Enhanced Exon Skipping and Dystrophin Production in a Mouse Model of Duchenne Muscular Dystrophy with EEV-PMO Treatment

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March 16, 2022

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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 ENTRADA STORY

We are driven to transform the lives of patients by establishing Endosomal Escape Vehicle* therapeutics as a new class of medicines and become the world's foremost intracellular therapeutics company

Platform

A proprietary, modular and broadly applicable intracellular delivery platform with potential in a wide range of therapeutic areas

Portfolio

Diverse set of oligonucleotide applications and an expanding pipeline, including intracellular oligonucleotides, antibodies and enzymes

People

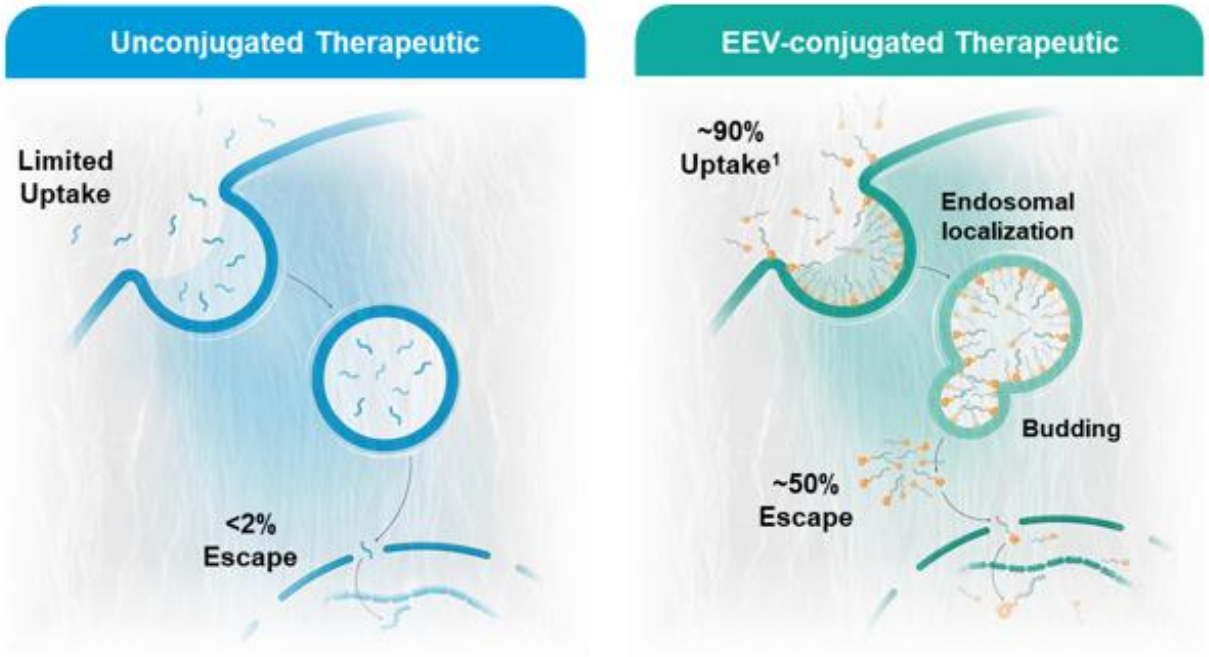
Led by a team of experts with extensive experience across drug discovery, development and company management

We are harnessing our EEV Platform to develop intracellular therapeutics that are designed to engage previously undruggable targets and revolutionize the treatment of devastating diseases

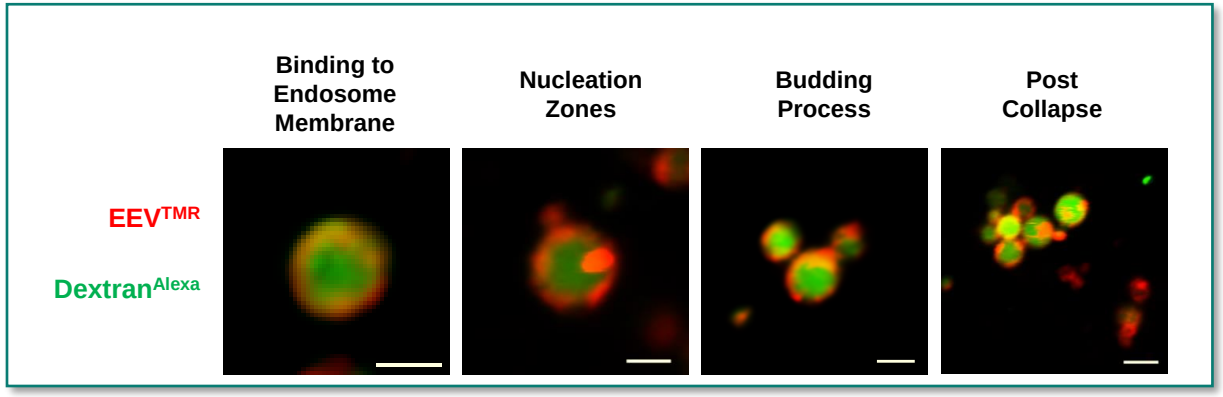
* Endosomal Escape Vehicles are referred to as EEVs

ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

The EEV Platform aims 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.



- Cyclic structure designed to **extend half life and increase stability**
- Phospholipid binding potentially **enables broad biodistribution to all cells**
- Unique chemistry results in **improved uptake and endosomal escape**

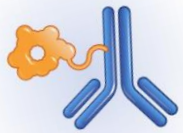


Qian, Z. et al. *ACS Chem. Biol.* **2013**, 423; Qian, Z. et al. *Biochemistry* **2014**, 4034; Qian, Z. et al. *Biochemistry* **2016**, 2601; Sahni, A. et al. *ACS Chem. Biol.* **2020**, 2485; Pei, D. *Acc. Chem. Res.* **2022**, 309.

