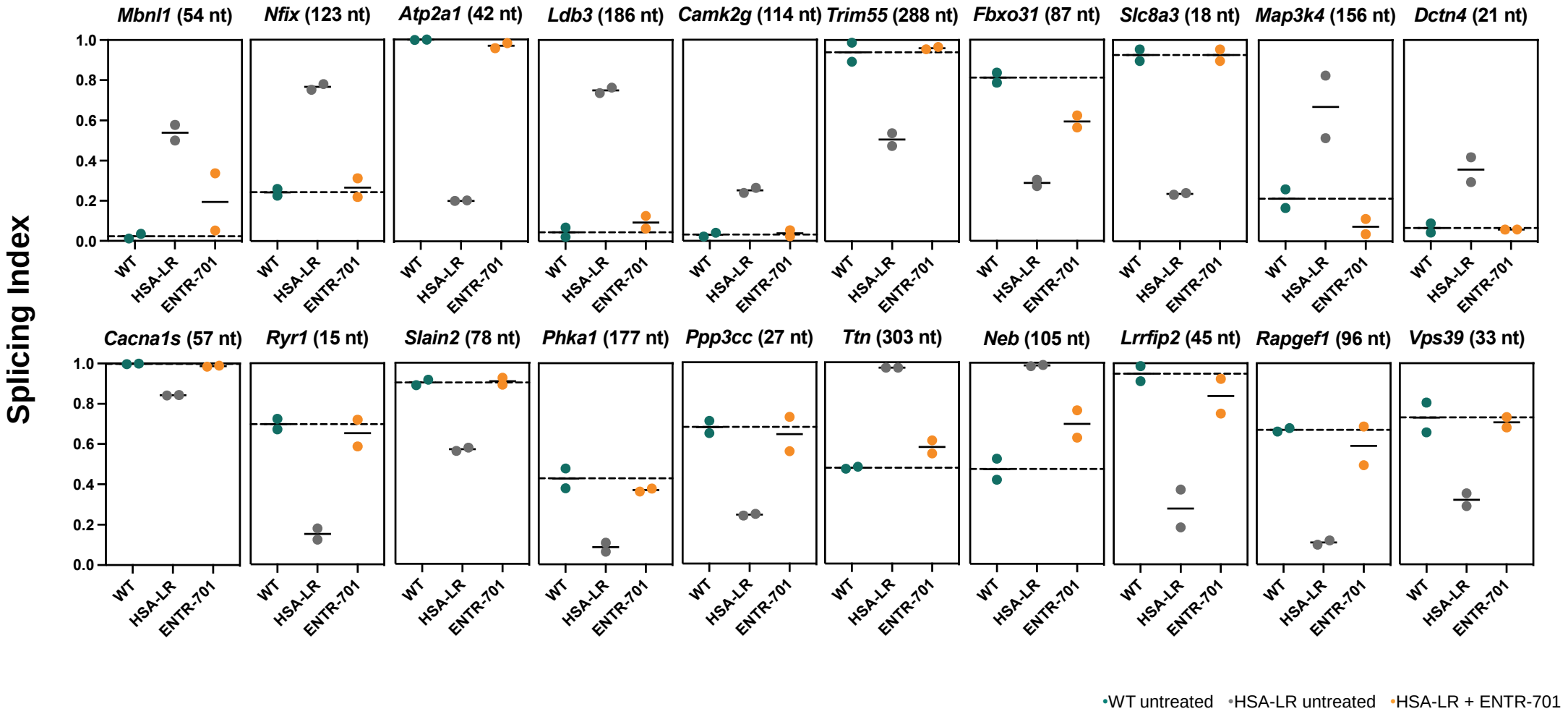
HSA-r(CUG)220 Reduction



- 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** mice carry 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. **WT**, wild type.

ENTR-701 CORRECTED SPLICEOPATHY IN HSA-LR MICE

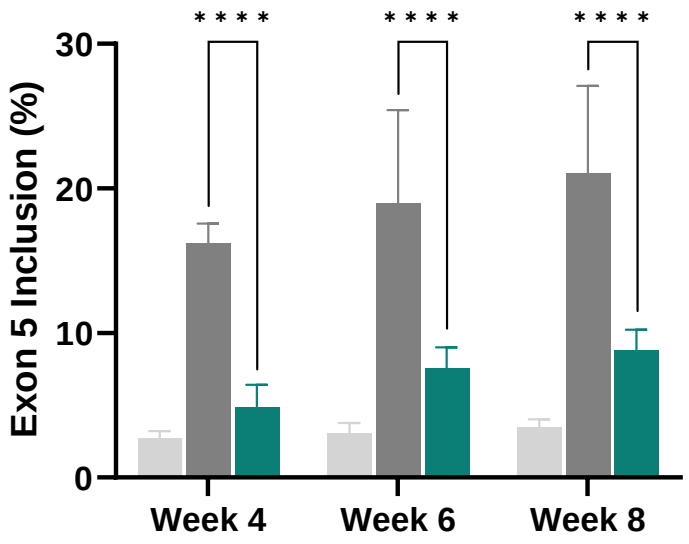


DM1-affected splicing events analyzed by RNA-seq; **ENTR-701** is the clinical candidate selected for DM1, composed of CUG-repeat blocking PMO conjugated to EEV

