Development and Optimization of the Endosomal Escape Vehicle (EEV™) Platform to Enhance the Intracellular Delivery of Oligonucleotides

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TIDES ASIA, March 19, 2024

entrada THERAPEUTICS

DISCLAIMER

This presentation includes express and implied “forward-looking statements.” Forward looking statements include all statements that are not historical facts, and in some cases, can be identified by terms such as “may,” “might,” “will,” “could,” “would,” “should,” “expect,” “intend,” “plan,” “objective,” “anticipate,” “believe,” “estimate,” “predict,” “potential,” “continue,” “ongoing,” or the negative of these terms, or other comparable terminology intended to identify statements about the future. Forward-looking statements contained in this presentation include, but are not limited to, statements about our product development activities and clinical trials, our regulatory filings and approvals, statements related to our ability to initiate and recruit for a healthy volunteer trial for ENTR-601-44 in the United Kingdom with first subject dosed in September 2023, expectations regarding the timing of data from our Phase 1 trial for ENTR-601-44 in the second half of 2024, the ability to resolve the clinical hold for ENTR-601-44 and subsequent activities, expectations regarding the timing or content of any update regarding our regulatory filings, expectations regarding the safety and therapeutic benefits of ENTR-601-44, our ability to develop and advance our current and future product candidates and discovery programs, our ability to establish and maintain collaborations or strategic relationships, our ability to raise additional funding, the rate and degree of market acceptance and clinical utility of our product candidates, the potential of our EEV product candidates and EEV platform, the ability and willingness of our third-party collaborators to continue research and development activities relating to our product candidates, including our Vertex partnership for ENTR-701, our collaborators’ ability to protect our intellectual property for our products, and the sufficiency of our cash resources through 2025. By their nature, these statements are subject to numerous risks and uncertainties, including factors beyond our control, that could cause actual results, performance or achievement to differ materially and adversely from those anticipated or implied in the statements. You should not rely upon forward-looking statements as predictions of future events. Although our management believes that the expectations reflected in our statements are reasonable, we cannot guarantee that the future results, performance or events and circumstances described in the forward-looking statements will be achieved or occur. Recipients are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date such statements are made and should not be construed as statements of fact.

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.

OUR MISSION

To Treat Devastating Diseases with Intracellular Therapeutics

entrada THERAPEUTICS

A DIFFERENTIATED AND EXPANDING PIPELINE

Entrada’s pipeline includes a diverse array of high potential and high value assets;
Each disease has a substantial patient population with a significant unmet medical need

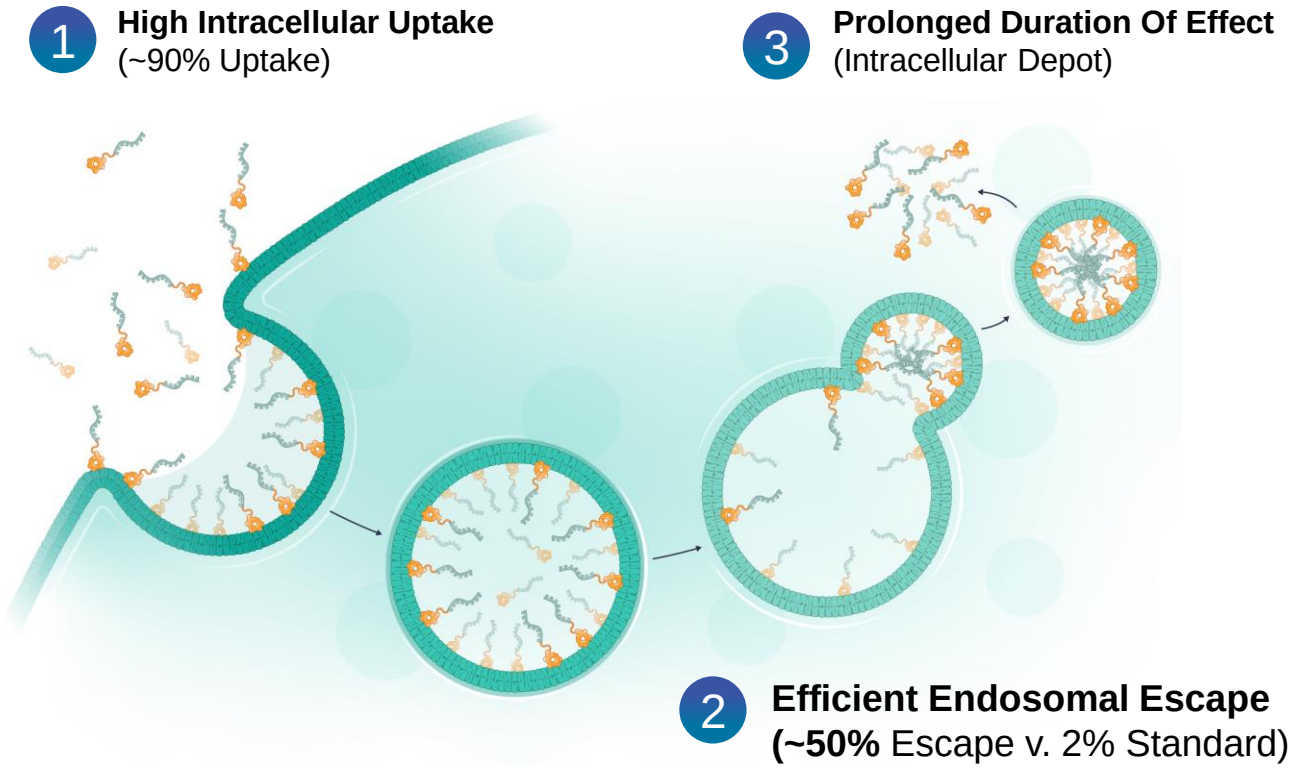
Disease/Condition		Discovery	Lead Optimization	IND Enabling	Clinical
Neuromuscular	DMD	ENTR-601-44			Trial ongoing; SAD data expected H2 2024
		ENTR-601-45			On track to file CTA/IND in Q4 2024
		ENTR-601-50			Clinical candidate selected in Q4 2023; CTA/IND filing is expected in 2025
		Exon 51			
	DM1	VX-670 (formerly ENTR-701)			VERTEX Phase 1/2 trial initiated
	POMPE	ERT/Oligonucleotide			
Beyond Neuromuscular		Immunology			
		Ocular			
Platform Expansion		Gene editing			
		mRNA delivery			
		Antibody/Protein			

EEV™ PLATFORM DEVELOPMENT AND OPTIMIZATION

Endosomal Escape Vehicle (EEV™) Therapeutics

- 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**

Entrada seeks to solve a fundamental problem: a lack of efficient cellular uptake and escape from the endosome; Both are critical to intracellular target engagement and therapeutic benefit

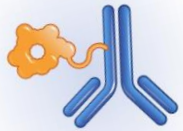


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

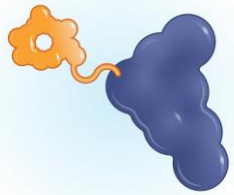
A BROADLY APPLICABLE PLATFORM

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

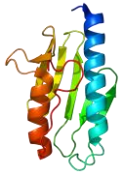
Antibodies



Enzymes

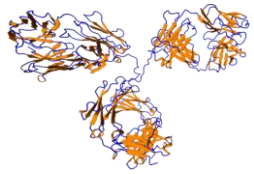


Oligonucleotides



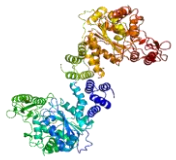
550-600 KDa

Hybrid frataxin



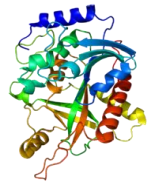
150 KDa

Antibody



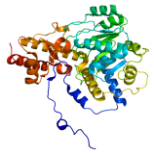
98 KDa

Thymidine phosphorylase



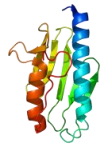
96 KDa

Purine nucleoside phosphorylase



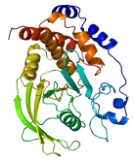
86 KDa

Alanine-glyoxylate aminotransferase



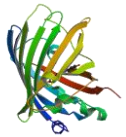
46 KDa

Human frataxin



37 KDa

PTP1B catalytic domain



32 KDa

EGFP

16 KDa

Nanobody

6 KDa

Oligonucleotide

1-3 KDa

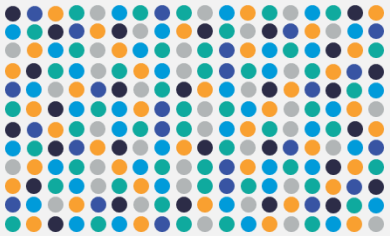
Various peptide cargos

EGFP, enhanced green fluorescent protein.

