Endosomal Escape Vehicle-Oligonucleotide Conjugates for the Targeted Upregulation and Downregulation of Gene Expression

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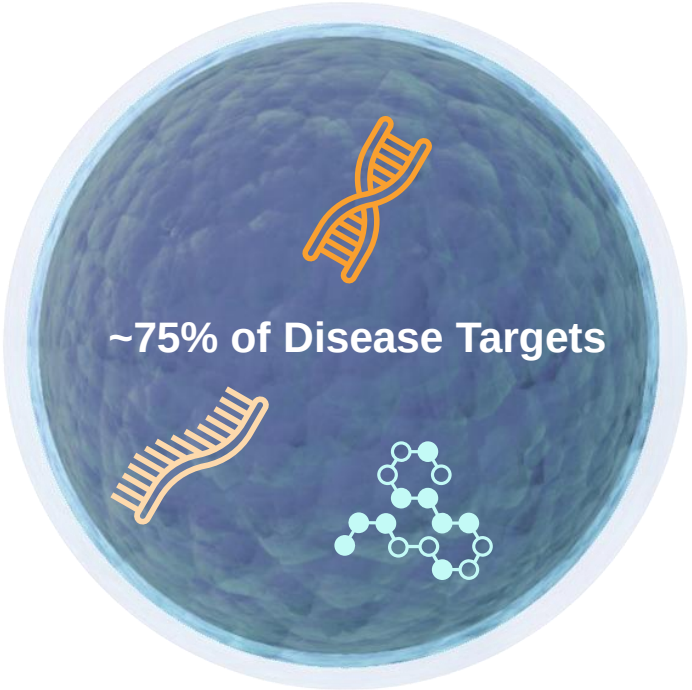
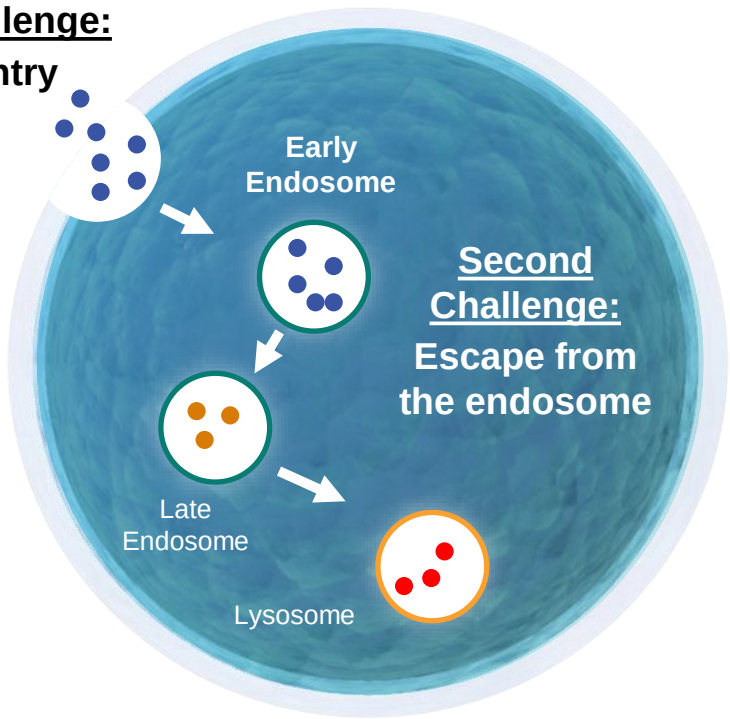
ENTRADA'S MISSION

