



Endosomal Escape Vehicle- Oligonucleotide Conjugates for the Targeted Upregulation and Downregulation of Gene Expression

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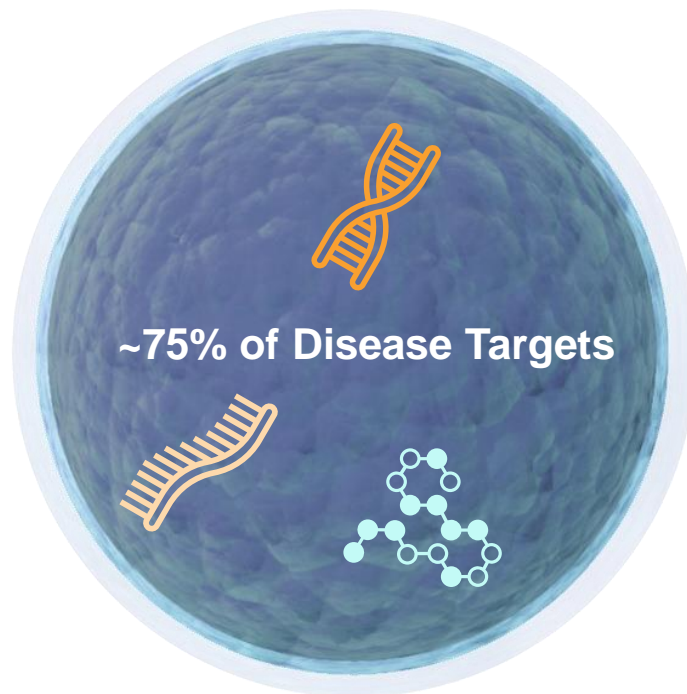
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ENTRADA'S MISSION

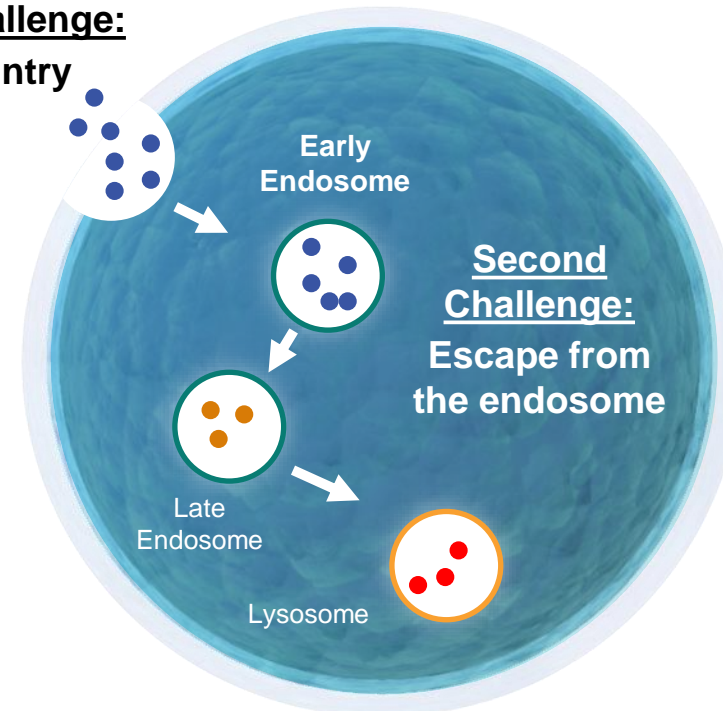
*Treating Devastating Diseases With
Intracellular Therapeutics*

THE NEED FOR INTRACELLULAR THERAPEUTICS

Approximately 75% of all disease-causing targets are intracellular and difficult to reach, representing a significant issue when developing effective therapies



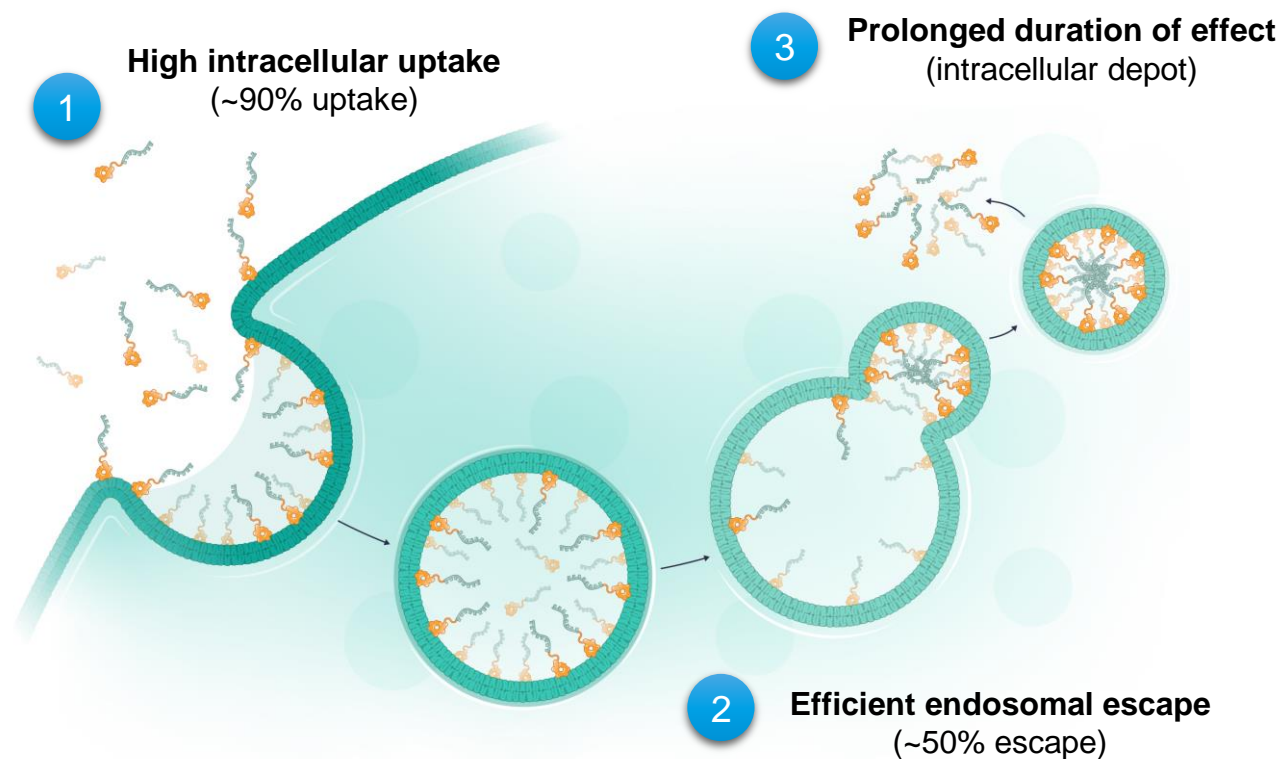
First Challenge:
Cell Entry



**The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem:
Lack of efficient cellular uptake and escape from the endosome**

