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

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2024 ASGCT Breakthroughs in Muscular Dystrophy

entrada THERAPEUTICS

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OUR MISSION

To Treat Devastating Diseases with Intracellular Therapeutics

entrada THERAPEUTICS

Meet Max and his family, living with Duchenne muscular dystrophy

EEV™ PLATFORM

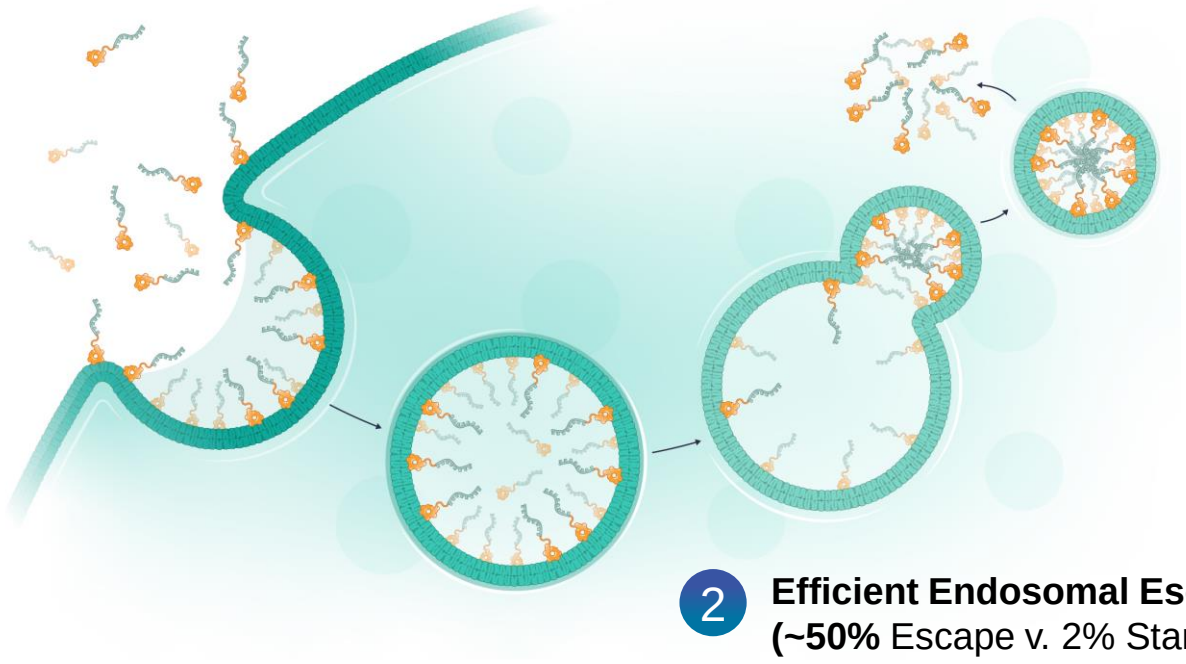
Endosomal Escape Vehicle (EEV™) Therapeutics

- 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)

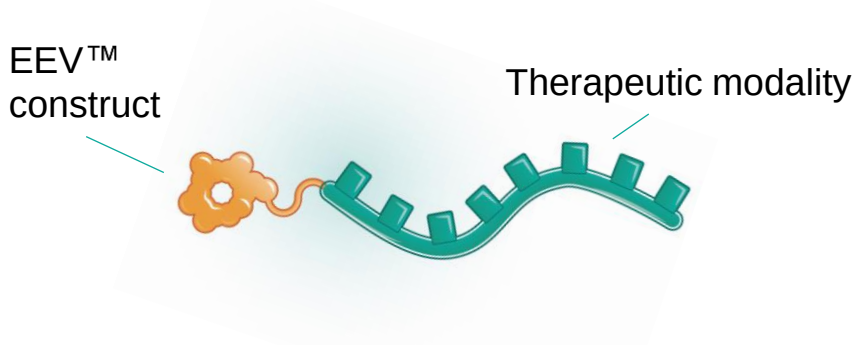


2 **Efficient Endosomal Escape**
(~50% Escape v. 2% Standard)

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.

EEV LIBRARY: SCREENING AND OPTIMIZATION

Discovery Engine for Intracellular Therapeutics



- Cyclic peptide library design and combinatorial synthesis to generate **EEV library**
- Delivery and counter-screening assays enabled for in vitro **high throughput screening**
- Functional screening of lead EEVs in vivo to select for **pharmacodynamic activity** in target tissues
- Optimize **conjugation chemistry** for desired therapeutic modality

Screening Cascade for EEV Candidates

Chemically-diverse >500 member EEV library

EEVs with robust target cell uptake and efficacy

In vitro **functional validation** in relevant cell types with therapeutic payload

Well-tolerated EEVs with desired tissue functional delivery

Assess in vivo **functional delivery** in wild-type and disease models

Fit-for-purpose EEV candidate for target indication

Identify EEV candidate with desired **therapeutic profile**

EEV, endosomal escape vehicle.