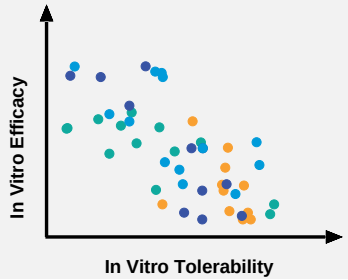
DISCOVERY ENGINE FOR EEV THERAPEUTICS
EEV-OLIGO EXAMPLE

Fit-for-purpose EEVs can be designed for target indications and modalities via iterative optimizations of EEV peptides through medicinal chemistry, *in vitro* and *in vivo* screenings

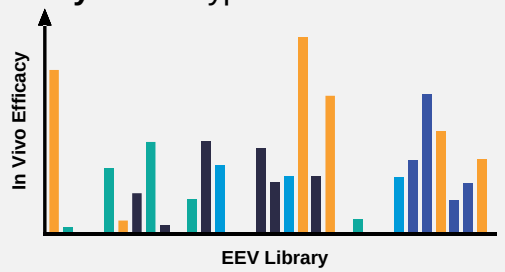
Chemically diverse macrocyclic **EEV library** generated through medicinal and combinatorial chemistry



In vitro **delivery and counter-screening** in relevant cell types with therapeutic payload



In vivo screening to assess **functional delivery and pharmacodynamic activity** in wild-type and disease models

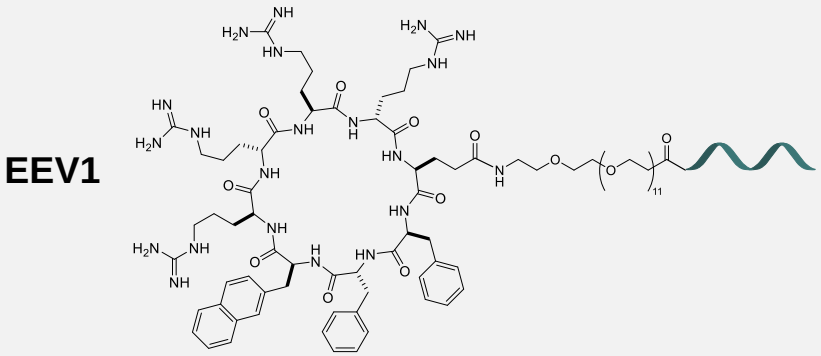


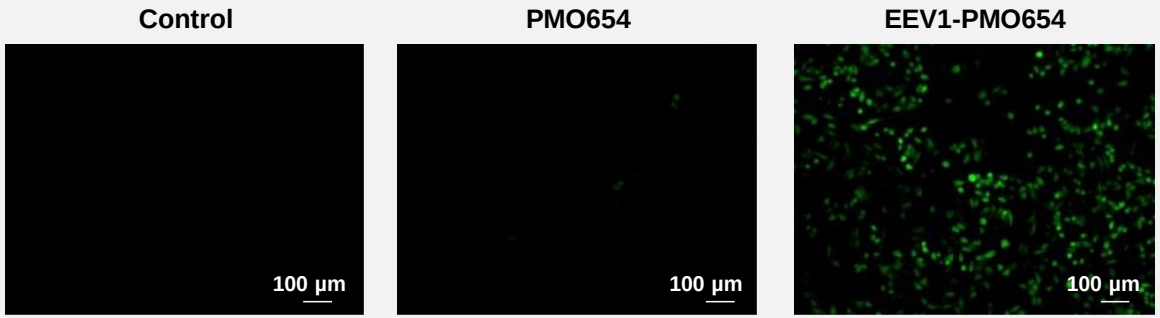
EEV, endosomal escape vehicle.

OLIGO DELIVERY WITH FIRST GENERATION EEV
EEV1 EXAMPLE

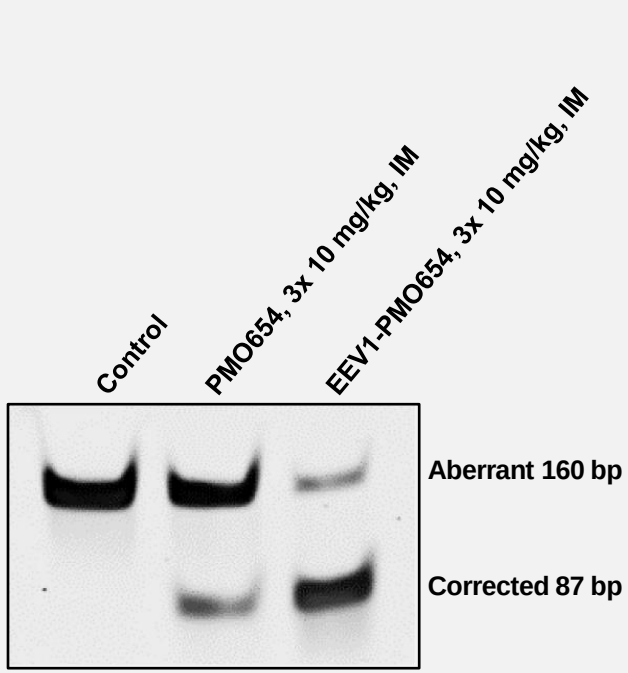
A first-generation EEV1 peptide-PMO construct enhanced splice correction *in vitro* and after local injection, demonstrated functional delivery of oligonucleotides

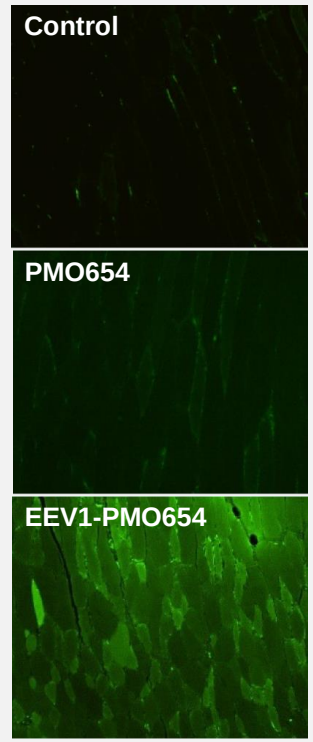
Splicing Correction in HeLa EGFP-654 Cells