Entrada seeks to solve a fundamental problem: lack of efficient cellular uptake and escape from the endosome; Both are critical to intracellular target engagement and therapeutic benefit

- 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**



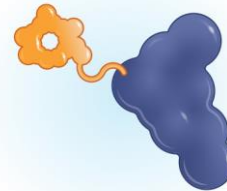
A BROADLY APPLICABLE PLATFORM

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

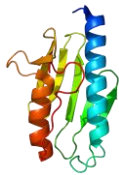
Antibodies



Enzymes

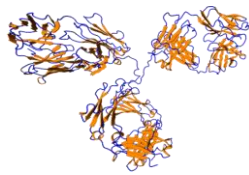


Oligonucleotides



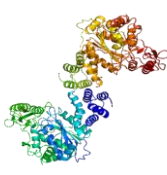
550-600 KDa

Hybrid frataxin



150 KDa

Antibody



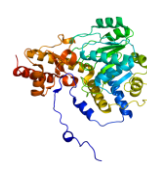
98 KDa

Thymidine
phosphorylase



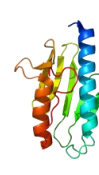
96 KDa

Purine
nucleoside
phosphorylase



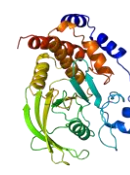
86 KDa

Alanine-
glyoxylate
aminotransferase



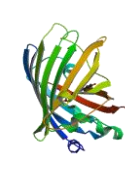
46 KDa

Human frataxin



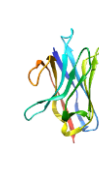
37 KDa

PTP1B
catalytic
domain



32 KDa

EGFP



16 KDa

Nanobody



6 KDa

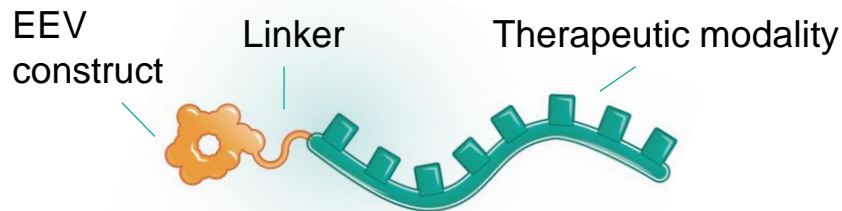
Oligonucleotide



1-3 KDa

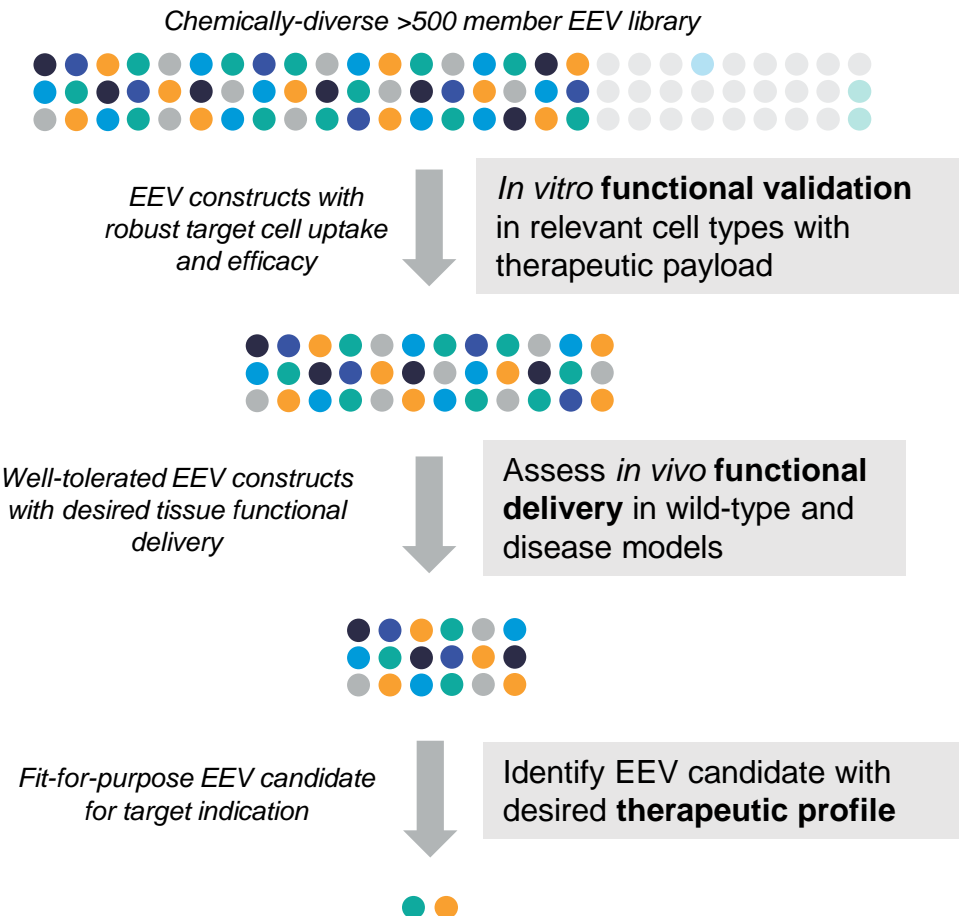
Various
peptide cargos

Discovery Engine for Intracellular Therapeutics



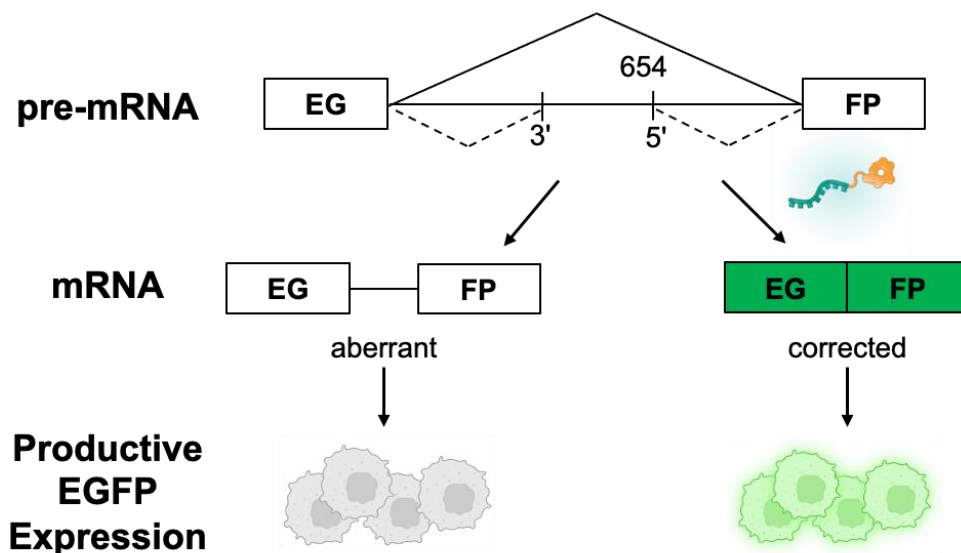
- Cyclic peptide library design and combinatorial synthesis to generate **EEV library**
- Delivery and counter-screening assays enabled for *in vitro* **high throughput screening**
- Functional screening of lead EEV constructs *in vivo* to select for **pharmacodynamic activity** in target tissues
- Optimize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

Screening Cascade for EEV Candidates

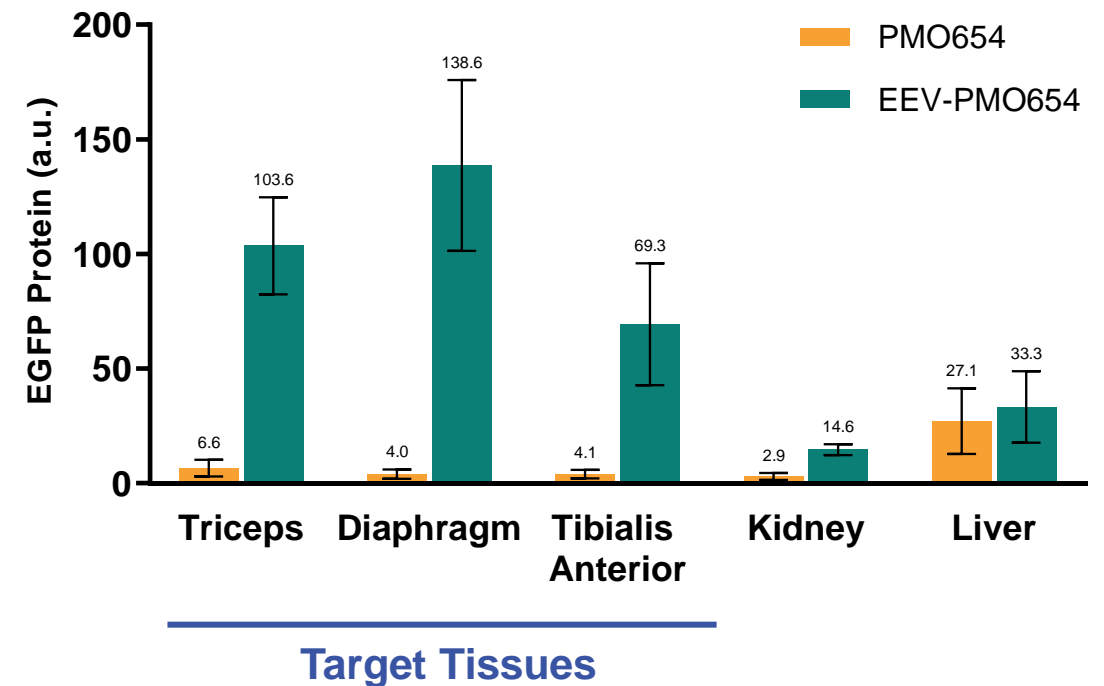


Entrada's EEV-therapeutics can be designed to enhance functional delivery to target tissues

EGFP-654 Transgenic Mice



Functional Delivery to Target Tissues



TRANSLATION FROM UPTAKE TO OUTCOMES

EEV-therapeutics possess favorable pharmacological properties: significant uptake in target tissues, efficient intracellular delivery and potent pharmacodynamic outcomes