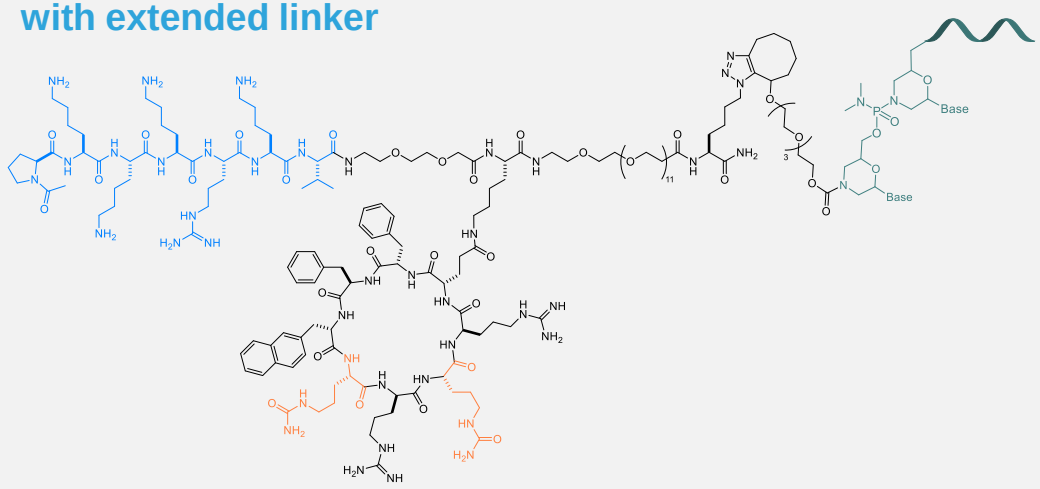
OPTIMIZATION OF EEV FOR MUSCLE DELIVERY

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

EEV3-PMO654 Structure and Medicinal Chemistry

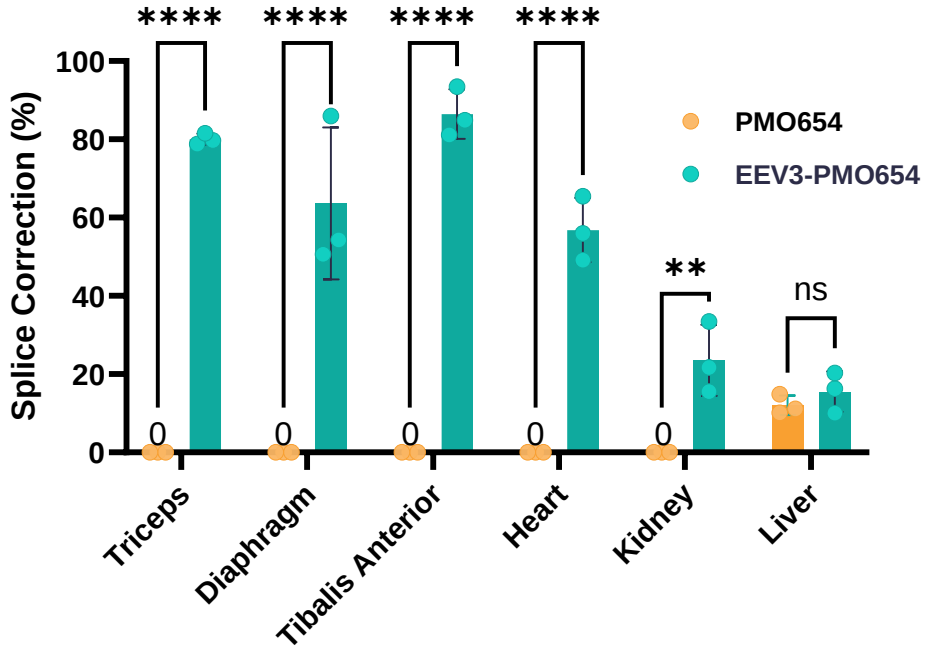
Exocyclic peptide sequence with extended linker

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.

DUCHENNE MUSCULAR DYSTROPHY

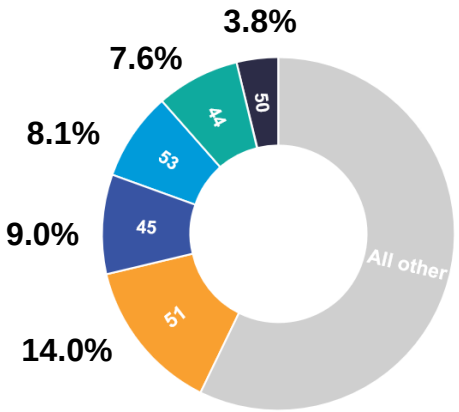
SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

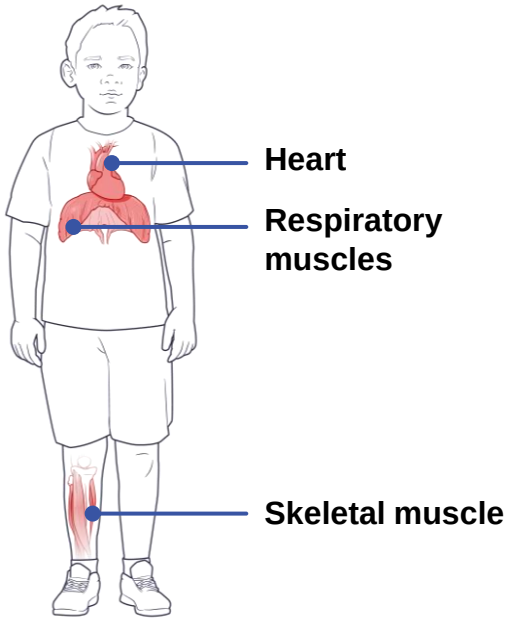
Duchenne is caused by **mutations in the *DMD* gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

Exon skipping therapeutics have been approved based on **modest improvement in dystrophin levels ranging from ~1% to 6%**[4-7]

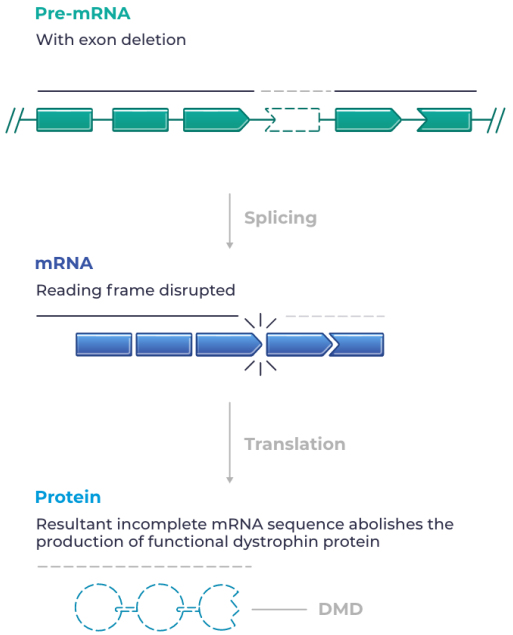
Approximately **41,000** people in the **U.S.**[1] **and Europe**[2] have Duchenne

