Endosomal Escape Vehicle (EEV™)-Conjugated Phosphorodiamidate Morpholino Oligomer (PMO) Therapeutics For The Treatment Of Duchenne Muscular Dystrophy

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4th Novel Conjugates Summit, March 26, 2025

entrada THERAPEUTICS

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This presentation contains forward-looking statements that involve substantial risks and uncertainties. All statements, other than statements of historical facts, contained in this presentation, including statements regarding the Company's strategy, future operations, prospects and plans, objectives of management, the validation and differentiation of Entrada’s approach and EEV platform and its ability to provide a potential treatment for patients, expectations regarding the Company’s planned Phase 1/2 multiple ascending dose clinical studies of ENTR-601-44, -45, and -50, including their initiation in the UK in 2025, expectations regarding significant accumulation of exon skipping and dystrophin production in patients, expectations regarding the importance of endosomal escape to therapeutic index optimization, the translatability of the data from the Phase 1 clinical study for ENTR-601-44 to our planned DMD clinical studies, expectations regarding the ability of the Company’s preclinical studies and clinical studies to demonstrate safety and efficacy of its therapeutic candidates, and other positive results, expectations regarding the approvals and specific protocols for the Company’s planned Phase 1/2 clinical studies for ENTR-601-44, -45, and -50, the timing of regulatory filings for the planned Phase 1/2 clinical studies for ENTR-601-50 in the second half of 2025 and ENTR-601-51 in 2026, the ability to recruit for, enroll, and complete a global Phase 1/2 study for ENTR-601-44, -45, -50, and -51, the ability to recruit for, enroll, and complete a Phase 1b study for ENTR-601-44 in the US, the potential of its EEV product candidates and EEV platform, including the potential for ENTR-601-44, -45, -50, and -51 to be transformative treatment options, the continued development and advancement of ENTR-601-44, -45, -50, and -51 for the treatment of Duchenne and the partnered product VX-670 for the treatment of myotonic dystrophy type 1, and the sufficiency of the Company’s cash resources extending into 2027, constitute forward-looking statements within the meaning of The Private Securities Litigation Reform Act of 1995. The words “anticipate,” “believe,” “continue,” “could,” “estimate,” “expect,” “intend,” “may,” “might,” “objective,” “ongoing,” “plan,” “predict,” “project,” “potential,” “should,” or “would,” or the negative of these terms, or other comparable terminology are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. The Company may not actually achieve the plans, intentions or expectations disclosed in these forward-looking statements, and you should not place undue reliance on these forward-looking statements. Actual results or events could differ materially from the plans, intentions and expectations disclosed in these forward-looking statements as a result of various important factors, including: uncertainties inherent in the identification and development of product candidates, including the conduct of research activities and the initiation and completion of preclinical studies and clinical studies; uncertainties as to the availability and timing of results from preclinical and clinical studies; timing of and expectations regarding the Company’s ability to submit and obtain regulatory authorization and initiate clinical studies; whether results from preclinical studies will be predictive of the results of later preclinical studies and clinical studies; whether earlier clinical data will be predictive of later clinical data; our ability to establish and maintain collaborations or strategic relationships; whether the Company's cash resources will be sufficient to fund the Company's foreseeable and unforeseeable operating expenses and capital expenditure requirements; as well as the risks and uncertainties identified in the Company's filings with the SEC, including the Company's most recent Form 10-K and in subsequent filings the Company may make with the SEC. In addition, the forward-looking statements included in this presentation represent the Company's views as of the date of this presentation. The Company anticipates that subsequent events and developments will cause its views to change. However, while the Company may elect to update these forward-looking statements at some point in the future, it specifically disclaims any obligation to do so. These forward-looking statements should not be relied upon as representing the Company's views as of any date subsequent to the date of this presentation.

entrada THERAPEUTICS

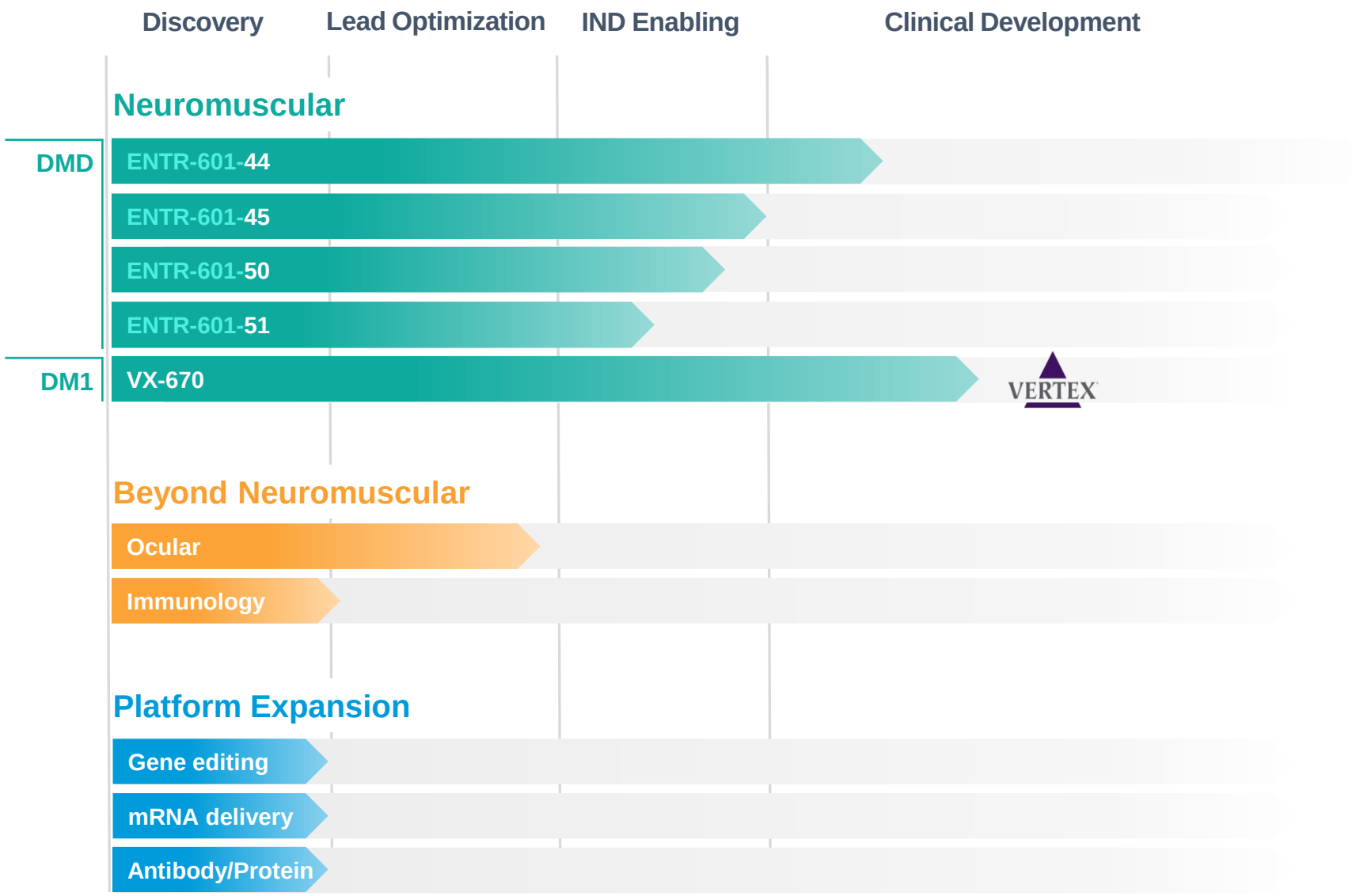
OUR MISSION:

To Treat Devastating Diseases With Intracellular Therapeutics

An Expanding Pipeline of Intracellular Therapeutics

Entrada's pipeline includes a diverse array of high potential and high value assets

Each target disease has a substantial patient population with a significant unmet medical need