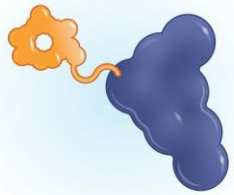
A BROADLY APPLICABLE PLATFORM

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

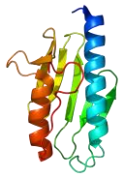
Antibodies



Enzymes

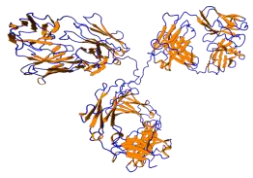


Oligonucleotides



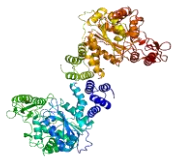
550-600 KDa

Hybrid frataxin



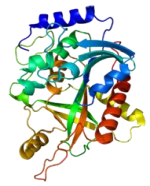
150 KDa

Antibody



98 KDa

Thymidine phosphorylase

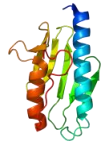


96 KDa

Purine nucleoside phosphorylase

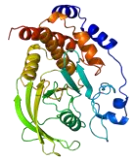
86 KDa

Alanine-glyoxylate aminotransferase



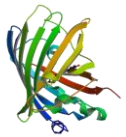
46 KDa

Human frataxin



37 KDa

PTP1B Catalytic domain



32 KDa

EGFP

16 KDa

Nanobody

6 KDa

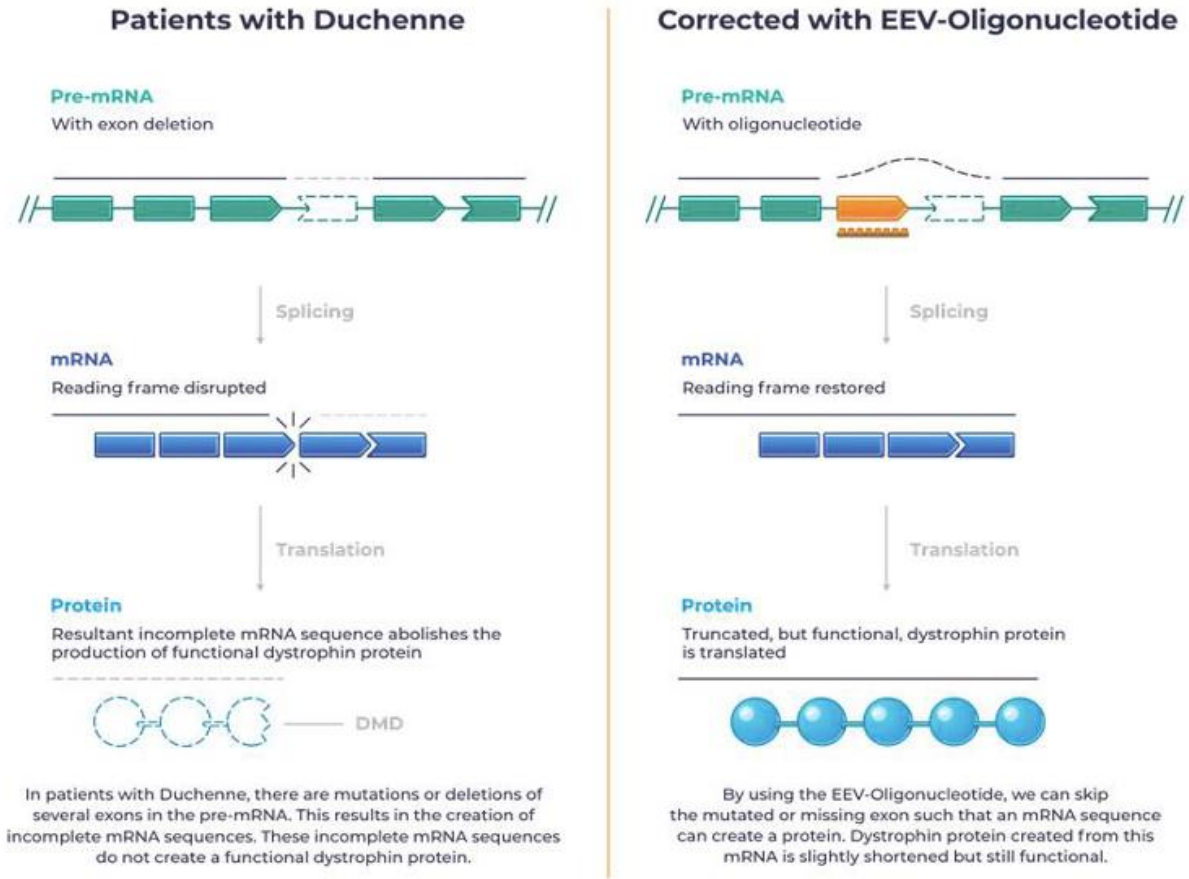
Oligonucleotide

1-3 KDa

Various peptide cargos

DUCHENNE MUSCULAR DYSTROPHY

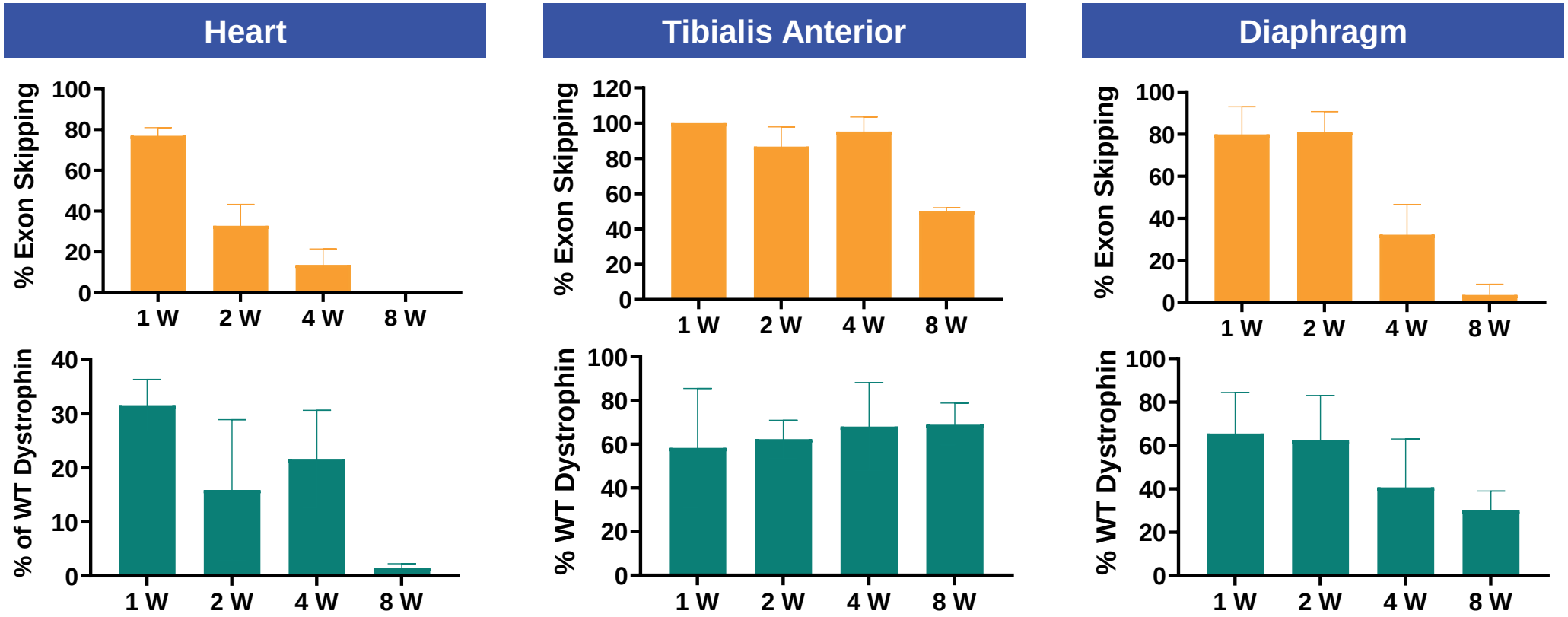
Duchenne muscular dystrophy (DMD) is a progressive, devastating muscle wasting disease with significant unmet need



