Therapeutic Potential of ENTR-601-44, an Endosomal Escape Vehicle (EEV™)-Oligonucleotide Conjugate for the Treatment of Exon 44 Skip Amenable Duchenne Muscular Dystrophy

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2025 Solve FSHD

entrada THERAPEUTICS

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EEV™ PLATFORM

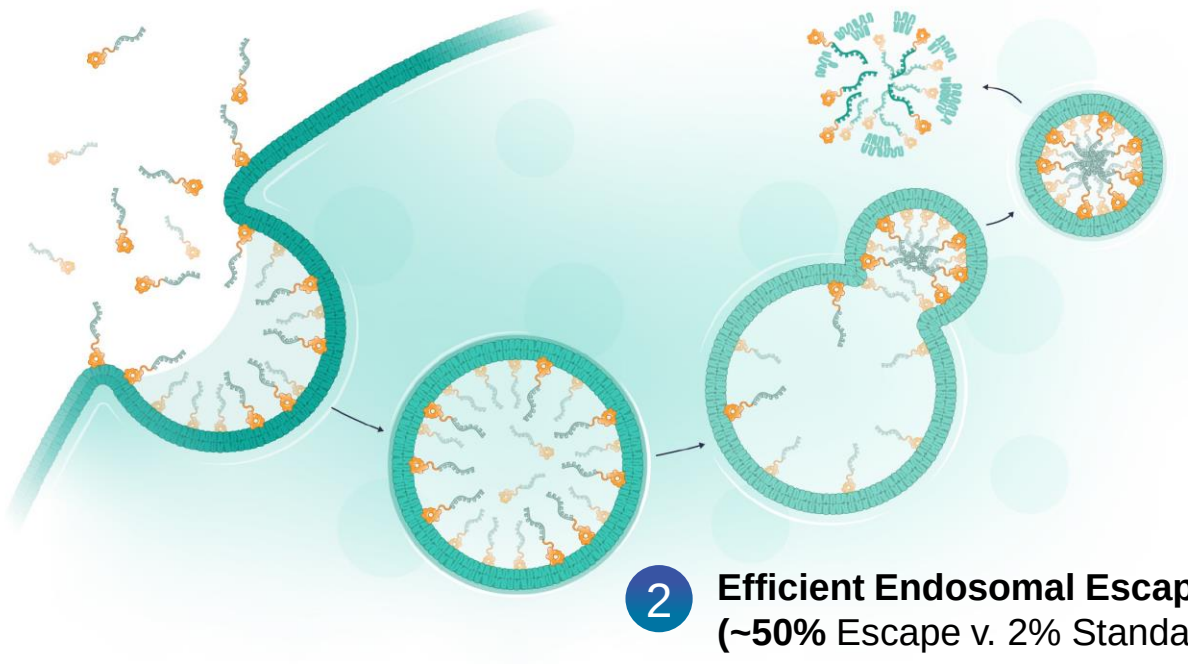
Endosomal Escape Vehicle (EEV™) Therapeutics

- 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**

The EEV Platform 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.

1 **High Intracellular Uptake**
(~90% Uptake)

3 **Prolonged Duration Of Effect**
(Intracellular Depot)



2 **Efficient Endosomal Escape**
(~50% Escape v. 2% Standard)

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.