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



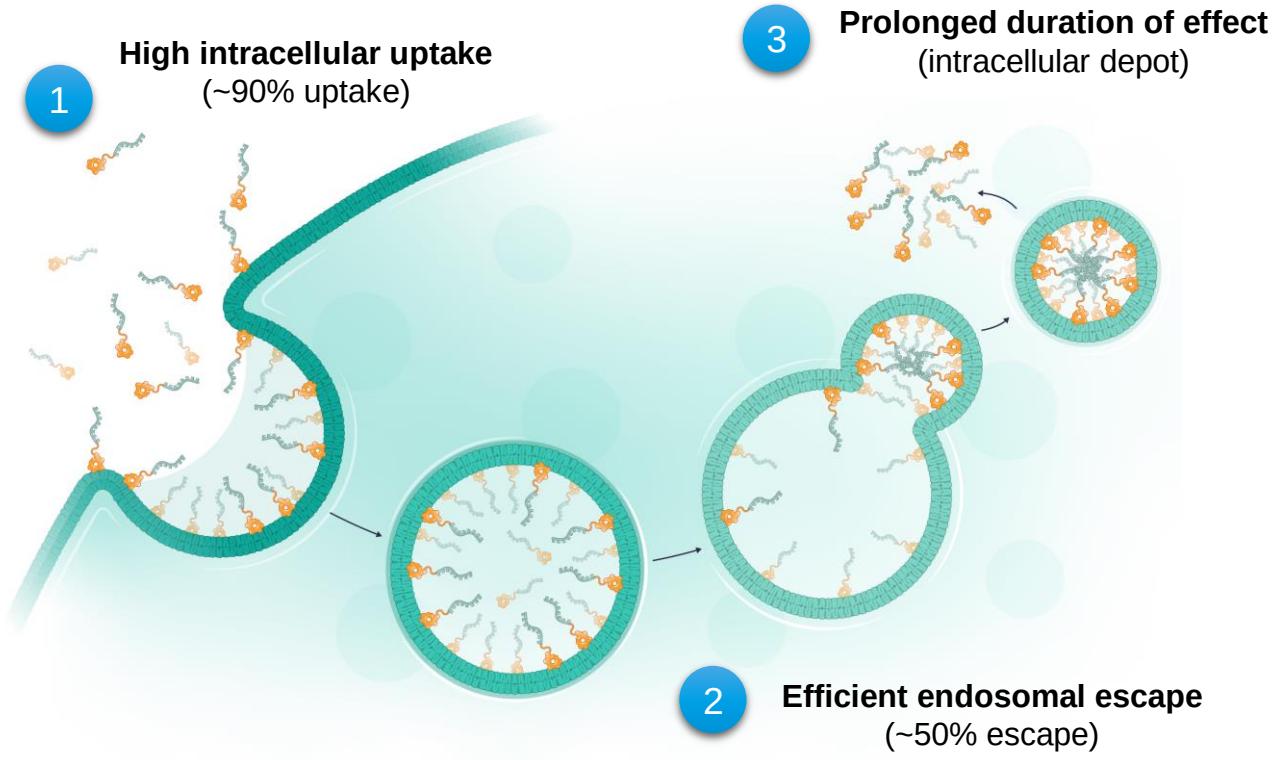


The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome

ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

Entrada 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**

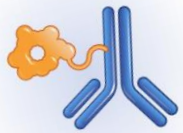


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

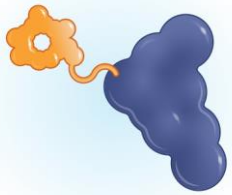
A BROADLY APPLICABLE PLATFORM

Entrada has demonstrated intracellular uptake of unique moieties ranging from 1 kDa to 600 kDa

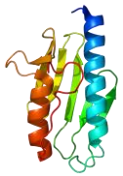
Antibodies



Enzymes

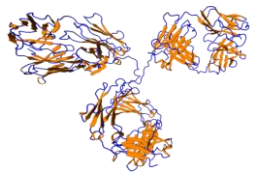


Oligonucleotides



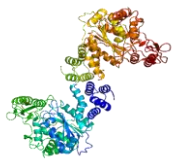
550-600 KDa

Hybrid frataxin



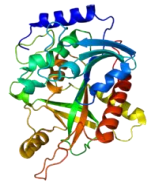
150 KDa

Antibody



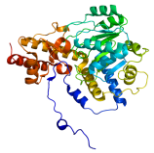
98 KDa

Thymidine phosphorylase



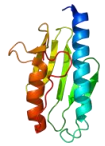
96 KDa

Purine nucleoside phosphorylase



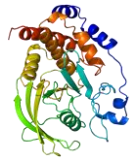
86 KDa

Alanine-glyoxylate aminotransferase



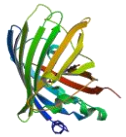
46 KDa

Human frataxin



37 KDa

PTP1B catalytic domain



32 KDa

EGFP

16 KDa

Nanobody

6 KDa

Oligonucleotide

1-3 KDa

Various peptide cargos

DUCHENNE MUSCULAR DYSTROPHY (DMD)

SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

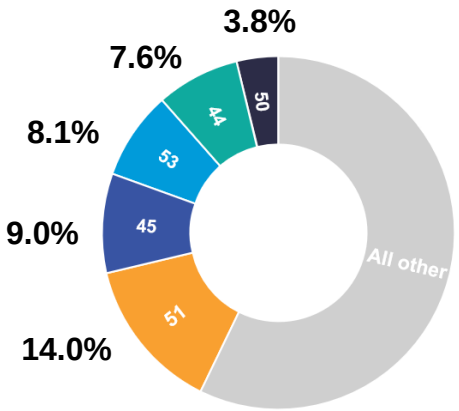
Duchenne is caused by **mutations in the DMD gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

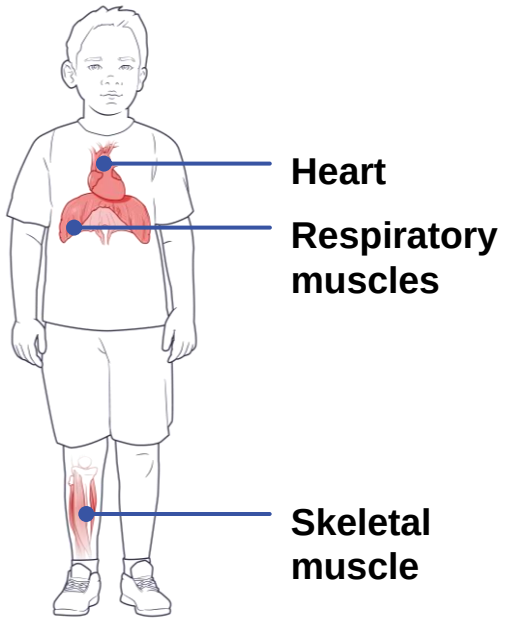
~30,000

people in the **U.S. and Europe** have Duchenne[1]