- Duchenne muscular dystrophy (DMD) is a rare, X-linked neuromuscular disorder caused by mutation in the DMD gene
- ~30,000 patients in the US and Europe
- Loss of functional dystrophin results in progressive muscle wasting, respiratory insufficiency, and cardiomyopathy
- Patients with DMD typically lose ambulation in their teens and respiratory/ cardiac complications are the most frequent cause of death
- Corticosteroids are the current standard of care
- Exon skipping therapeutics using phosphorodiamidate morpholino oligomer (PMO) chemistry for patients with mutations amenable to skipping exons 51, 53 and 45 have been 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; there is currently no approved therapy for patients with mutations amenable to exon 44 skipping

HIGH AND PERSISTENT EXON SKIPPING AND DYSTROPHIN LEVELS AFTER A SINGLE DOSE

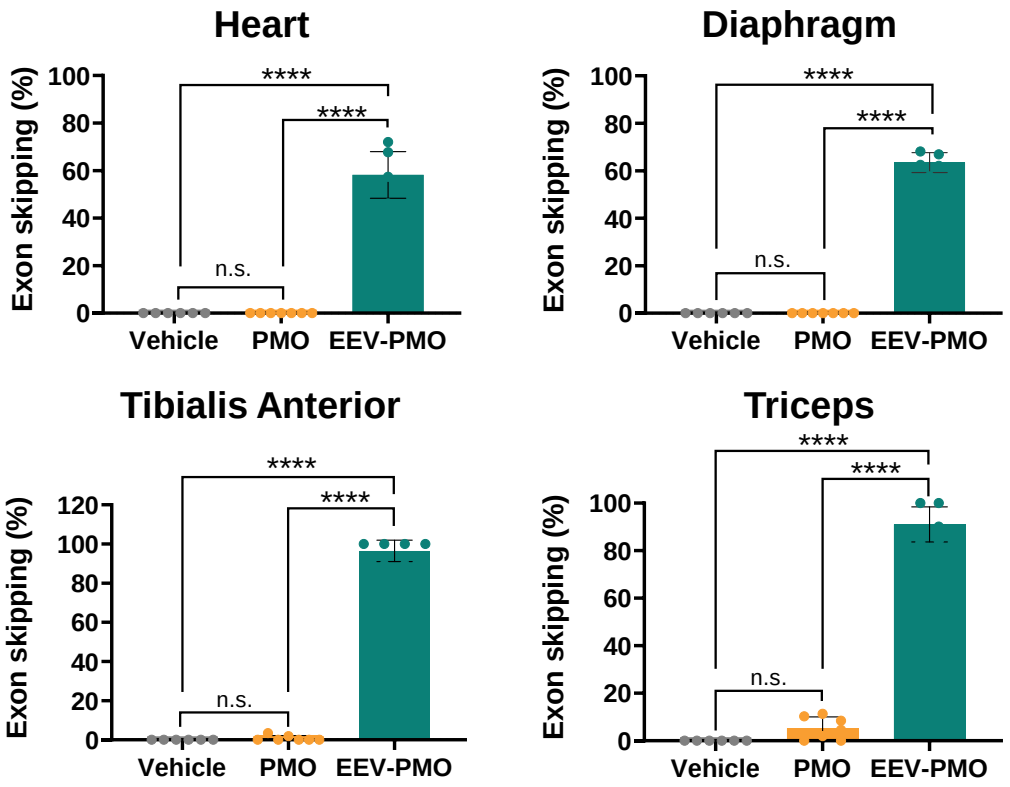
High levels of exon 23 skipping and dystrophin correction observed up to 8 weeks after a single IV dose of EEV-PMO-23 in *mdx* mice



