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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INTRODUCTION

- Currently approved antisense phosphorodiamidate morpholino oligomer (PMO)–mediated exon-skipping therapies for Duchenne muscular dystrophy (DMD) produce only a very modest amount of dystrophin in skeletal muscle, likely due to poor cell entry and limited escape from the endosome in target muscle cells.[1-4]
- To enhance PMO delivery to muscle, we designed a family of proprietary cyclic cell-penetrating peptides that form the core of the Endosomal Escape Vehicle (EEV™) platform[5] (**Figure 1**).
  - Results of preliminary studies in *mdx* mice demonstrated that antisense EEV-PMO constructs produce dystrophin in skeletal and cardiac muscle by exon skipping.[6]
- ENTR-601-44 is a *DMD* exon 44–skipping PMO conjugated to the EEV platform and is currently being investigated for the treatment of exon 44 skip-amenable DMD.
  - ENTR-601-44 has demonstrated exon skipping and dystrophin restoration in skeletal muscle cells derived from patients with DMD who have a *DMD* exon 44 skip-amenable mutation, as well as robust exon 44 skipping in cardiac and skeletal muscle of human dystrophin-expressing mice and nonhuman primates.[7]
- Here, we present the first in-human clinical results following a single intravenous (IV) infusion of ENTR-601-44 in healthy male volunteers.

MATERIALS AND METHODS

Study Design

- Study ENTR-601-44-101 was a single-center, phase 1, double-blind, placebo-controlled trial to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) in healthy male volunteers (**Figure 2**).
- Participants were administered a single IV dose of ENTR-601-44 (0.75, 1.5, 3.0, or 6.0 mg/kg) or placebo following randomization and assessed up to 41 (±2) days post-administration.

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.

Assessments

- Safety and tolerability of ENTR-601-44 were the primary endpoints of the study and were assessed on each study visit day.
  - The US Food & Drug Administration–qualified kidney safety composite measure biomarker panel[8,9] was also assessed.
- Plasma PK and urine excretion of ENTR-601-44 and its final metabolite (PMO-44) were secondary assessments, and samples were collected each study visit day. ENTR-601-44, all intermediate metabolites, and the final metabolite are metabolically active; the final metabolite was monitored because preclinical data indicated that it had the longest persistence in plasma, tissues, and urine.
- Exploratory assessments included PD parameters: ENTR-601-44 and PMO-44 metabolite concentration and exon 44 skipping in skeletal muscle (biceps brachii) following a needle biopsy 3 days post-administration.

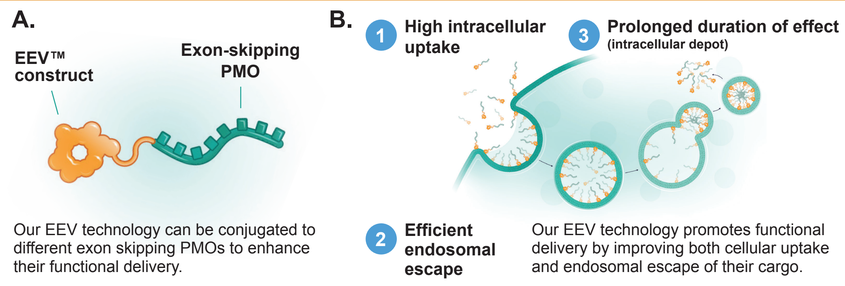
Statistical Analysis

- Adverse events (AEs) and PK parameters were reported with descriptive statistics.
- A statistical analysis was conducted using the Mann-Whitney U test to assess the differences in exon skipping between the placebo and treatment groups.

OBJECTIVE

- To assess safety, PK, and target engagement of ENTR-601-44 in healthy male volunteers.

Figure 1. EEV-PMO Construct Structure and Mechanism of Action.