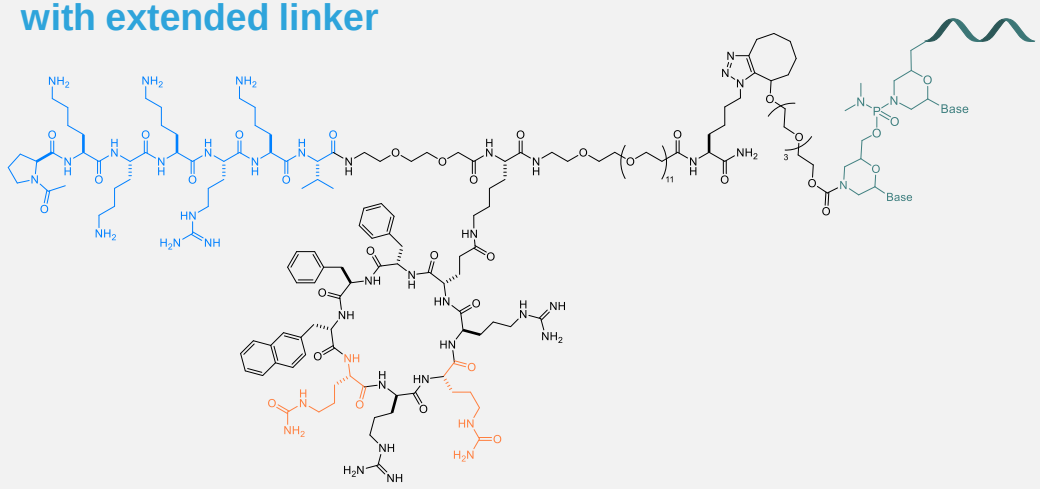
OPTIMIZATION OF EEV FOR MUSCLE DELIVERY

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

EEV3-PMO654 Structure and Medicinal Chemistry

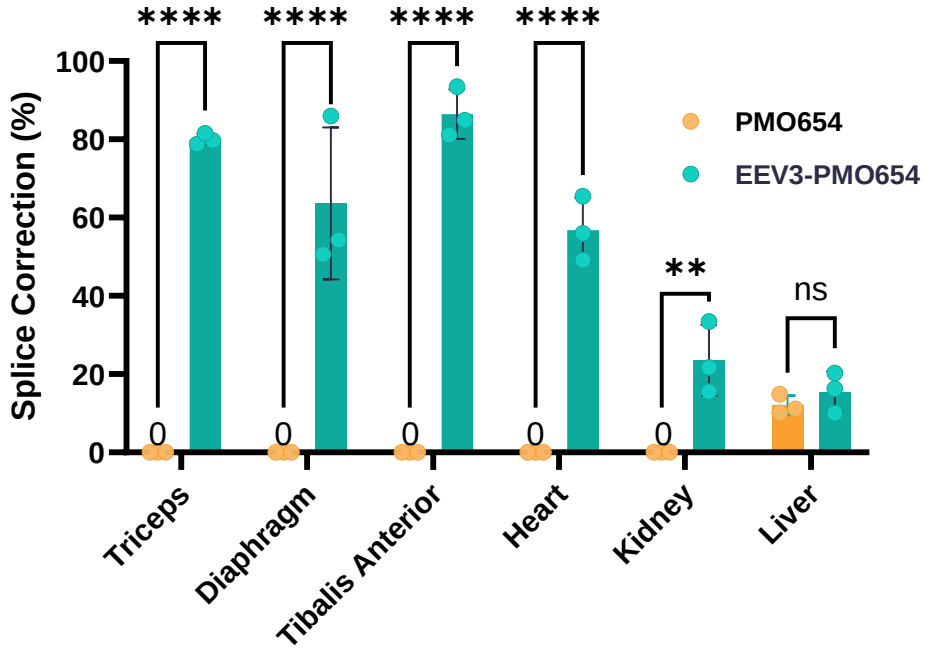
Exocyclic peptide sequence with extended linker

Conjugation with PMO



Substitution of positively charged arginine residues with neutral charged citrullines

Enhanced Functional Delivery to Muscle



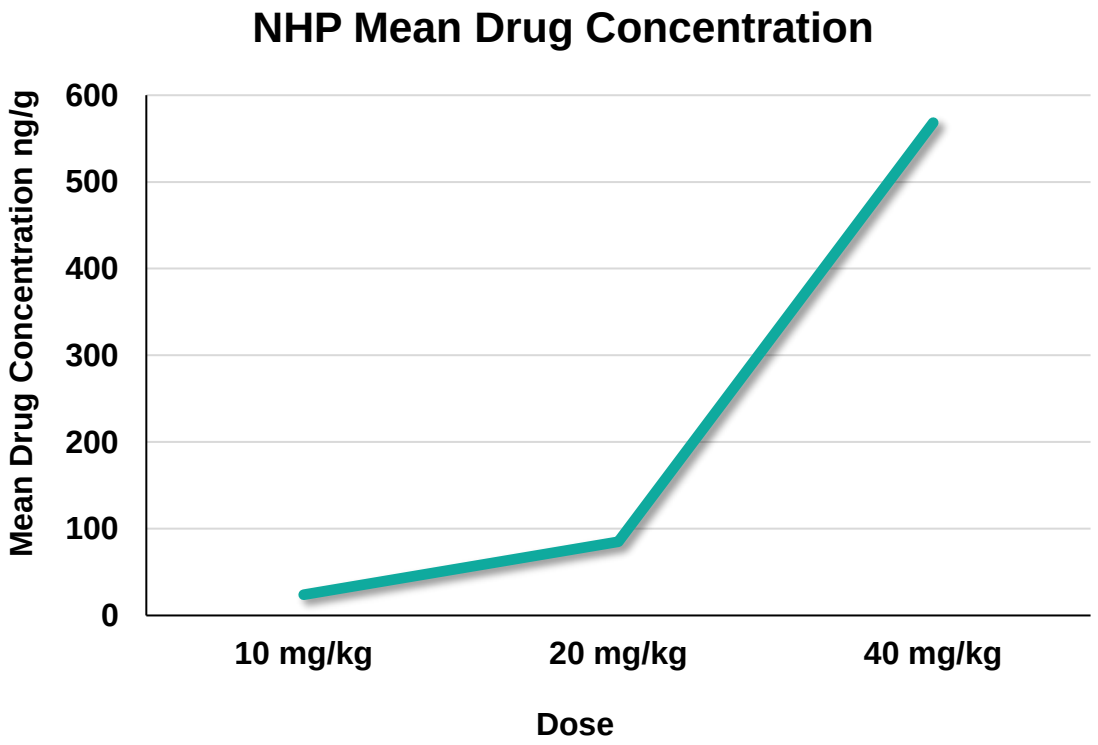
- *EGFP654* mice were evaluated for splice correction 7 days following three weekly 10 mg/kg IV injections of PMO654 or EEV3-PMO654

