Delivery of Antisense Oligonucleotides to Satellite Cells in Preclinical Models of Duchenne Muscular Dystrophy

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Entrada Therapeutics

MDA Clinical & Scientific Conference | March 8–11, 2026 | Orlando, FL

Meet Andrew and JJ, living with Duchenne muscular dystrophy

entrada THERAPEUTICS

Disclosure

- Mahasweta Girgenrath, PhD, is an employee of Entrada Therapeutics.

OUR MISSION:

To Treat Devastating Diseases with Intracellular Therapeutics

We're proud to share the stories of JJ, Andrew, Max and Franklin – all living with Duchenne Muscular Dystrophy

EEV™ Platform

Understanding EEV endosomal escape platform advantages

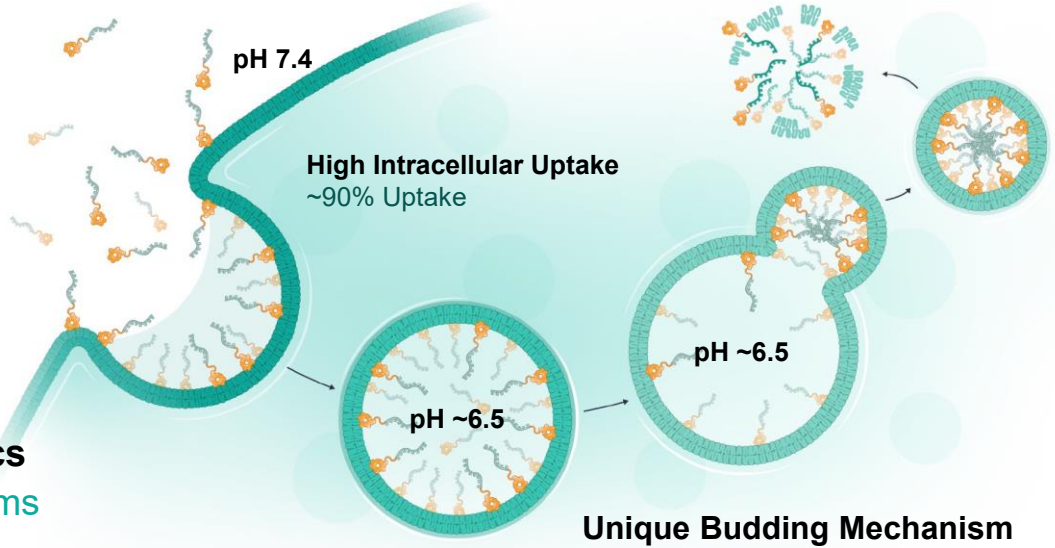
Unique pH-dependent membrane binding affinity enhances endosomal escape

Cyclic structure
Extended half-life and increased stability

Phospholipid binding
EEV constructs have broad biodistribution to all cells and have higher affinity to phospholipid bilayer than traditional CPPs

Consistent and predictable pharmacokinetics
Same EEV construct used across initial programs

Efficient Endosomal Escape
~25-50-fold increase in endosomal escape vs. other competitive approaches



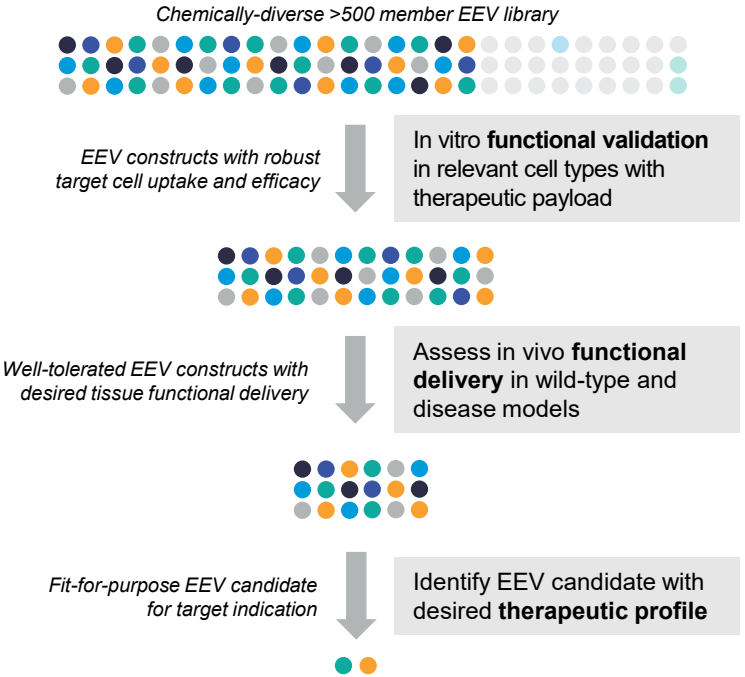
Qian Z. et al. *ACS Chem. Biol*. 2013; Qian Z. et al. *Biochemistry* 2014; Qian Z. et al. *Biochemistry 2016*; Sahni A. et al. *ACS Chem Biol*. 2020; Pei D. *Acc Chem Res*. 2022.