Tissue Uptake in Muscle



- ✓ Skeletal muscle
- ✓ Cardiac muscle

Intracellular Delivery

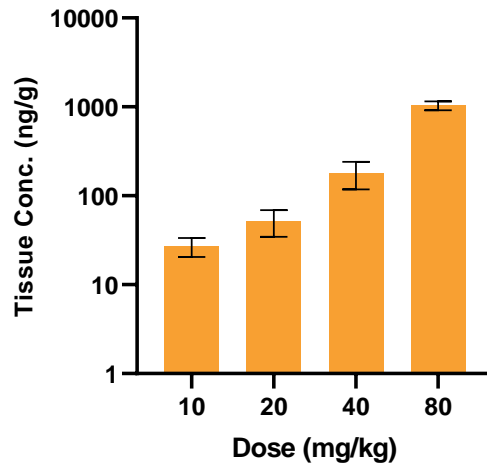


- ✓ Endosomal escape
- ✓ Nuclear localization

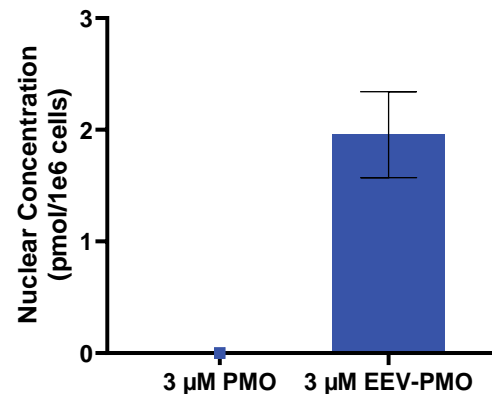
Pharmacodynamic Outcome



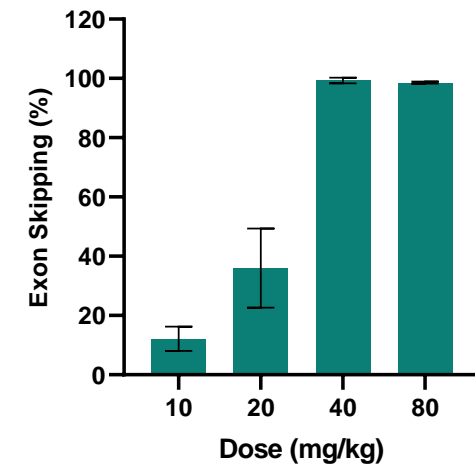
- ✓ Rapid, dose-dependent efficacy
- ✓ Duration of at least 12 weeks



IV, hDMD mice, 5-day p.i.



24-hour incubation

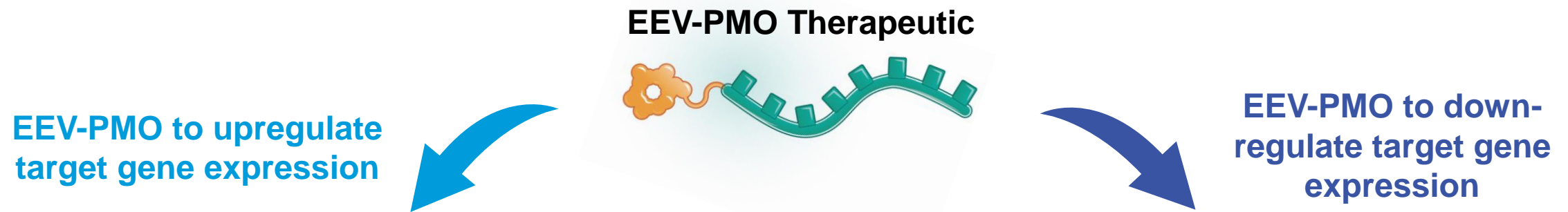


IV, hDMD mice, 5-day p.i.

hDMD mice express full-length human dystrophin gene. p.i. post injection; shown as mean ± standard deviation.

THERAPEUTIC APPLICABILITY OF EEV-PMO CONJUGATES

EEV-PMO conjugates can be customized to functionally deliver splice modulating oligonucleotide therapeutics to target tissues and cell types



Duchenne Muscular Dystrophy (DMD)

- Currently approved unconjugated exon skipping PMO therapies were designed to restore the dystrophin mRNA reading frame and produce dystrophin protein but have shown modest improvements
- Conjugated of the EEV platform to exon skipping PMOs may enhance functional delivery to skeletal and cardiac muscle

Interferon regulatory factor 5 (IRF5)

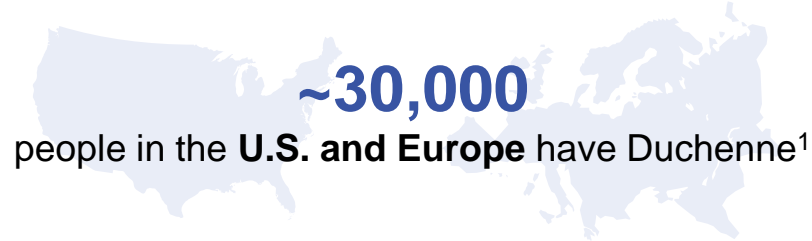
- IRF5 is a transcription factor that promotes production of several proinflammatory cytokines in macrophages, and overexpression of IRF5 has been implicated in several autoimmune and inflammatory diseases
- Exon skipping PMOs that prevents transcription of IRF5 mRNA may decrease IRF5 levels

DUCHENNE MUSCULAR DYSTROPHY (DMD)