>40% of patients with Duchenne[3] have mutations amenable to exon skipping of exons 44, 45, 50, 51 and 53

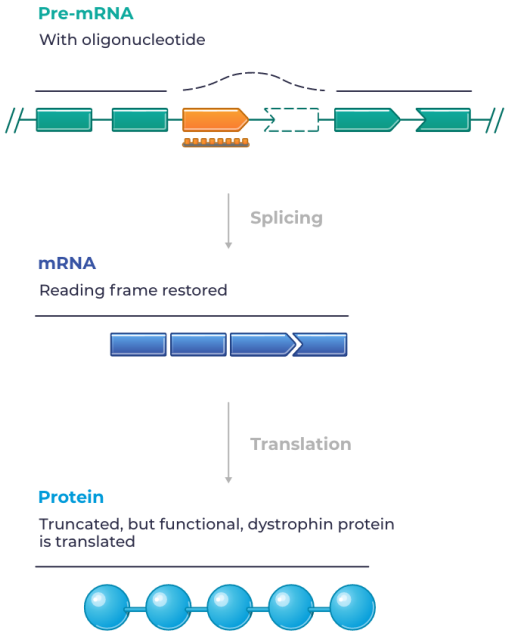




Patients with Duchenne



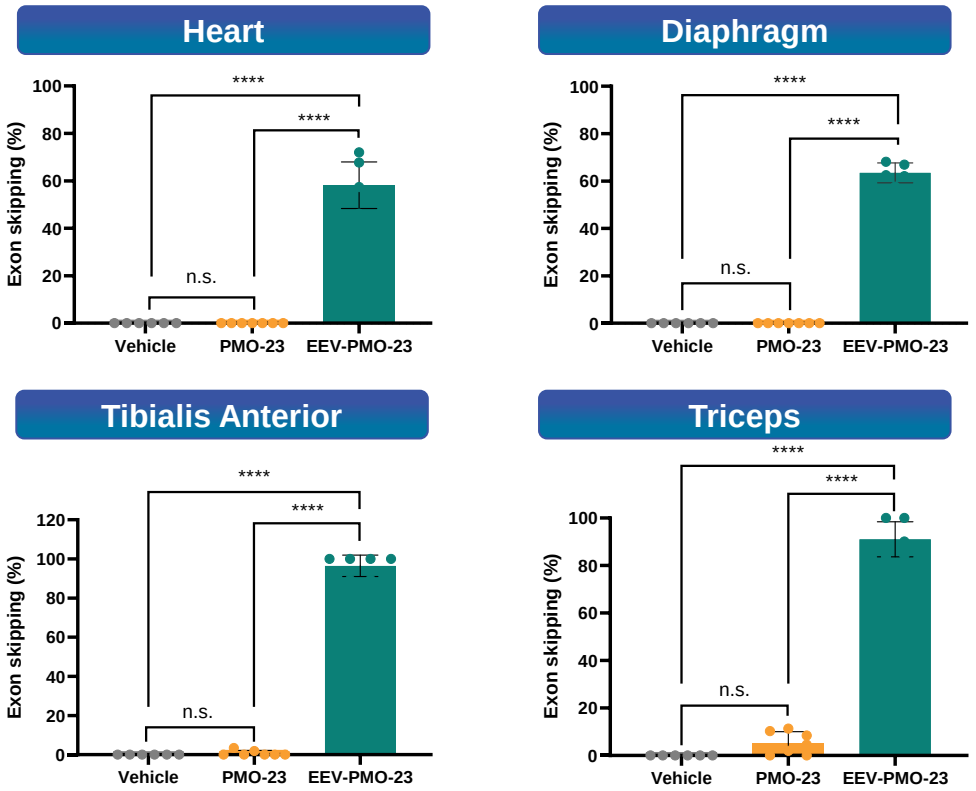
EEV-Oligonucleotide Approach



1. Parent Project Muscular Dystrophy. https://www.parentprojectmd.org/about-duchenne/. Accessed August 18, 2023. **2.** Europeans Medicines Agency. https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3202375. Accessed August 18, 2023. **3.** Bladen, C.L. et al. *Hum Mutat*. 2015. **4.** AMONDYS 45 PI. **5.** VILTEPSO PI. **6.** VYONDYS 53 PI. **7.** EXONDYS 51 PI. **DMD**, Duchenne muscular dystrophy; **EEV**, endosomal escape vehicle

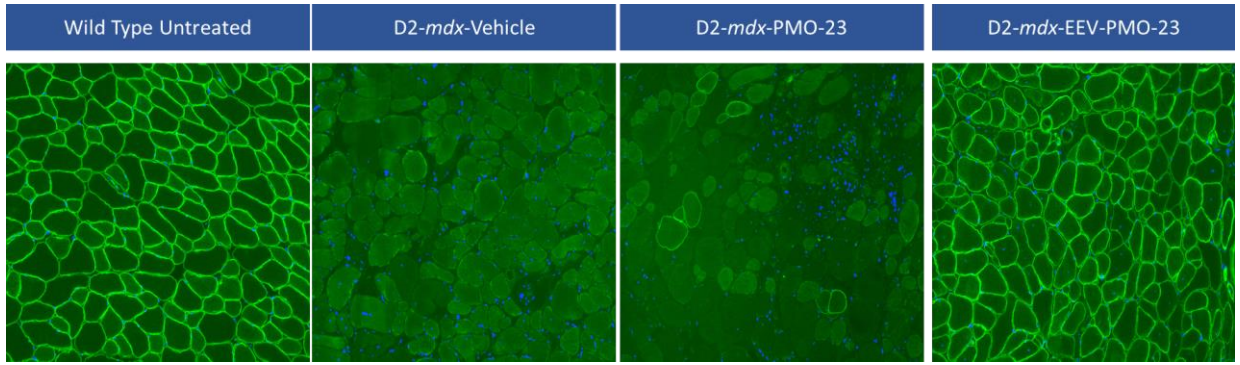
EEV-PMO RESTORES MUSCLE INTEGRITY
D2-*mdx* Mice