EEV™ PLATFORM

ENDOSOMAL ESCAPE VEHICLE (EEV™)-BASED THERAPIES

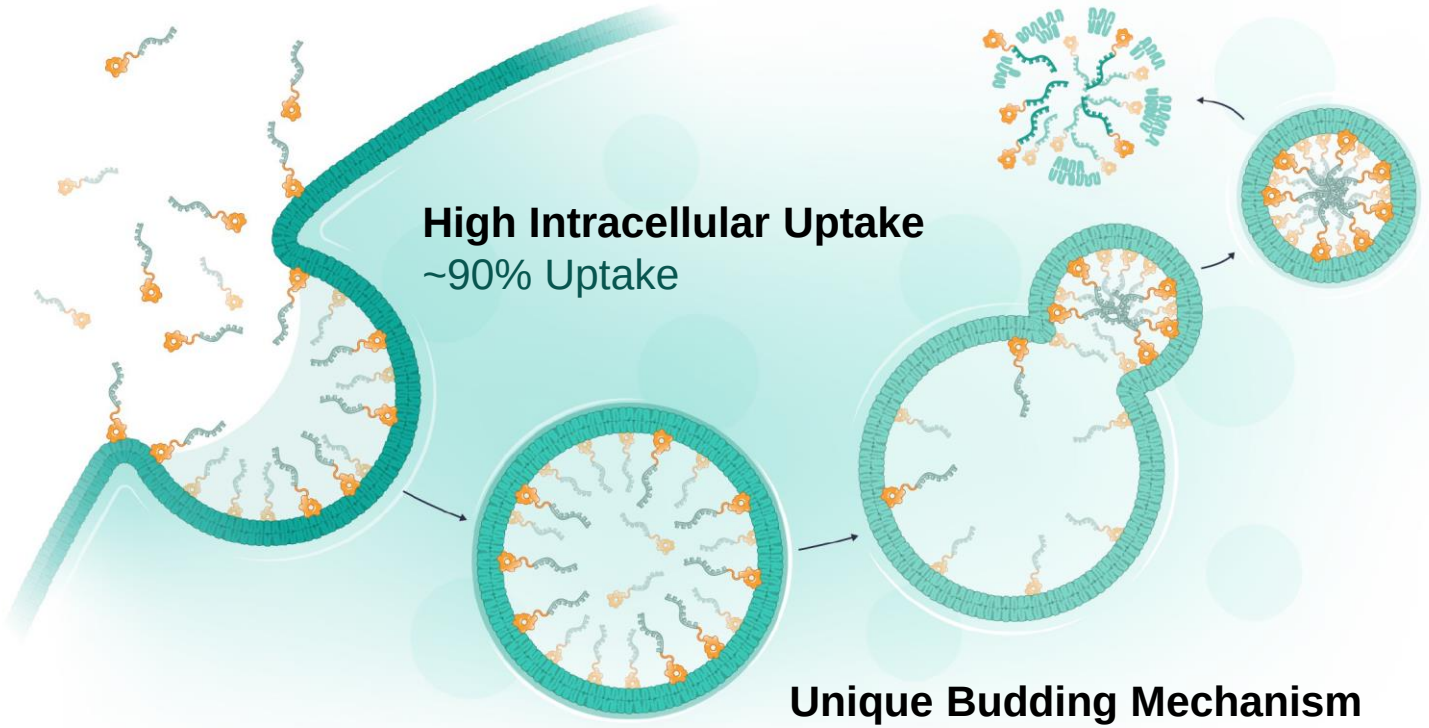
Unique chemistry
Improved uptake and endosomal escape

Cyclic structure
Extended half-life and increased stability

Phospholipid binding
Broad biodistribution to all cells

Consistent and predictable pharmacokinetics
Same EEV used across initial programs

Efficient Endosomal Escape
~50% Escape vs. ~2% Standard

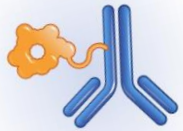


Qian, Z. et al. ACS Chem. Biol. 2013; Qian, Z. et al. Biochemistry 2014; Qian, Z. et al. Biochemistry 2016; Sahni, A. et al. ACS Chem. Biol. 2020; Pei, D. Acc. Chem. Res. 2022.

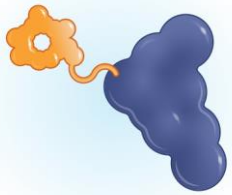
A BROADLY APPLICABLE PLATFORM

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

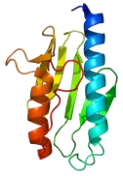
Antibodies



Enzymes

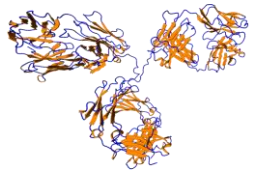


Oligonucleotides



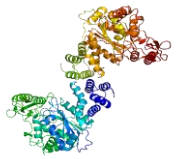
550-600 KDa

Hybrid frataxin



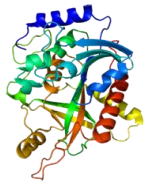
150 KDa

Antibody



98 KDa

Thymidine phosphorylase

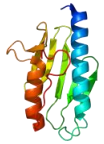


96 KDa

Purine nucleoside phosphorylase

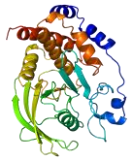
86 KDa

Alanine-glyoxylate aminotransferase



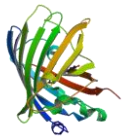
46 KDa

Human frataxin



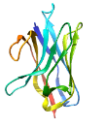
37 KDa

PTP1B catalytic domain



32 KDa

EGFP



16 KDa

Nanobody

6 KDa

Oligonucleotide

1-3 KDa

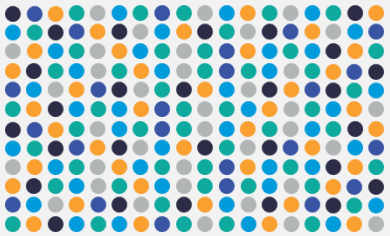
Various peptide cargos

EGFP, enhanced green fluorescent protein.

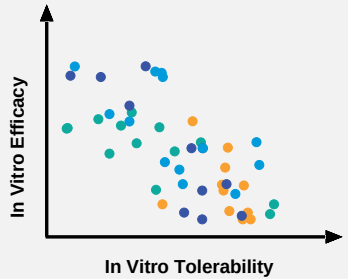
DISCOVERY ENGINE FOR EEV THERAPEUTICS
EEV-OLIGO EXAMPLE

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

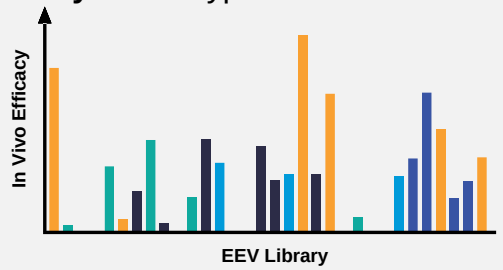
Chemically diverse macrocyclic **EEV library** generated through medicinal and combinatorial chemistry



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

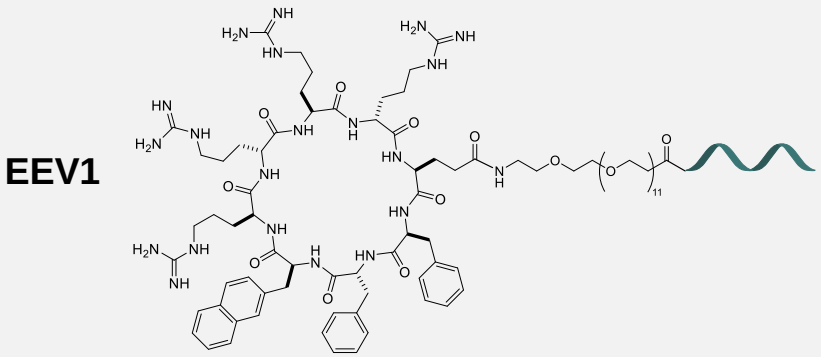


EEV, endosomal escape vehicle.

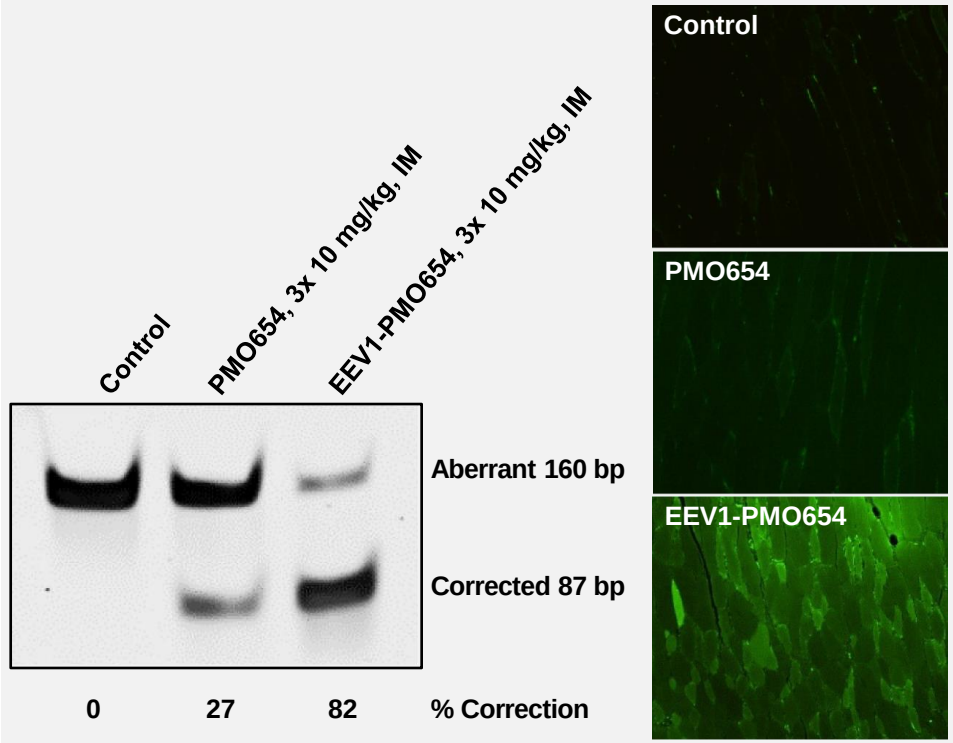
OLIGO DELIVERY WITH FIRST GENERATION EEV
EEV1 EXAMPLE