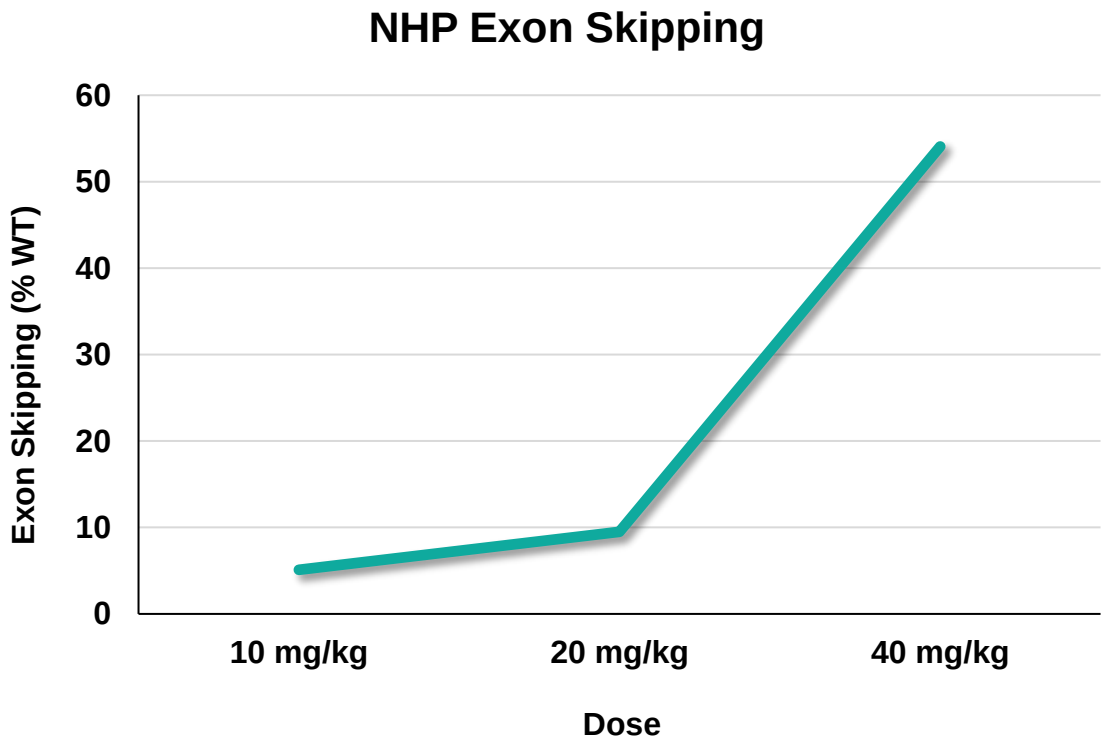
p<0.01, ****p<0.0001; values are shown as mean ± standard deviation. **EEV, endosomal escape vehicle; **IV,** intravenous; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601-44 PRECLINICAL STUDIES

DOSE-DEPENDENT PK AND PD IN NHP

NHP data demonstrated exponential increases at higher doses; A close correlation between drug concentration and exon skipping was observed*



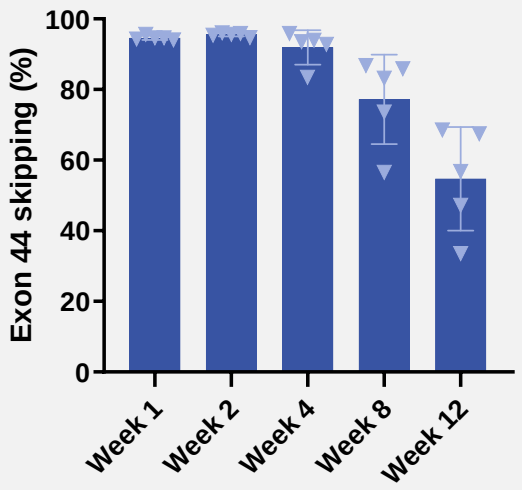


Single dose, bicep biopsy at 48 hours post-infusion; *R^2=0.9996; NHP: Non-human primates.

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

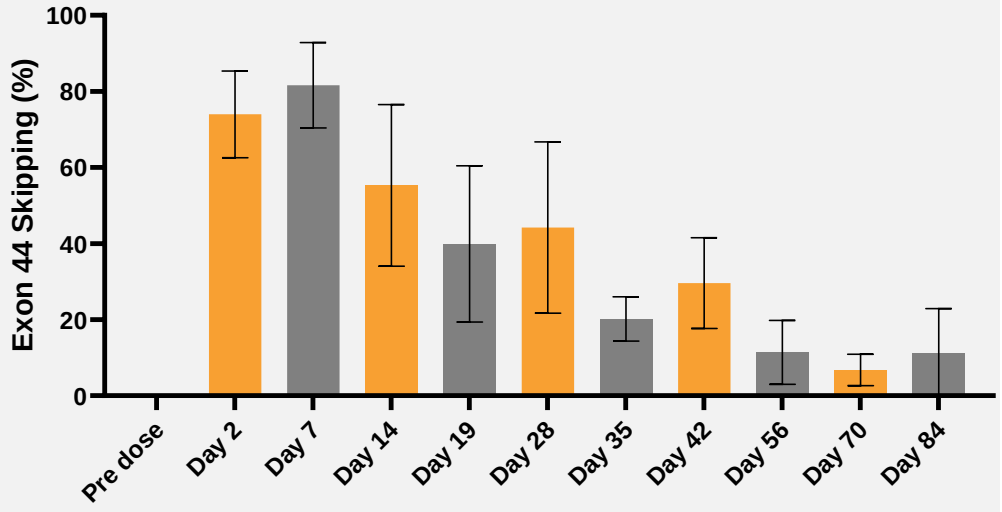
Significant patient benefit is implied by data in the mouse and the monkey at clinically relevant levels; *in vitro* data suggests much higher target engagement in patient cells