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 EEV constructs in vivo to select for **pharmacodynamic activity** in target tissues
- Optimize **conjugation chemistry** for desired therapeutic modality

Screening Cascade for EEV Candidates



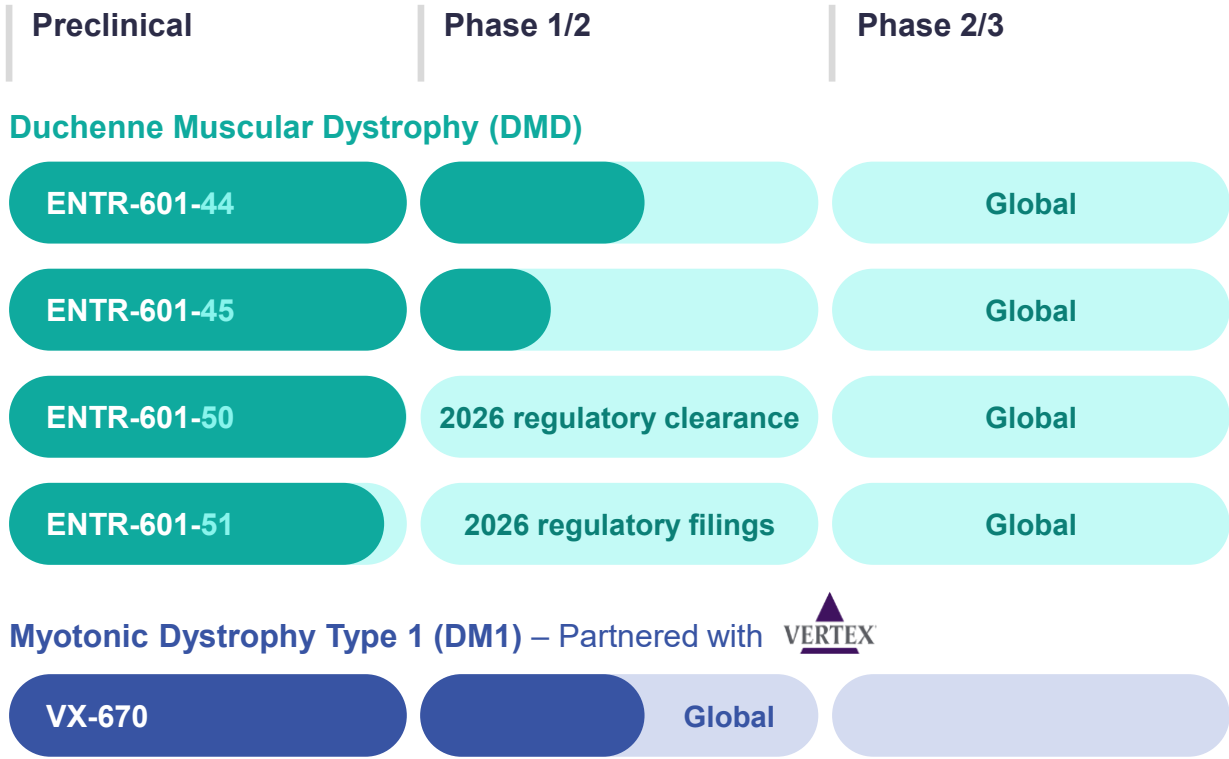
EEV, endosomal escape vehicle.

Advancing 5 clinical stage programs by end of 2026

Phase 1/2 data from ex-U.S. DMD studies to support Accelerated Approval regulatory filings in the U.S.

Our pipeline includes programs to address unmet needs in DMD and DM1

February 2026

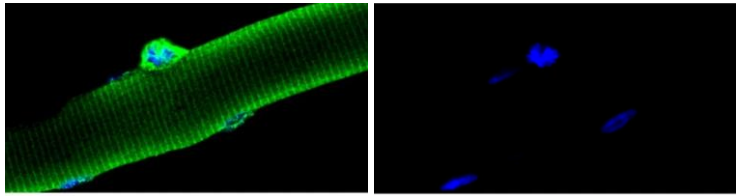


Delivery of PMO to Satellite Cells

Delivering to Muscle Satellite Cells —Why Does It Matter

Muscle satellite cells: therapeutic target with potential in several neuromuscular disorders