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



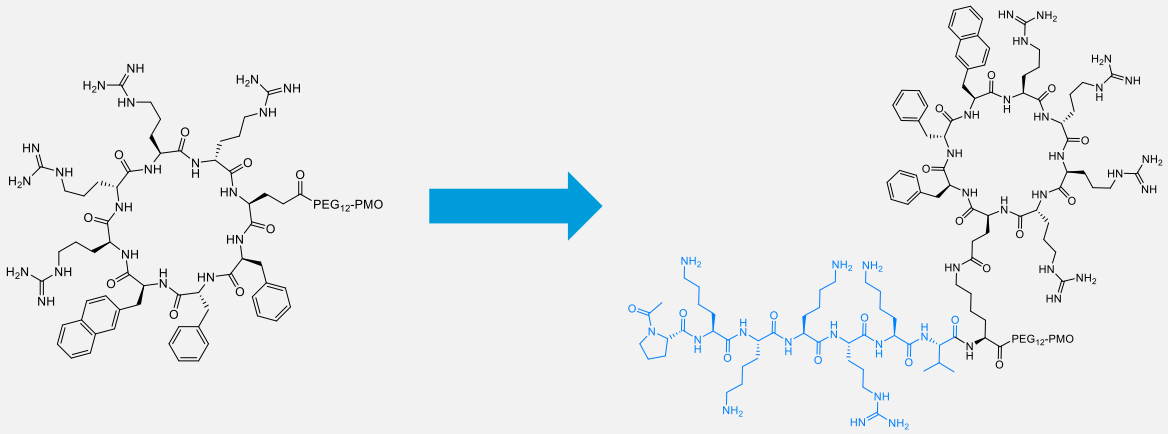
[a]Treatment of HeLa EGFP-654 cells with antisense oligonucleotides, such as PMOs, could switch the splicing and restore expression of EGFP; **EEV**, endosomal escape vehicle; **PMO**, phosphorodiamidate morpholino oligomer; **IM**, intramuscular; Qian, Z. et al. *Biochemistry* 2014, 2016; Li, X. et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

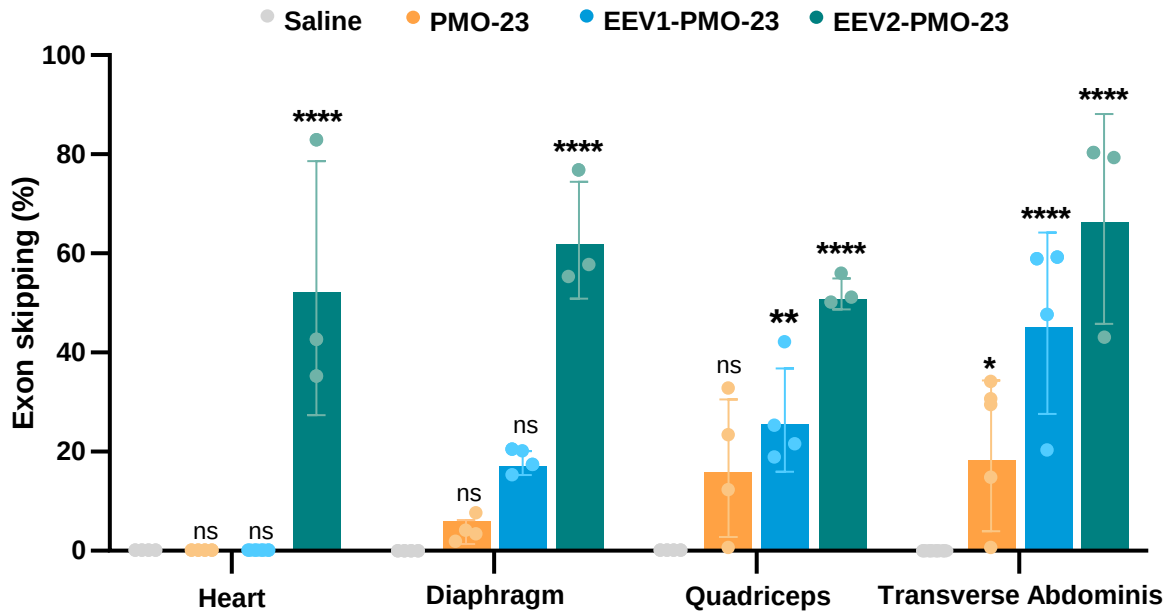
Structure of EEV2 Construct

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.

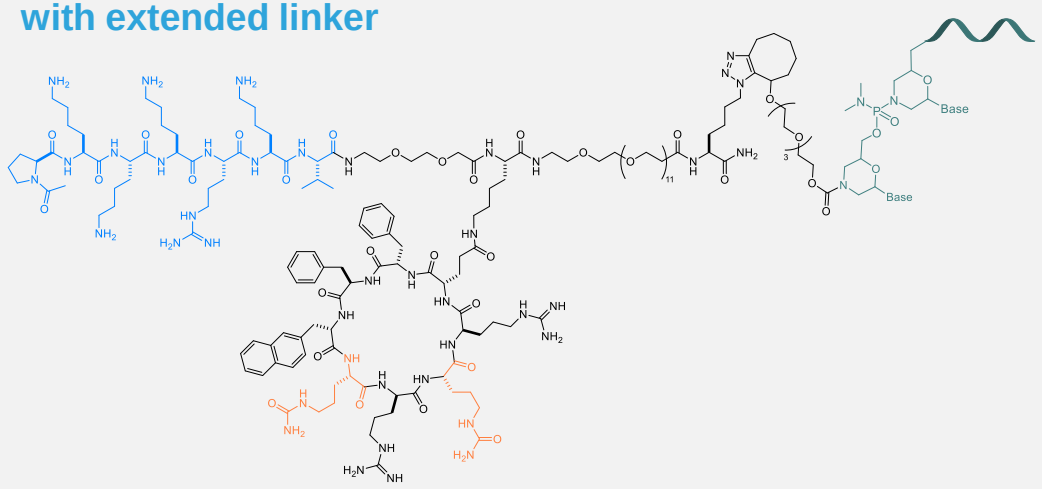
OPTIMIZATION OF EEV FOR MUSCLE DELIVERY
EEV3 Example

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

EEV3-PMO654 Structure and Medicinal Chemistry

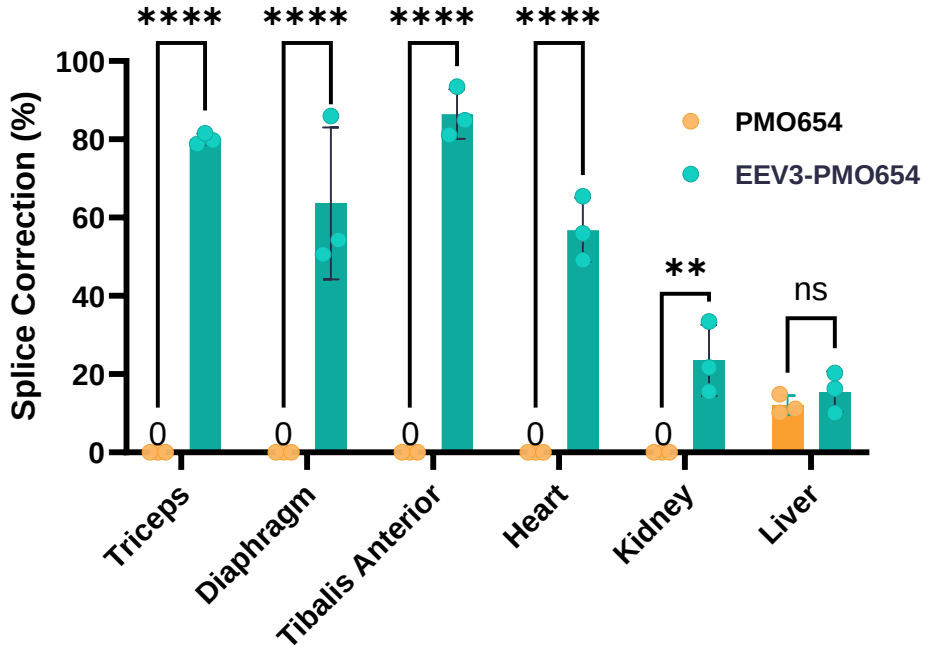
Exocyclic peptide sequence with extended linker

