Endosomal Escape Vehicles (EEV™) to Enhance the Functional Delivery of Oligonucleotides in Preclinical Models of Neuromuscular Diseases

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Solve FSHD
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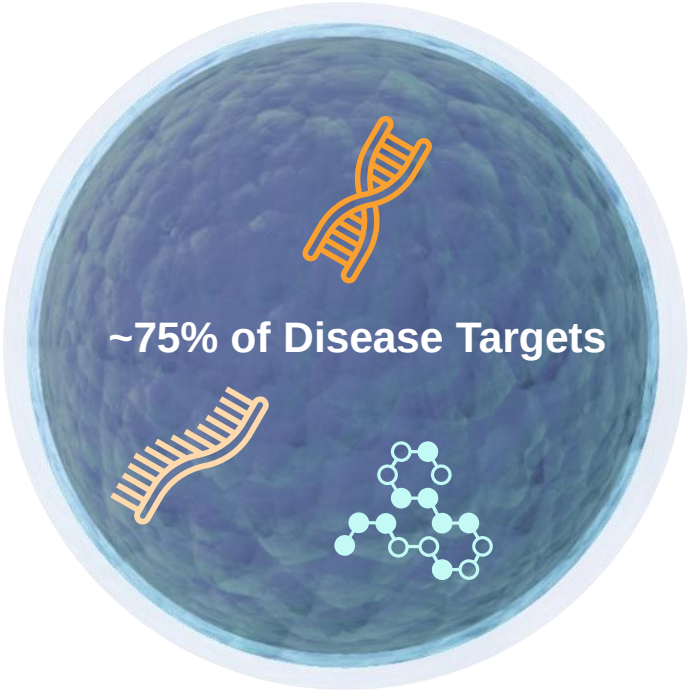
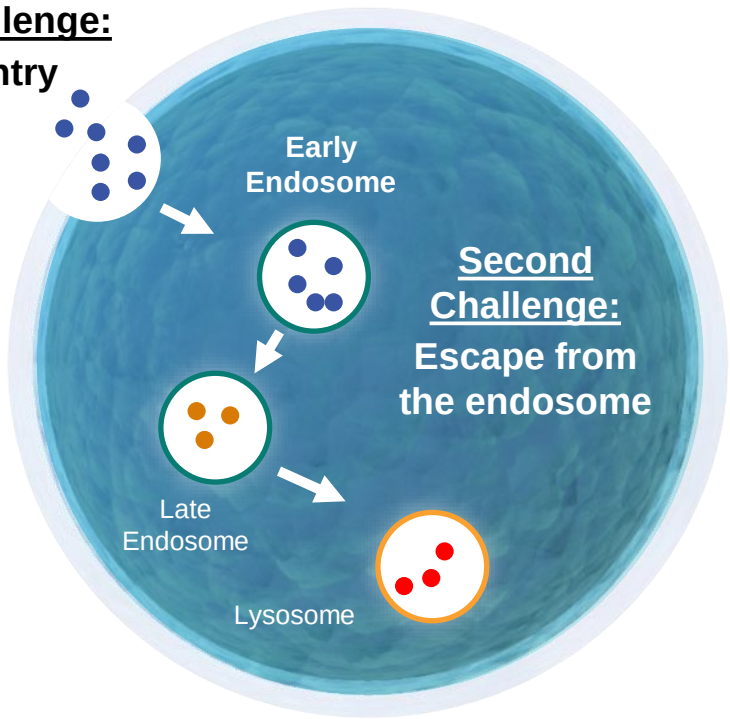
THE NEED FOR INTRACELLULAR THERAPEUTICS

Approximately 75% of all disease-causing targets are intracellular and difficult to reach, representing a significant issue when developing effective therapies