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

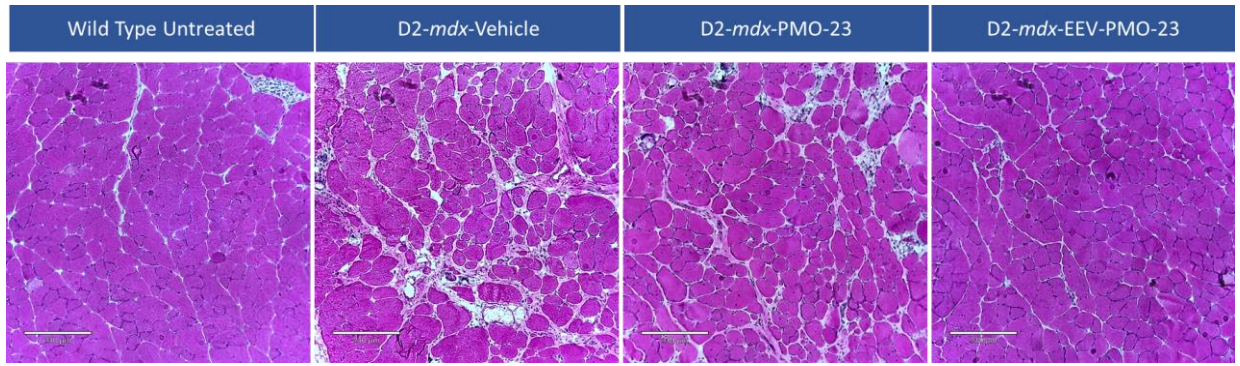


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)



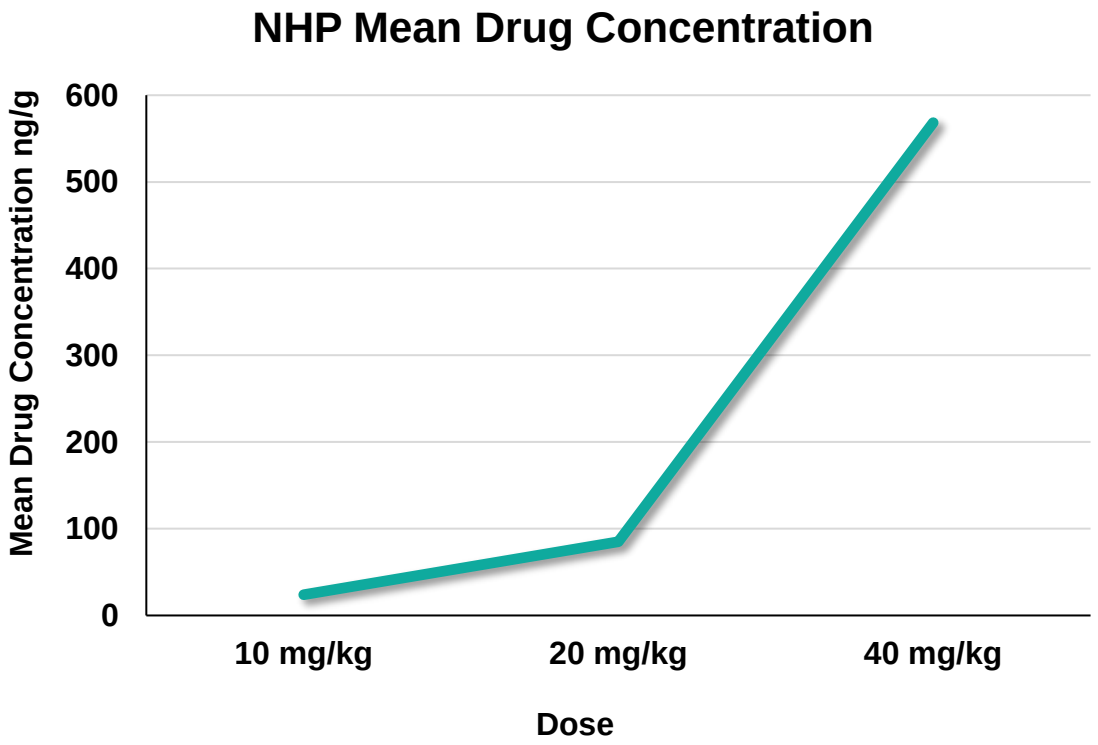
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose