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

+

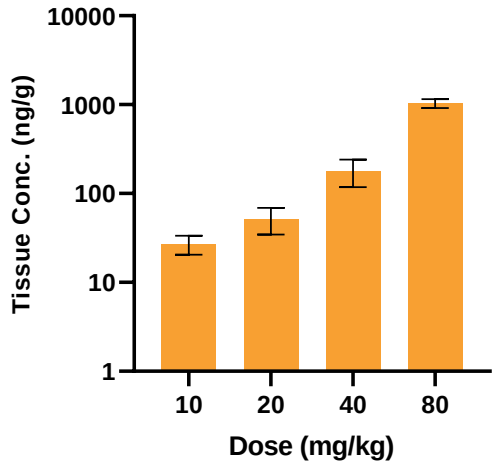
Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

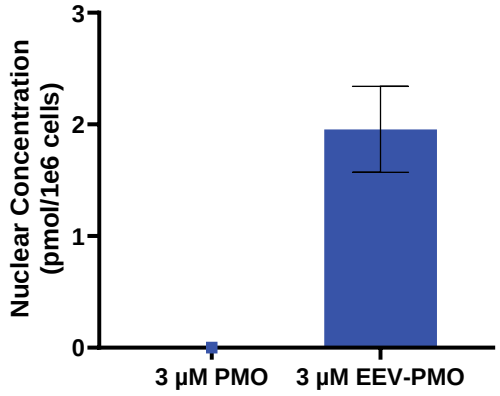
=

Pharmacodynamic Outcome

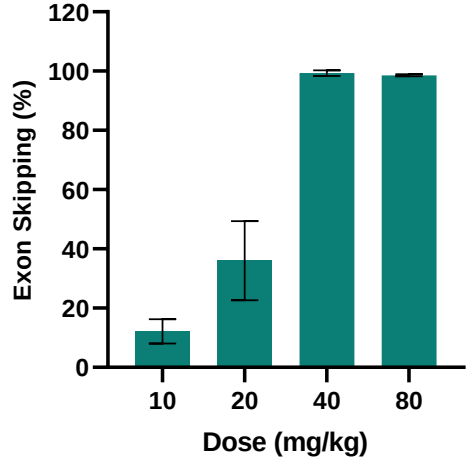
- ✓ Rapid, dose-dependent response
- ✓ Duration of at least 12 weeks



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

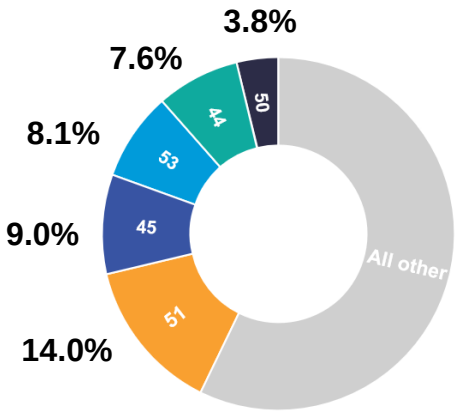
SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

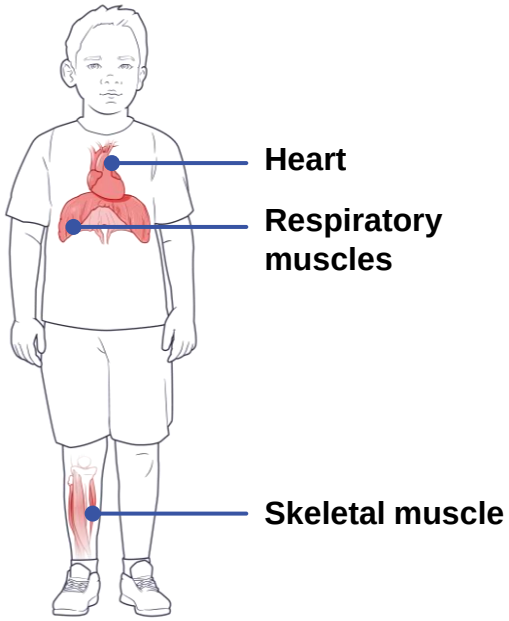
Duchenne is caused by **mutations in the *DMD* gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

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

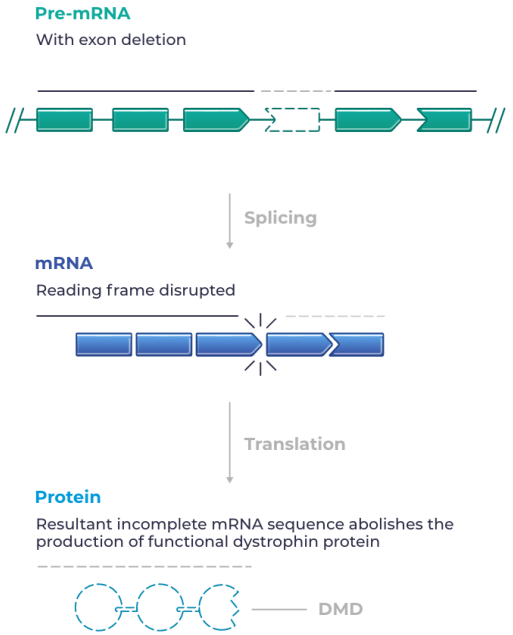
Approximately 41,000
people in the **U.S.**[1] **and** in **Europe**[2]
have Duchenne