Three Daily IM Doses of EEV1-PMO654





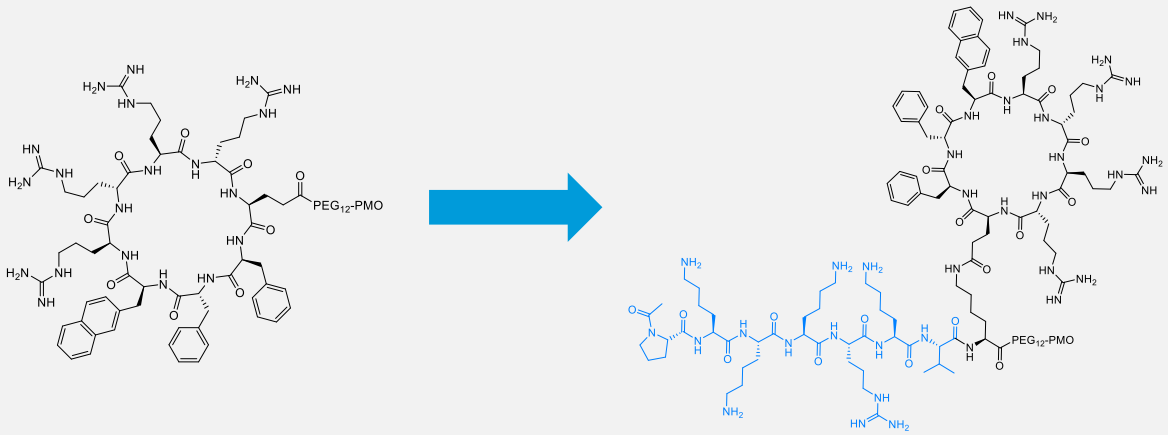
[a]Treatment of HeLa EGFP-654 cells with antisense oligonucleotides, such as PMOs, could switch the splicing and restore expression of EGFP; **EEV**, endosomal escape vehicle; **PMO**, phosphorodiamidate morpholino oligomer; **IM**, intramuscular; Qian, Z. et al. *Biochemistry* 2014, 2016; Li, X. et al. *Mol. Ther. Nucleic Acids* 2023.

ENHANCED OLIGONUCLEOTIDE DELIVERY
EEV2 EXAMPLE

The addition of an exocyclic peptide sequence to a first-generation EEV1 peptide improved exon skipping in skeletal and cardiac muscle of *mdx* mice after intravenous injection

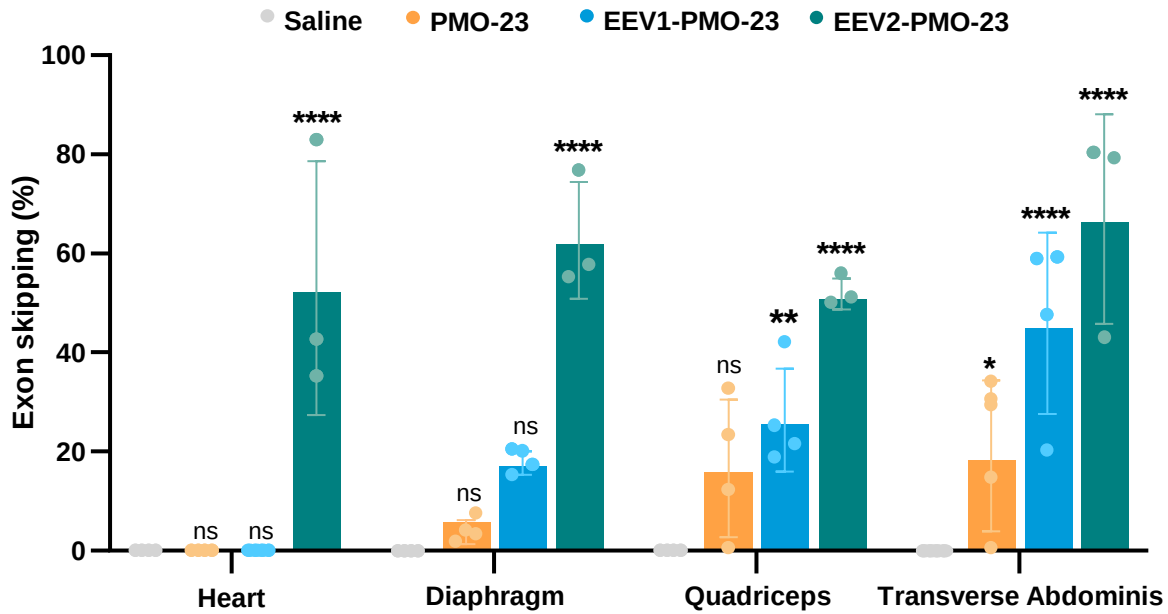
Structure of EEV2 Construct

EEV1 + exocyclic peptide sequence = EEV2



- To create the EEV2 construct, EEV1 was modified to include an exocyclic peptide sequence to improve delivery to the nucleus

Higher In Vivo Exon Skipping with EEV2 vs. EEV1



- *mdx* mice were evaluated for exon skipping (via RT-PCR) 7 days following a single 20-mg/kg IV injection of saline, PMO-23, EEV1-PMO-23, or EEV2-PMO-23

p<0.01, ****p<0.0001 vs. Vehicle. Values are shown as mean ± standard deviation. *mdx* is a DMD mouse model with a nonsense mutation in DMD exon 23; **EEV, endosomal escape vehicle; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer; **RT-PCR,** reverse transcription polymerase chain reaction; Li, X. et al. *Mol. Ther. Nucleic Acids* 2023.

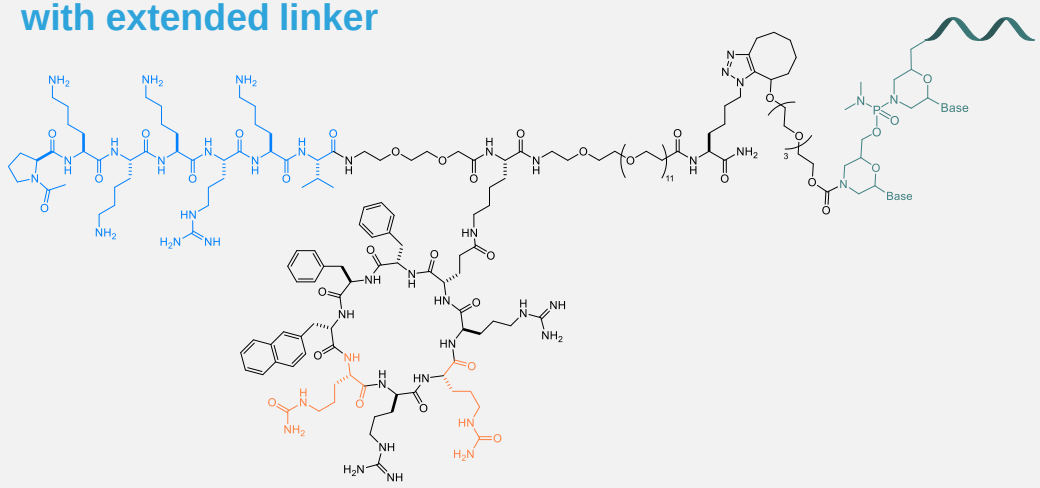
OPTIMIZATION OF EEV FOR MUSCLE DELIVERY
EEV3 Example

Rational substitution of cationic residues with a surrogate results in robust functional delivery to skeletal and cardiac muscle

EEV3-PMO654 Structure and Medicinal Chemistry

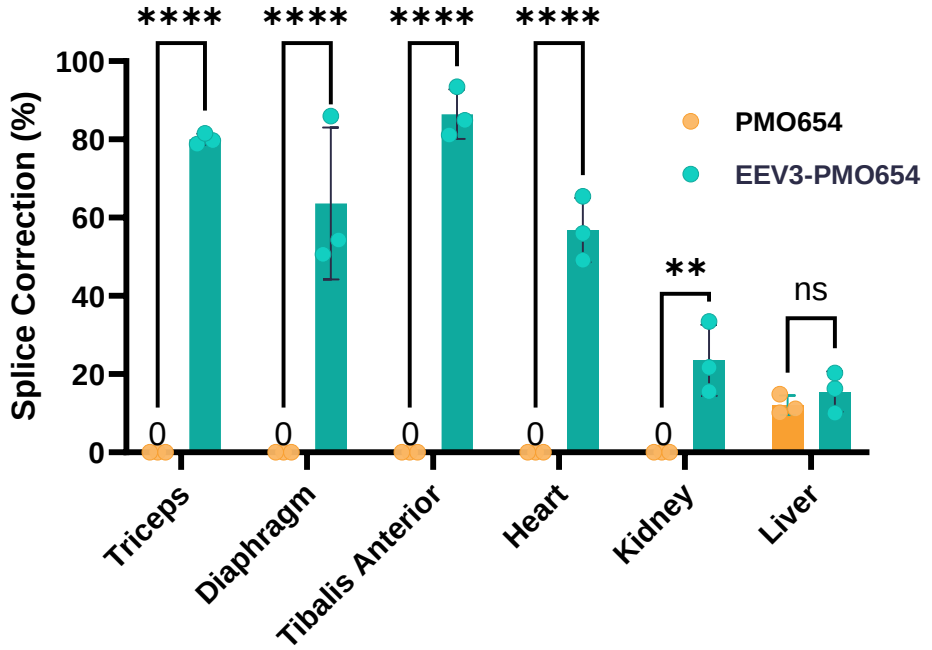
Exocyclic peptide sequence with extended linker