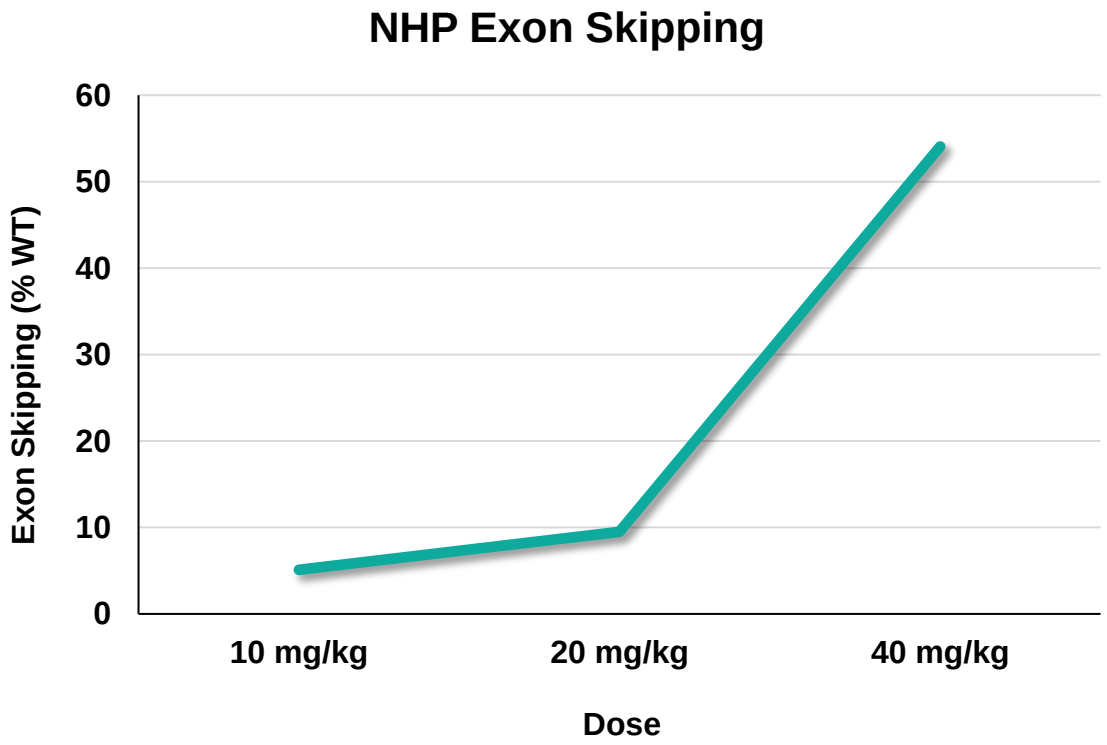
EEV, Endosomal Escape Vehicle; **PMO-23**, mouse *Dmd* exon 23 skipping phosphorodiamidate morpholino oligomer; **D2-*mdx*** is a DMD mouse model with a nonsense mutation in DMD exon 23 on a DBA/2J background and better recapitulates disease pathology (Fukada, S. et al. *Am. J. Path.* 2010, Coley, W .D. et al. *Hum. Mol. Genet.* 2016). ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

ENTR-601-44 PRECLINICAL STUDIES

DOSE-DEPENDENT PK/PD IN NHP

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



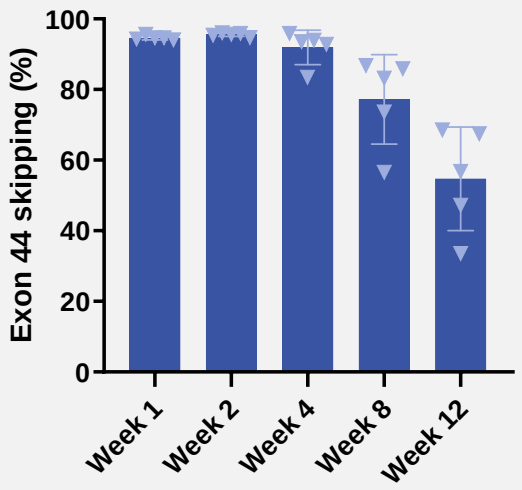


Single dose, bicep biopsy at 48 hours post-infusion; *R^2=0.9996; NHP: Non-human primates.

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

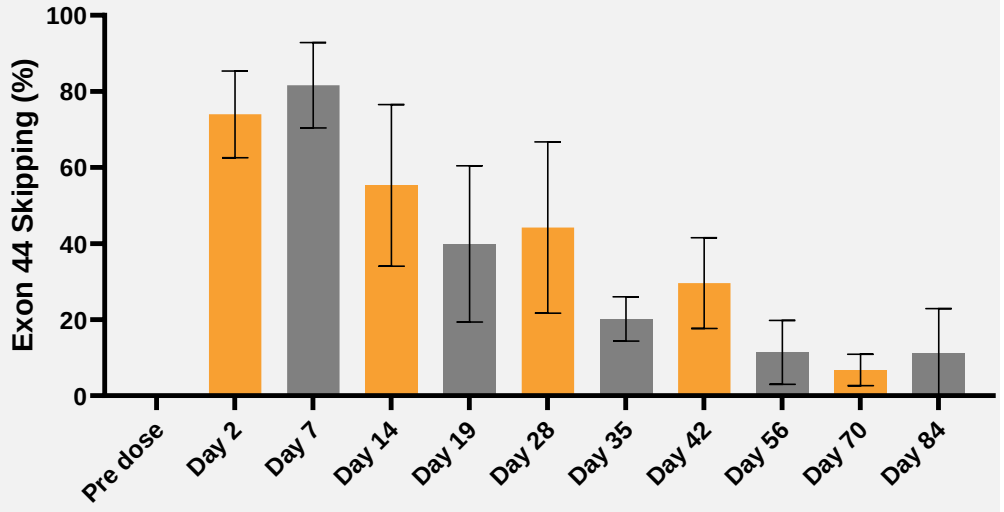
Significant patient benefit is implied by data in the mouse and the monkey at clinically relevant levels; *in vitro* data suggests much higher target engagement in patient cells

Exon 44 Skipping in hDMD Mouse



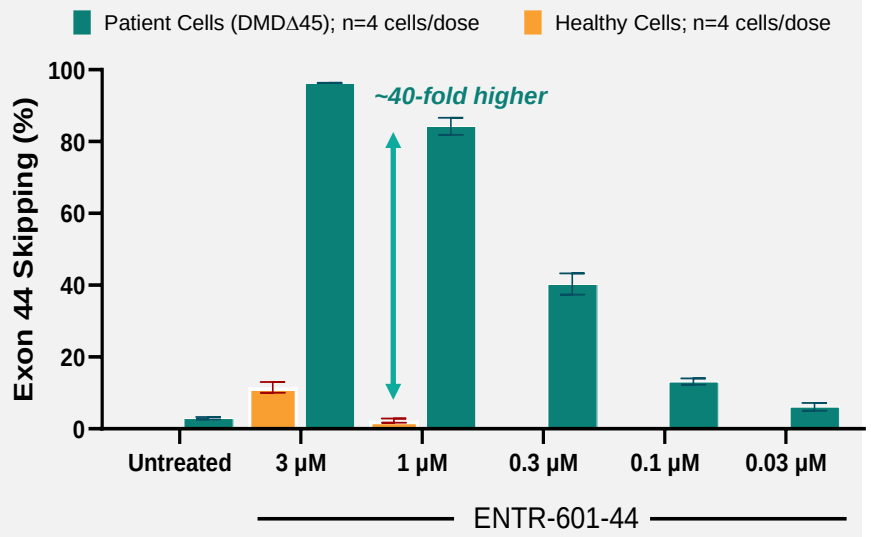
- Single IV 80 mg/kg dose of ENTR-601-44
- Tibialis Anterior