- ***mdx* mice** are the canonical model used in DMD research and carry a nonsense mutation in exon 23 of the DMD gene (*Sicinski et al. Science* ***1989***)
- Percent exon skipping and dystrophin protein expression in various muscle groups post a single IV dose of EEV-PMO at 40 mg/kg in *mdx* mice

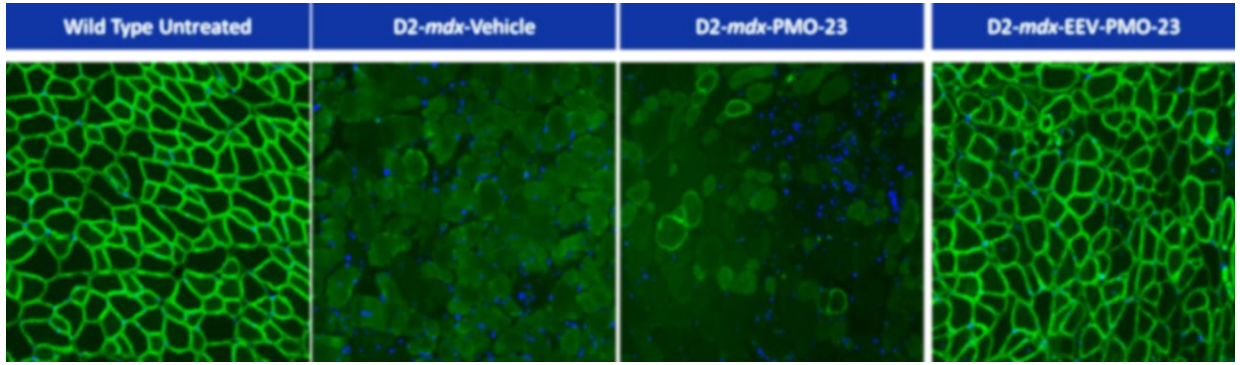
REPEAT EEV-PMO TREATMENT RESTORES MUSCLE INTEGRITY IN D2-*mdx* MICE

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

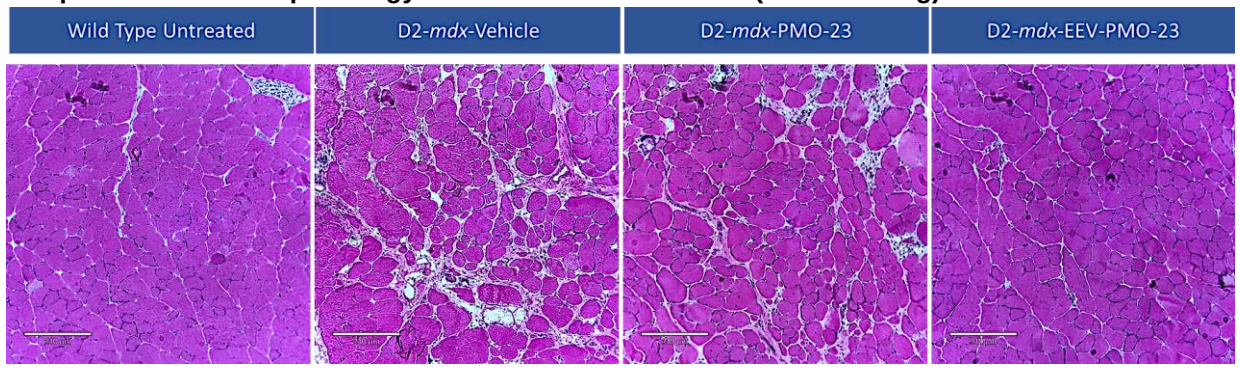


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



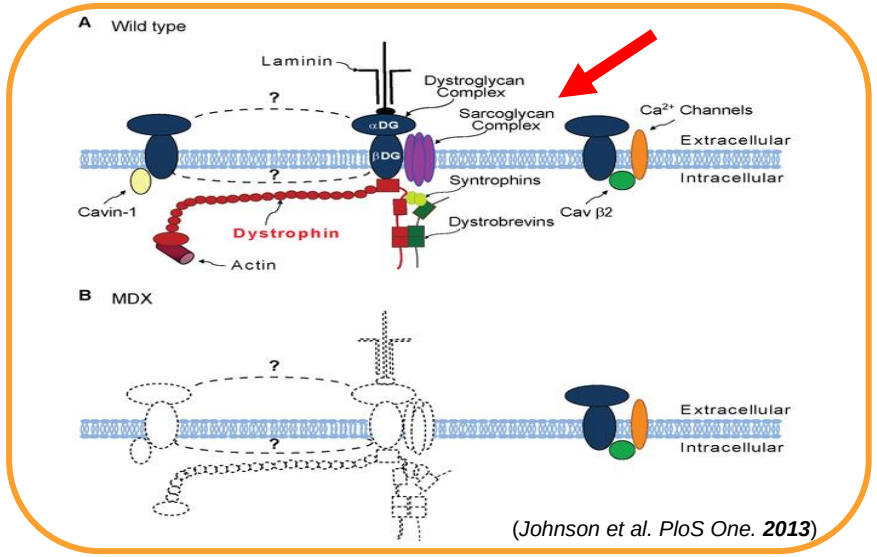
- **D2-*mdx* mice** is a DMD mouse model on DBA/2J background and better recapitulate disease pathology (*Fukada et al. Am. J. Path.* ***2010***)
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO or 20 mg/kg PMO equivalent of EEV-PMO, and the data were collected ~4 weeks after the last dose