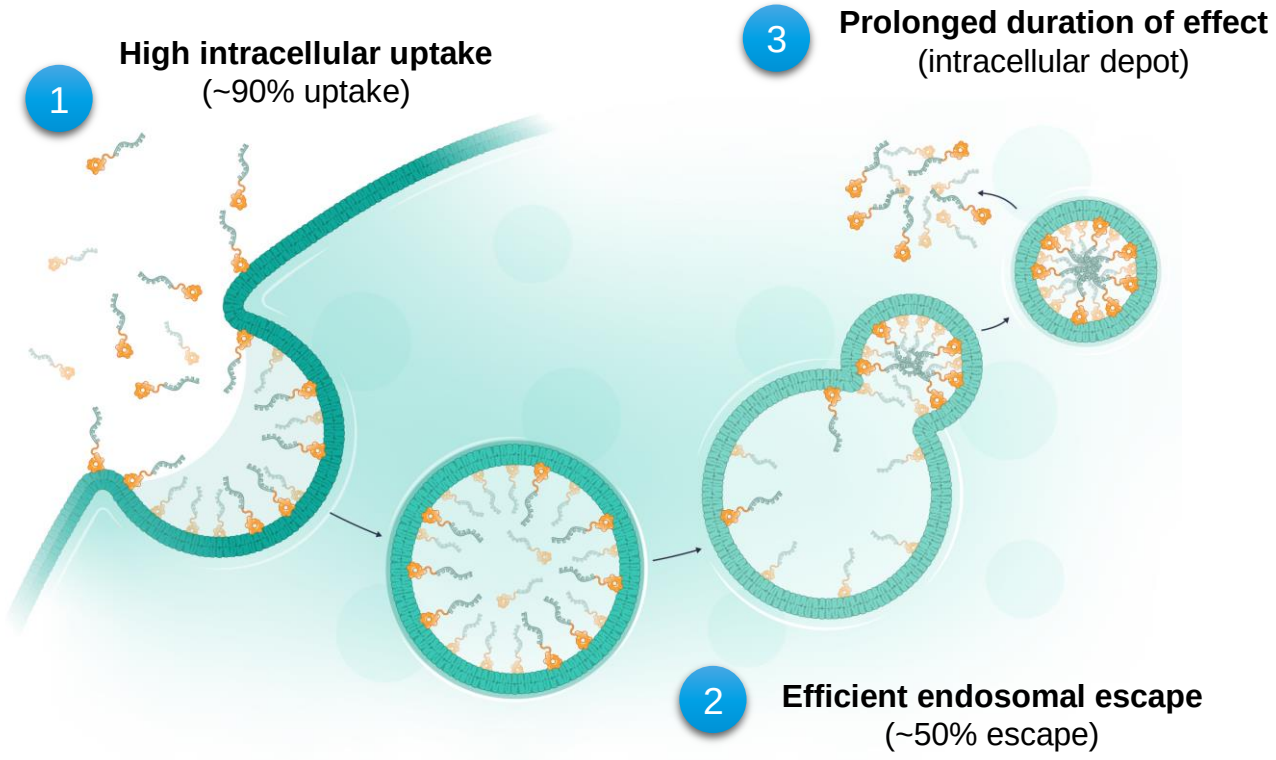


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

Entrada 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**



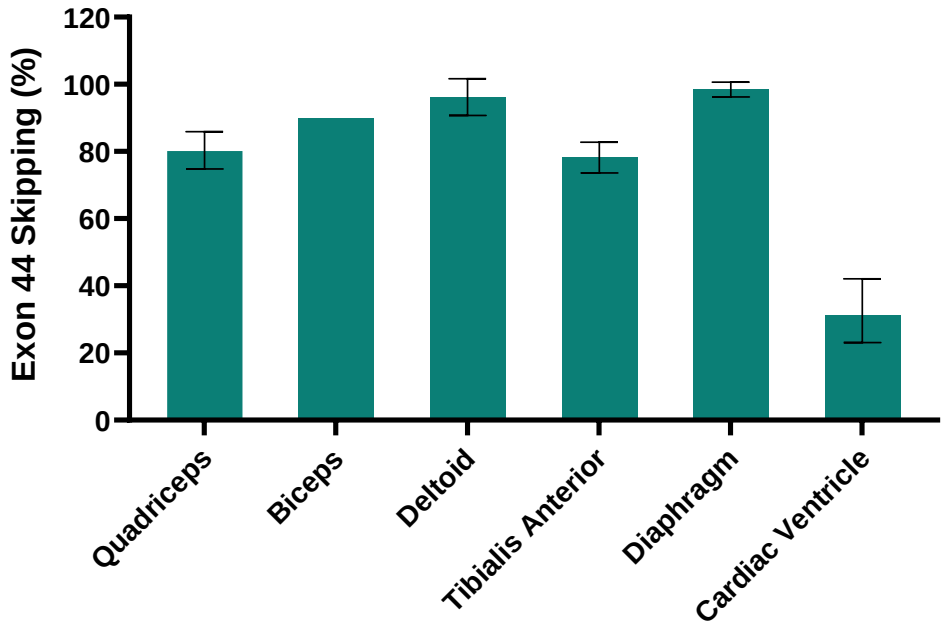
Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