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

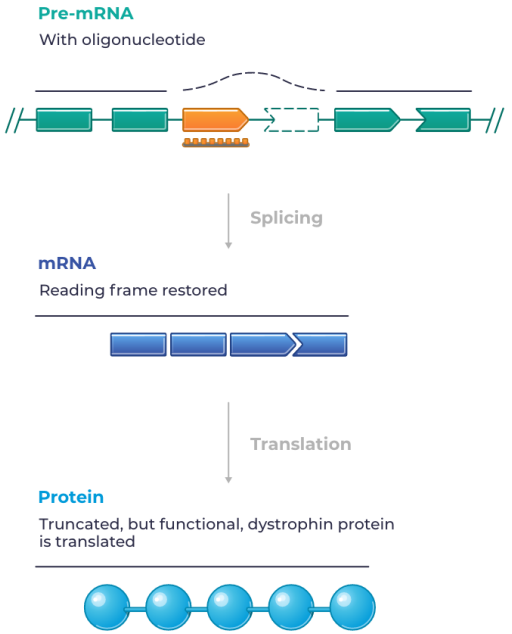




Patients with Duchenne



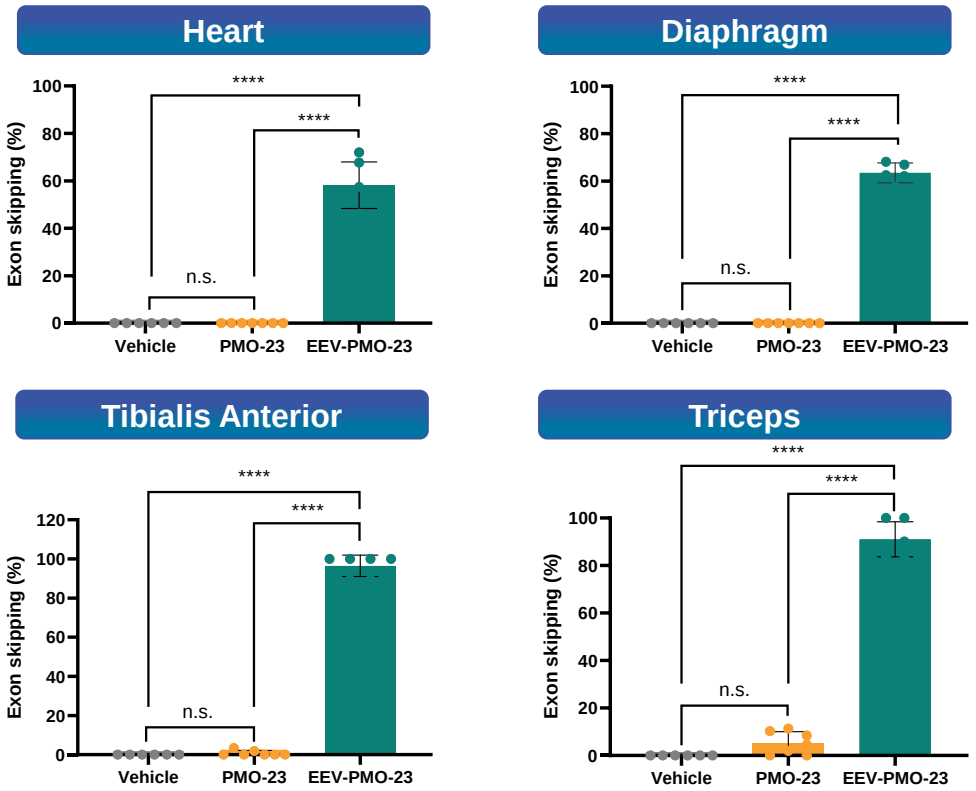
EEV-Oligonucleotide Approach



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

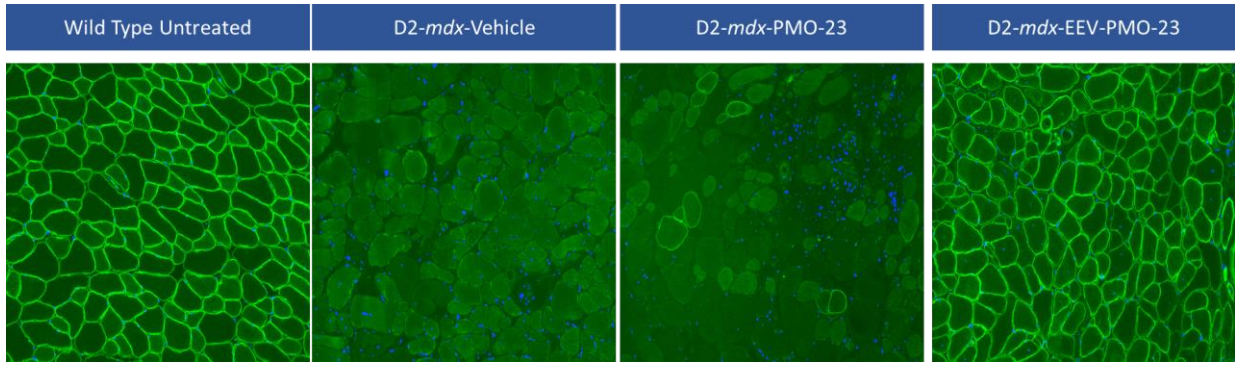
EEV-PMO RESTORES MUSCLE INTEGRITY
D2-*mdx* Mice

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

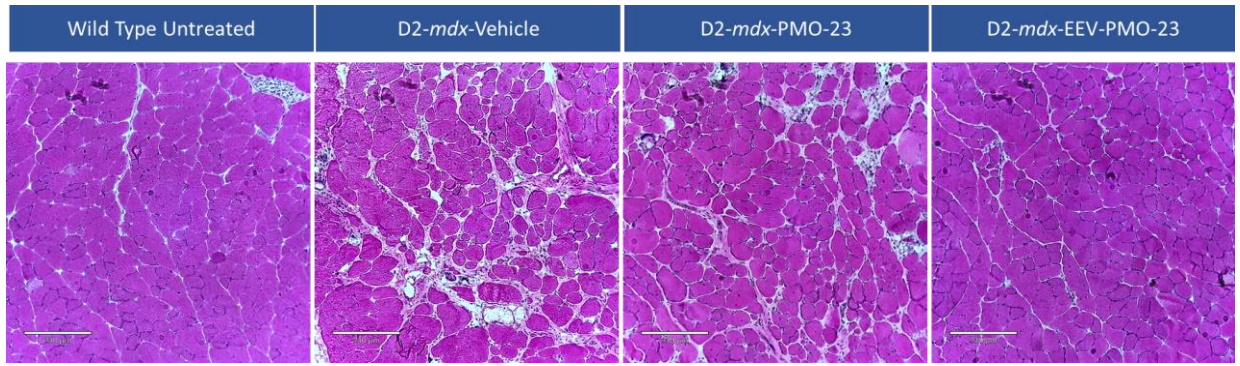


Broad dystrophin expression and restoration of muscle integrity after four monthly IV doses of EEV-PMO-23 in D2-*mdx* mice

Representative Immunofluorescence of Gastrocnemius Muscle (Dystrophin Staining in Green)



Representative Histopathology of Gastrocnemius Muscle (H&E Staining)

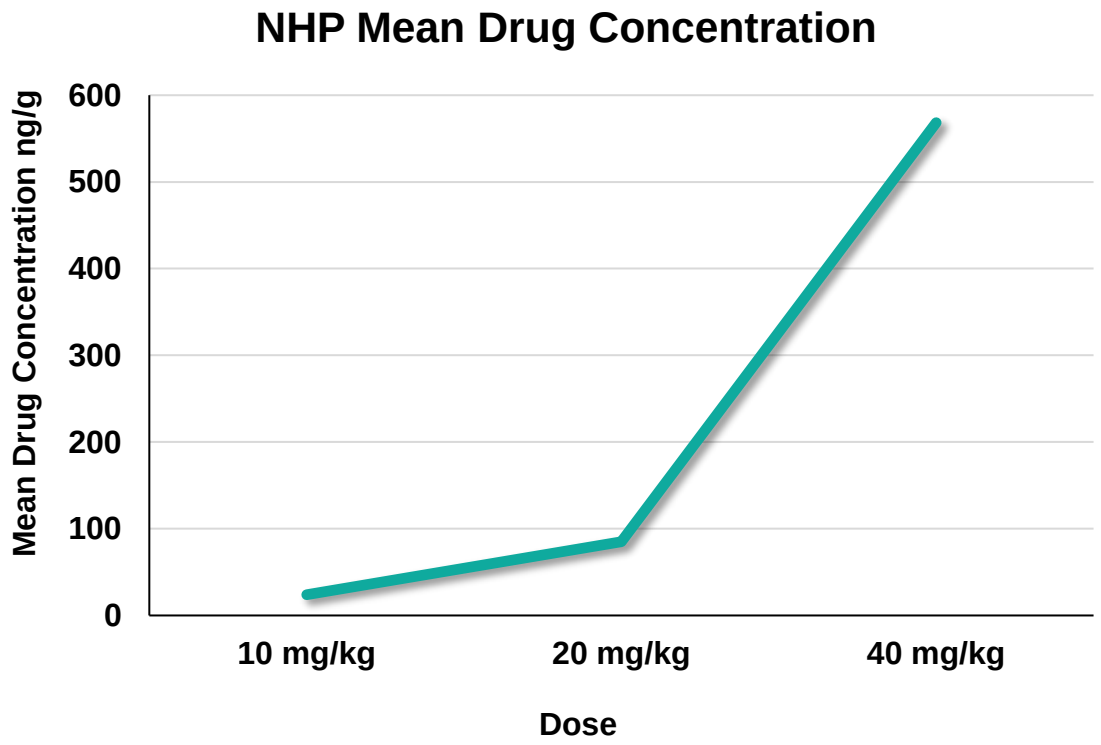


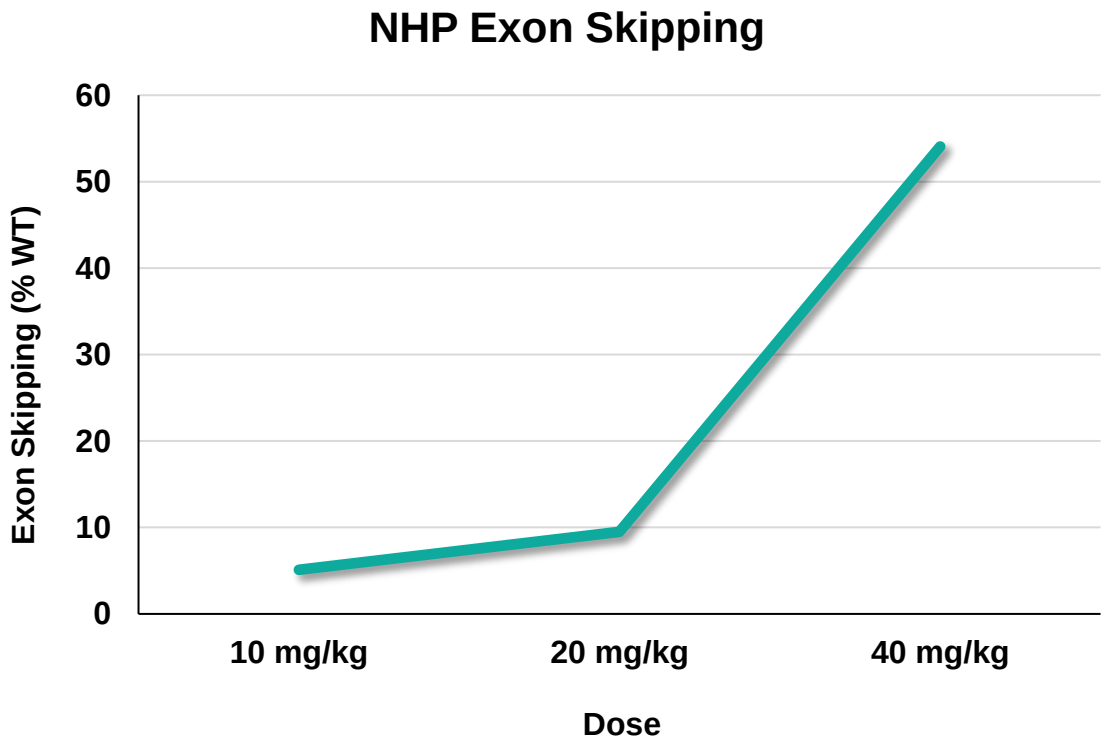
- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose

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.