Conjugation with PMO



Substitution of positively charged arginine residues with neutral charged citrullines

Enhanced Functional Delivery to Muscle



- *EGFP654* mice were evaluated for splice correction 7 days following three weekly 10 mg/kg IV injections of PMO654 or EEV3-PMO654

p<0.01, ****p<0.0001; values are shown as mean ± standard deviation. **EEV, endosomal escape vehicle; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer.

TRANSLATION FROM UPTAKE TO OUTCOMES

Murine Example

EEV-therapeutic candidates have demonstrated favorable pharmacological properties: efficient intracellular delivery, significant uptake in target tissues and potent pharmacodynamic outcomes

Tissue Uptake in Muscle

- ✓ Skeletal muscle
- ✓ Cardiac muscle

+

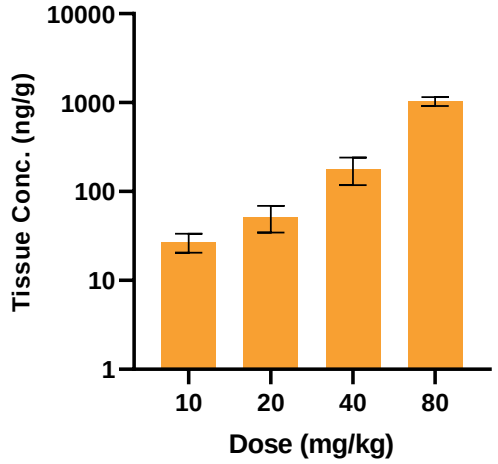
Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

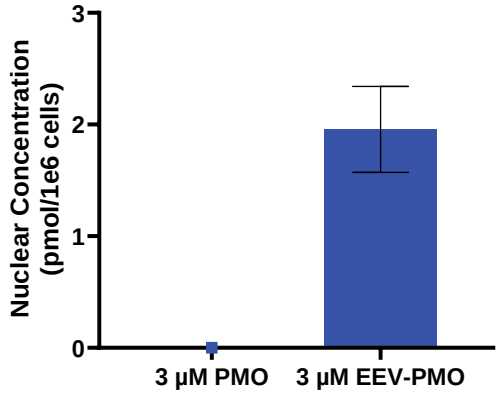
=

Pharmacodynamic Outcome

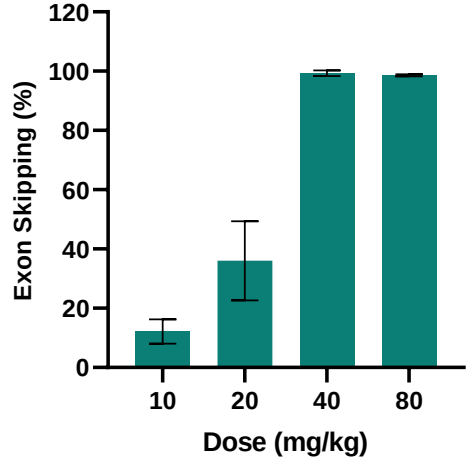
- ✓ Rapid, dose-dependent response
- ✓ Duration of at least 12 weeks



IV, hDMD mice, 5-day post injection



24-hour incubation

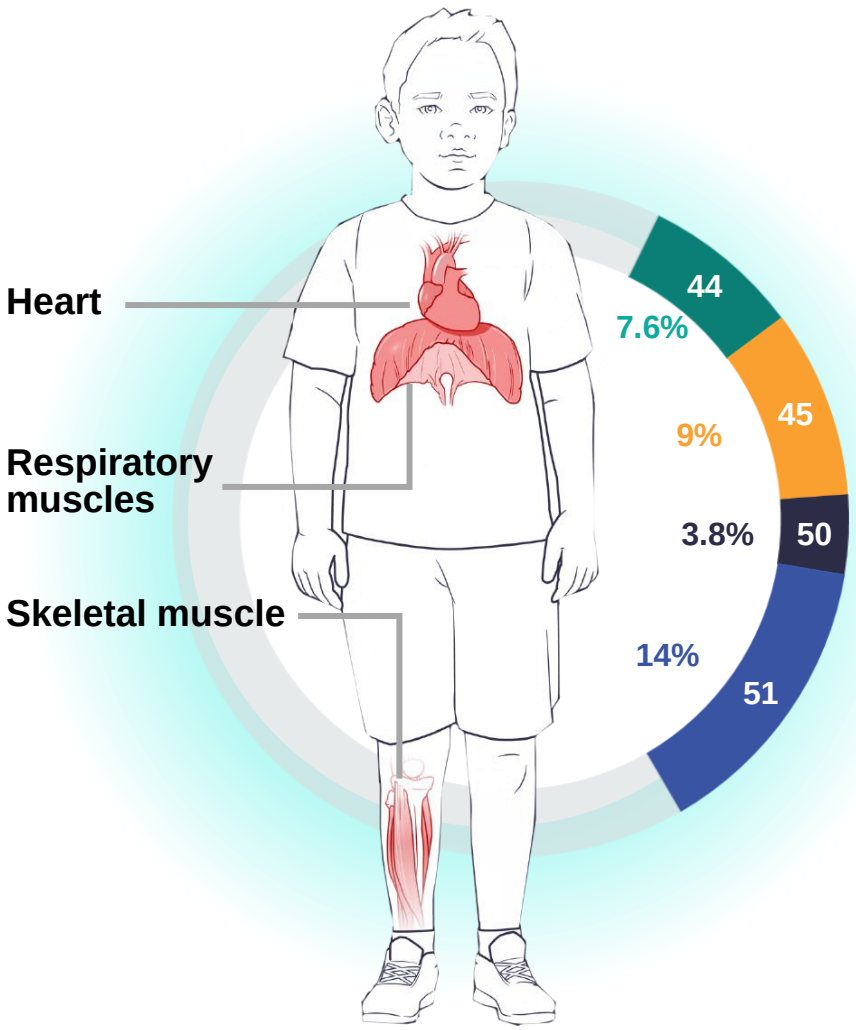


IV, hDMD mice, 5-day post injection

hDMD mice express full-length human dystrophin gene; **IV,** intravenous; **PMO**, phosphorodiamidate morpholino oligomer.