DUCHENNE MUSCULAR DYSTROPHY (DMD)

ENTR-601-44 FOR EXON 44 SKIP AMENABLE DMD

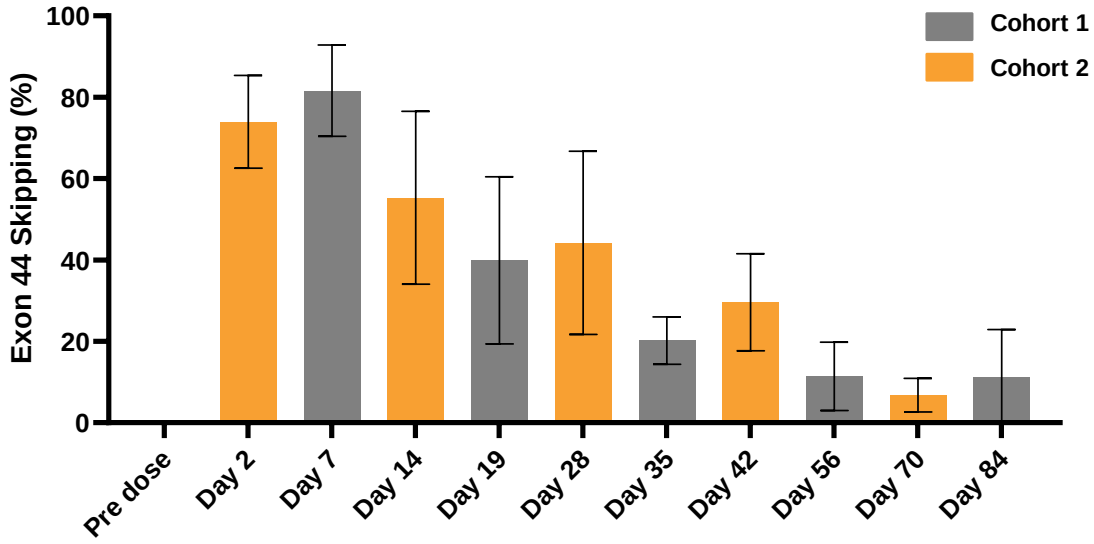
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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