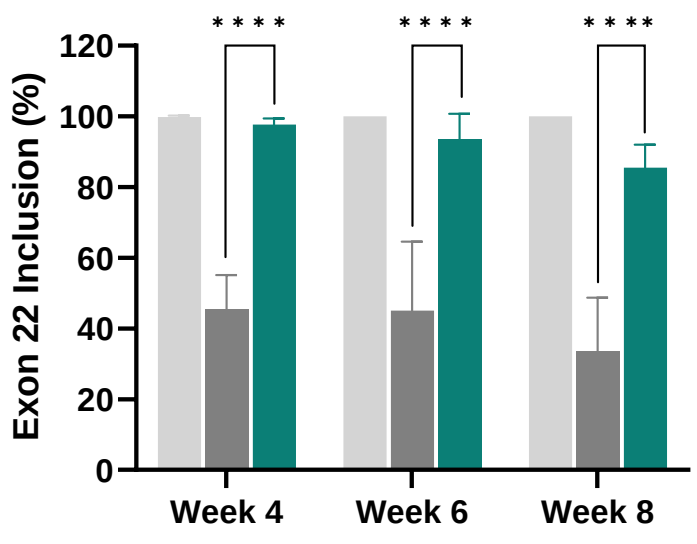
DURABILITY OF ENTR-701 IN HSA-LR MICE

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

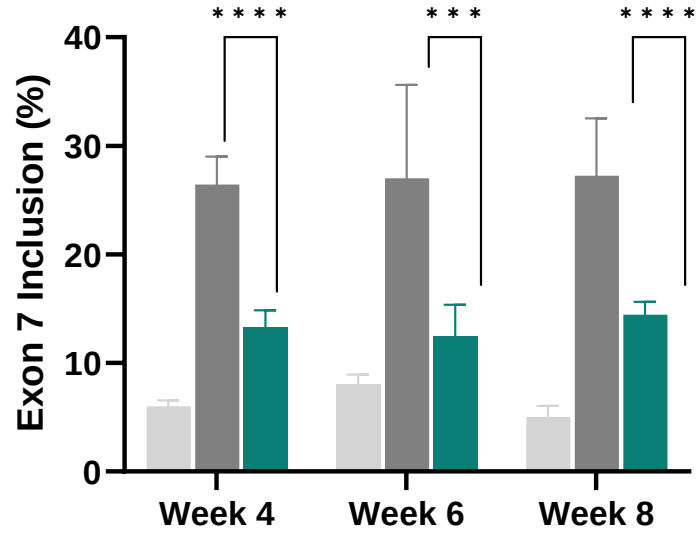
Mbnl1 Exon 5 Inclusion



Atp2a1 Exon 22 Inclusion



Nfix Exon 7 Inclusion



Wild type | HSA-LR + Vehicle | HSA-LR + ENTR-701

- 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**

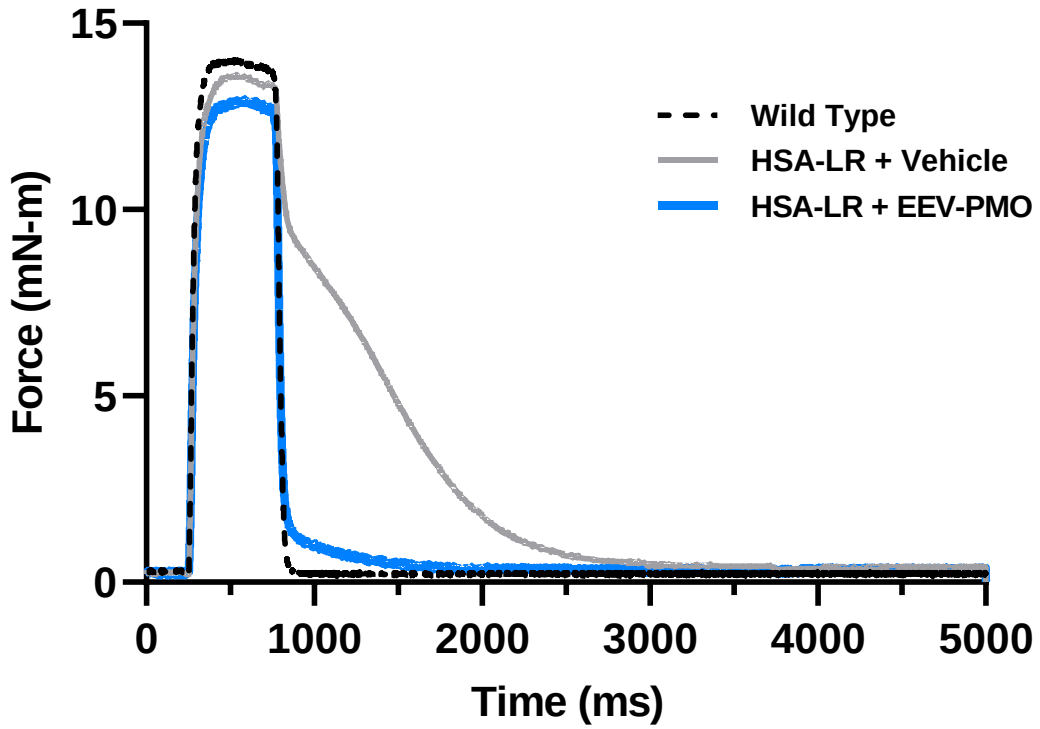
HSA-LR Mouse: **ENTR-701 Treated**

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

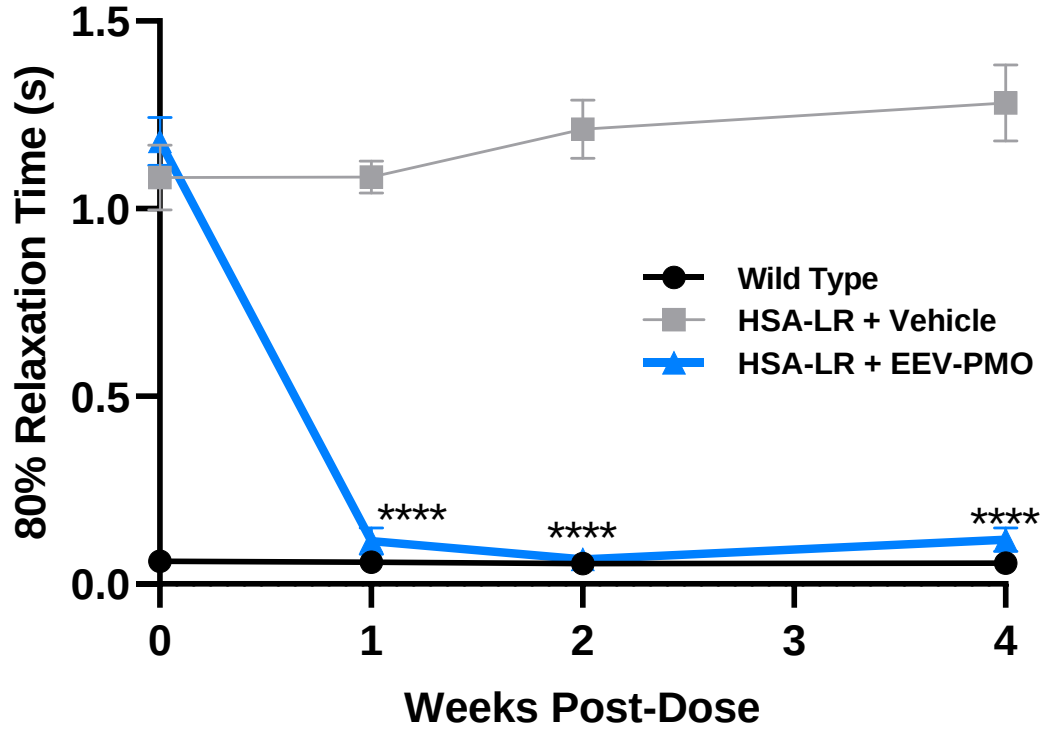
PROLONGED MUSCLE RELAXATION OBSERVED IN HSA-LR MICE

Muscle relaxation after tetanic contraction of plantar flexor muscle group was rescued in 7 days and sustained for 4 weeks following a single dose of a CUG-repeat blocking EEV-PMO

Muscle Relaxation



Durable Muscle Relaxation



EEV-PMO is composed of CUG-repeat blocking PMO conjugated our EEV platform; Muscle relaxation was assessed by stimulating sciatic nerve using 200 ms trains of supramaximal stimuli at 150 Hz. ****p<0.0001 for EEV-PMO compared to untreated; shown as mean ± SEM.

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**

OUR DIFFERENTIATED AND EXPANDING PIPELINE

Entrada’s pipeline includes a diverse array of high potential and high value assets;
We plan to leverage early learnings to advance subsequent programs

Disease/Condition	Discovery	Preclinical	Clinical
Neuromuscular Disease: DMD	ENTR-601-44 Exon 44 Skipping Oligonucleotide		
	Exon 45 Skipping Oligonucleotide		
Neuromuscular Disease: DM1	CUG Steric Blocker Oligonucleotide		
Neuromuscular Disease: Pompe	GYS1 Knockdown Oligonucleotide		
Neurodegenerative Diseases	CD33 Exon 2 Skipping Oligonucleotide		
Inflammatory Diseases	IRF5 Knockdown Oligonucleotide		
Solid Tumors	β-catenin Degrader Antibody		
MNGIE	ENTR-501 Enzyme Replacement		

ACKNOWLEDGEMENTS

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