DUCHENNE MUSCULAR DYSTROPHY

SIGNIFICANT UNMET NEED IN DMD



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

~40,000

people in the **U.S. and Europe** have Duchenne[1,2]

Duchenne Franchise

ENTR-601-44 **Phase 1**
Phase 1 data expected H2 2024

ENTR-601-45 **IND Enabling**
CTA/IND filing expected in Q4 2024

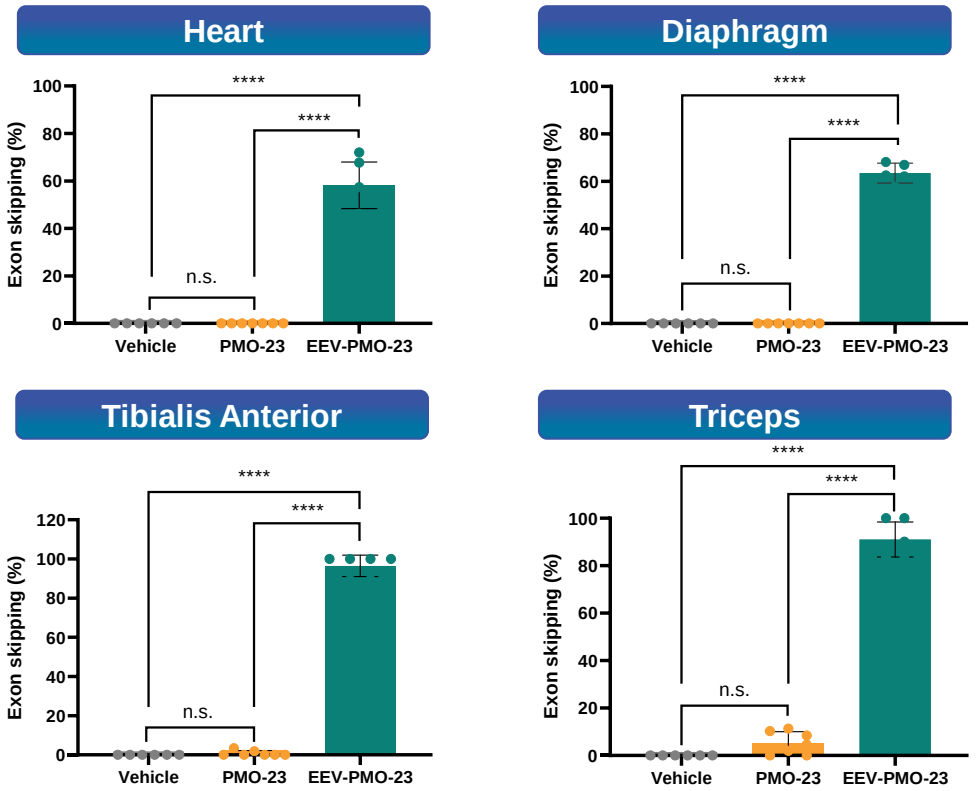
ENTR-601-50 **IND Enabling**
CTA/IND filing expected in 2025

Exon 51 **Lead Optimization**
Candidate selection expected in H1 2024

[1]Parent Project Muscular Dystrophy: About Duchenne. [2]Europeans Medicines Agency: Orphan designation for the treatment of Duchenne muscular dystrophy. Bladen, C.L. et al *HUMAN MUTATION*, 2015.

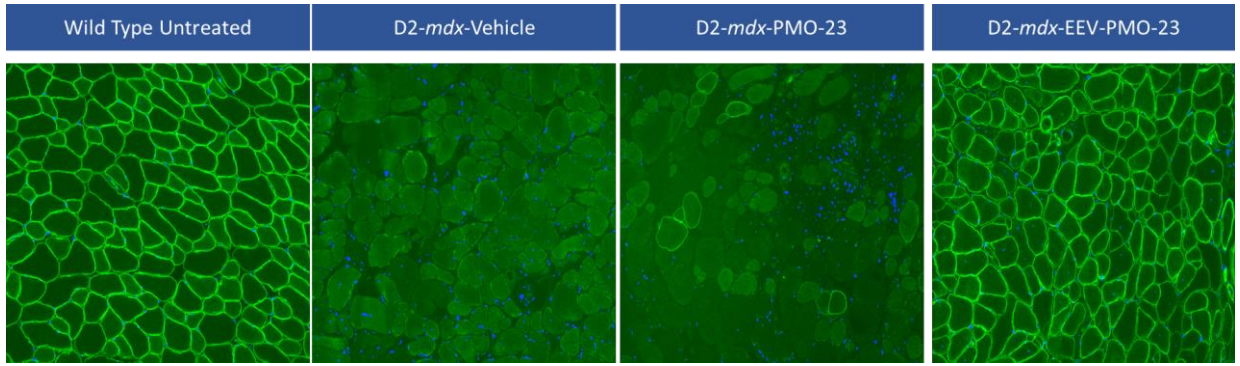
EEV-PMO RESTORES MUSCLE INTEGRITY
D2-*mdx* Mice

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

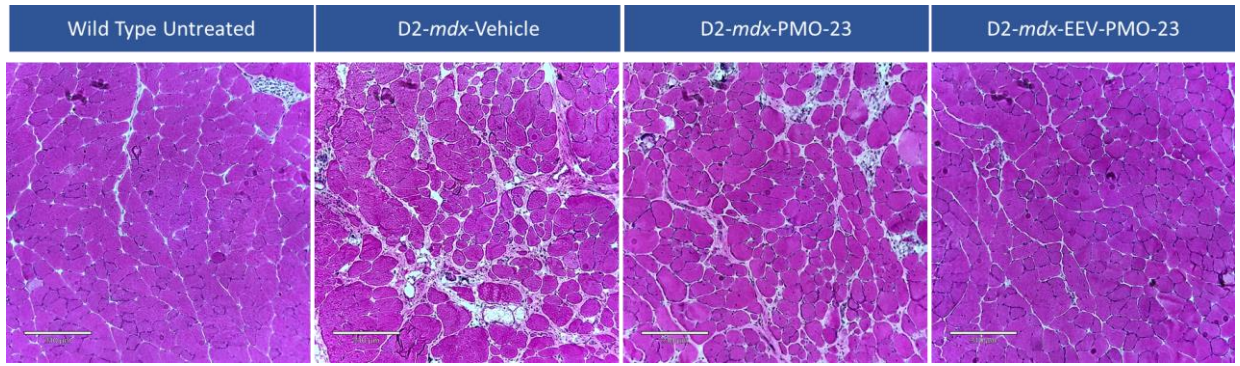


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

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 on a DBA/2J background and better recapitulates disease pathology (Fukada, S. et al. *Am. J. Path.* 2010, Coley, W .D. et al. *Hum. Mol. Genet.* 2016). ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

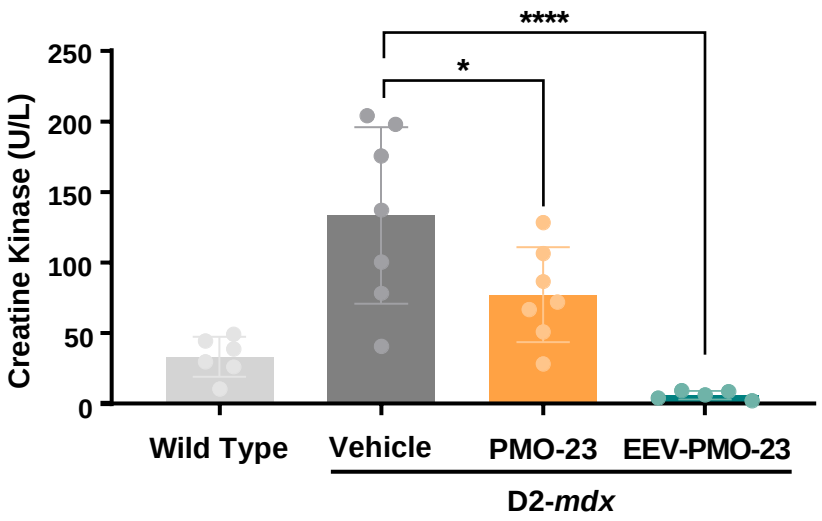
EEV-PMO RESULTS IN FUNCTIONAL IMPROVEMENT