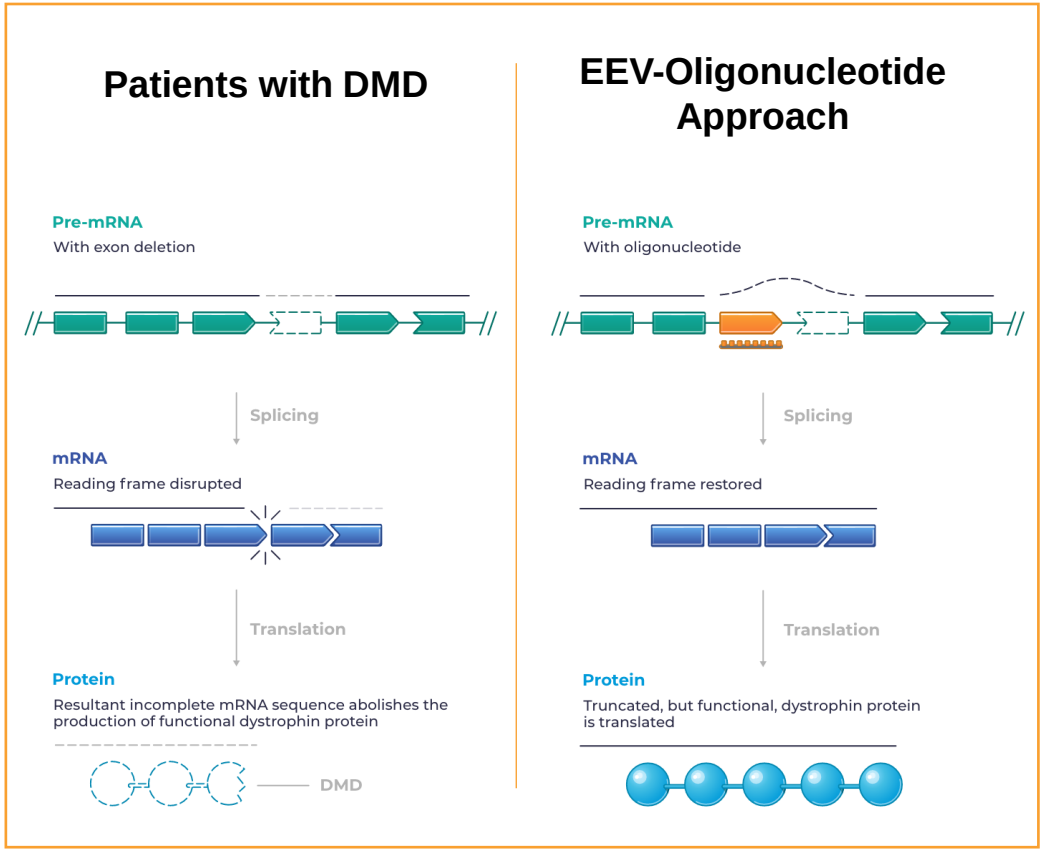
>40% of patients with Duchenne

have mutations amenable to exon skipping of exons 44, 45, 50, 51 and 53





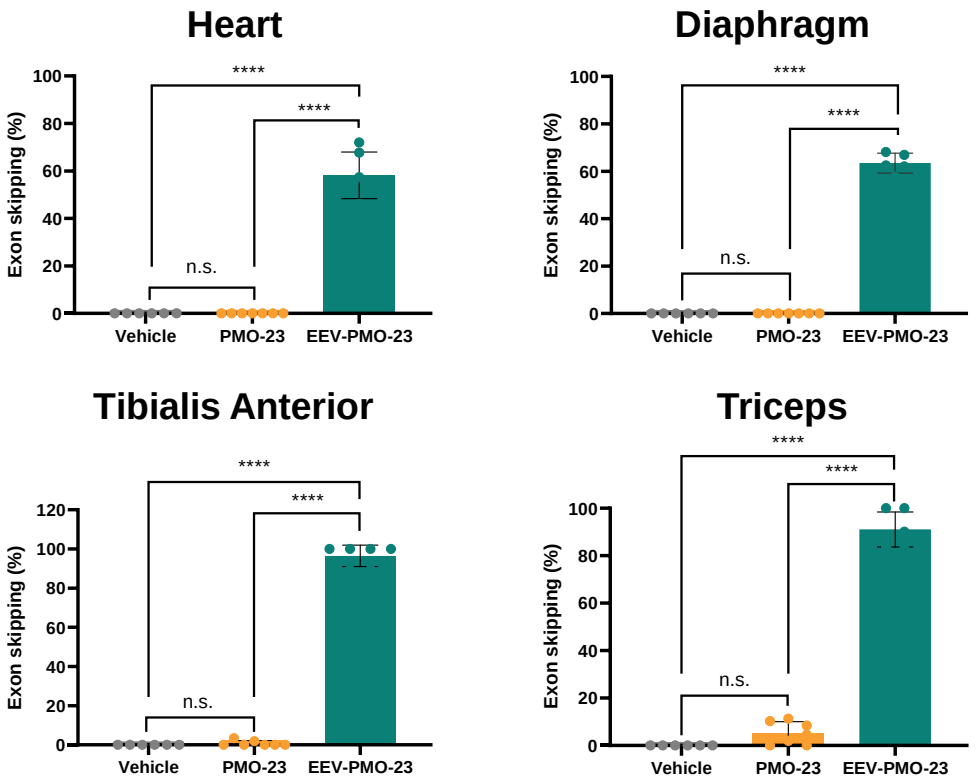
Exon skipping therapeutics have been approved based on **modest improvement in dystrophin levels ranging from ~1 to 6%**



[1]Crispi V, Matsakas A. Duchenne muscular dystrophy: genome editing gives new hope for treatment. Postgraduate Medical Journal. 2018;94(1111):296-304. doi: 10.1136/postgradmedj-2017-135377.