n.s. not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

REPEAT EEV-PMO TREATMENT RESTORES α-SARCOGLYCAN AT THE SARCOLEMMA

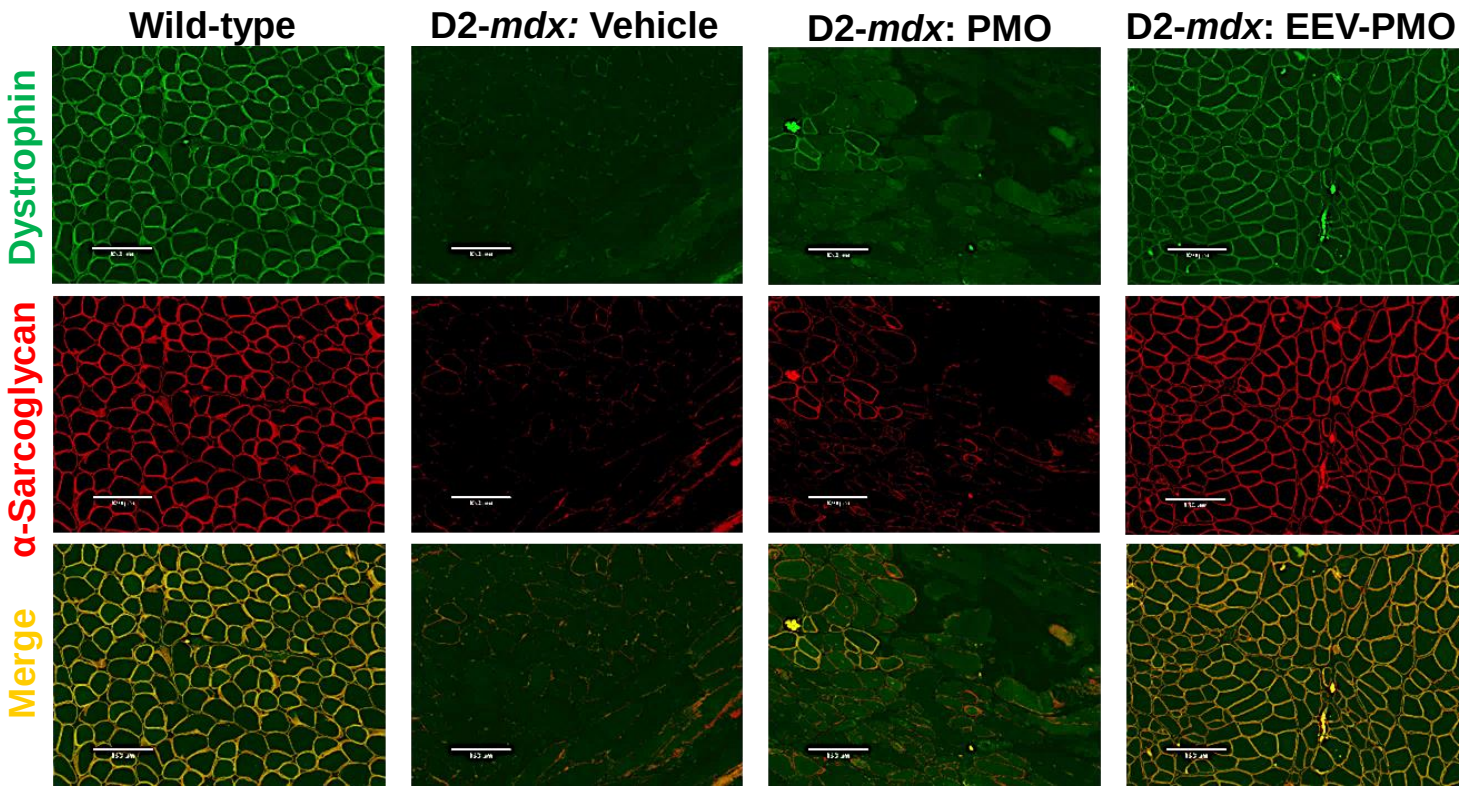
Repeat EEV-PMO-23 treatment resulted in functional restoration of dystrophin and DGC complex protein α-sarcoglycan after four monthly IV doses in D2-*mdx* mice

Schematic of DGC complex



(*Johnson et al. PloS One.* ***2013***)

- In the absence of dystrophin, α-sarcoglycan fails to correctly localize to the dystrophin-glycoprotein complex (DGC) causing weakening of the plasma membrane

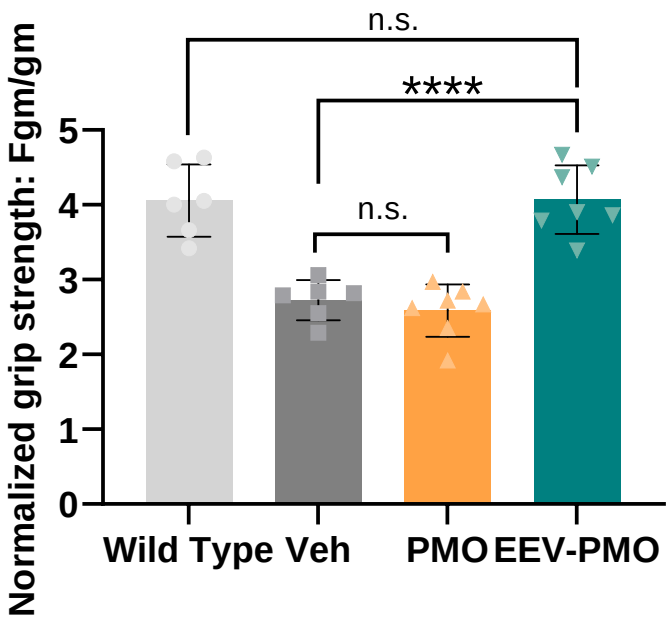


- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO or 20 mg/kg PMO equivalent of EEV-PMO, and the data were collected ~4 weeks after the last dose