D2-*mdx* Mice

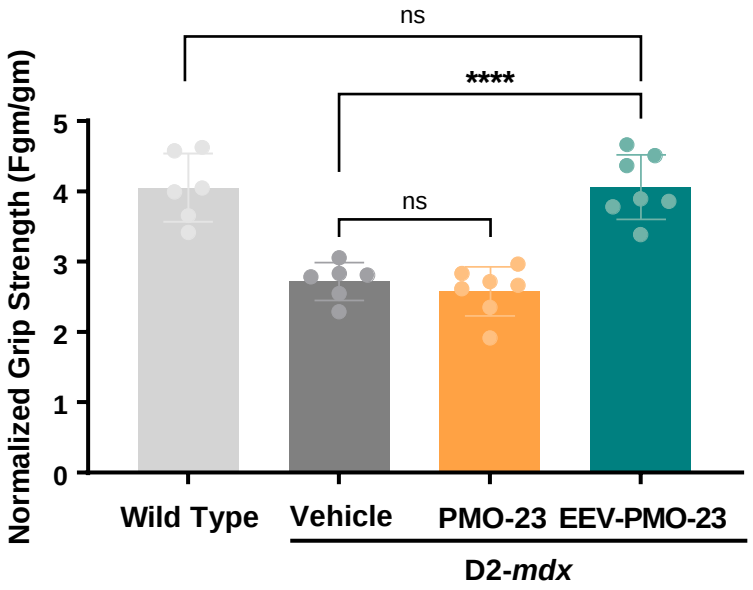
Repeat EEV-PMO-23 treatment normalized serum CK levels and showed significant improvements in muscle function when compared to PMO alone after four monthly IV doses in D2-*mdx* mice

*p<0.05, **p<0.01, ****p<0.0001

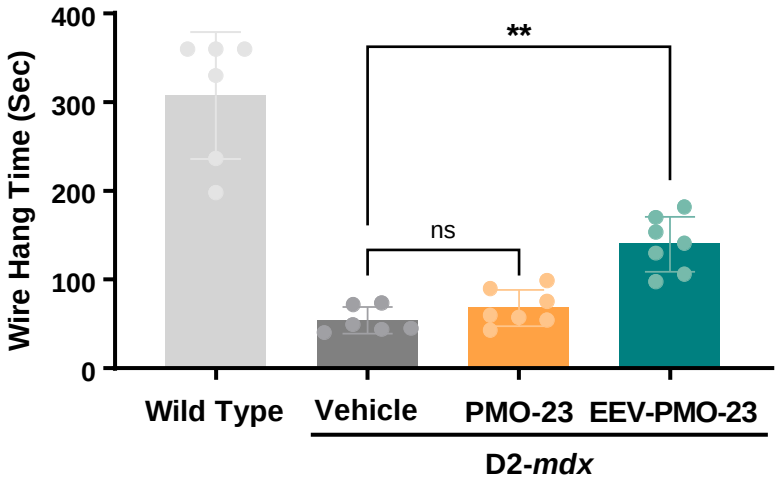
Serum CK Levels



Grip Strength



Wire Hang Time

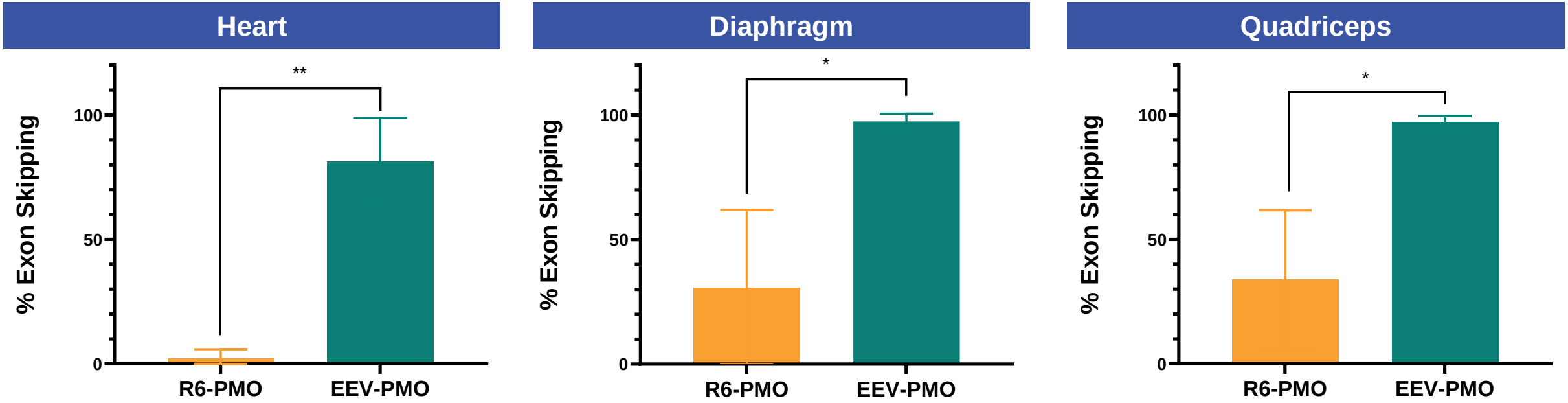


- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~2 weeks after the last dose

Values are shown as mean ± standard deviation. **D2-*mdx*** is a DMD mouse model with a nonsense mutation in *DMD* exon 23 (Coley, W.D. et al. *Hum. Mol. Genet*. 2016). The D2-*mdx* model is on a DBA/2J background and better recapitulates disease pathology (Fukada, S. et al. *Am. J. Path.* 2010). **CK**, creatine kinase; **EEV**, endosomal escape vehicle; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer; **PMO-23**, mouse *DMD* exon 23 skipping phosphorodiamidate morpholino oligomer.

SUPERIOR TO ALTERNATIVE PEPTIDES
R6-PMO Example

EEV-PMO significantly improved exon 23 skipping after 3 days in *mdx* mice as compared to competitive R6-PMO



- EEV-PMO-23 demonstrates significantly improved PD effects after single 40 mg/kg IV dose in *mdx* mice

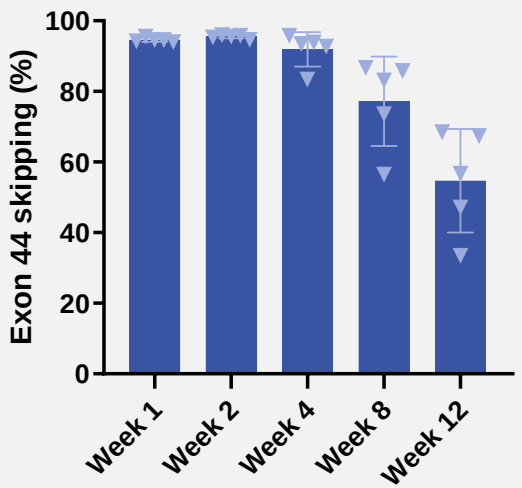
Values are shown as mean ± standard deviation; *p<0.05, **p<0.01; ***mdx*** is a DMD mouse model with a nonsense mutation in *DMD* exon 23; **PMO-23**, mouse *DMD* exon 23 skipping phosphorodiamidate morpholino oligomer; **IV,** intravenous.

ENTR-601-44

CONSISTENT AND DURABLE EFFICACY OF EEV-PMO WAS DEMONSTRATED ACROSS SPECIES

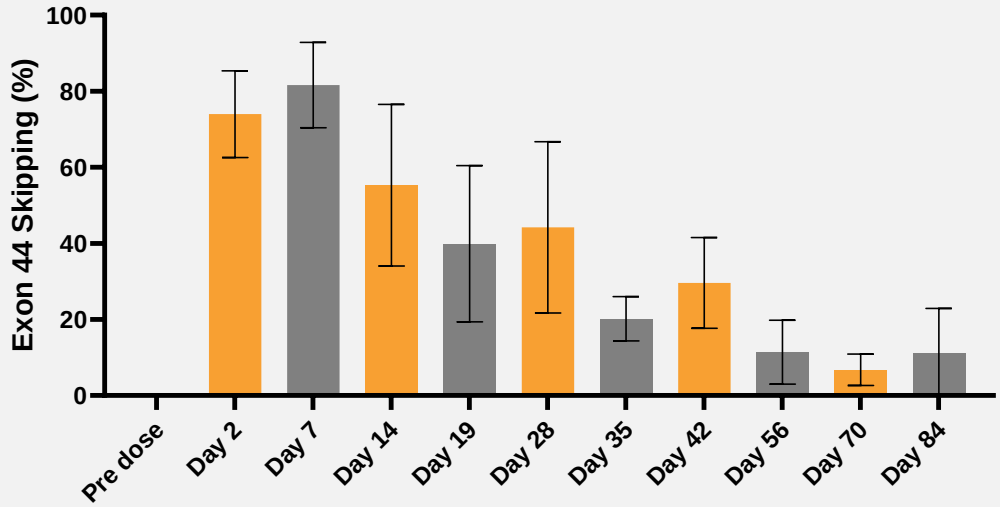
Significant patient benefit is implied by data in the mouse and the monkey at clinically relevant levels; *in vitro* data suggests much higher target engagement in patient cells