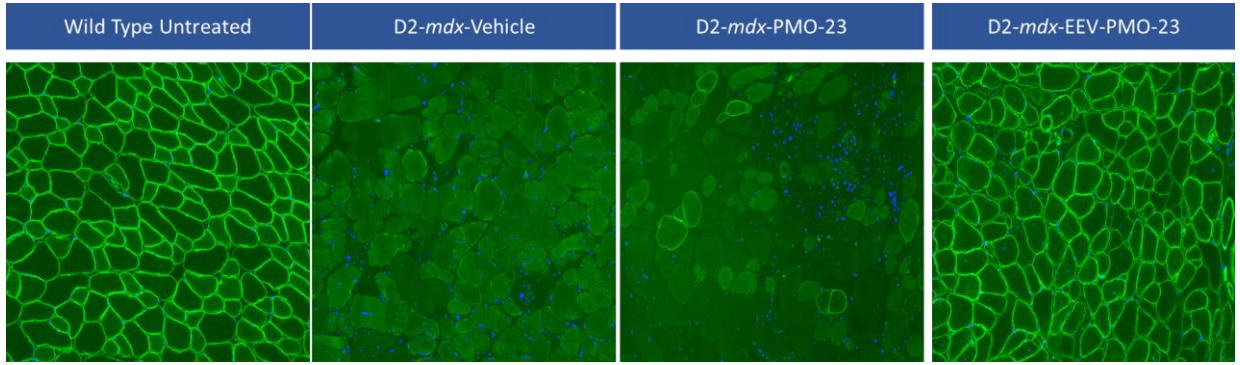
REPEAT EEV-PMO TREATMENT IN D2-*mdx* MICE

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

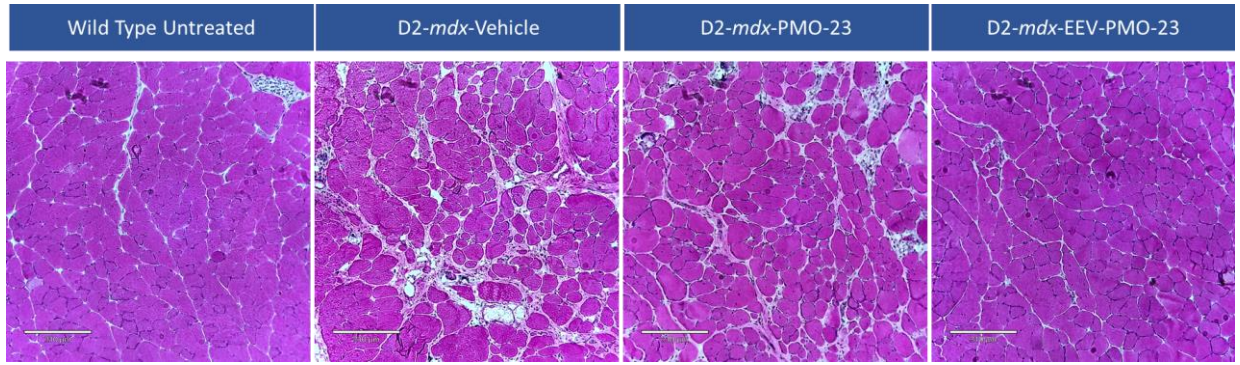


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



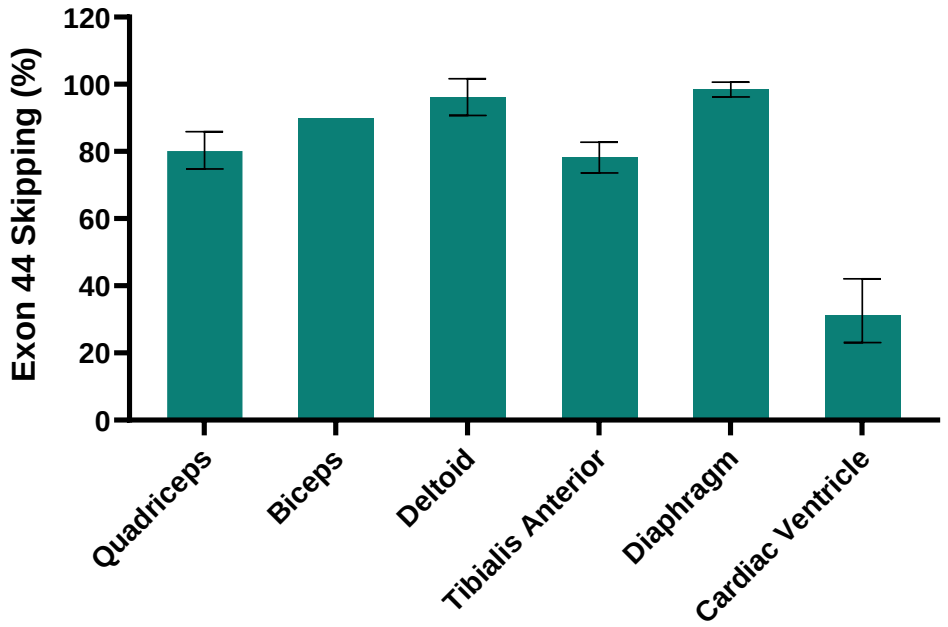
- D2-*mdx* is a DMD mouse model with a nonsense mutation in *Dmd* exon 23 (Coley et al. Hum. Mol. Genet. 2016)

EEV, Endosomal Escape Vehicle; **PMO-23**, mouse *Dmd* exon 23 skipping phosphorodiamidate morpholino oligomer; 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. ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