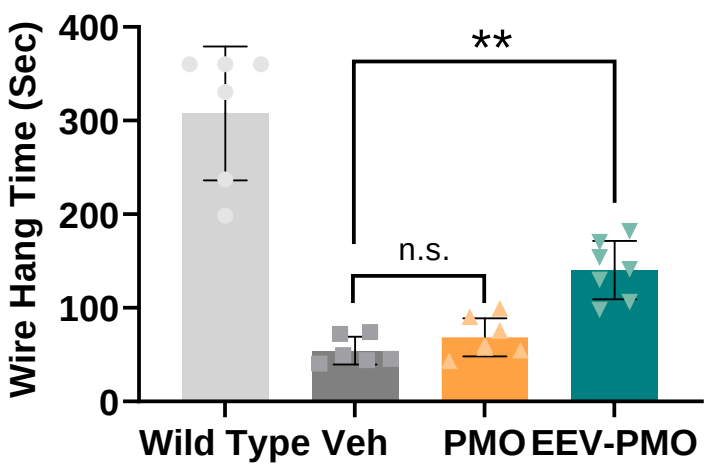
REPEAT EEV-PMO TREATMENT RESULTS IN CK CORRECTION AND FUNCTIONAL IMPROVEMENT

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

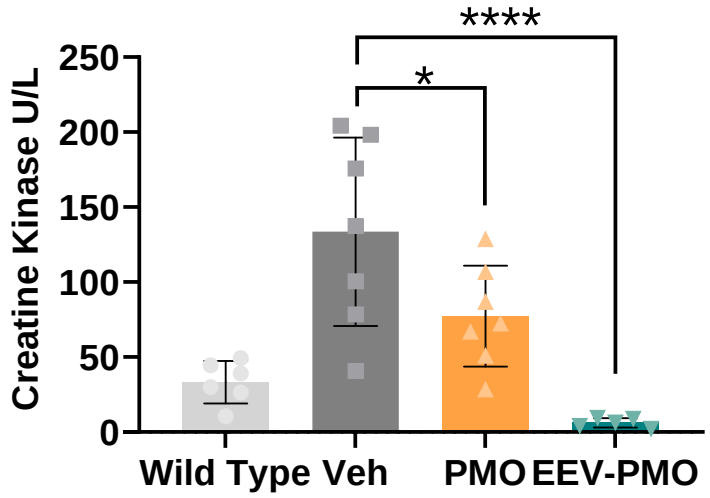
Grip Strength



Wire Hang Time



Serum CK Levels

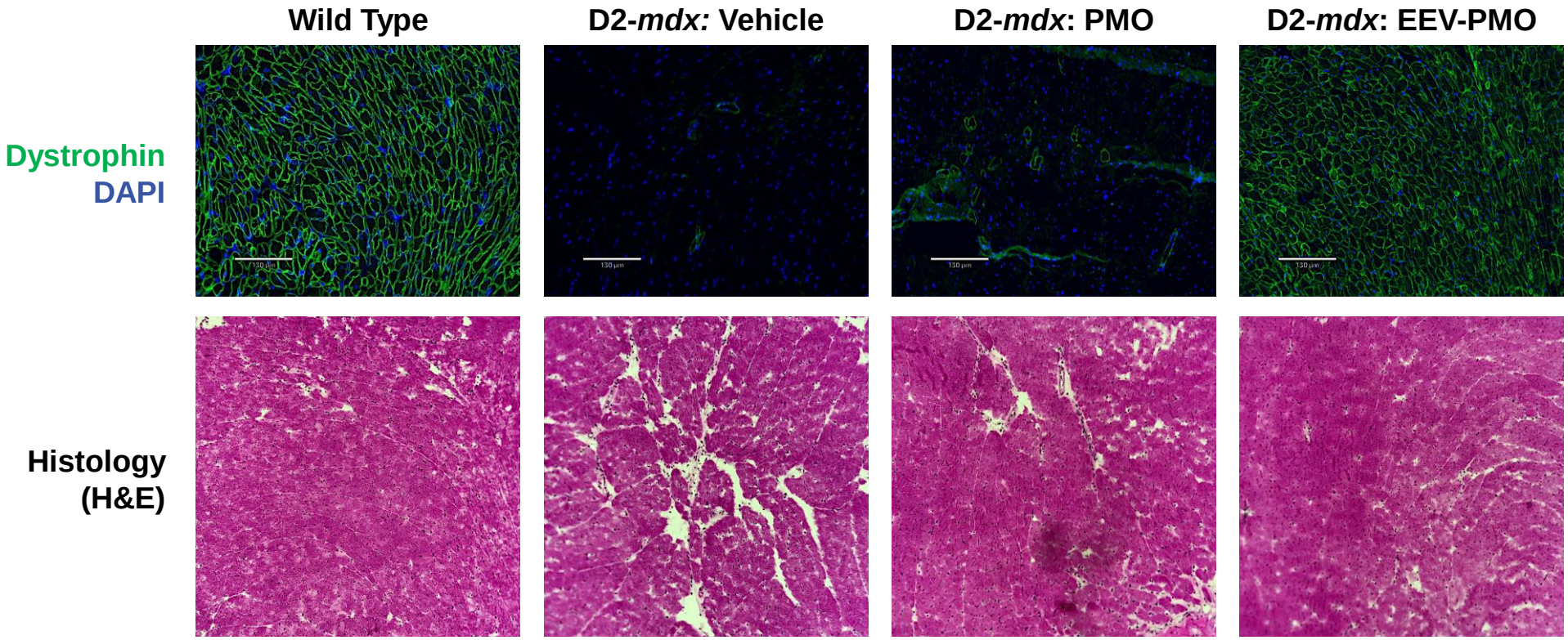


- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO or 20 mg/kg PMO equivalent of EEV-PMO, and the data were collected ~2 weeks after the last dose

n.s. not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

REPEAT EEV-PMO TREATMENT CORRECTS DYSTROPHIN AND CARDIAC PATHOLOGY

Repeat EEV-PMO-23 treatment corrected dystrophin expression and cardiac pathology after four monthly IV doses in D2-*mdx* mice

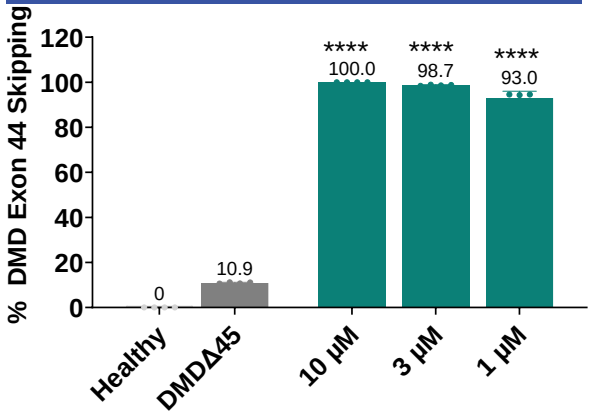


- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO or 20 mg/kg PMO equivalent of EEV-PMO, and the data were collected ~4 weeks after the last dose

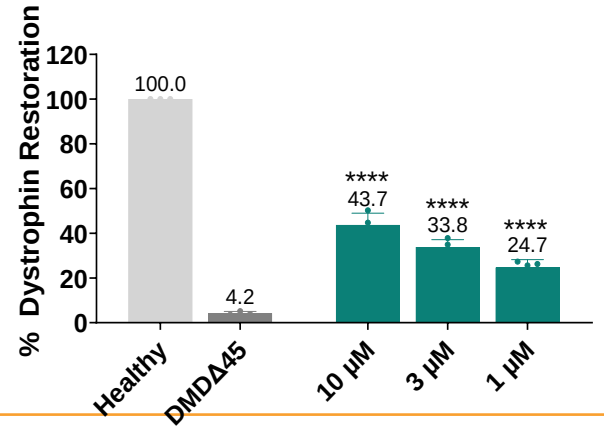