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

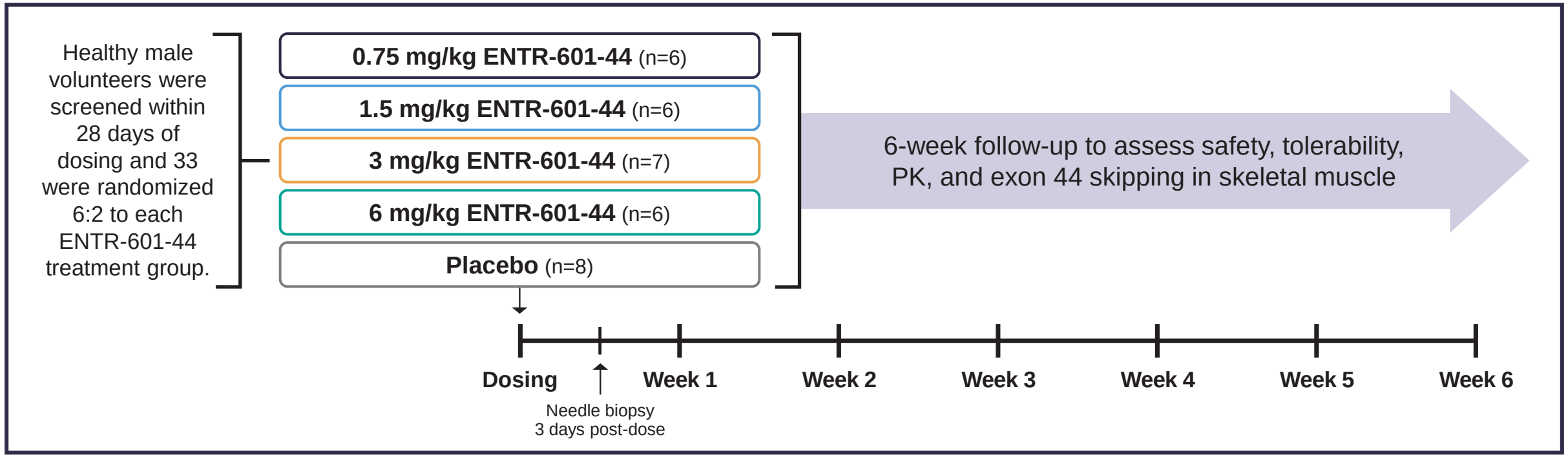


- Robust dose-dependent exon 44 skipping was observed in DMD patient-derived muscle cells harboring an exon 44 skip-amenable mutation

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: ADDITIONAL SAFETY ASSESSMENTS

No clinically relevant adverse findings were observed with laboratory values, hematology, blood analysis, or urinalysis following a single IV dose of ENTR-601-44

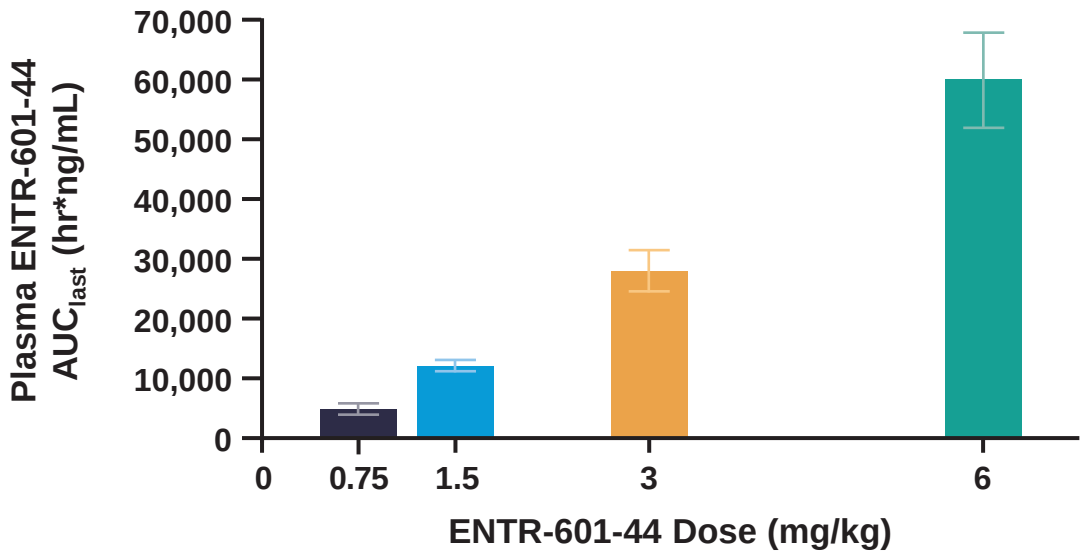
- No clinically significant findings were observed with laboratory values, including renal function, coagulation parameters, hematology, blood analysis, or urinalysis.
  - Notably, no clinically significant changes from baseline values were observed in any renal function–related findings (blood urea nitrogen, creatine, cystatin-C, magnesium, estimated glomerular filtration rate) or in creatine kinase.
- FDA-qualified urinary kidney biomarkers were measured at multiple timepoints per volunteer. At the highest dose tested (6 mg/kg), the results were below the 5% preset threshold at all timepoints measured, suggesting no kidney related toxicities were associated with drug exposure.
- No clinically significant changes or patterns in electrocardiogram or vital signs were observed during the study.