DOSE-DEPENDENT PK/PD IN NHPS

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



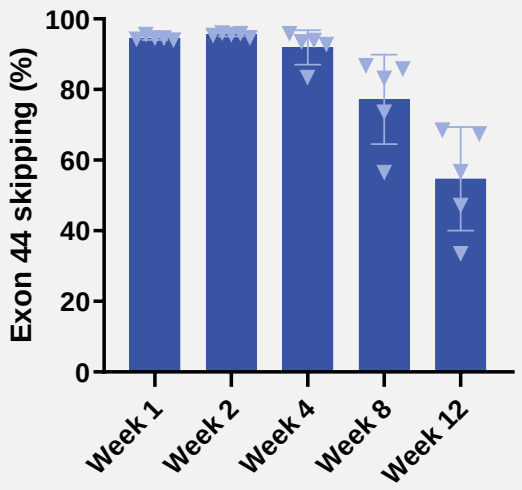


Single dose, bicep biopsy at 48 hours post-infusion; *R^2=0.9996; NHP: Non-human primates.

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

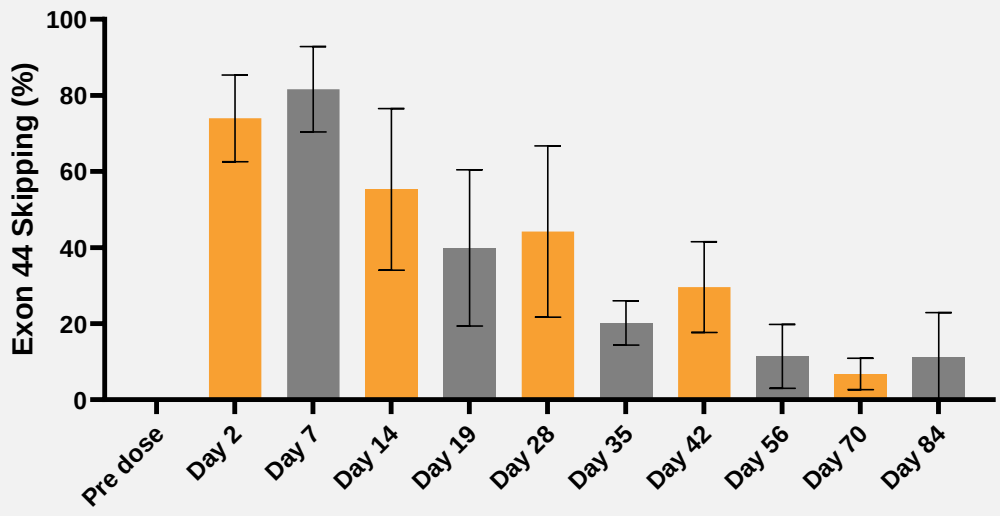
Significant potential for patient benefit is supported by ENTR-601-44 data in the mouse and the NHP at clinically relevant levels; *in vitro* data suggest higher target engagement in patient cells

Exon 44 Skipping in hDMD Mouse



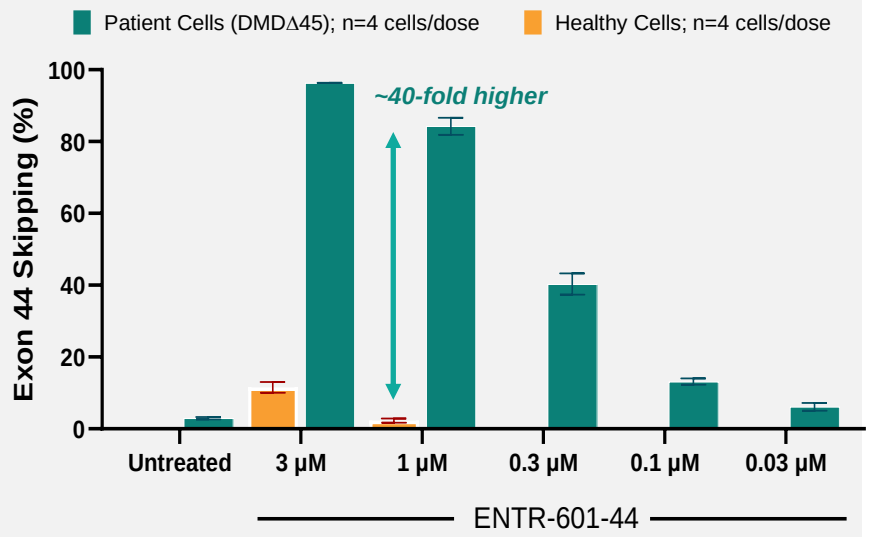
- Single IV 80 mg/kg dose (PMO equivalent) of ENTR-601-44
- Tibialis Anterior

Exon 44 Skipping in Monkey



- Post IV infusion of single 35 mg/kg dose (PMO equivalent) 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 IN HEALTHY HUMAN SUBJECTS

Study ENTR-601-44-101 met all study objectives and supports further evaluation of ENTR-601-44 in patients with Duchenne muscular dystrophy amenable to exon 44 skipping

Healthy male volunteers were randomized to receive ENTR-601-44 (n=25) or placebo (n=8)

- Single IV dose of 0.75, 1.5, 3.0, or 6.0 mg ENTR-601-44
- 24 of 25 ENTR-601-44-treated subjects completed study (physician's decision; not AE-related)

Favorable safety and tolerability profile of ENTR-601-44

- No AEs related to study drug
- No severe or serious AEs were reported in any dose group throughout the study
- No adverse findings or clinically relevant changes to any biomarkers of renal toxicity at the highest dose tested (6 mg/kg)

Exon 44 skipping in skeletal muscle indicates significant target engagement

- Statistically significant *DMD* exon 44 skipping was observed with a single IV dose of 6 mg/kg ENTR-601-44
- Dose-dependent concentrations of the final PMO-44 metabolite in skeletal muscle were observed in the 3 and 6 mg/kg dose groups

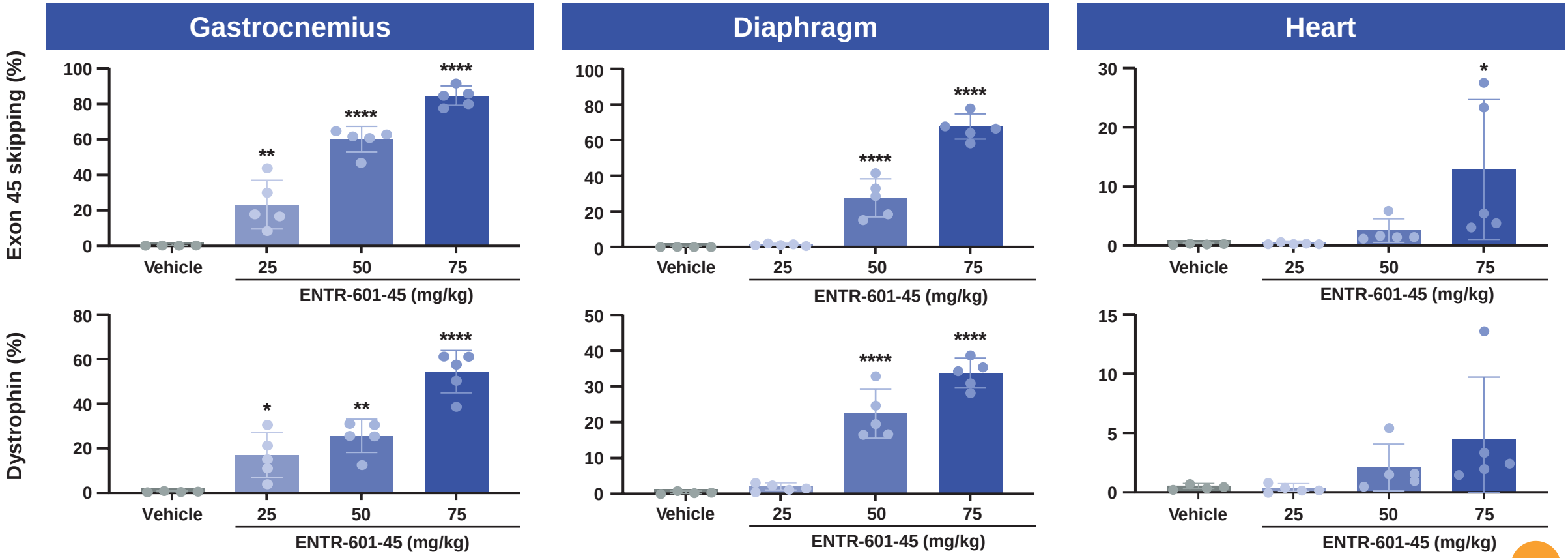
Healthy male volunteers aged 18-55 were screened within 28 days of dosing. Muscle concentrations and exon skipping were assessed using a needle muscle biopsy taken from biceps brachii 72 hours (±4 hours) post-dose of ENTR-601-44. Safety, tolerability, and pharmacokinetic parameters were assessed at each study visit through 6 weeks post-dose. **IV,** intravenous**; AE,** adverse event**; DMD**, Duchenne muscular dystrophy