SIGNIFICANT PROMISE OF ENTR-601-44 IN PATIENT CELLS AND TRANSGENIC MOUSE MODELS

Robust dose-dependent exon 44 skipping and dystrophin protein restoration were observed in DMD patient-derived muscle cells treated with ENTR-601-44

Exon Skipping



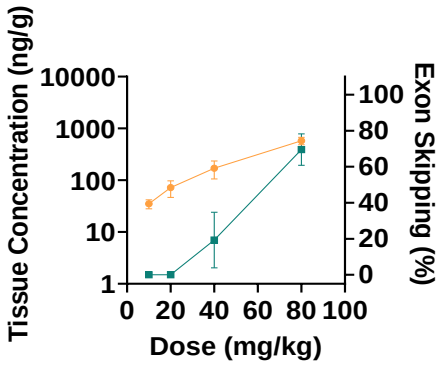
Dystrophin



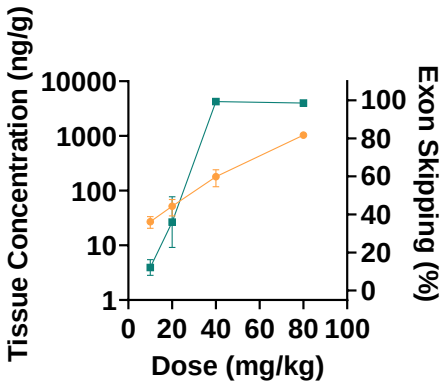
****p<0.0001

Dose-dependent tissue exposure and exon skipping in cardiac and skeletal muscles in a transgenic mouse carrying the full-length human DMD gene

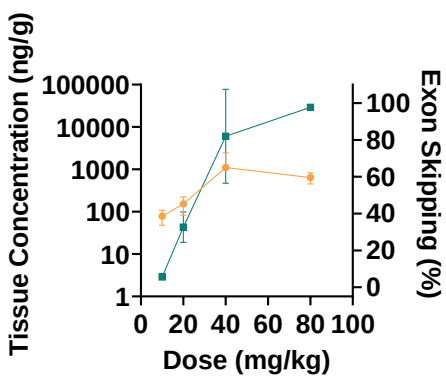
Heart



Tibialis Anterior



Diaphragm



Tissue Concentration (ng/g) Exon Skipping (%)

- **<u>hDMD transgenic mice</u>** express full-length human dystrophin gene which allows for preclinical testing (target engagement) of human sequence-specific PMO for DMD transcript correction
- Exon skipping and tissue concentrations in various muscles groups assessed 5-day post 10, 20, 40 and 80 mg/kg IV dosage