- **Quiescent satellite cells are historically challenging to access by therapeutic modalities**
  - Current DMD treatments mainly target dystrophic myofibers and have limited correction of satellite cell compartment
  - The loss of dystrophin disrupts muscle regeneration by causing mitotic defects in satellite cells, favoring symmetric division and hyperplasia over the necessary asymmetric division[1]
  - Dystrophin absence also leads to acquired senescence in an increased number of satellite cells, diminishing their proliferative capacity and ultimately impairing muscle regeneration[2]
  - Efficacy of Adeno Associated Viruses (AAV) is reduced in proliferating myoblasts and nearly absent in quiescent satellite cells, indicating limited potential for supporting muscle regeneration and post-natal growth[3]
  - Receptor mediated delivery to quiescent satellite cells is limited by the expression of specific receptor (e.g. Tfr1) on satellite cell membrane[4]



Bentzinger C, et al. *Stem Cell Rest Ther.* 2010;1:27 https://doi.org/10.1186/scrt27. Springer Nature. Reprinted with permission.

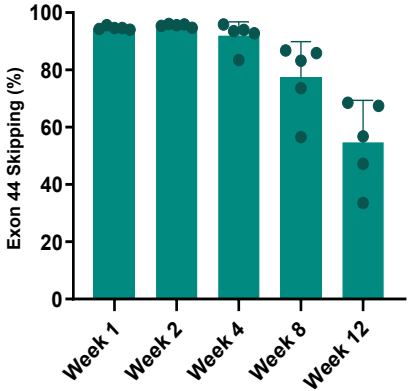
Efficient delivery of EEV-PMO to quiescent satellite cells could enable early disease intervention.

1. Kodippili K & Rudnicki MA. *Front Physiol*. 2023. 2. Cardone N, et al. *Acta Neuropathol Commun*. 2023. 3. Arnett AL, et al. *Mol Ther Method Clin Dev*. 2014.
4. Ding H, et al, *J Cachexia Sarcopenia Muscle*. 2021.

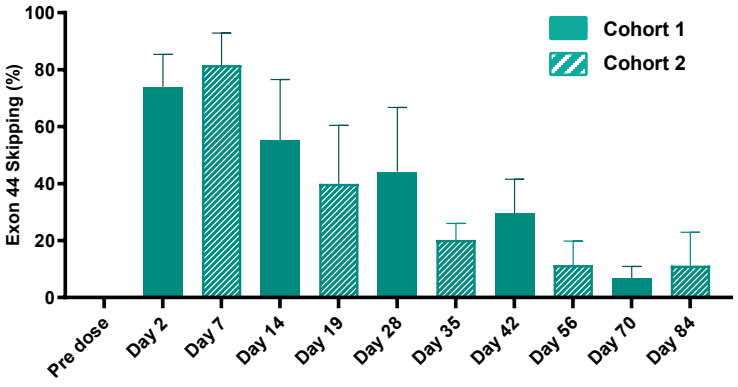
Consistent and Durable Efficacy of EEV-PMO Was Demonstrated Across Species

Robust exon skipping and dystrophin protein expression observed several weeks after a single dose of EEV-PMO treatment in mouse-, NPH-, and patient-derived cells

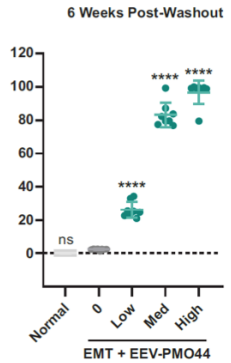
Exon 44 Skipping in hDMD Mouse

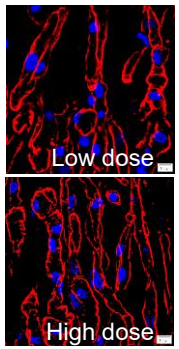


Exon 44 Skipping in NPH



Exon 44 Skipping in Healthy and Patient-Derived 3D Engineered Muscle Tissue





Δ45 DMD

Δ45 DMD

Single IV dose of 80 mg/kg of ENTR-601-44 showed robust exon skipping in skeletal muscle for at least 12 weeks post treatment