Exon 44 Skipping in hDMD Mouse



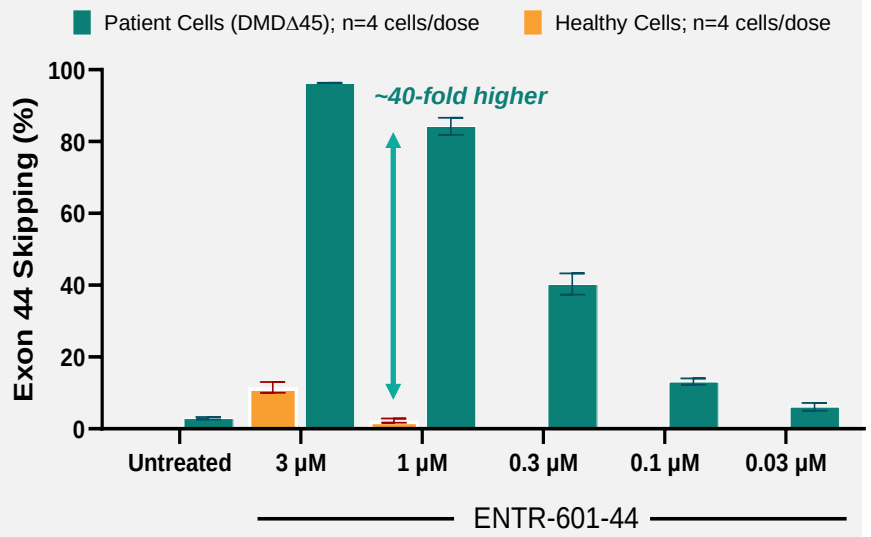
- Single IV 80 mg/kg dose of ENTR-601-44
- Tibialis Anterior

Exon 44 Skipping in Monkey



- Post IV infusion of single 45 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



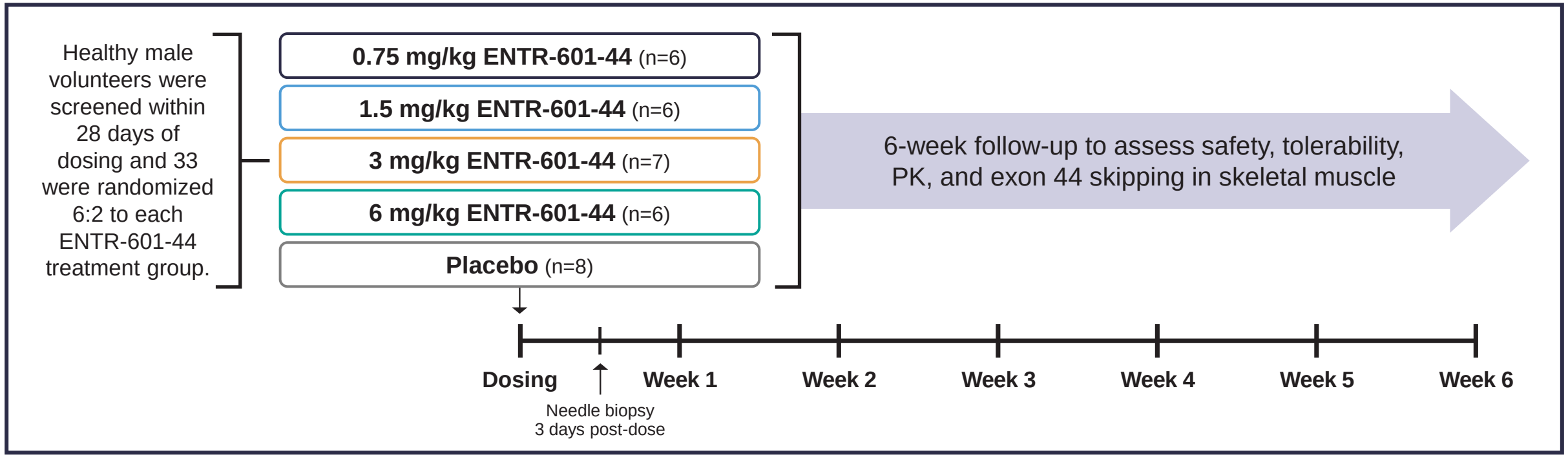
- Robust dose-dependent exon 44 skipping was observed in DMD patient-derived muscle cells harboring an exon 44 skip-amenable mutation

SOLVE FSHD

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-601-44-101 PHASE 1 STUDY

ENTR-601-44-101: STUDY DESIGN



Key Inclusion Criteria

- Healthy males aged 18–55 years, inclusive.
- Body mass index (BMI) of 18.0 to 32.0 kg/m², inclusive, and a minimum weight of 50 kg at screening.

Key Exclusion Criteria

- No current or prior history of clinically significant illness, organ transplant, cardiac disease, hypertension, long QT syndrome, hepatitis B, or diabetes.

Volunteers were required to stay overnight between dosing and Week 1, as well as 1 day at Weeks 2, 3, and 4 study visits. Volunteers underwent a needle biopsy on the biceps brachii 3 days after dosing. Safety, tolerability, and PK parameters were assessed at each study visit. **IV**, intravenous; **PK**, pharmacokinetics. **BMI**, body mass index; **PD**, pharmacodynamics; **PK**, pharmacokinetics; **QoL**, quality of life.

ENTR-601-44-101: OVERALL SAFETY AND TOLERABILITY

A single IV dose of ENTR-601-44 was well-tolerated in healthy human volunteers up to a dose of 6 mg/kg. No treatment-related adverse events were reported in the study.