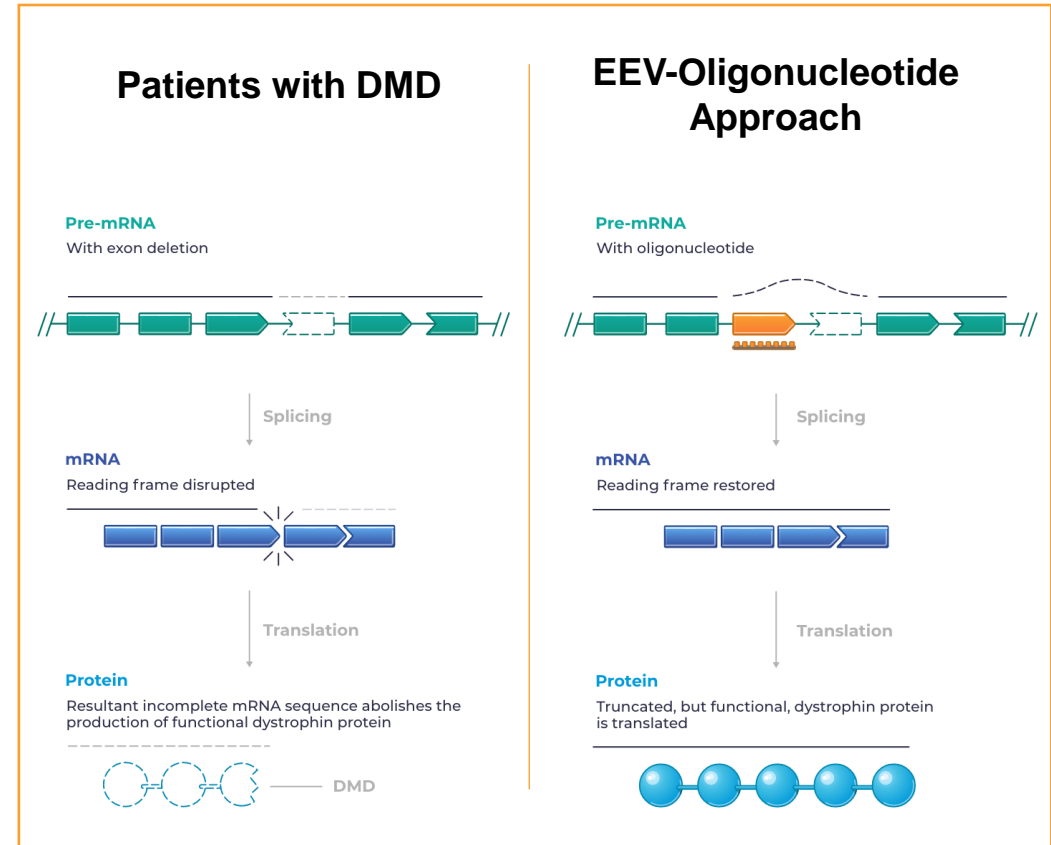
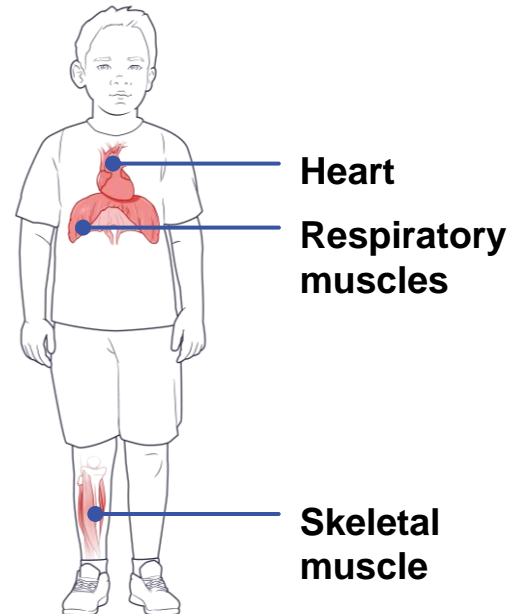
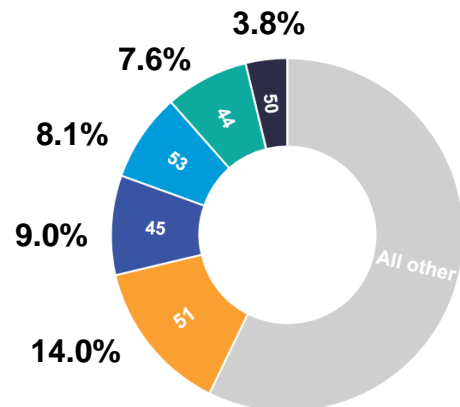
SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