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, post a single treatment

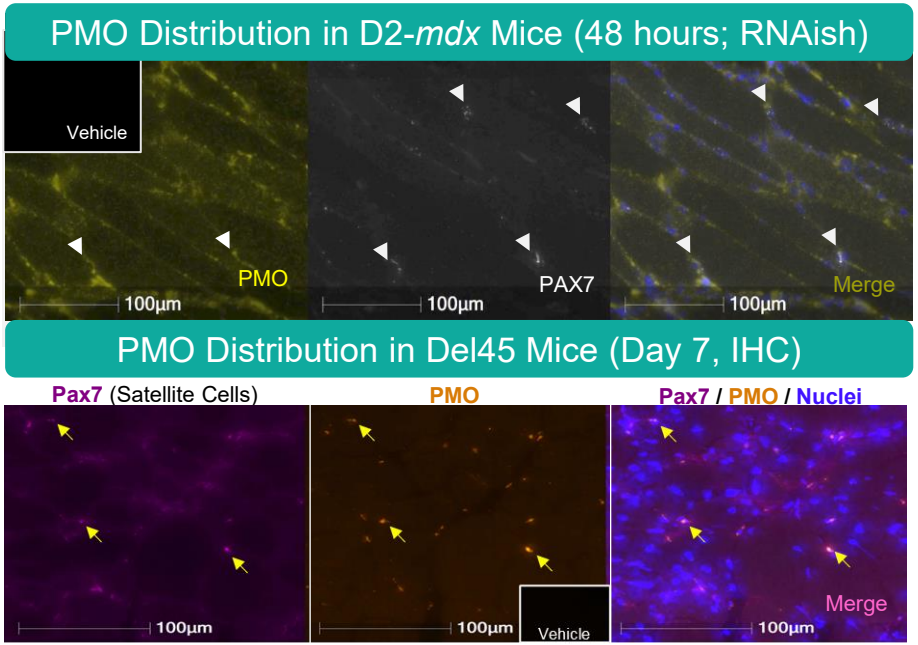
Robust dose-dependent exon 44 skipping was observed in DMD patient-derived engineered muscle tissue 6 weeks post a single treatment

hDMD transgenic mice (left) express full-length human dystrophin gene ('t Hoen AC, 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; NPH, normal human primates.

Distribution of EEV-PMO-23 and -44 Exon Skippers to Satellite Cells

EEV-PMO efficiently co-localizes with quiescent satellite cells (Pax7 positive) at 48 hours; Qualitative data demonstrate that co-localization lasts at least 1-week post-dose

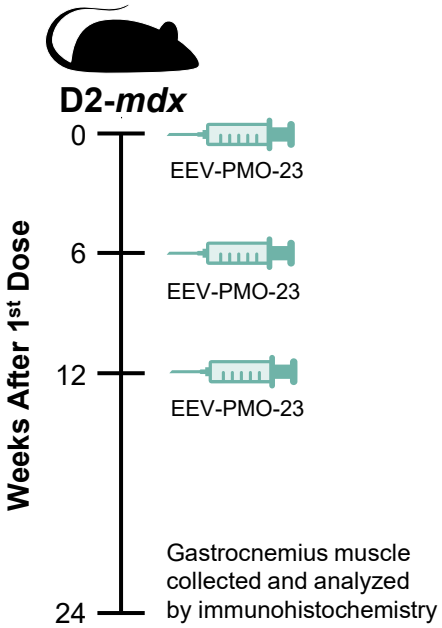
- **Two independent molecular techniques were utilized to determine EEV-PMO distribution within specific cell lineages across muscle tissue**
  - RNA-ISH: Highly selective and sensitive technique to assess specific cell lineages across muscle tissue (top panel)
  - IHC assessment (bottom panel)
- **Analysis of RNA-ISH data confirms that PMO-23 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-Del45 mice with PMO-44 at 7 days**