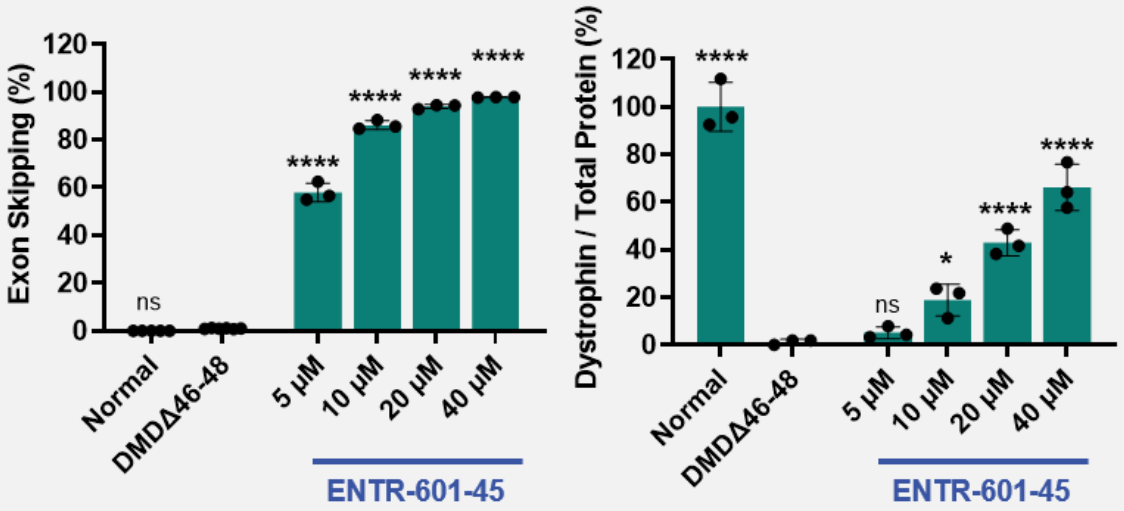
- Post IV infusion of 35 mg/kg (PMO equivalent), robust exon 44 skipping observed in biceps in the ENTR-601-44 treated NHP (n=3 per cohort) for at least 12 weeks

ENTR-601-44 is a DMD exon 44 skipping PMO conjugated to the EEV platform. **NHP**, non-human primates; shown as mean ± standard deviation.

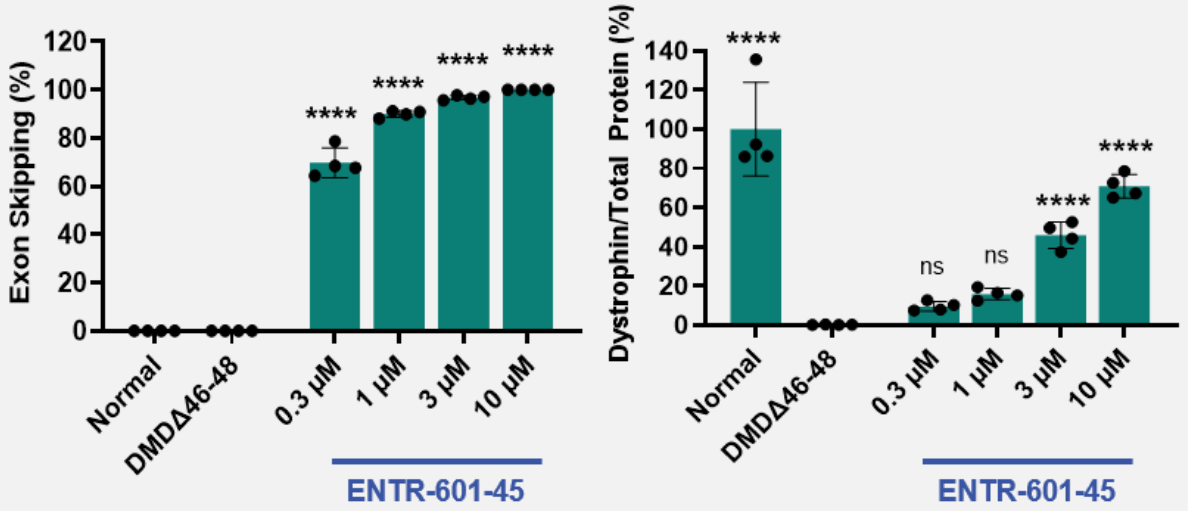
ENTR-601-45 FOR EXON 45 SKIP AMENABLE DMD