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%**



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

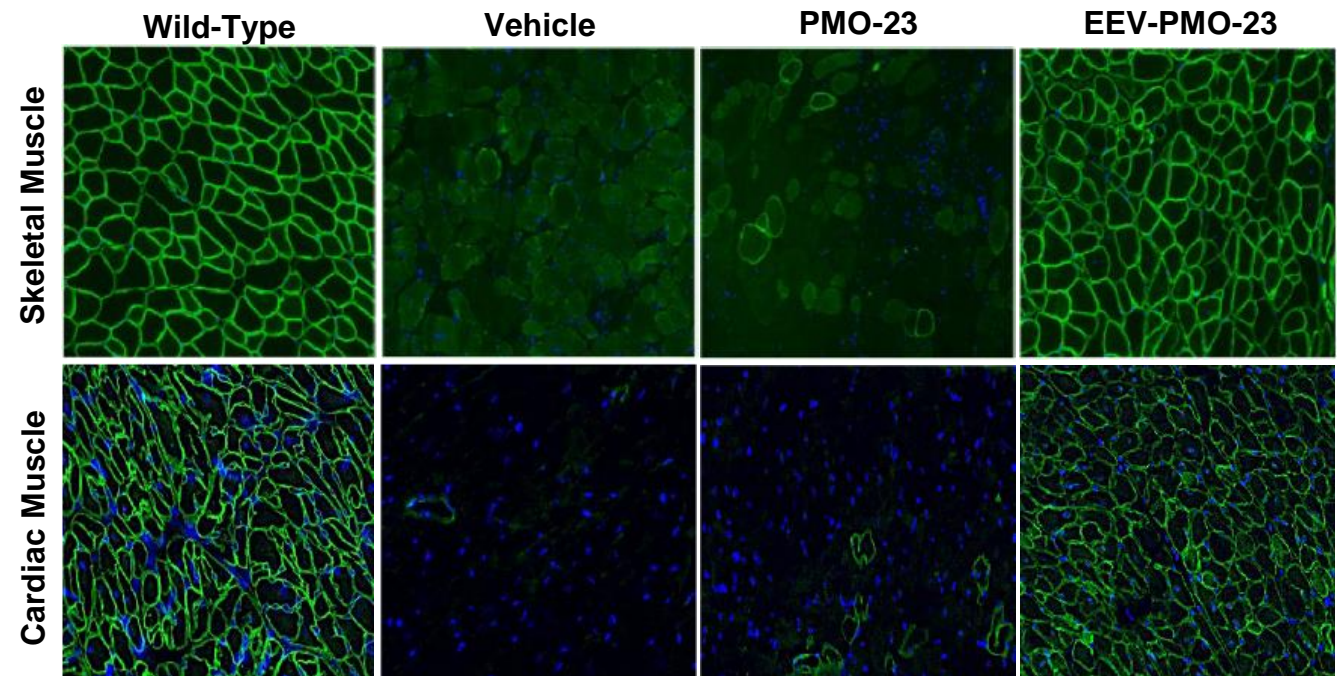
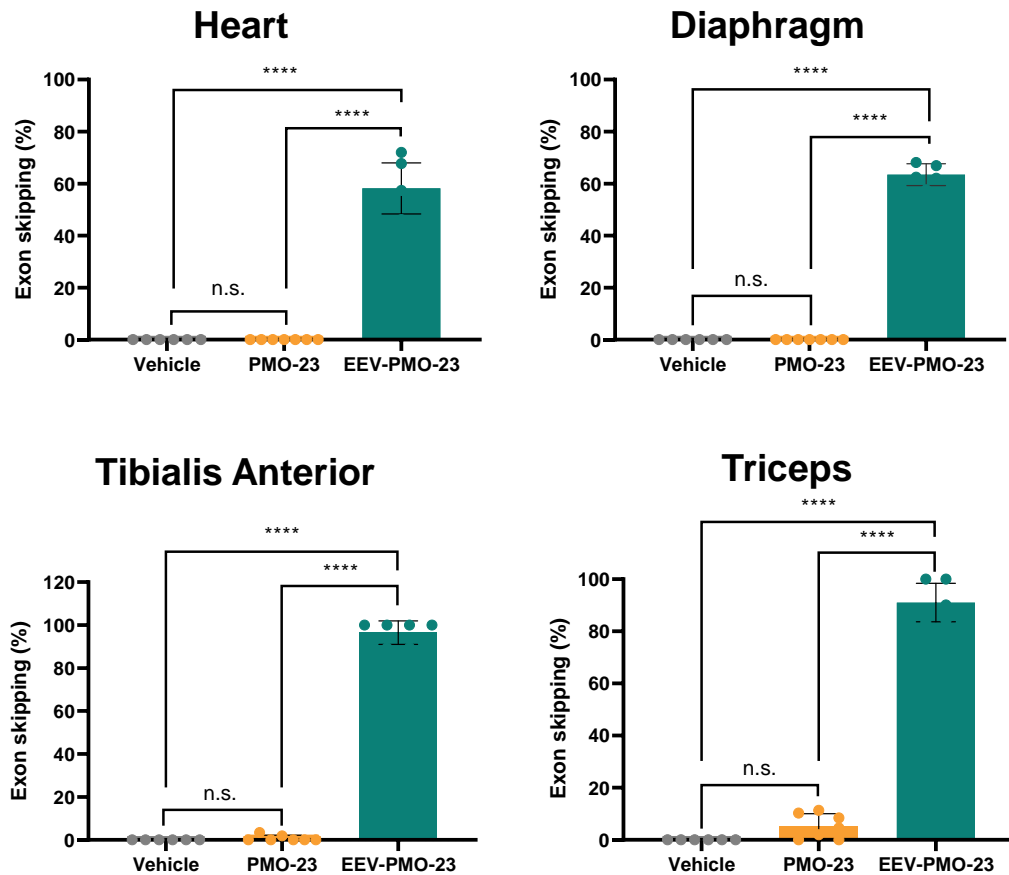