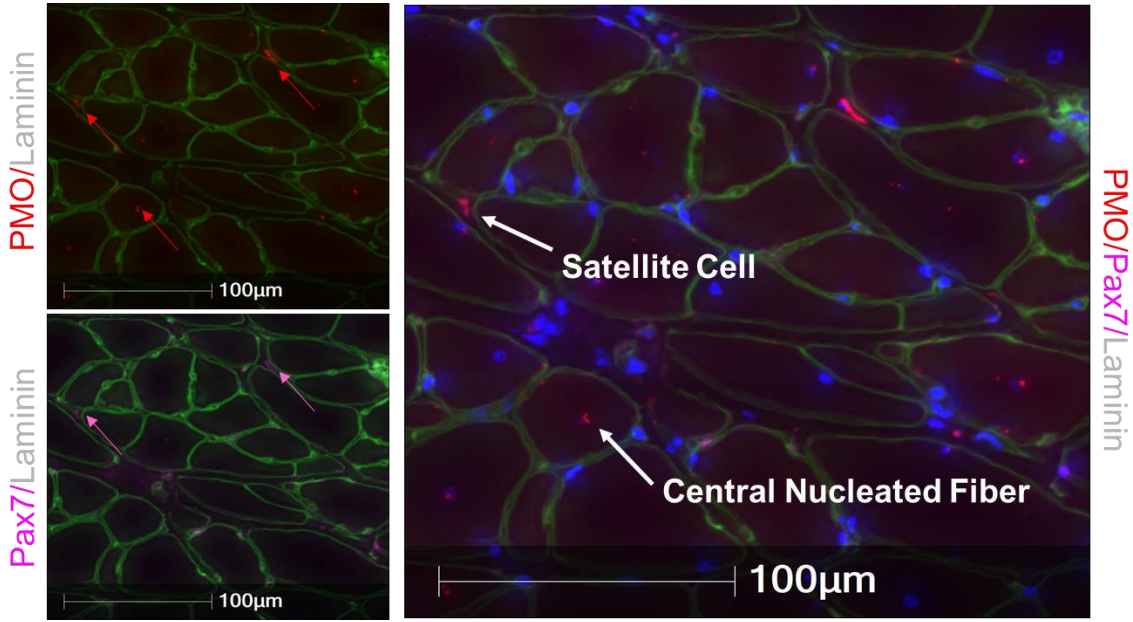


(Top right) D2-*mdx* mice were treated with a single IV dose of EEV-PMO-23; Gastrocnemius was collected at 48 hours and analyzed by RNA-ISH. (Bottom right) hDMD mice were treated with a single IV dose of EEV-PMO-44; Gastrocnemius was collected at 7 days and tissues analyzed by IHC. Pax7 is a satellite cell marker (Seale, P. et al *Cell* 2000). EEV, Endosomal Escape Vehicle; hDMD, human Duchenne muscular dystrophy; IHC, immunohistochemistry; IV, intravenous; PMO, phosphorodiamidate morpholino oligomers; RNA-ISH: RNA in situ hybridization image analysis.

PMO-23 Persists in Satellite Cells of *D2-mdx* Mice 12 Weeks After Final Dose

PMO-23 co-localizes with satellite cells and newly regenerated centrally nucleated fibers 12 weeks post washout after 3 Q6W doses

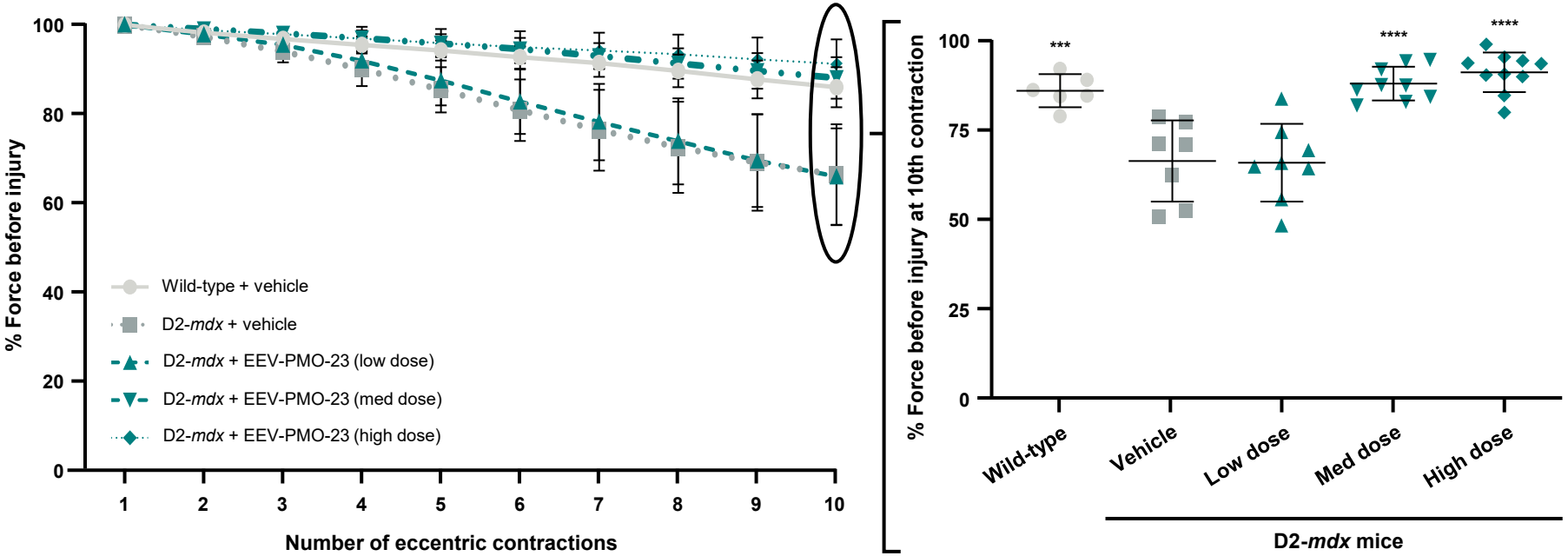




D2-*mdx* mice were treated with 3 IV doses of EEV-PMO-23 administered Q6W; Gastrocnemius was collected 12 weeks after the 3rd dose and analyzed by immunohistochemistry.
Q6W: every six weeks.