ENTR-601-45

ENTR-601-45 EFFICACY IN del44hDMD.*mdx* MICE

Three Q6W doses of ENTR-601-45 produced robust human *DMD* exon 45 skipping and dystrophin production 6 weeks after the third dose

*$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$ vs. Vehicle



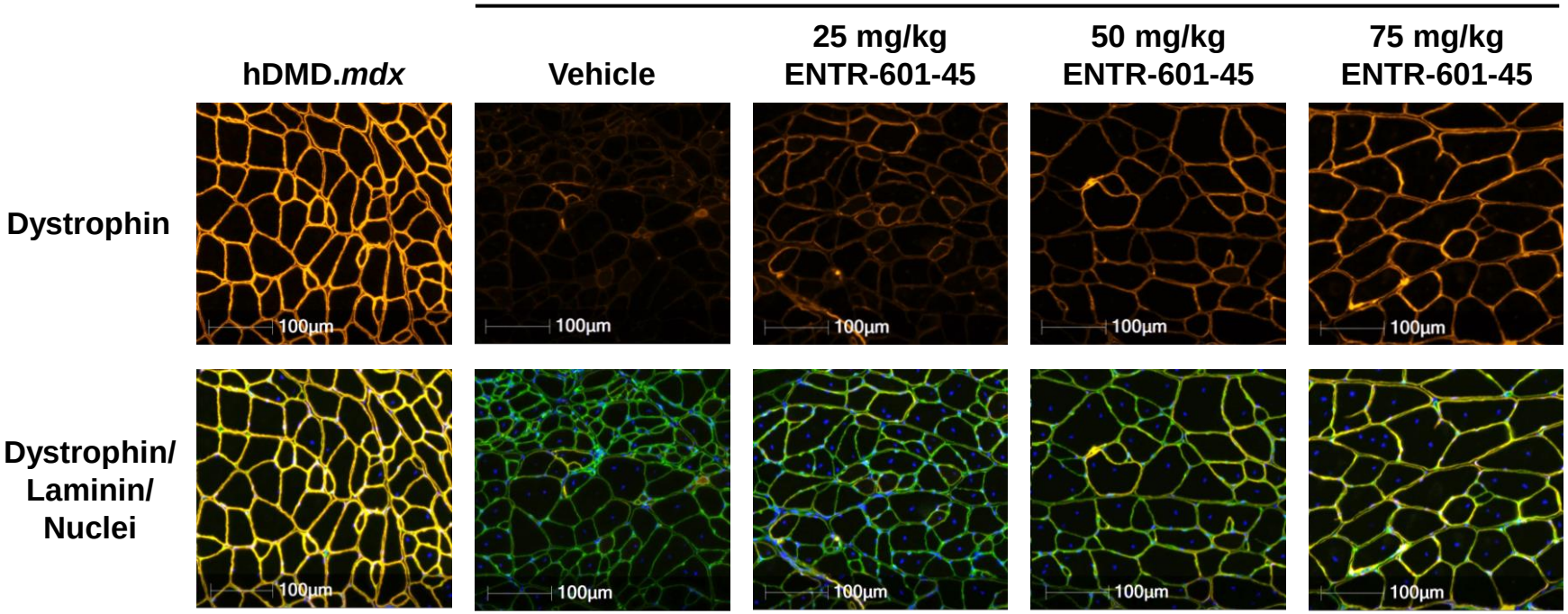
del44hDMD.*mdx* mice were treated with three Q6W IV injections of ENTR-601-45 or vehicle. Human *DMD* exon 45 skipping (top) and dystrophin protein expression (bottom) were analyzed in the gastrocnemius, diaphragm, and heart 6 weeks after the final dose. Percent dystrophin protein restoration is normalized to total protein and normalized to hDMD.*mdx* controls. Data shown as mean ± standard deviation. One-way ANOVA was used for statistical comparison; **ANOVA**, analysis of variance; **hDMD**, human dystrophin transgene; **IV**, intravenous; **Q6W**, every 6 weeks.

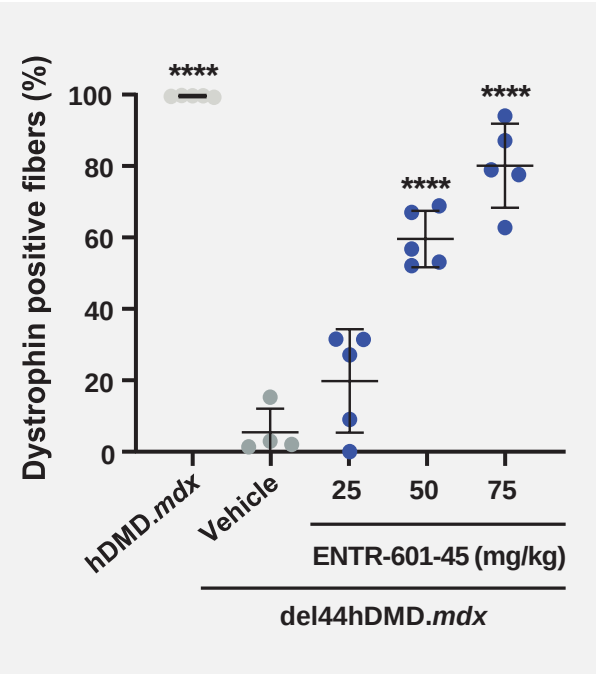
DYSTROPHIN LOCALIZATION WITH ENTR-601-45 IN del44hDMD.*mdx* Mice

ENTR-601-45 produced dose-dependent increases in dystrophin-positive muscle fibers localized to the sarcolemma of del44hDMD.*mdx* mice 6 weeks following the third Q6W dose

****$p \leq 0.0001$ vs. Vehicle

del44hDMD.*mdx* Skeletal Muscle





- del44hDMD.*mdx* mice were treated with three Q6W IV injections of ENTR-601-45 or vehicle. Dystrophin protein distribution and cellular localization was analyzed by immunofluorescence in the gastrocnemius 6 weeks after the final dose.

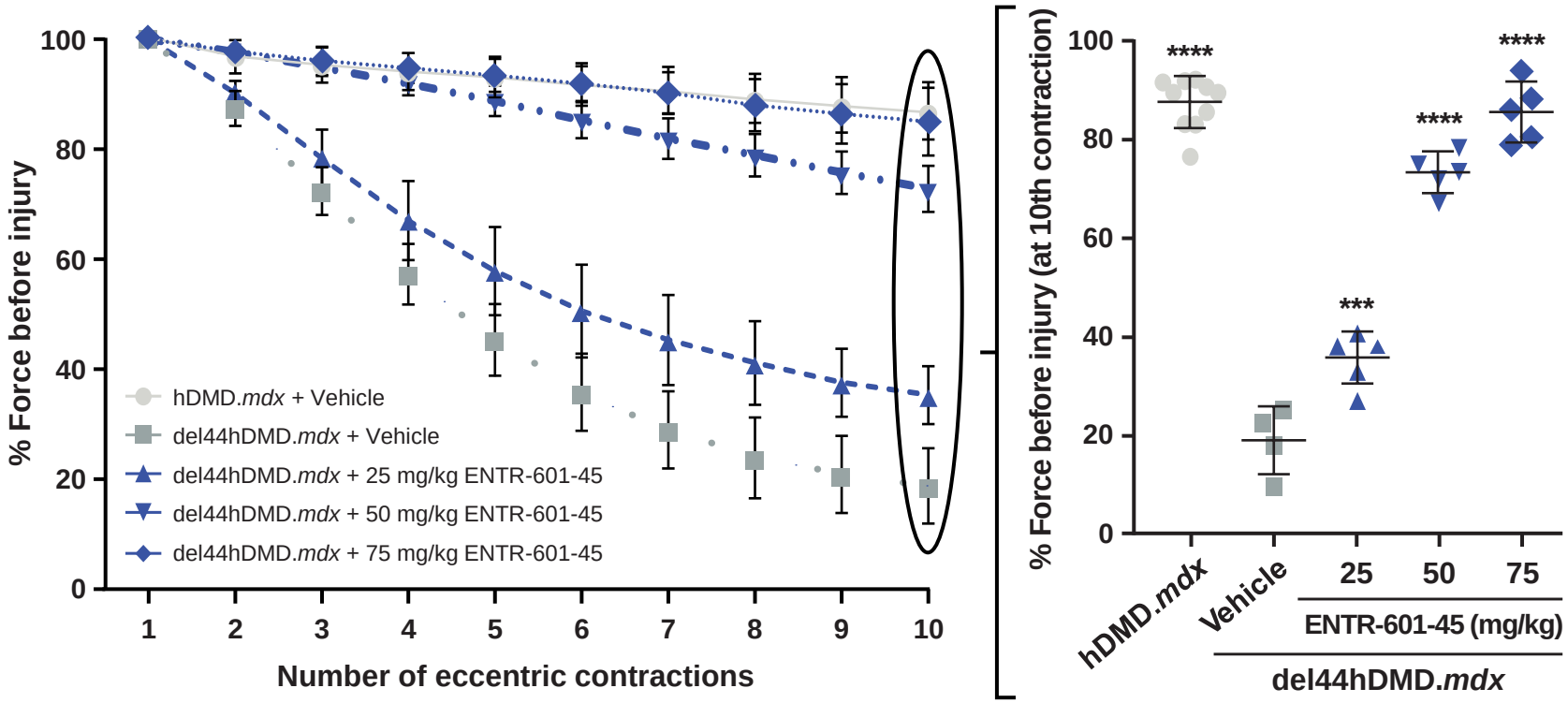
Quantification via Halo Image Analysis Software is shown as the percentage of dystrophin-positive muscle fibers relative to the total number of muscle fibers as determined by laminin staining (green) and dystrophin staining (red); co-localization to the sarcolemma appears yellow and nuclei appear blue. Data shown as mean ± standard deviation. One-way ANOVA was used for statistical comparison; **ANOVA**, analysis of variance; **hDMD**, human dystrophin transgene; **IV**, intravenous; **Q6W**, every 6 weeks.