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



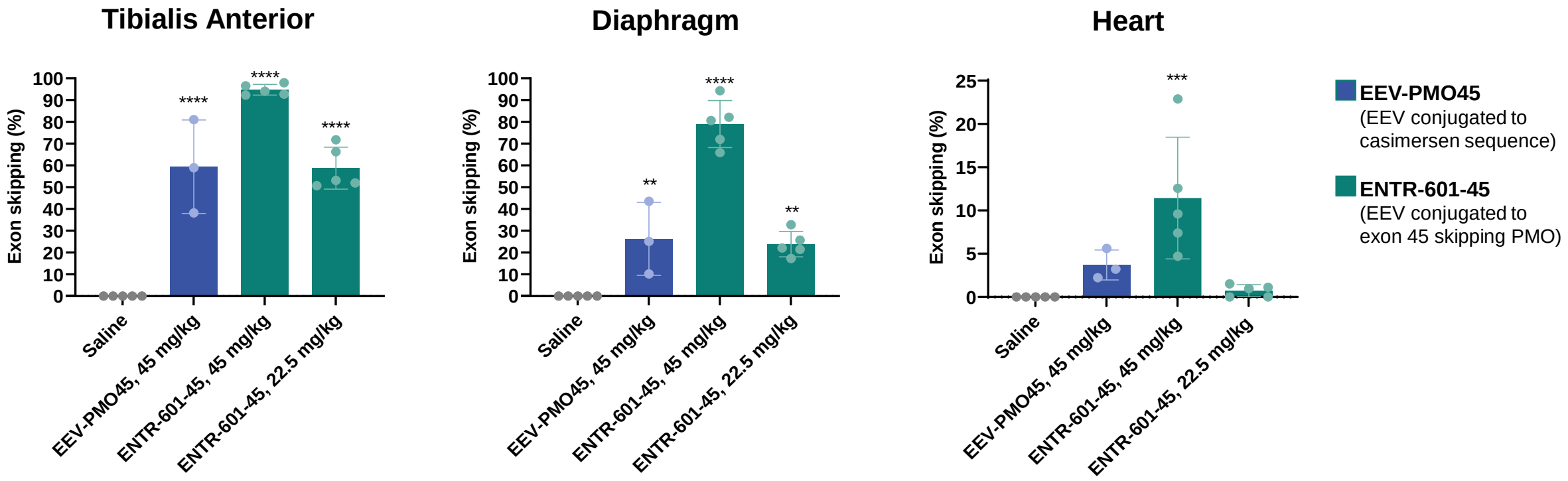
ENTR-601-45 in Cardiac Muscle Cells



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 HUMANIZED 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



- 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

Casimersen is an exon 45 skipping PMO approved in the US. 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.

ENTRADA'S DMD PIPELINE

Entrada is advancing new therapeutic options for people living with exon 44 and exon 45 skip amenable DMD

- **ENTR-601-44 produced robust exon skipping and dystrophin production in several preclinical models of DMD**
  - Durable dystrophin production over 12+ weeks from a single injection of ENTR-601-44 was observed in the NHP
  - 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.