EEV-PMO-23 Improves Muscle Function in *D2-mdx* Mice

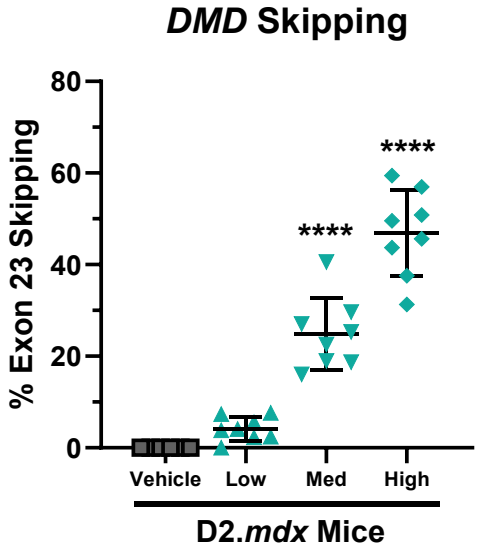
Three Q6W doses of EEV-PMO (medium and high dose) maintain a significantly improved tetanic force and restored the membrane stability to wild-type mice

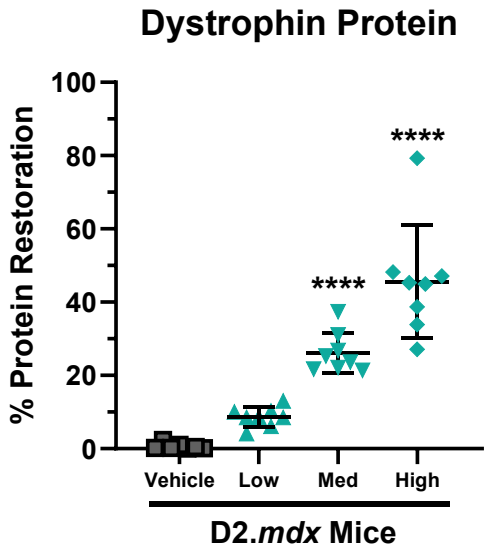


D2-*mdx* mice were treated with 3 Q6W IV injections of EEV-PMO-23 (PMO designed to skip mouse exon-23) or vehicle. ECC-induced muscle force loss generated by repeated ECC contraction of the gastrocnemius muscle was assessed. 12 weeks after the third dose. Data (mean ± standard deviation) shown across 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. One-way ANOVA was used for statistical comparison. ***$p<0.001$, ****$p<0.0001$. ECC, eccentric force; IV, intravenous; med, medium; Q6W, every 6 weeks.

EEV-PMO-23 Leads to a Longer Duration of Exon Skipping and Enhanced Dystrophin Expression

Gastrocnemius muscle show sustained and continued exon-skipping and dystrophin expression 12 weeks post 3 doses administered 6 weeks apart