ENTR-601-44 FOR EXON 44 SKIP AMENABLE DMD

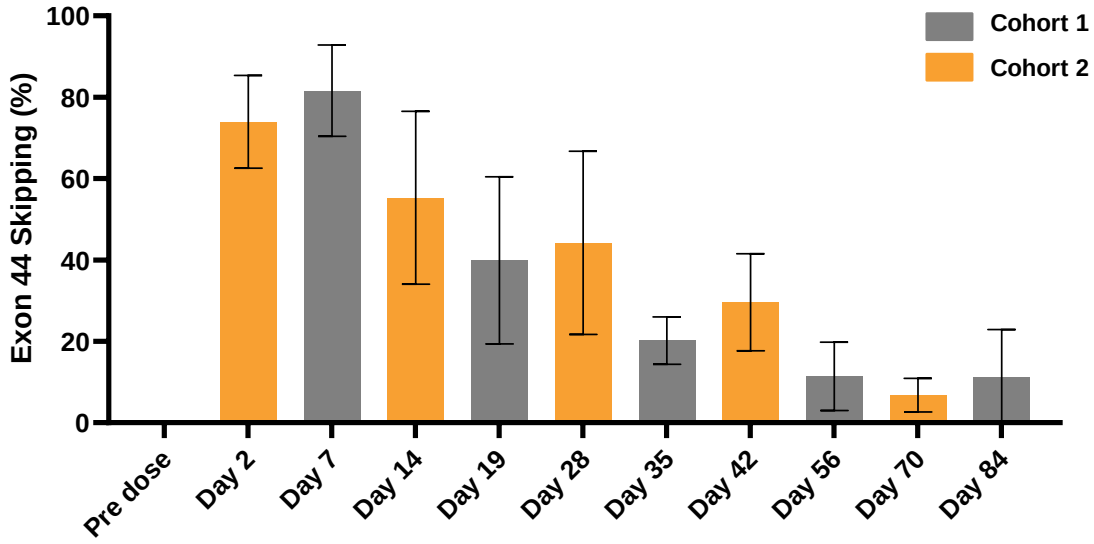
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

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



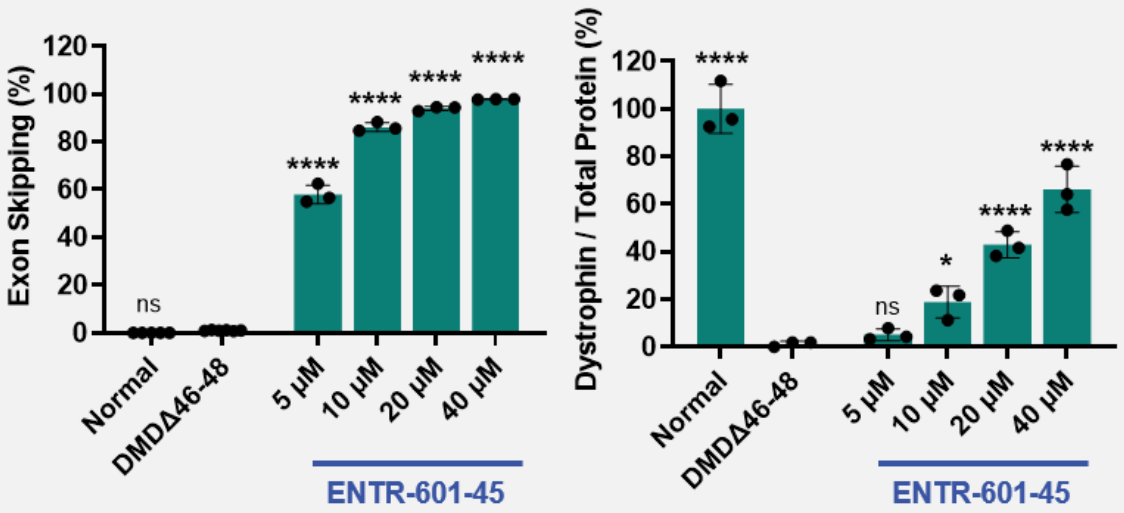
- Post IV infusion of 35 mg/kg (PMO equivalent), robust exon 44 skipping observed in biceps in the ENTR-601-44 treated NHP (n=3 per cohort) for at least 12 weeks

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

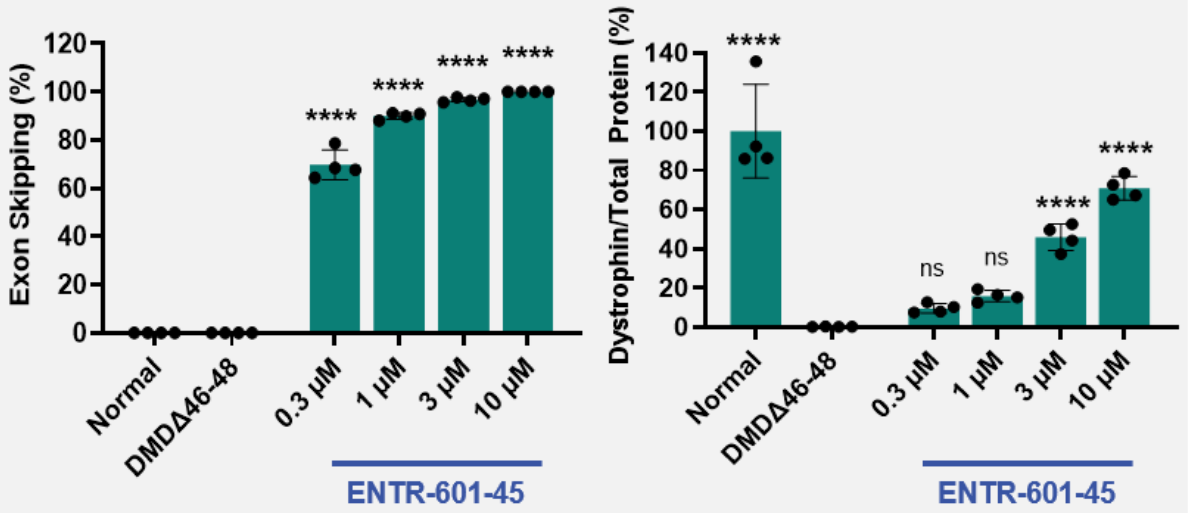
ENTR-601-45 FOR EXON 45 SKIP AMENABLE DMD