ENTR-601-45 IMPROVES MUSCLE FUNCTION IN del44hDMD.*mdx* Mice

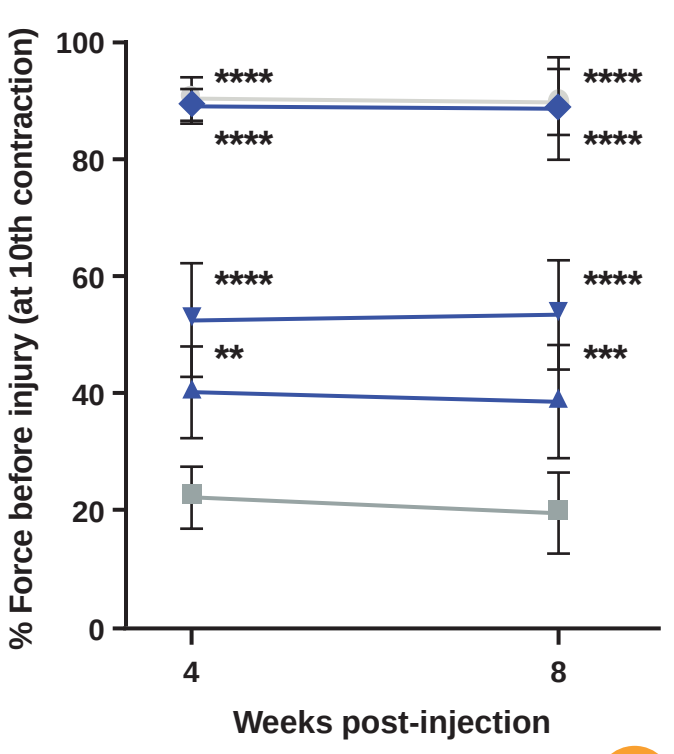
A dose-dependent increase in resistance to membrane damage was observed following the tenth contraction, which was maintained until at least 8 weeks after the third Q6W dose of ENTR-601-45

***p*<0.01, ****p*<0.001, *****p*<0.0001 vs. Vehicle

Skeletal Muscle Membrane Stability



Stability After Washout

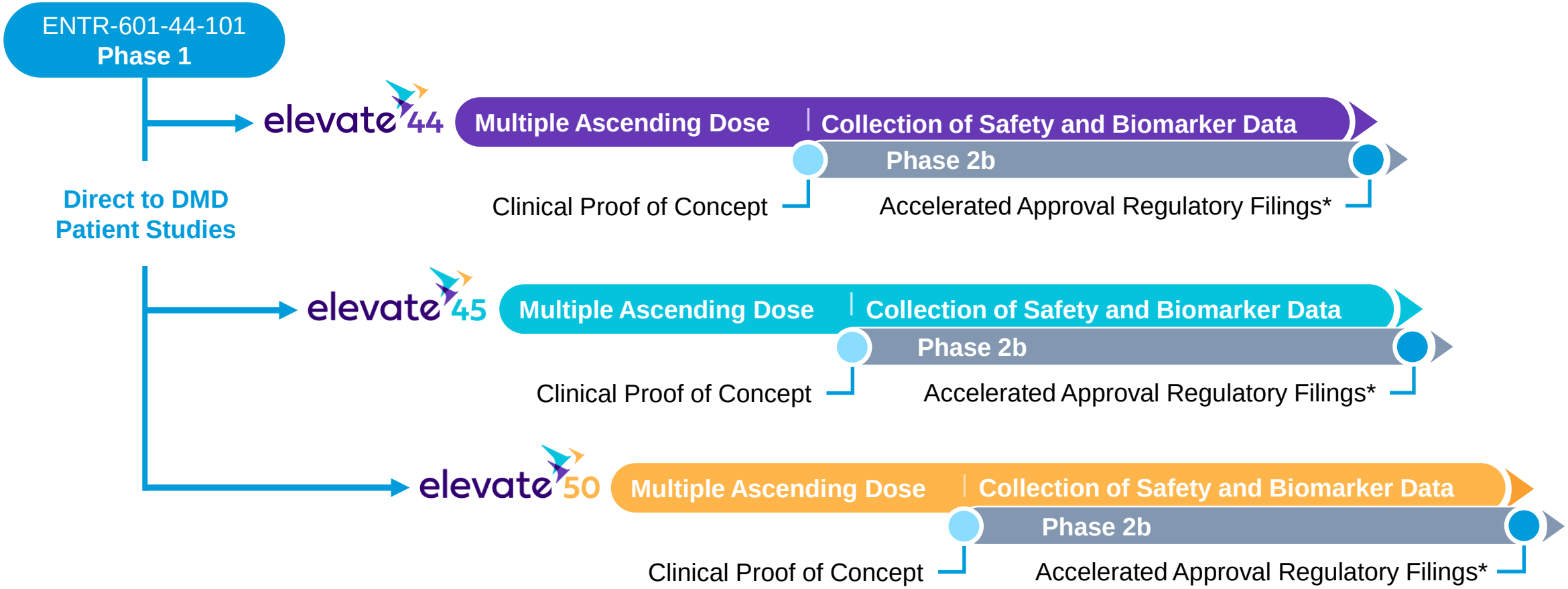


del44hDMD.*mdx* mice were treated with three Q6W IV injections of ENTR-601-45 or vehicle. ECC-induced muscle force loss generated by repeated ECC contraction of the gastrocnemius muscle was assessed 5 weeks (left/center) or 4 and 8 weeks (right) after the third dose. Data (mean ± standard deviation) shown across 10 ECC contractions normalized into a percentage of the initial force before any ECC contractions and as the percentage of force retained after the 10th contraction. Vehicle-treated hDMD.*mdx* mice were used as a control group for normal muscle function. One-way ANOVA was used for statistical comparison to vehicle-treated del44hDMD.*mdx* mice.
ECC, eccentric force; **hDMD**, human dystrophin transgene; **IV**, intravenous; **Q6W**, every 6 weeks.

DMD CLINICAL DEVELOPMENT PLAN

CLINICAL STRATEGY IS DESIGNED FOR EFFICIENT REGULATORY PATH

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.

PLATFORM EXPANSION

MULTIPLE PIPELINE EXPANSION OPPORTUNITIES

Entrada's flexible approach to intracellular therapeutics enables pipeline expansion by leveraging new moieties and by targeting additional therapeutic areas

TARGET	DNA	RNA			PROTEINS		
APPROACH	Gene Editing	RNA Editing	RNA Splicing	RNA Blocking	RNA Silencing	Protein Replacement	Protein Inhibition	Protein Degradation
GOAL	Deliver CRISPR enzyme and repair gene function with guide RNA	Deliver oligonucleotide therapeutics for RNA editing	Modify RNA via exon/intron splicing to activate protein expression	Block trinucleotide repeats in RNA to inhibit adverse binding	Silence or knockdown RNA to prevent protein expression	Replace proteins and enzymes	Inhibit protein signaling pathways	Degrade disease-causing proteins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