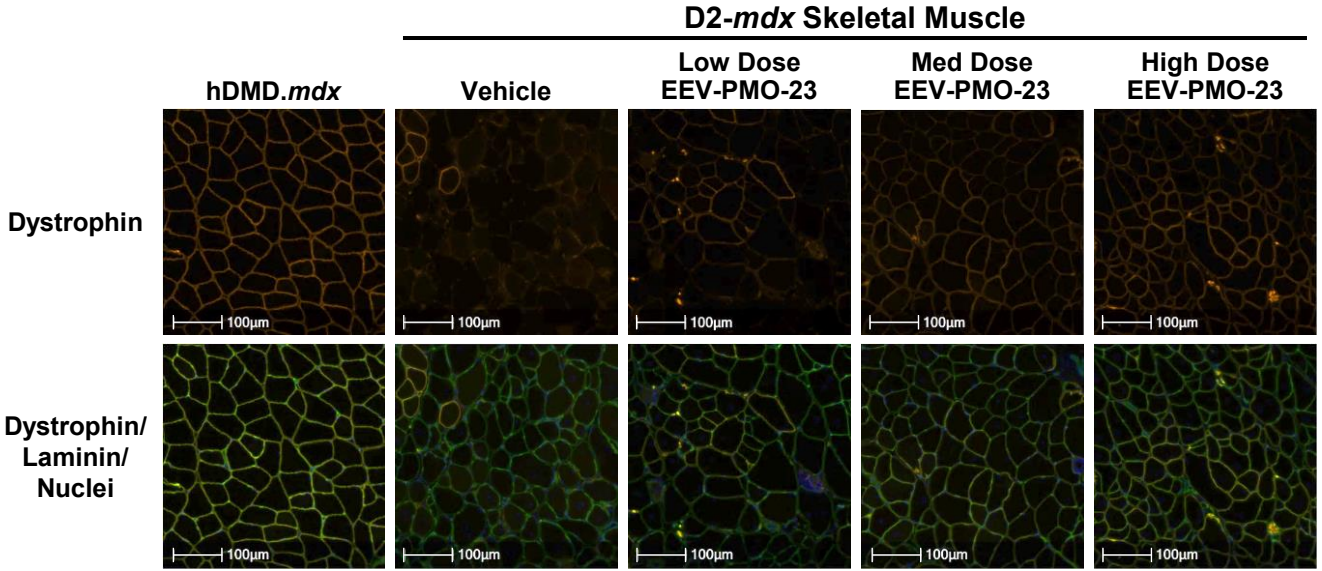


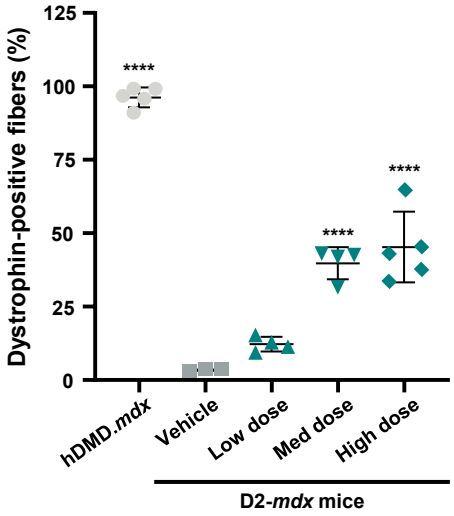


D2-*mdx* mice were treated with 3 Q6W IV injections of EEV-PMO-23 (PMO designed to skip mouse exon-23) or vehicle. Exon skipping and dystrophin restoration was assessed. 12 weeks after the third dose. Exon skipping with labchip- 0%(vehicle), 4.154% (low), 46.86%(high). Dystrophin protein expression using Jess- 0.629%(vehicle), 8.667%(low), 26.15%(medium), 45.58%(high).

Dystrophin Localization EEV-PMO-23 in D2-*mdx* Mice

Three Q6W doses of EEV-PMO-23 produced dose-dependent increases in dystrophin-positive muscle fibers localized to the sarcolemma of D2-*mdx* mice 12 weeks after the third dose

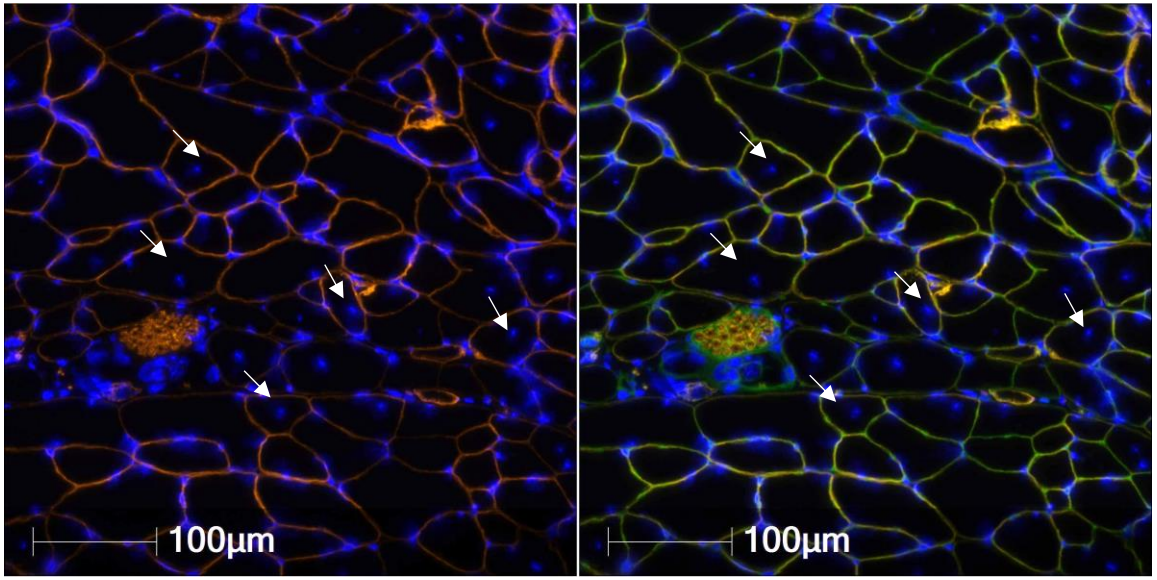




D2-*mdx* mice were treated with a three Q6W IV doses of EEV-PMO-23 or vehicle. Dystrophin protein distribution and cellular localization were analyzed by immunofluorescence in the gastrocnemius 12 weeks post-dose. Quantification via Halo Image Analysis Software is shown as the percentage of dystrophin-positive 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. ****$p \leq 0.0001$; ANOVA, analysis of variance; hDMD, human dystrophin transgene; IV, intravenous; med, medium.