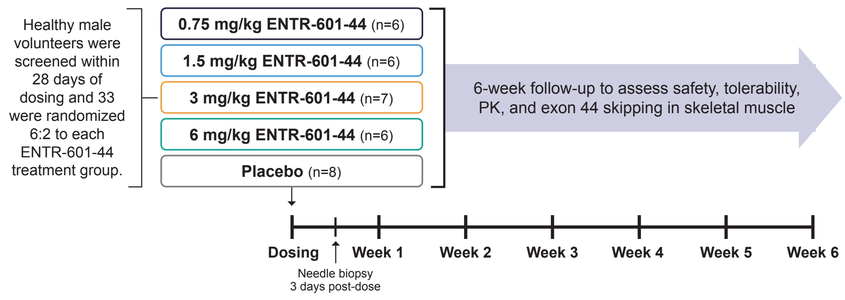


Our EEV technology can be conjugated to different exon skipping PMOs to enhance their functional delivery.

Our EEV technology promotes functional delivery by improving both cellular uptake and endosomal escape of their cargo.

(A) Structure of EEV-PMO construct consisting of a cyclic cell-penetrating peptide-based EEV construct and exon-skipping PMO. **(B)** Mechanism of EEV construct-mediated delivery of PMOs. EEV, Endosomal Escape Vehicle; PMO, phosphorodiamidate morpholino oligomer.

Figure 2. ENTR-601-44-101 Study Design.



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.

RESULTS

Safety and Tolerability of ENTR-601-44

- Healthy male volunteers (N=33) were randomized to receive ENTR-601-44 (n=25) or placebo (n=2 per treatment arm; n=8 pooled) (**Table 1**). At screening, volunteers had a mean (SD) age of 32.6 (8.1) years and BMI of 25.2 (2.9) kg/m².
- 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.

Table 1. Healthy Male Volunteer Disposition and Adverse Events.

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)

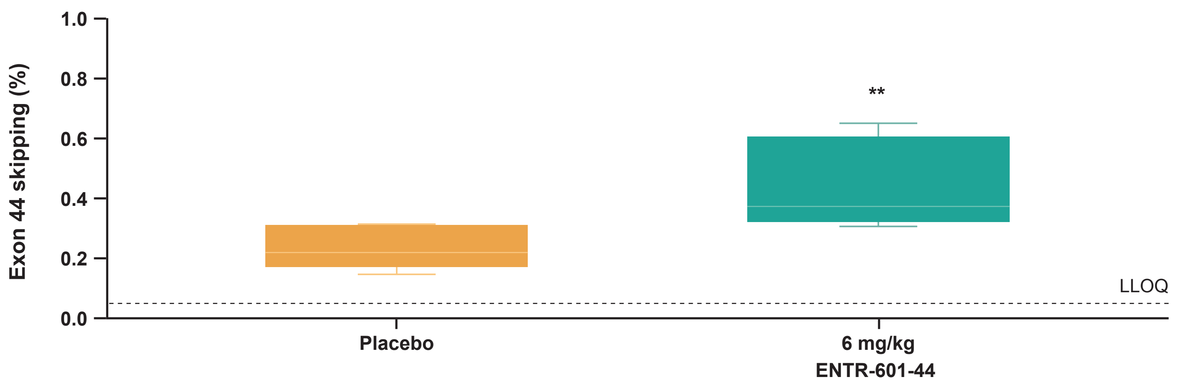
Safety and tolerability were assessed at each study visit following a single IV dose of ENTR-601-44 or placebo. AE, adverse event; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

- 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.

DMD Exon 44 Skipping in Skeletal Muscle

- Statistically significant DMD exon 44 skipping was observed with a single IV dose of 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%) (**Figure 3**). No other ENTR-601-44 dose group was statistically significant in comparison with placebo.

Figure 3. *DMD* Exon 44 Skipping Following a Single IV dose of 6 mg/kg ENTR-601-44.