Exon 44 Skipping in hDMD Mouse



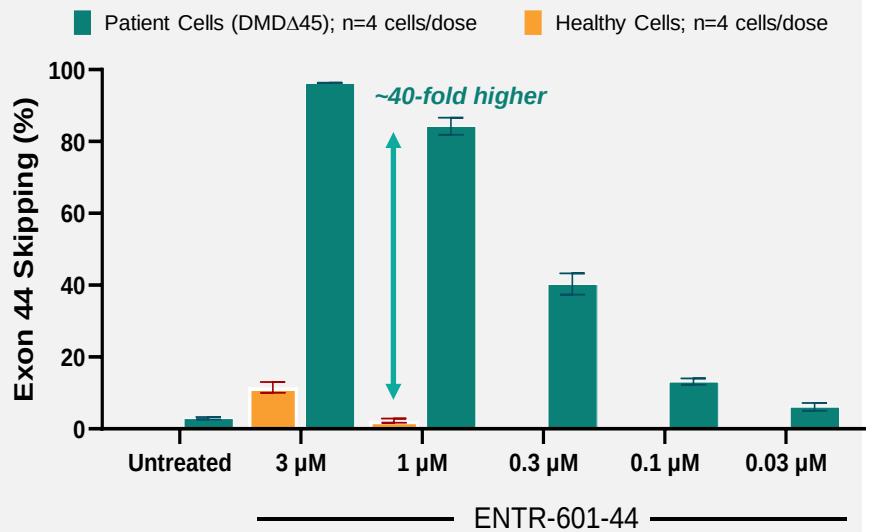
- Single IV 60 mg/kg dose of ENTR-601-44
- Tibialis Anterior

Exon 44 Skipping in Monkey



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

Exon 44 Skipping in Healthy and Patient-Derived Muscle Cells



- Robust dose-dependent exon 44 skipping was observed in DMD patient-derived muscle cells harboring an exon 44 skip-amenable mutation

hDMD transgenic mice (left) express full-length human dystrophin gene ('t Hoen, A.C. et al. *J. Biol. Chem.* 2008). **DMDΔ45** (right) are immortalized myoblasts from DMD patients harboring an out-of-frame exon 45 deletion and further differentiated into myotubes. Values are shown as mean ± standard deviation. **ENTR-601-44** is a DMD exon 44 skipping EEV-oligonucleotide construct. **DMD**, Duchenne muscular dystrophy; **hDMD,** human Duchenne muscular dystrophy; **IV,** intravenous.

ENTR-601-44 Data Summary

Significant patient benefit is implied by data in the mouse and the monkey at clinically relevant levels; *in vitro* data suggests much higher target engagement in patient cells

- ✓ High levels of 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 observed results in functional improvement in D2-*mdx* mouse
- ✓ Extended circulating half-life and durable exon skipping over 12+ weeks from a single injection of ENTR-601-44 was observed in the NHP

ENTR-601-44-101:
Phase 1 clinical trial ongoing

- First participant dosed in September 2023
- Completed dosing of cohorts 1, 2 and 3
- Data anticipated in the H2 2024
- Phase 1 clinical data will support a global clinical trial in patients*

*MAD/Phase 2b study is subject to regulatory feedback and the outcome of the SAD study.

ENTR-601-44 Clinical Strategy

First-in-Human Trial

Data expected H2 2024

Single Ascending Dose (SAD) Study
in Healthy Volunteers (ENTR-601-44-101)

- **First subject dosed in Q3 2023**
- **Completed dosing for cohort 1, 2 and 3**
- **~40 subjects**

Outcome Measures

- Safety and tolerability
- Evaluation of PK and PD
- Target engagement as measured via exon skipping

Multiple Ascending Dose/Phase 2b (Global)

Regulatory filings expected in Q4 2024

Multiple Ascending Dose (MAD) Study*
in Exon 44 Skipping Amenable Patients

Phase 2b Study*
in Exon 44 Skipping Amenable Patients

Target Product Profile:

- ***Double digit dystrophin improvement from baseline***
- ***Dosing interval ≥ every 6 weeks***

Dose Selection

File for Accelerated Approval

Phase 2b | **Open-label Extension**

Outcome Measures

- Safety and tolerability
- Evaluation of PK and PD
- Evaluation of exon skipping and dystrophin production (skeletal muscle)

Primary Efficacy Measures

- Change in dystrophin level (skeletal muscle)

Secondary/Exploratory Efficacy Measures

- NSAA (North Star Ambulatory Assessment), timed function tests, and other measures of function (e.g., PUL 2.0; wearable device)
- Other parameters may include cardiac MRI, FVC, QoL

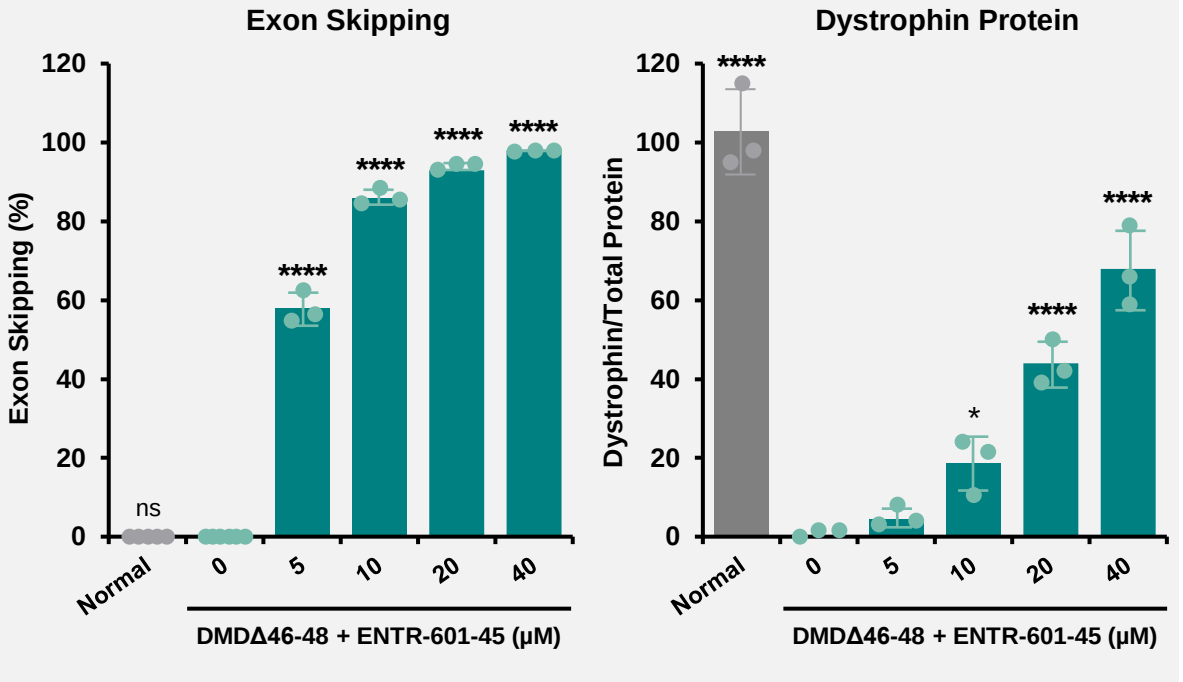
*MAD/Phase 2b study is subject to regulatory feedback and the outcome of the SAD study.