¹Crispi V, Matsakas A. Duchenne muscular dystrophy: genome editing gives new hope for treatment. Postgraduate Medical Journal. 2018;94(1111):296-304.

REPEAT EEV-PMO TREATMENT IN 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

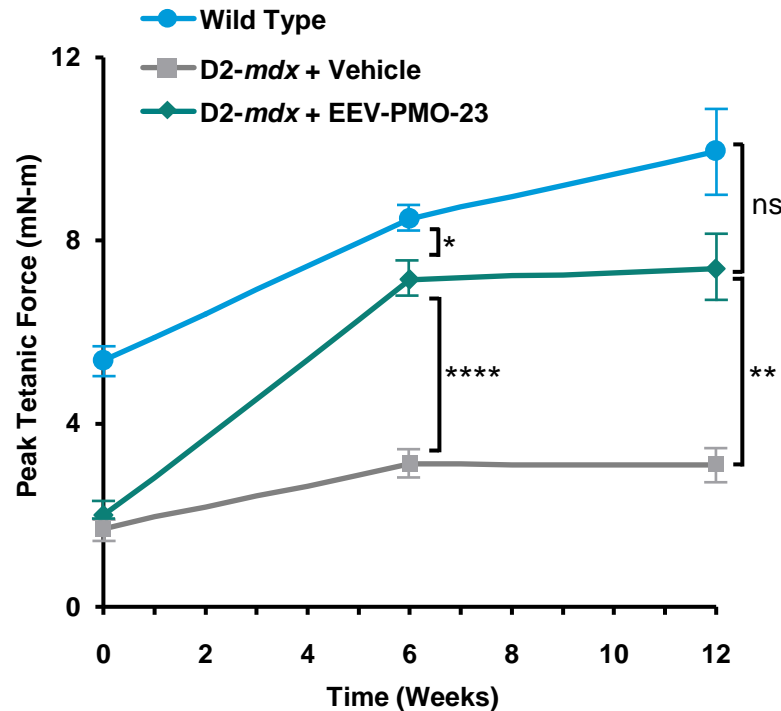


- D2-*mdx* mice (male, n=6-7) were treated with 4 monthly doses of either vehicle, 20 mg/kg unconjugated PMO-23 or 20 mg/kg PMO-23 equivalent of EEV-PMO-23, and the data were collected ~4 weeks after the last dose.

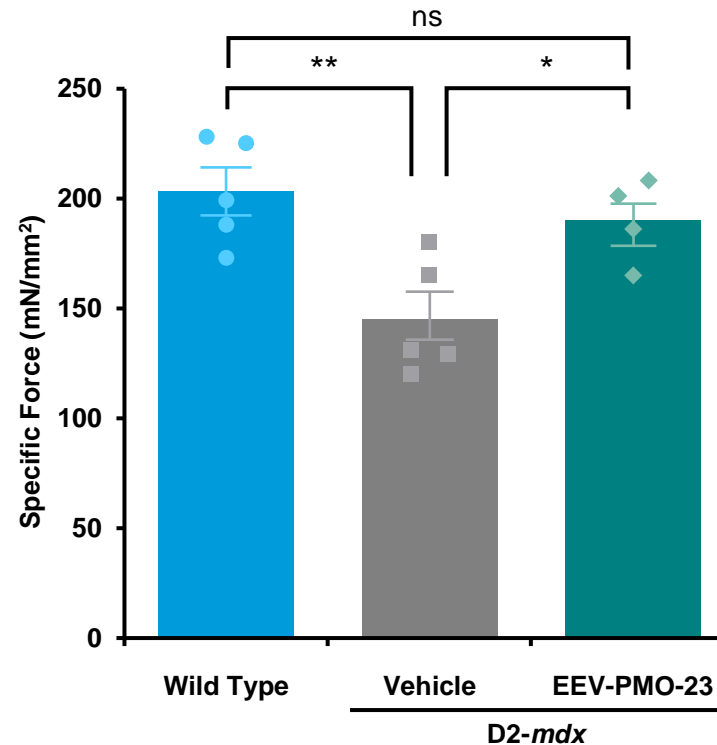
REPEAT EEV-PMO TREATMENT RESULTS IN IMPROVED MUSCLE CONTRACTILITY

Bi-weekly treatment with EEV-PMO-23 improved skeletal muscle contractile force in D2-*mdx* mice and was not significantly different than wild type mice at Week 12