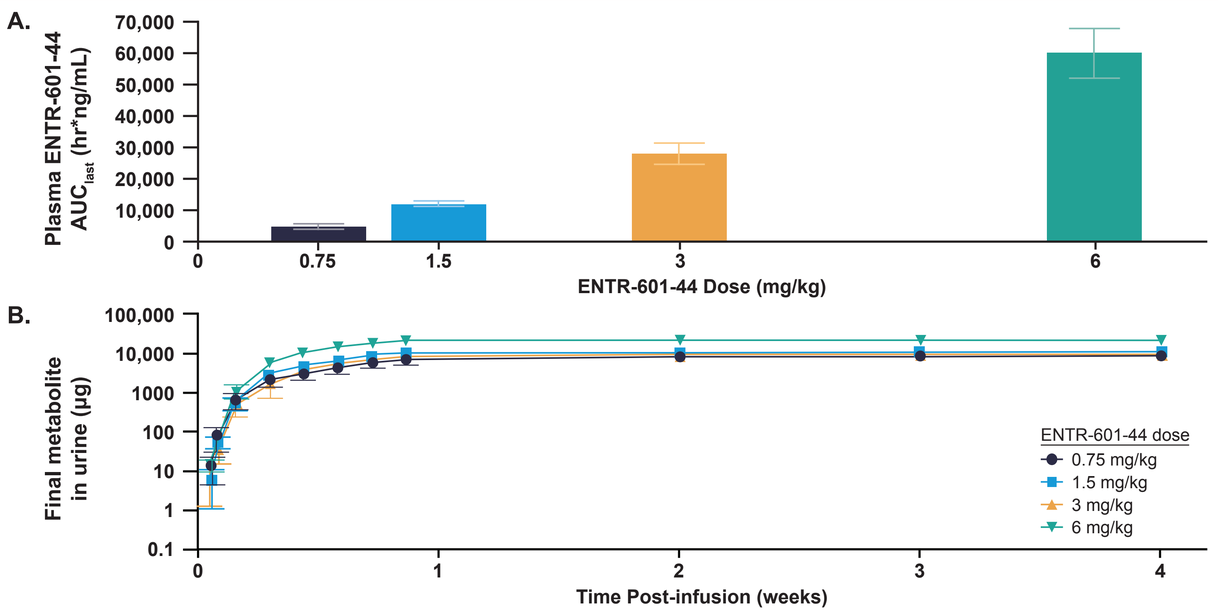


Exon skipping was assessed by digital droplet polymerase chain reaction using a tissue sample from a needle muscle biopsy taken from biceps brachii 72 hours (±4 hours) post-dose. Box and whisker plot illustration: the boxes represent the IQR and median. Whiskers show the smallest and largest values within 1.5 times the IQR. $^{**}p<0.005$ vs. placebo using Mann-Whitney U test. IQR, interquartile range; IV, intravenous; LLOQ, lower level of quantification.

Pharmacokinetics of ENTR-601-44

- Dose-dependent increases in plasma concentration (area under the plasma concentration-time curve to the last measurable plasma concentration [AUC_{last}]) were observed following a single IV dose of ENTR-601-44 (**Figure 4A**).
  - In addition, there was a dose-dependent increase in mean maximum (range) concentration (C_{max}) 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.
- Dose-dependent increases of the final PMO-44 metabolite were observed in urine (**Figure 4B**).
  - Urinary excretion of final metabolite is consistent with preclinical data, which demonstrate urinary excretion as the primary route of elimination.
  - The urinary excretion was similar between cohorts up to 3 mg/kg (geometric mean 39.2%, range 31.7% to 48.1%), with an overproportional excretion at 6 mg/kg (geometric mean 50.8%, range 47.4% to 60.2%).
  - This suggests saturation of receptor-mediated re-uptake in human kidney on a dose-adjusted basis, contributing to lower dose-proportional renal exposure and lower possible renal toxicity in comparison with non-clinical models.