- At screening, volunteers had a mean (SD) age of 32.6 (8.1) years and BMI of 25.2 (2.9) kg/m^2
- All randomized volunteers completed dosing and 6-week follow-up except for one in the 3 mg/kg group who withdrew from the study prior to receiving treatment (physician's decision)
- No AEs were deemed related to study drug by the investigator. The most common AE was headache (n=7; 5 were mild and 2 were moderate).
  - All AEs resolved by study completion
  - No severe or serious AEs were reported in any dose group throughout the study

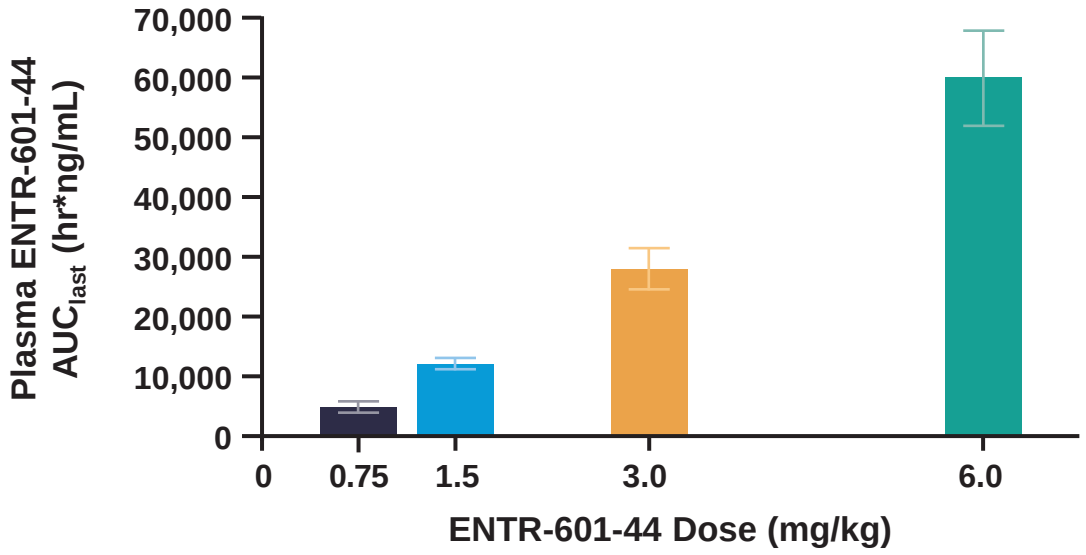
n (%)	Pooled placebo (N=8)	ENTR-601-44				
		0.75 mg/kg (n=6)	1.5 mg/kg (n=6)	3.0 mg/kg (n=7)	6.0 mg/kg (n=6)	Total (N=25)
Randomized	8 (100)	6 (100)	6 (100)	7 (100)	6 (100)	25 (100)
Dosed	8 (100)	6 (100)	6 (100)	6 (85.7)	6 (100)	24 (96)
Completed study	8 (100)	6 (100)	6 (100)	6 (85.7)	6 (100)	24 (96)
Any TEAE	1 (12.5)	5 (83.3)	2 (33.3)	3 (50)	3 (50)	13 (54)
Treatment-related TEAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe AEs	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
SAEs	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Safety and tolerability were assessed at each study visit following a single IV dose of ENTR-601-44 or placebo. **AE**, adverse event; **SAE**, serious adverse event; **TEAE**, treatment-emergent adverse event.

ENTR-601-44-101: PHARMACOKINETICS

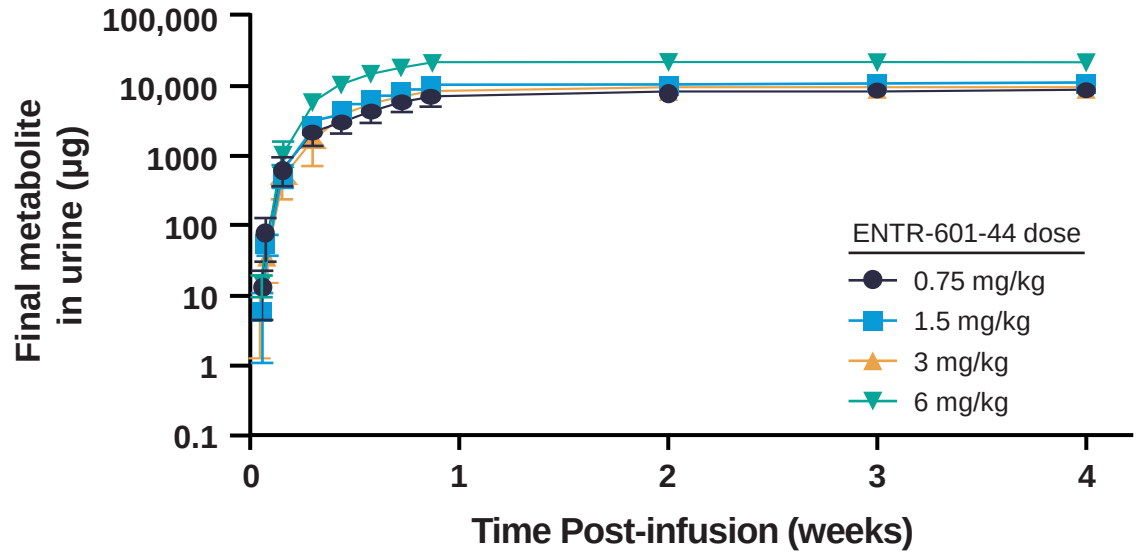
Pharmacokinetic analysis of ENTR-601-44 demonstrates dose-dependent increases in plasma concentration and urinary excretion.