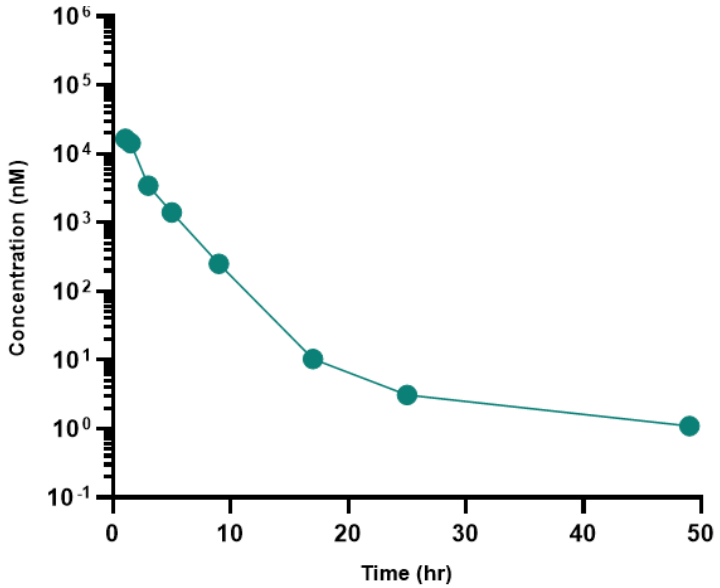
't Hoen, P.A. et al. *J. Biol. Chem.* **2008**, 5899.

ENTR-601-44 IN NHP CONFIRMS LEAD CANDIDATE

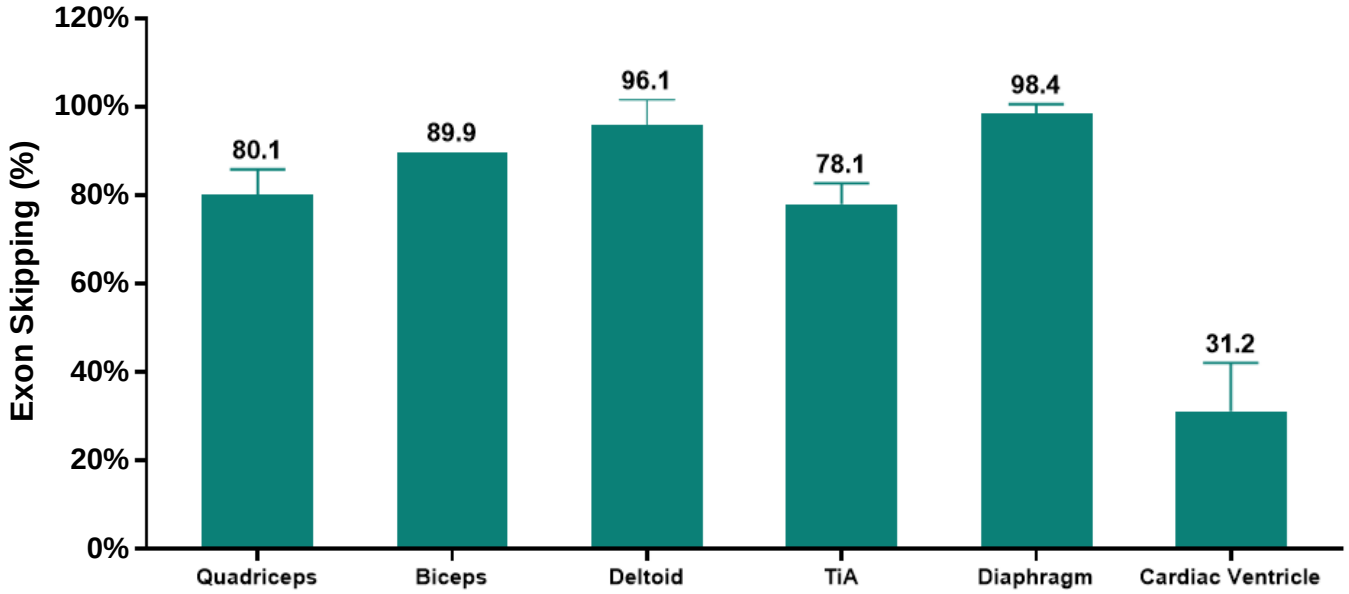
An extended circulating half-life for ENTR-601-44 was observed in the NHP

A single 30 mg/kg IV dose of ENTR-601-44 resulted in meaningful levels of exon skipping in both skeletal muscles and the heart of the NHP which provides confidence in translational potential

ENTR-601-44 in NHP Plasma



ENTR-601-44 Exon Skipping in NHP Muscle



- At 7 days post 1 hour IV infusion at 30 mg/kg, robust exon 44 skipping observed across different muscle groups isolated from the ENTR-601-44 treated NHP

ENTRADA DMD DATA SUMMARY

Entrada's *in vivo* data are consistent and promising; These advances represent a robust set of translational data

- Promising exon skipping across *mdx*, D2-*mdx*, human dystrophin mouse and NHP studies
- Exon skipping translates to promising dystrophin production in heart and skeletal muscles
- Dystrophin production is sufficient to result in functional improvement
- Durable dystrophin production over 4+ weeks after a single injection
- ENTR-601-44 candidate selected on the basis of exon skipping and tolerability with IND planned in 2H 2022
- Second candidate for patients with DMD that have mutations amenable to skipping of exon 45 is planned
- The robust distribution of oligonucleotides in target tissues supports development of EEV-conjugated oligonucleotide therapies for myotonic dystrophy type 1 (DM1) and potentially other neuromuscular diseases

ACKNOWLEDGEMENTS

Thank you!

(www.Entradatx.com)

- *Discovery Research*
- *Neuromuscular*
- *Pharmacology*
- *Product Development*

The Entrada team at the 2021 MDA Muscle Walk