ENTR-601-45

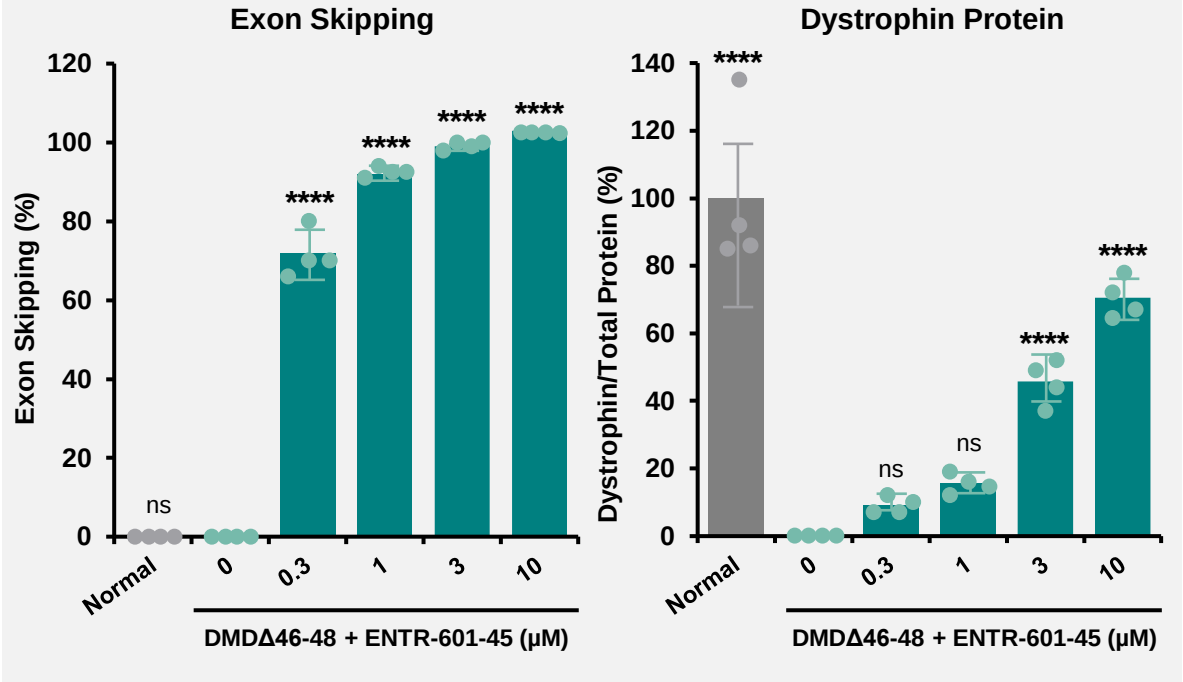
ENTR-601-45 IN VITRO EFFICACY

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



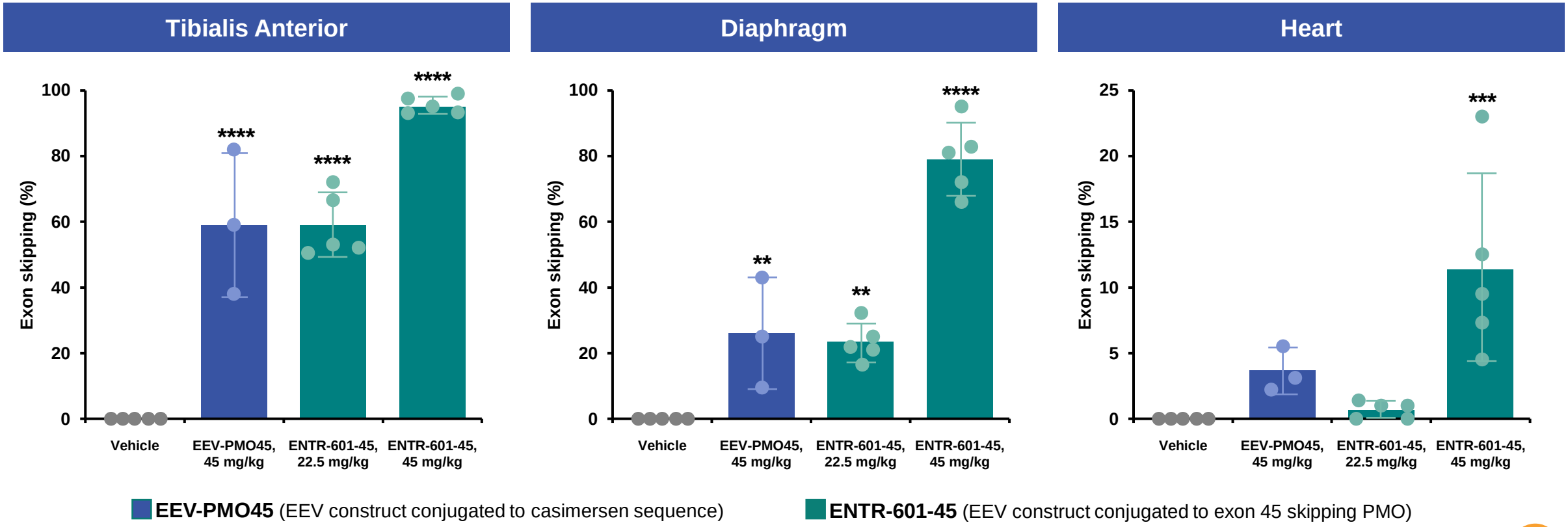
- DMD patient-derived skeletal (n=3) and cardiac (n=4) muscle cells (DMDΔ46-48) were treated with ENTR-601-45 for 24 hours

*p<0.05, ****p<0.0001 vs. untreated DMDΔ46-48 cells. Values are shown as mean ± standard deviation. **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide construct. **DMDΔ46-48** induced pluripotent stem cell-derived skeletal and cardiac muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601-45 TARGET ENGAGEMENT IN hDMD MICE

A single dose of 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

*p<0.05, **p<0.01, ****p<0.0001 vs. Vehicle



Values are shown as mean ± standard deviation (n=3-5). **ENTR-601-45** is a DMD exon 45 skipping EEV-oligonucleotide construct. **hDMD** transgenic mice express full-length human dystrophin gene ('t Hoen, P.A. et al. *J. Biol. Chem.* 2008). Casimersen is an exon 45 skipping PMO approved in the US. Concentrations provided are PMO equivalent.
DMD, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle; **hDMD,** human Duchenne muscular dystrophy; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601-45 Data Summary

ENTR-601-45 consistently demonstrated robust *in vitro* and *in vivo* data;
Regulatory submissions planned in Q4 2024

- **Patient-derived Cells**
  - ENTR-601-45 showed robust exon skipping and dystrophin protein production in patient-derived cardiac and skeletal muscle cells
- **DMD mouse models**
  - High levels of exon skipping were measured in hDMD mouse heart and skeletal muscle tissue
  - Exon 44 deletion mouse amenable to exon 45 skipping has been generated and population is being expanded externally
- **Process development**
  - Non-GMP ENTR-601-45 generated to support GLP toxicology studies
  - GMP drug substance production complete

Next Steps

- Planning for a global MAD trial in Duchenne patients
- Regulatory submissions expected in Q4 2024

PIPELINE EXAPNSION

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	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	**RNA Splicing**	Modify RNA via exon/intron splicing to activate protein expression
RNA	**RNA Blocking**	Block trinucleotide repeats in RNA to inhibit adverse binding
RNA	**RNA Silencing**	Silence or knockdown RNA to prevent protein expression
PROTEIN	**Protein Replacement**	Replace proteins and enzymes
PROTEIN	**Protein Inhibition**	Inhibit protein signaling pathways
PROTEIN	**Protein Degradation**	Degrade disease-causing proteins

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