Plasma Concentration



- Dose-dependent increase in mean C_{max} (range) of 3530 (2970–4530), 7380 (6750–8000), 15,400 (12,400–18,500), and 30,900 (26,300–34,200) ng/mL in the 0.75, 1.5, 3.0, and 6.0 mg/kg dose groups, respectively

Urinary Excretion of Final PMO-44 Metabolite



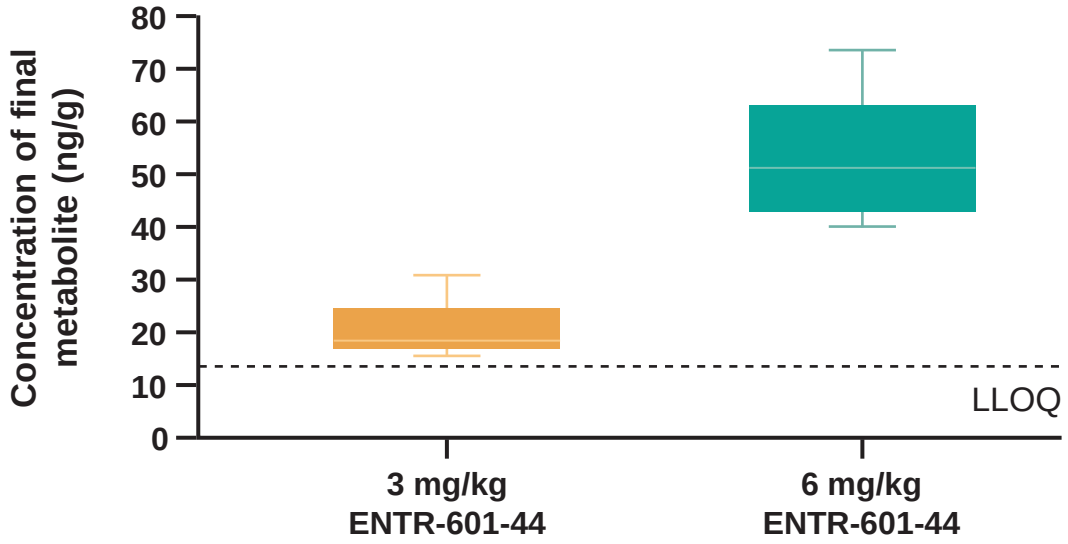
- Urinary excretion of the final metabolite is consistent with preclinical data, which demonstrate urinary excretion as the primary route of elimination.

(Left) Blood samples for PK assessment were collected at 2 hours pre-dose and post-end of infusion: 5 minutes, 1h, 4h, 8h, 16h, 24h, and every 24 hours after. Additional samples were taken at follow-up study visits. **(Right)** 24-hour urine samples for PK assessment were collected the day prior to dosing and every 24 hours after. Additional samples were taken at follow-up study visits. Data shown as mean ± standard deviation. **AUClast**, area under the plasma concentration-time curve to the last measurable plasma concentration; **Cmax**, maximum concentration **NHP**, normal human primate; **PK**, pharmacokinetics; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601-44-101: MUSCLE CONCENTRATION AND EXON SKIPPING

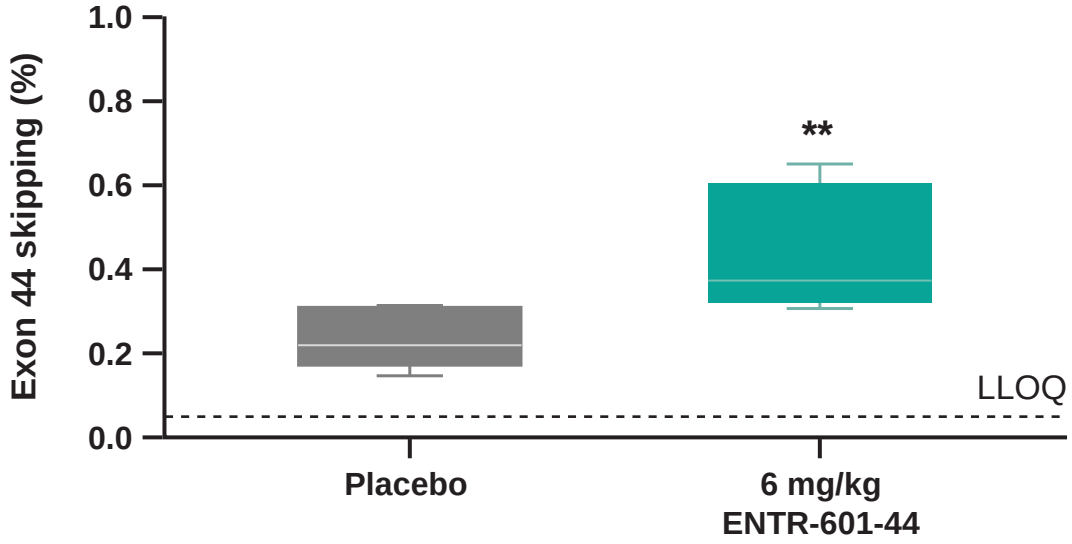
Dose-dependent increases in muscle concentration and *DMD* exon 44 skipping were observed 72 hours following a single IV dose of ENTR-601-44

Skeletal Muscle Concentration



- All six volunteers in the 6 mg/kg dose group had detectable levels of PMO-44 metabolite in skeletal muscle (mean 52.4 ng/g, range 40.0–73.5 ng/g)
- Concentrations of PMO-44 metabolite were below LLOQ in 3 of 6 volunteers in the 3 mg/kg dose group and all volunteers in the 0.75 and 1.5 mg/kg dose groups