Strong Expression of Dystrophin Is Observed in Many Centrally Nucleated Fibers

PMO-positive satellite cells may play a role in the formation of newly regenerated fibers that maintain high levels of dystrophin expression after a 12-week washout period



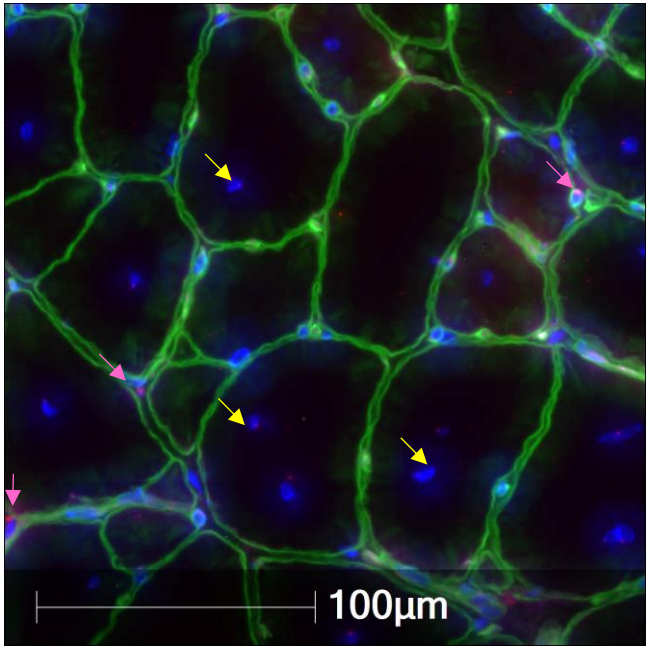
Nuclei/Dystrophin

Nuclei/Dystrophin/Laminin

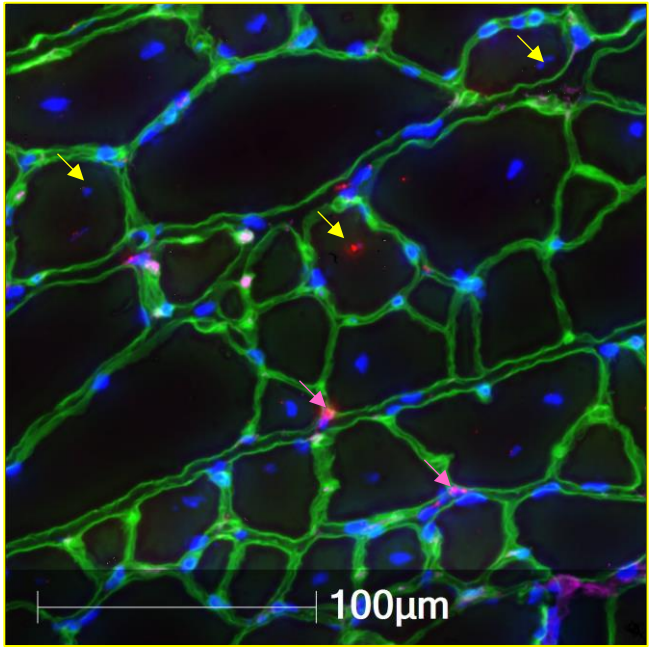
D2-*mdx* mice were treated with a three Q6W IV doses of EEV-PMO-23 or vehicle. Dystrophin protein distribution and cellular localization were analyzed by immunofluorescence in the gastrocnemius 12 weeks post-dose. Images were captured at a magnification of 40x, cropped and enlarged for visualization of centrally nucleated fibers (CNF). dystrophin-positive muscle fibers were visualized using dystrophin staining (red), and all fibers were visualized by laminin staining (green); co-localization to the sarcolemma appears yellow and nuclei appear blue.

EEV-PMO Is Detected in Pax7 Positive Cells and in CNFs in Multiple Models of DMD With Different Mutations

Del45hDMD-mdx mouse model



Del51hDMD-mdx mouse model



Yellow Arrow:: PMO⁺ CNF
Pink Arrow: PMO⁺/Pax7⁺

Del45hDMD.mdx mice were treated Q6x3 with tool compound, EEV-PMO-44, and analyzed six weeks post last dose. Del51hDMD.mdx mice were treated with a single dose of EEV-PMO-50 and analyzed 2 weeks after dosing . DMD, Duchenne muscular dystrophy; CNF, centrally nucleated fibers.

Key Takeaways

- EEV-PMO constructs effectively target the satellite cell compartment and muscle fibers in various DMD models, regardless of the PMO conjugate used. This observation highlights the platform capabilities of EEV constructs
- The targeted delivery of PMO to the satellite cell compartment may account for the prolonged effects observed in dystrophin expression, muscle regeneration, and overall muscle function
- Since satellite cells play a crucial role in postnatal muscle growth, targeting these cells in conjunction with myofibers could significantly improve the effectiveness of early interventions
- The capability to deliver therapeutic PMOs to muscle fibers and quiescent satellite cells presents potentially significant promise for treating DMD and other neuromuscular disorders

DMD, Duchenne muscular dystrophy; EEV, Endosomal Escape Vehicle; PMO, phosphorodiamidate morpholino oligomers.


Meet Max and his family,
living with Duchenne muscular dystrophy
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