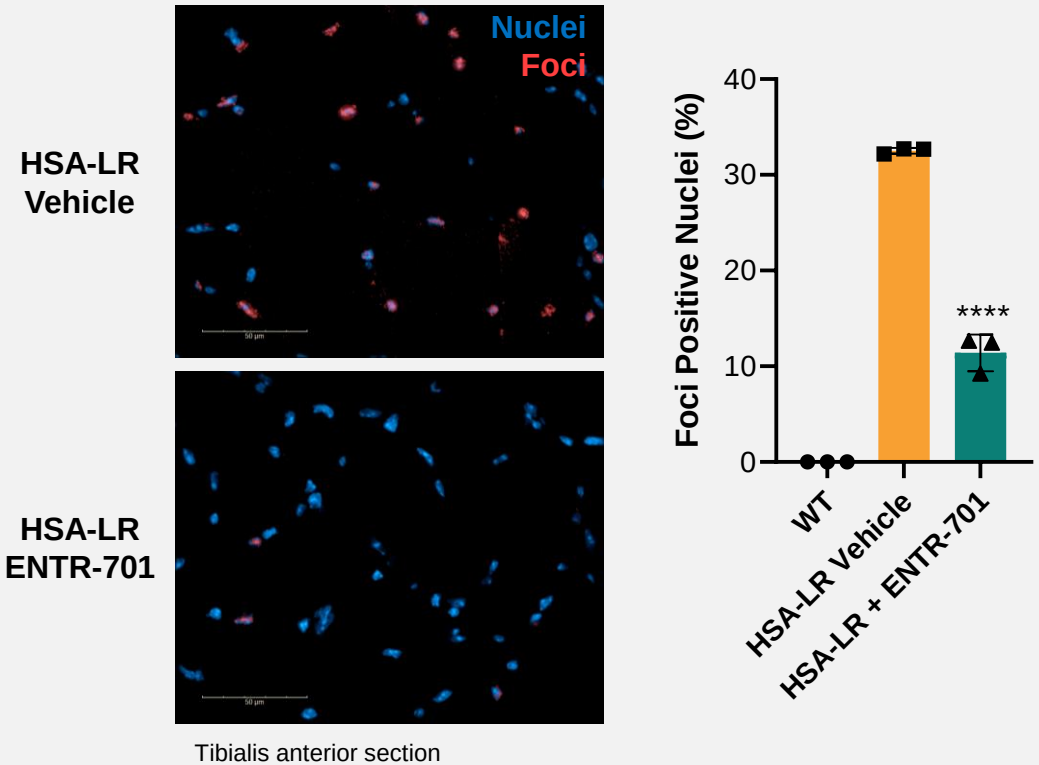
- **ENTR-601-45 IND filing is planned for H2 2024**
  - ENTR-601-45 showed robust exon skipping and dystrophin protein production in patient-derived cardiac and skeletal muscle cells
  - High levels of exon skipping were measured in hDMD mouse heart and skeletal muscle tissue

MYOTONIC DYSTROPHY TYPE 1 (DM1)

EFFICACY OF ENTR-701 IN HSA-LR MICE

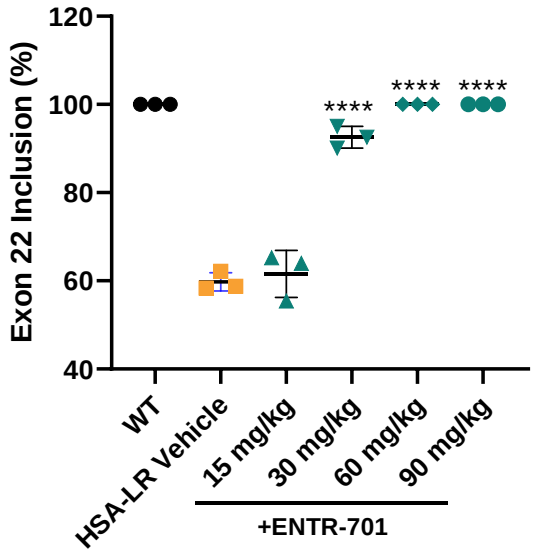
ENTR-701 treatment reduced nuclear foci and CUG-repeat expansion transcript level and corrected aberrant splicing in HSA-LR mice