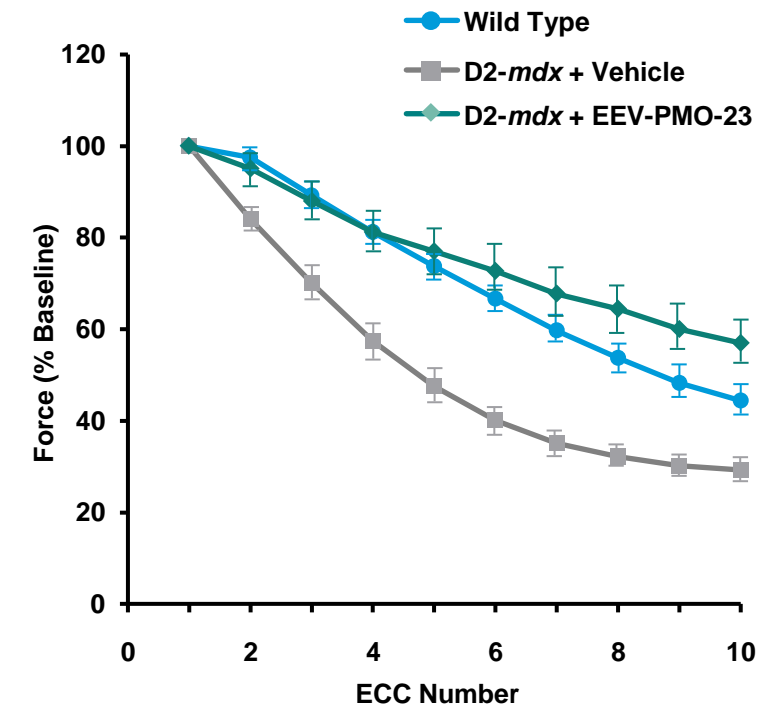
Longitudinal Tetanic Force



Week 12 Specific Force

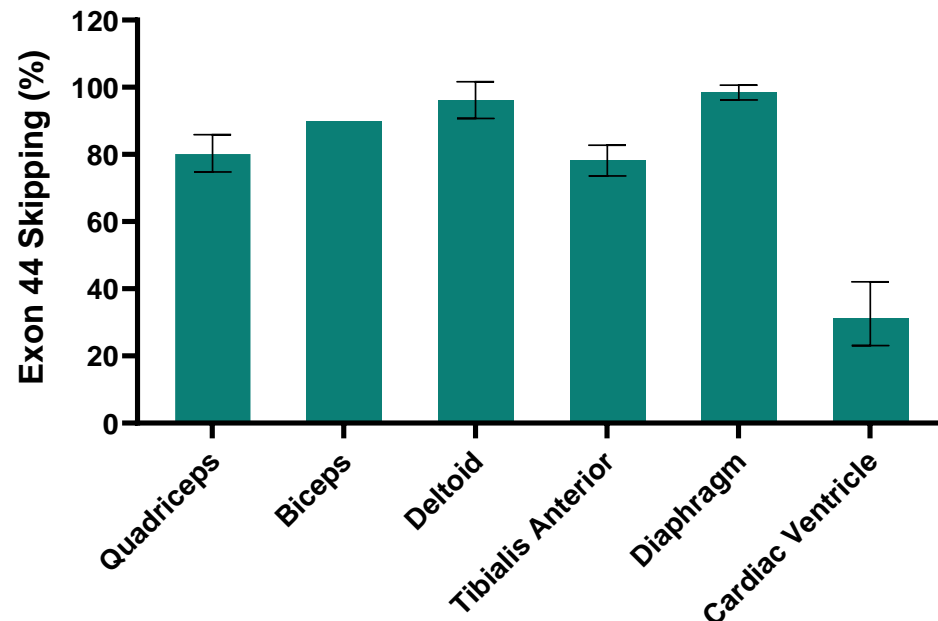


Repeated Muscle Contraction



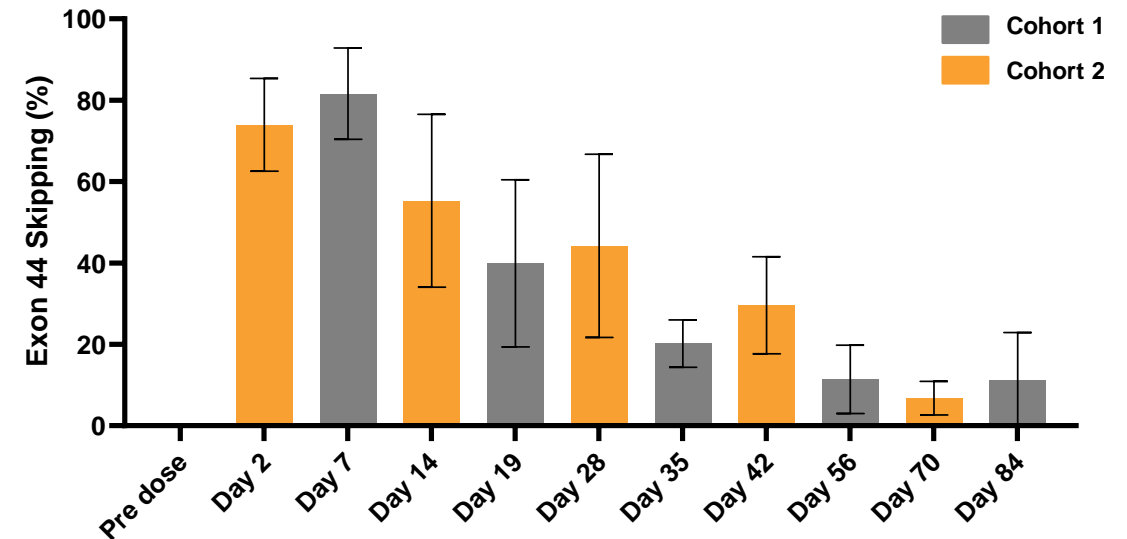
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