DMD Exon 44 Skipping

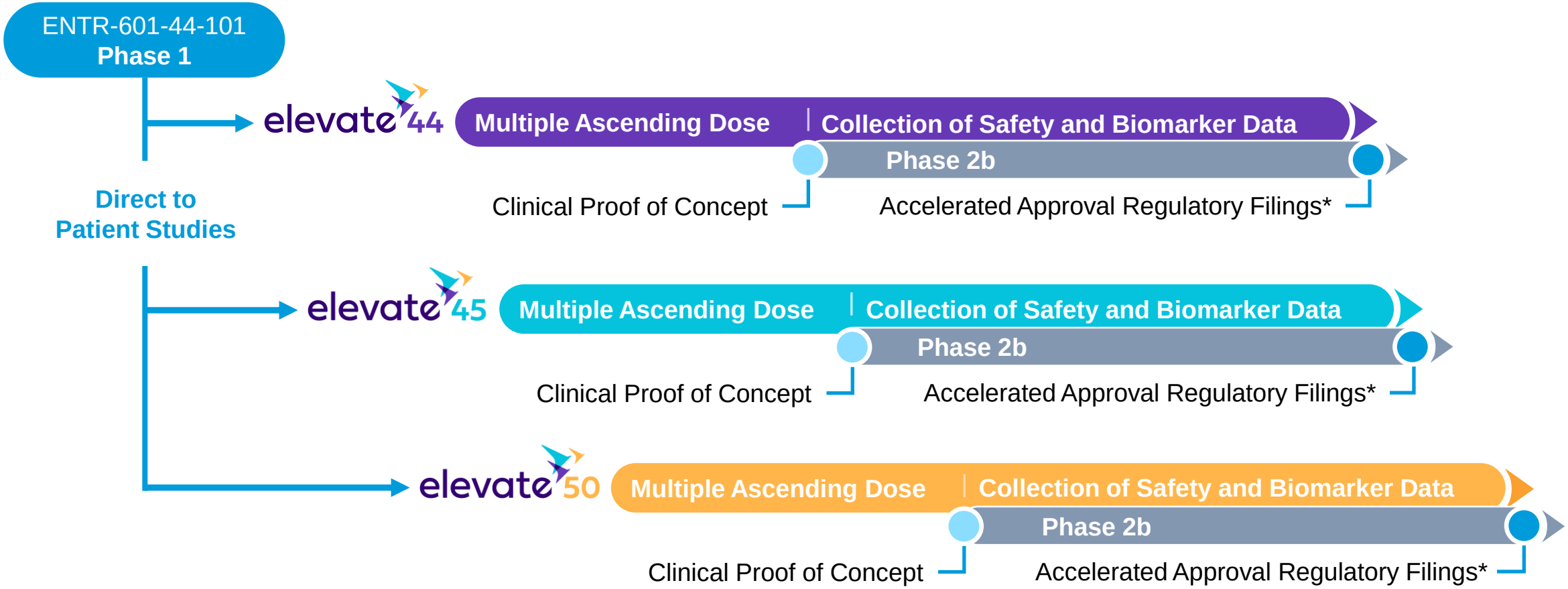


- Statistically significant *DMD* exon 44 skipping was observed with 6 mg/kg ENTR-601-44 (mean 0.44%, range 0.30%–0.65%) in comparison with placebo (mean 0.22%, range 0.14%–0.31%)
- No other ENTR-601-44 dose group was statistically significant in comparison with placebo.

Muscle concentrations and exon skipping were assessed using a needle muscle biopsy taken from biceps brachii 72 hours (±4 hours) post-dose of ENTR-601-44. Box and whisker plot illustration (right): the boxes represent the IQR and median. Whiskers show the smallest and largest values within 1.5 times the IQR. ***p*<0.005 vs. placebo using Mann-Whitney U test. **IQR**, interquartile range; **LLOQ**, lower level of quantification; **PMO**, phosphorodiamidate morpholino oligomer.

ENTR-601 CLINCAL DEVELOPMENT PROGRAM

All ENTR-601-series programs will follow a similar clinical and regulatory approach



Protocols pending regulatory feedback; *Potential for Accelerated Approval in the US, followed by confirmatory Phase 3 studies to obtain Full Approval in the US and ex-US countries.