Composite measurement of individual volunteers' samples were calculated by fold changes from baseline of urine creatinine-normalized values of six urinary biomarkers (clusterin [CLU], cystatin-c [CysC], kidney injury molecule-1 [KIM1], *N*-acetyl-β-D-glucosaminidase [NAG], neutrophil gelatinase-associated lipocalin [NGAL], and osteopontin [OPN]) using the following formula: $CMit = exp\ [(\log FC_{CLU,it} + \log FC_{OPN,it} + \log FC_{NAG,it} + \log FC_{KIM1,it} + \log FC_{CysC,it} + \log FC_{NGAL,it}) / 6]$. The geometric mean of ENTR-601-44–treated volunteers in each cohort was calculated at each post-dose timepoint ratio in relation to placebo calculated accordingly per the Kidney Safety CM Biomarker User's Guide (FINH, C-Path).

ENTR-601-44-101: PHARMACOKINETICS

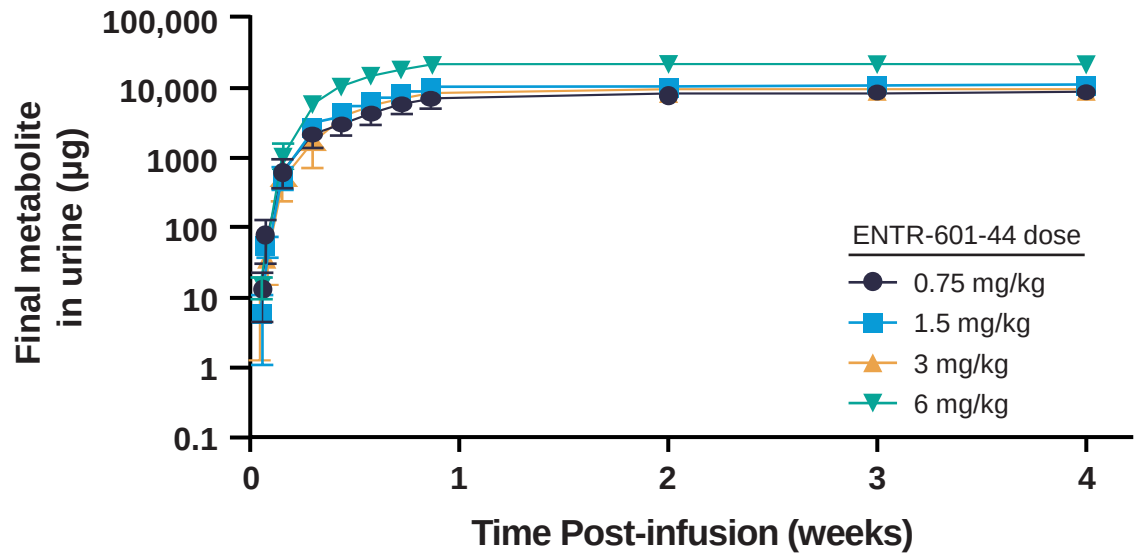
Pharmacokinetic analysis of ENTR-601-44 demonstrates dose-dependent increases in plasma concentration and urinary excretion.

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



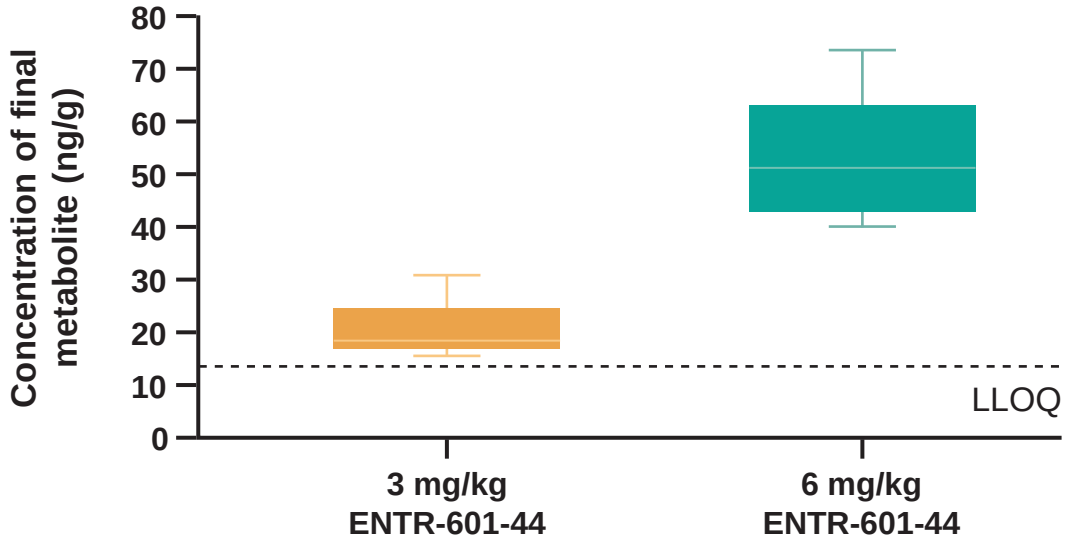
- Urinary excretion of the final metabolite is consistent with preclinical data, which demonstrate urinary excretion as the primary route of elimination