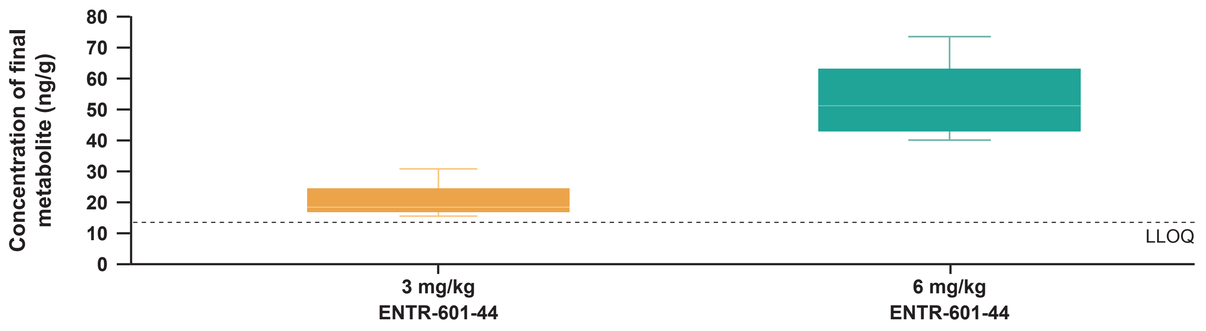
Figure 4. Pharmacokinetics of ENTR-601-44 Following a Single IV Dose.



Pharmacokinetics of ENTR-601-44. **(A)** Blood samples for PK assessment were collected at 2 hours pre-dose and post-end of infusion: 5 minutes, 1 h, 4 h, 8 h, 16 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h. Additional samples were taken at follow-up study visits. **(B)** 24-hour urine samples for PK assessment were collected the day prior to dosing and at 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h post-dose. Additional samples were taken at follow-up study visits. Data shown as mean ± standard deviation. AUC_{last}, area under the plasma concentration-time curve to the last measurable plasma concentration; NHP, normal human primate; PK, pharmacokinetics; PMO, phosphorodiamidate morpholino oligomer.

- Dose-dependent concentrations of the final PMO-44 metabolite in skeletal muscle were observed in the 3 and 6 mg/kg dose groups (**Figure 5**).
  - Three of six volunteers in the 3 mg/kg dose group had detectable concentrations of the final metabolite (above the lower limit of quantification [LLOQ=14 ng/g]) in skeletal muscle (geometric mean 20.5 ng/g, range 15.4–30.8 ng/g).
  - All six volunteers in the 6 mg/kg dose group had detectable levels of PMO-44 metabolite in skeletal muscle (geometric mean 52.4 ng/g, range 40.0–73.5 ng/g).
  - Concentrations of PMO-44 metabolite were below LLOQ in the 0.75 and 1.5 mg/kg dose groups.

Figure 5. Skeletal Muscle Concentration of ENTR-601-44 Metabolite.



Muscle concentrations of PMO-44 final metabolite were assessed using a needle muscle biopsy taken from biceps brachii 72 hours (±4 hours) post-dose of ENTR-601-44. Concentration of the final ENTR-601-44 metabolite was below LLOQ (14 ng/g) in the 0.75 and 1.5 mg/kg dose groups. LLOQ, lower level of quantification; PMO, phosphorodiamidate morpholino oligomer.

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CONCLUSIONS

- Study ENTR-601-44-101 met all study objectives in healthy male volunteers with no AEs related to ENTR-601-44 administration. Of note, there were no adverse findings or clinically relevant changes to any biomarkers of renal toxicity at the highest dose tested (6 mg/kg) during the study.
- Dose-dependent concentration of the final metabolite of ENTR-601-44 and significant exon 44 skipping were observed in skeletal muscle following administration of 6 mg/kg ENTR-601-44.
- These results demonstrate, for the first time, that the EEV platform can safely and effectively deliver oligonucleotide therapeutics to skeletal muscle with significant target engagement in human volunteers.
- Together with the robust efficacy of ENTR-601-44 observed in preclinical models, these clinical findings support further evaluation of ENTR-601-44 in patients with DMD amenable to exon 44 skipping.