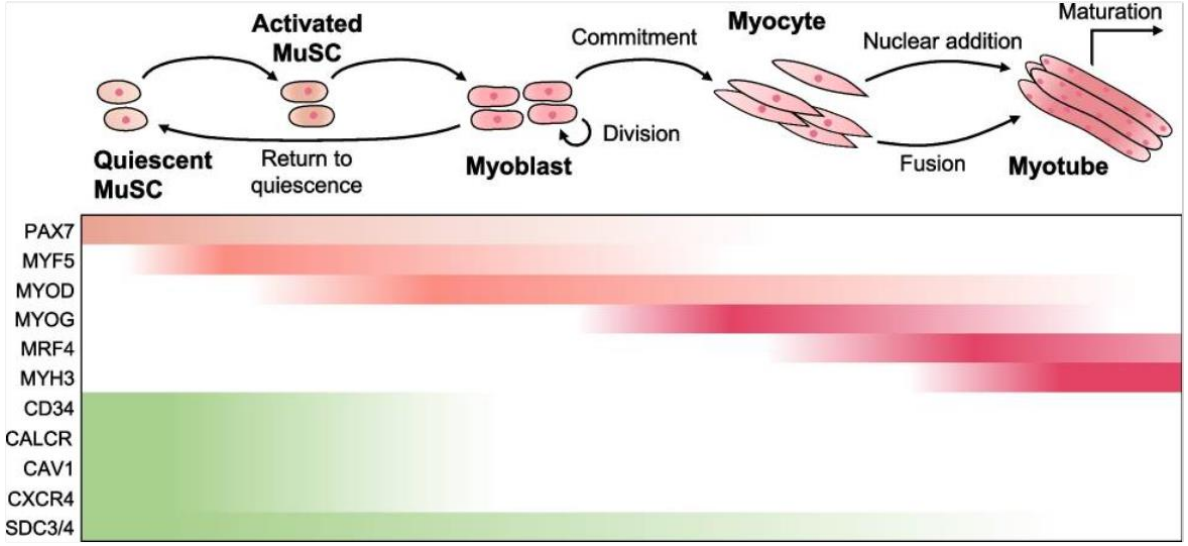
SATELLITE CELLS UPTAKE OF PMO

MUSCLE SATELLITE CELLS

Muscle satellite cells as a new therapeutic target with potential in several neuromuscular disorders

- **Satellite cells are muscle stem cells responsible for generating myoblasts for early muscle growth**
  - Mitotically quiescent in mature muscle, satellite cells maintain their own population by self renewal
  - In post-mitotic muscles, they can be activated to generate myoblast for homeostasis, repair, and hypertrophy
- **Quiescent satellite cells are historically challenging to access by therapeutic modalities**
- **EEV-mediated delivery to access quiescent satellite cells could enable early disease intervention**
  - Ability to deliver to myonuclei of muscle fibers and to quiescent satellite cells holds potential in several neuromuscular disorders
- **Currently evaluating satellite cell-opathies (primary and secondary) to identify attractive target-indication pairs, e.g., FSHD**

Developmental Stages of Muscle Satellite Cells



- **The developmental stages of muscle satellite cells can be delineated by various myogenic factors**
  - PAX7, a canonical myogenic marker essential for orchestrating proper muscle regeneration, is mainly expressed in **quiescent** state, and at a lower level in activated state
  - Stage-specific expression of myogenic factors provides tools for studying EEV-PMO uptake at different developmental stages of satellite cells

Image from Byun et al., Inflamm Regen, 2024

DISTRIBUTION OF EEV-PMO TO SATELLITE CELLS

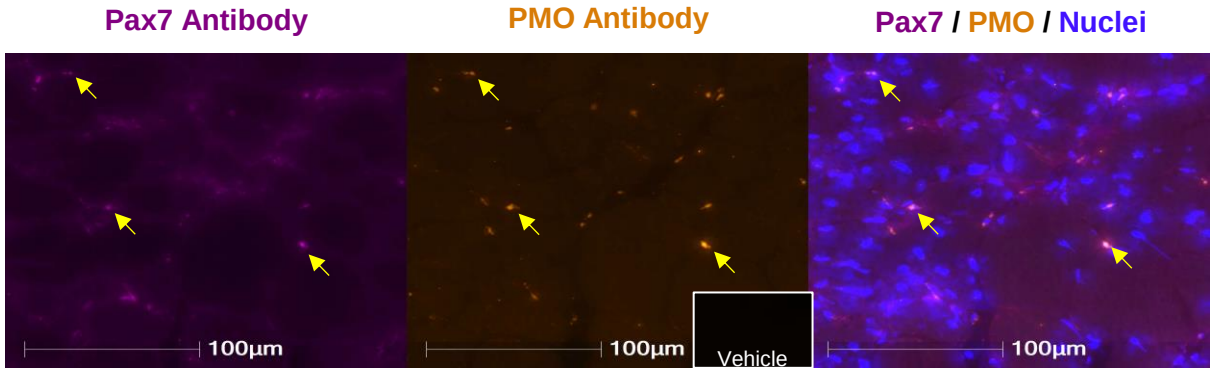
EEV-PMO shows 100% co-localization with quiescent satellite cells (Pax7 positive) at 48 hours; Qualitative data demonstrates that co-localization lasts at least 1-week post-dose

- **Two independent molecular techniques were utilized to determine EEV-PMO distribution within specific cell lineages across muscle tissue**
  - RNA-ISH: Highly selective and sensitive technique to assess specific cell lineages across muscle tissue
  - Immunohistochemistry (IHC) Assessment
- **Quantification analysis of RNA-ISH data confirms that EEV-PMO is co-localized in 100% of satellite cells at 48 hours**
  - Quantitative assessment confirms qualitative data (data not shown)
- **Qualitative assessment of IHC data demonstrates co-localization of satellite cells in hDMD mice with EEV-PMO at 7 days**
  - Quantitative analysis ongoing

PMO Distribution (48 hours, RNA-ISH Quantitation)

Treatment Group	% Pax7 Positive Cells	% Pax7 + PMO Positive Cells
Saline	1-10%	0%
EEV-PMO Treated	1-10%	1-10%

PMO Distribution (Day 7, IHC)



D2-*mdx* mice were treated with 80 mg/kg EEV-PMO by IV; Gastrocnemius was collected at 48 hours and analyzed by RNA-ISH (Top) hDMD mice were treated with 80 mg/kg EEV-PMO by IV; Gastrocnemius was collected at 7 days and tissues analyzed by histology (Bottom). **RNA-ISH**: RNA *in situ* Hybridization image analysis. **IHC**, Immunohistochemistry.

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