Nuclear Foci Reduction

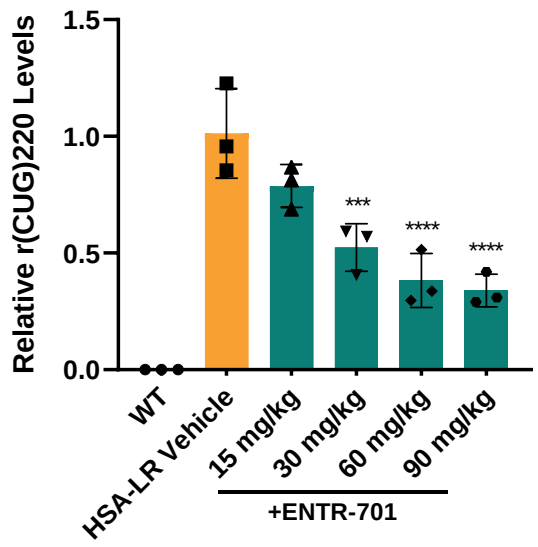


Tibialis anterior section

Atp2a1 Splicing Correction



HSA-r(CUG)220 Reduction

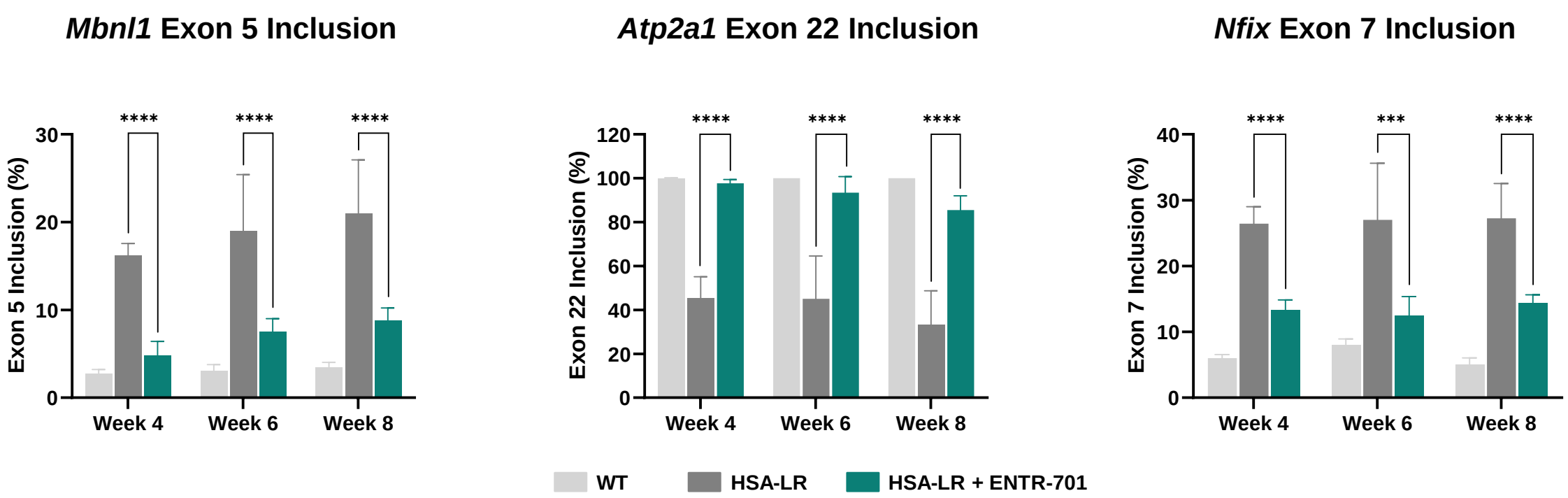


- HSA-LR mice were dosed with vehicle or ENTR-701 and taken down 1-week post injection; tibialis anterior samples analyzed as a representative skeletal group

[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 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 DURABILITY 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.

DM1 DATA SUMMARY

ENTR-701 demonstrated potential to treat DM1 via a CUG-repeat steric blocking approach both *in vitro* and *in vivo*

- Robust *in vitro* and *in vivo* data set demonstrating:
  - Highly specific reduction of pathogenic CUG-repeat containing mRNA
  - Reduction of nuclear foci
  - Correction of *Mbnl1* and downstream aberrant splicing
  - Correction of global transcriptome
- Single dose of ENTR-701 demonstrated durable splicing correction and amelioration of myotonia for at least 8 weeks post-dose in HSA-LR model

Announced collaboration with Vertex on December 2022 for the discovery and development of EEV-therapeutics for DM1

Note: Strategic Collaboration and License Agreement with Vertex is contingent on completion of review under HSR and other antitrust laws.

ACKNOWLEDGEMENTS

Thank you!

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