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



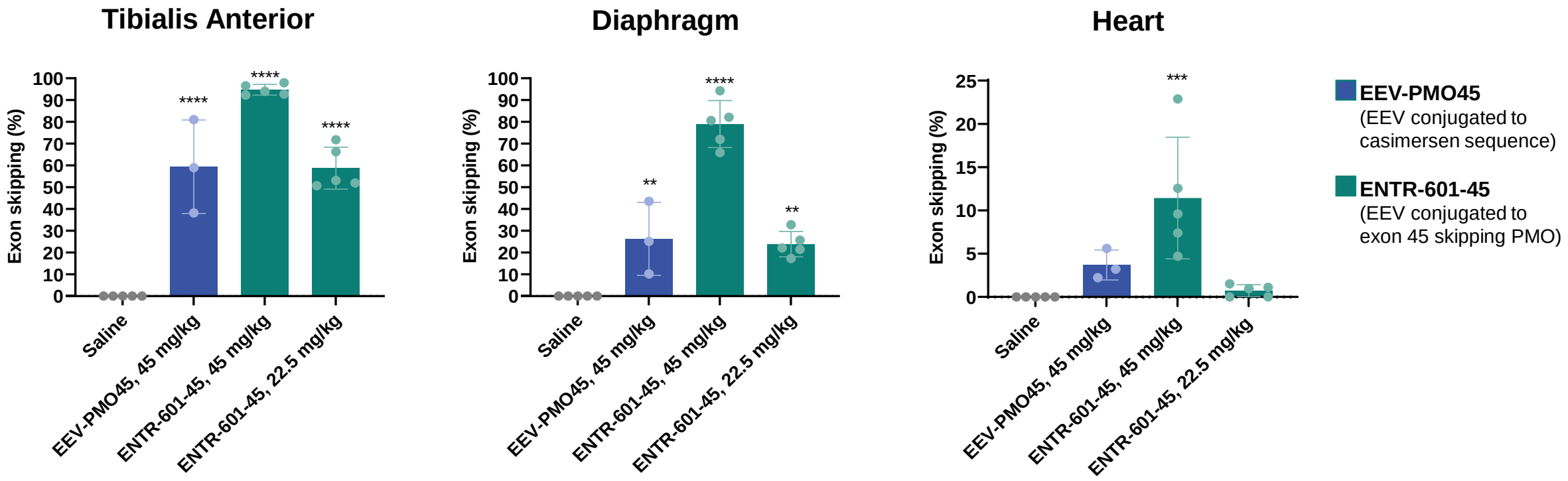
ENTR-601-45 in Cardiac Muscle Cells



ENTR-601-45 is a DMD exon 45 skipping PMO conjugated to the EEV platform. 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.

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

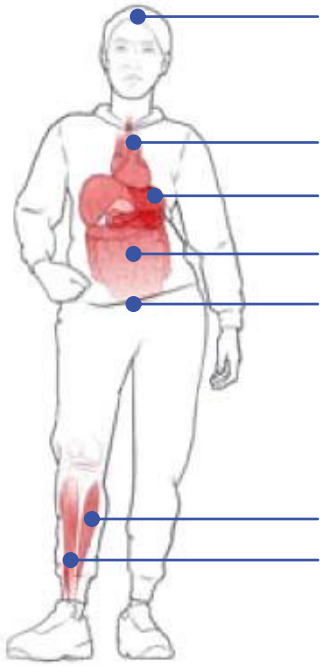
MYOTONIC DYSTROPHY TYPE 1 (DM1)

DM1 IS A DEBILITATING, MULTISYSTEMIC DISEASE WITH NO AVAILABLE TREATMENTS

40,000+
people in the **U.S.** have DM1[1]

50,000+
people in **Europe** have DM1[1]

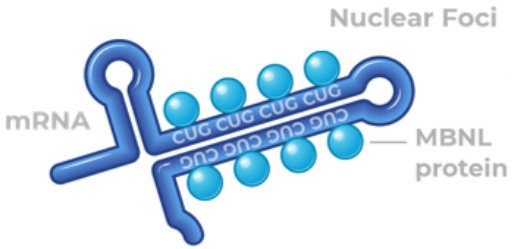
Symptoms include:



- Fatigue and excessive daytime sleepiness
- Cardiac conduction irregularities
- Respiratory muscle impairment
- Gastrointestinal complications
- Incontinence
- Generalized limb weakness
- Myotonia
  (delayed relaxation of skeletal muscle)

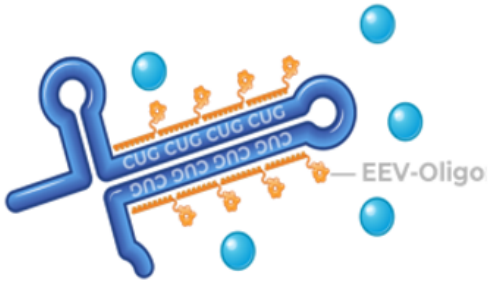
DM1 is caused by a mutation in the *dystrophia myotonica protein kinase (DMPK)* gene