(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

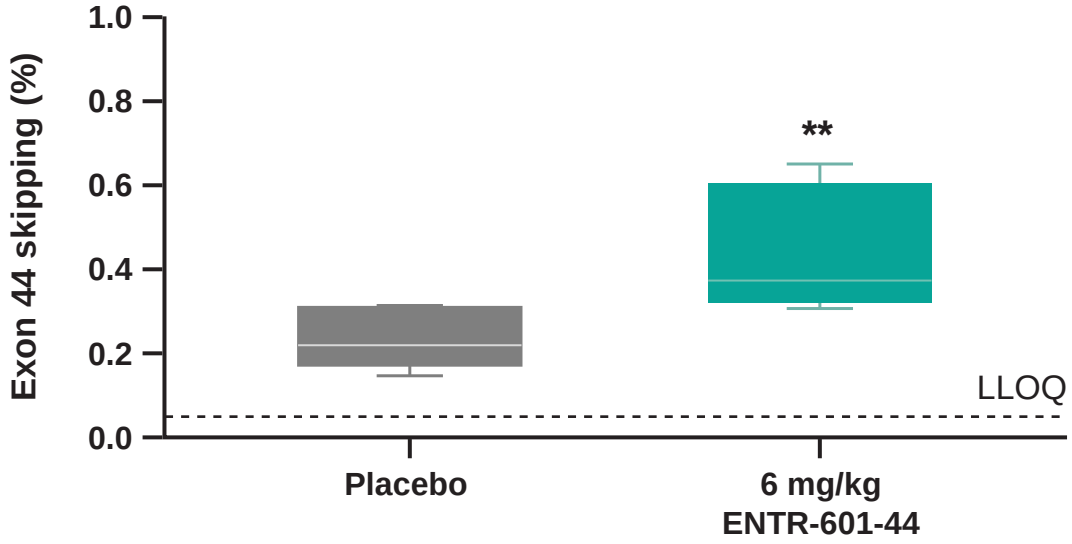
Dose-dependent increases in muscle concentration and *DMD* exon 44 skipping were observed 72 hours following a single IV dose of ENTR-601-44

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-601-44 CLINICAL PROGRAM

First-in-Human Trial

Completed

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

- **Study met all study objectives in healthy male volunteers with no AEs related to ENTR-601-44 administration**
- **No adverse findings or clinically relevant changes to any renal markers measured during the study**
- **Dose-dependent concentration and significant exon 44 skipping in skeletal muscle with 6 mg/kg ENTR-601-44**

Planned Multiple Ascending Dose/Phase 2b (Global)

Regulatory filings expected in Q4 2024

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

- **Juvenile patients**
- **3 MAD cohorts (final design TBD)**
- **Dosing interval ≥ every 6 weeks**

Phase 2b Study*
in Exon 44 Skipping Amenable Patients

Target Product Profile:
- **Double digit dystrophin improvement from baseline**
- **Dosing interval ≥ every 6 weeks**

File for Accelerated Approval

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)

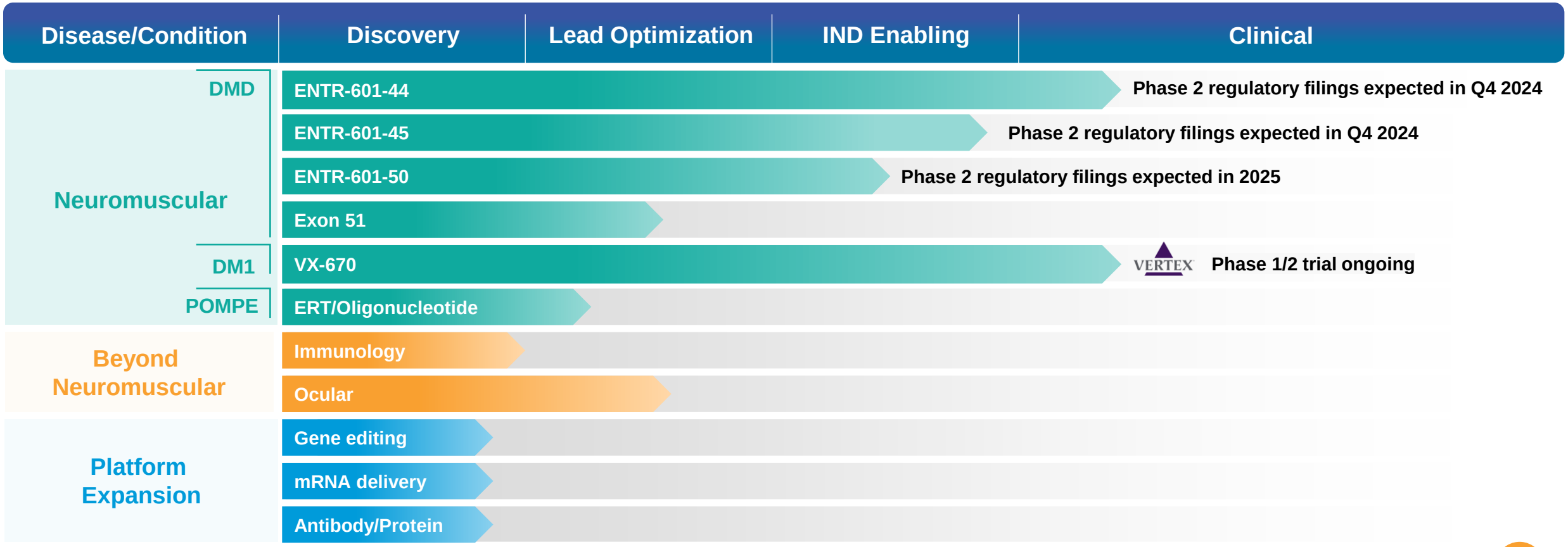
Secondary/Exploratory Efficacy Measures

- Change from baseline in the 10-meter walk/run
- Change from baseline in the timed rise from floor
- Other parameters may include NSAA, FVC, QoL

*MAD/Phase 2b study is subject to regulatory feedback. **ENTR-601-44** is a DMD exon 44 skipping EEV-oligonucleotide construct. **AE,** adverse event; **FVC**, forced vital capacity; **NSAA**, North Star Ambulatory Assessment: **PD**, pharmacodynamics; **PK**, pharmacokinetics; **Q,** quarter; **QoL**, quality of life; **TBD**, to be determined.

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



ASGCT Breakthroughs

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Meet Franklin and his family, living with Duchenne muscular dystrophy