Mutant *DMPK* mRNA



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

EEV-Oligonucleotide Approach



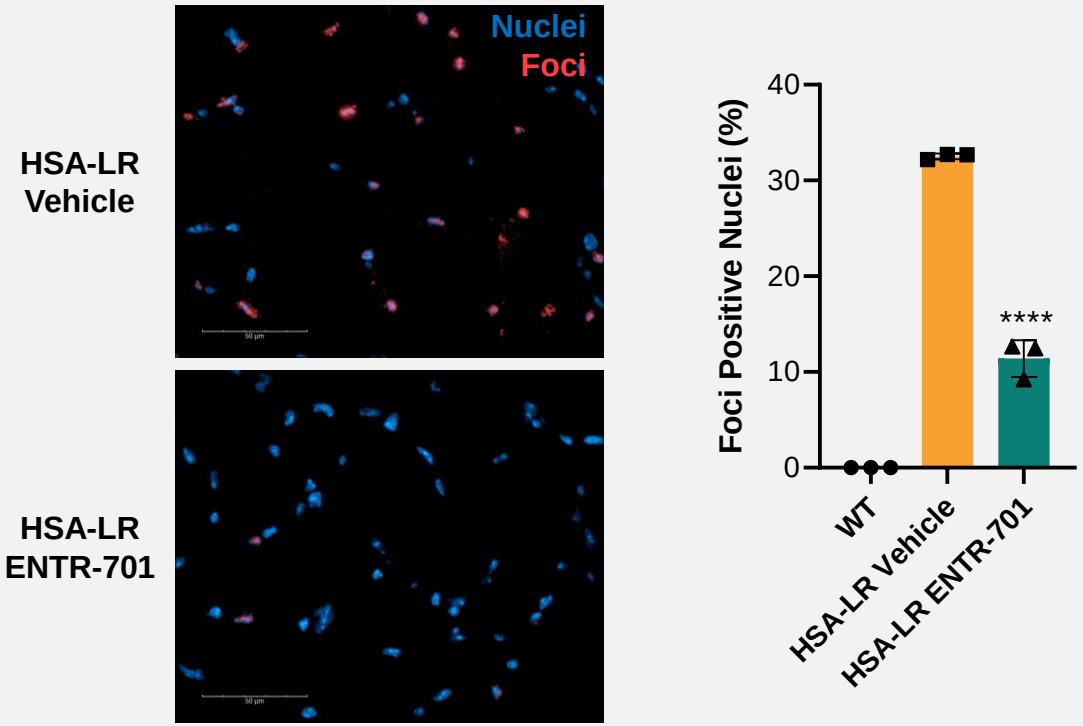
- Reduced nuclear foci
- Selective mRNA reduction
- Normal MBNL function
- Corrected spliceopathy

[1]Pascual-Gilabert M, López-Castel A, Artero R. Myotonic dystrophy type 1 drug development: a pipeline toward the market. *Drug Discovery Today*. 2021;26(7):1765-72. doi: 10.1016/j.drudis.2021.03.024.

ENTR-701 EFFICACY IN HSA-LR MICE

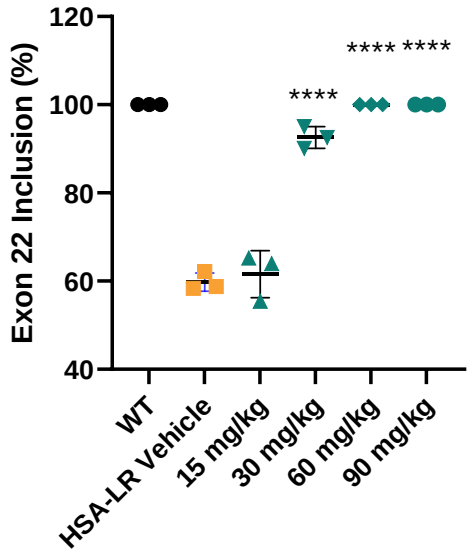
ENTR-701 treatment reduced nuclear foci and CUG-repeat expansion transcript level and corrected aberrant splicing in HSA-LR mice, in a dose dependent manner

Nuclear Foci Reduction

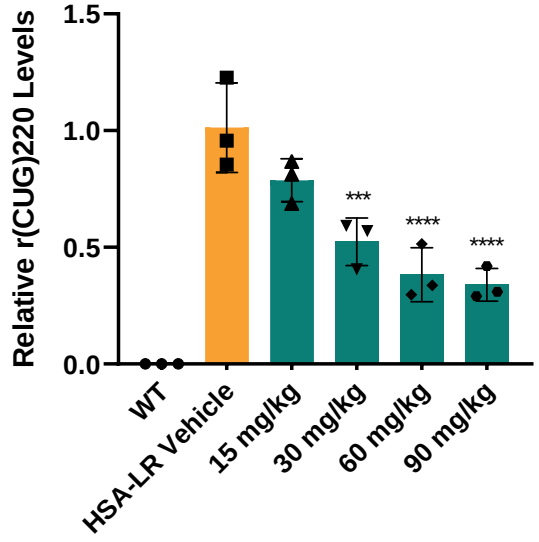


Tibialis anterior section

Atp2a1 Splicing Correction



HSA-r(CUG)220 Reduction



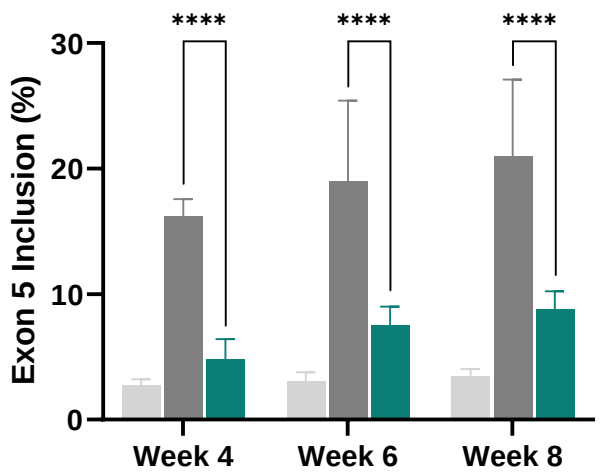
- **HSA-LR** model carries a transgene resulting in CUG repeat expansion and recapitulates DM1 phenotype and molecular pathology
- HSA-LR mice were dosed with vehicle or ENTR-701 and taken down 1-week post injection; tibialis anterior samples analyzed as a representative skeletal group

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.

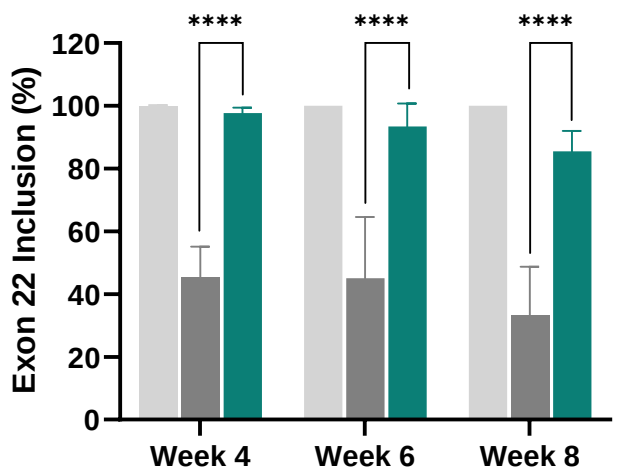
ENTR-701 DURABILITY IN HSA-LR MICE

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

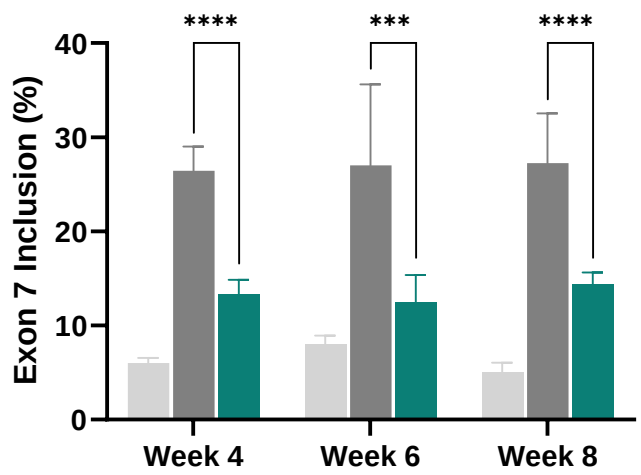
Mbnl1 Exon 5 Inclusion



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

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*

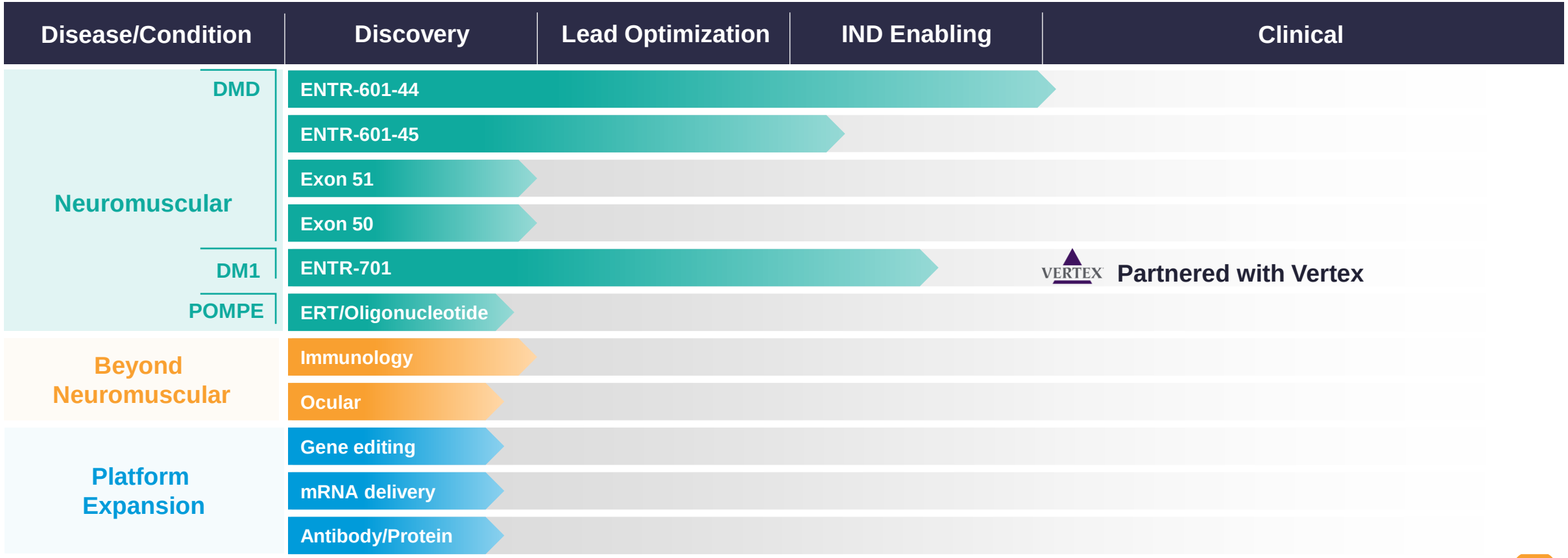
- Robust *in vitro* and *in vivo* data set demonstrating:
  - Highly specific reduction of pathogenic CUG-repeat containing mRNA
  - Reduction of nuclear foci
  - Correction of *Mbnl1* and downstream aberrant splicing
  - Correction of global transcriptome
- Single dose of ENTR-701 demonstrated durable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

Established transformational collaboration with Vertex for the discovery and development of EEV-therapeutics for the potential treatment of DM1

PIPELINE & DISCOVERY PROGRAMS

OUR DIFFERENTIATED AND EXPANDING PIPELINE

Entrada’s pipeline includes a diverse array of high potential and high value assets



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

Thank you!

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