Exon Skipping in NHP Muscles at Day 7



- At 7 days post IV infusion of 30 mg/kg (PMO equivalent), robust exon 44 skipping across different muscle groups isolated from the ENTR-601-44 treated NHP

Duration of Effect in NHP Biceps for at Least 12 Weeks



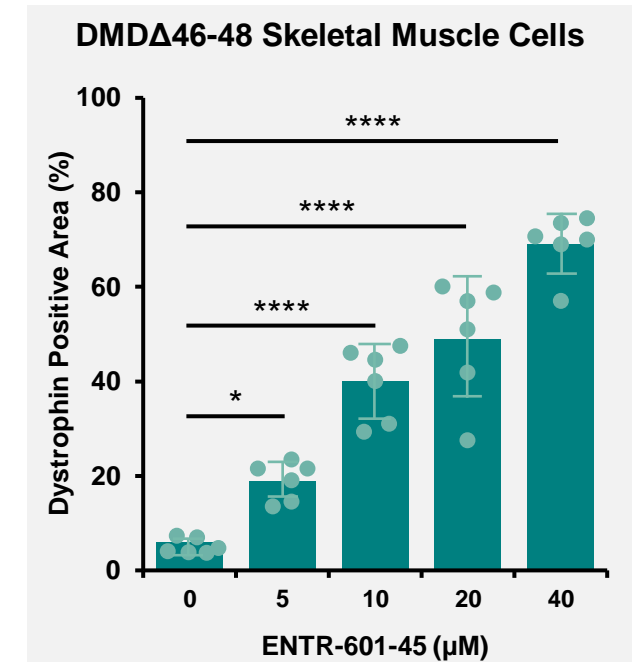
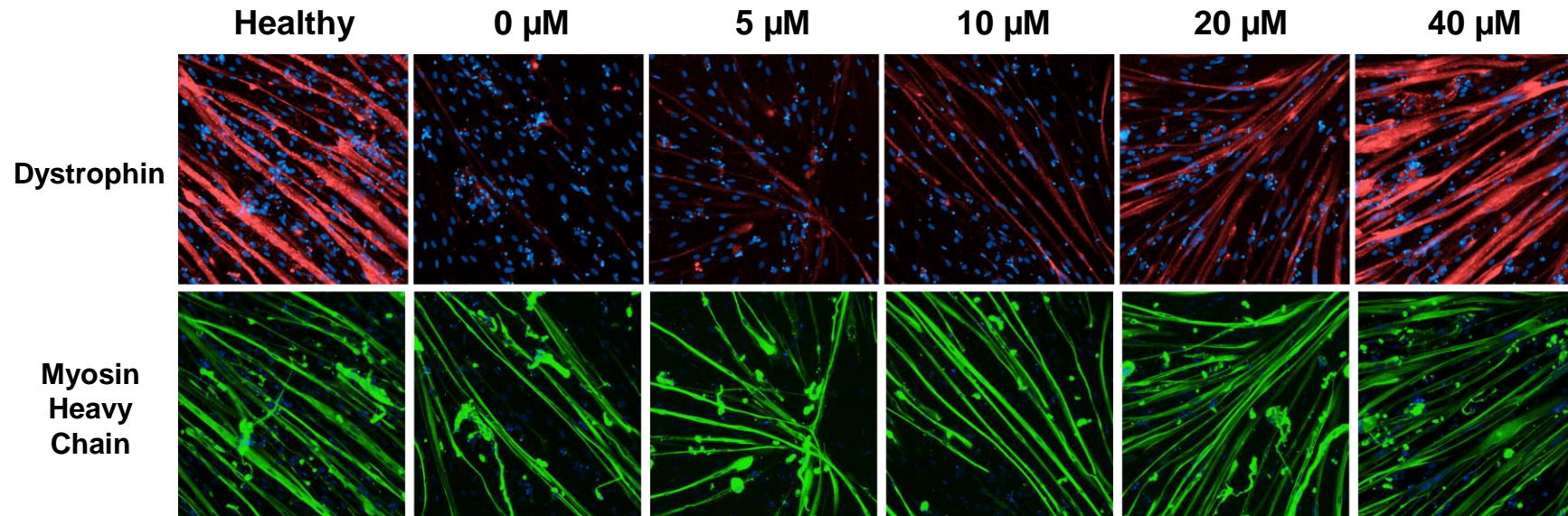
- Post IV infusion of 35 mg/kg (PMO equivalent), robust exon 44 skipping observed in biceps in the ENTR-601-44 treated NHP (n=3 per cohort) for at least 12 weeks

ENTR-601-44 is a DMD exon 44 skipping PMO conjugated to the EEV platform. NHP, non-human primates; shown as mean \pm standard deviation.

ENTR-601-45 IN SKELETAL MUSCLE CELLS

ENTR-601-45 produced dose-dependent dystrophin restoration in patient-derived skeletal muscle cells

DMD Δ 46-48 Skeletal Muscle Cells + ENTR-601-45

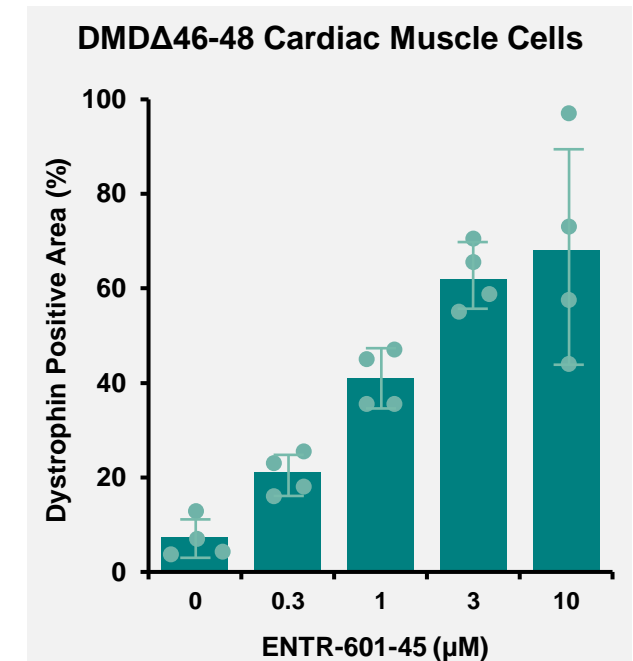
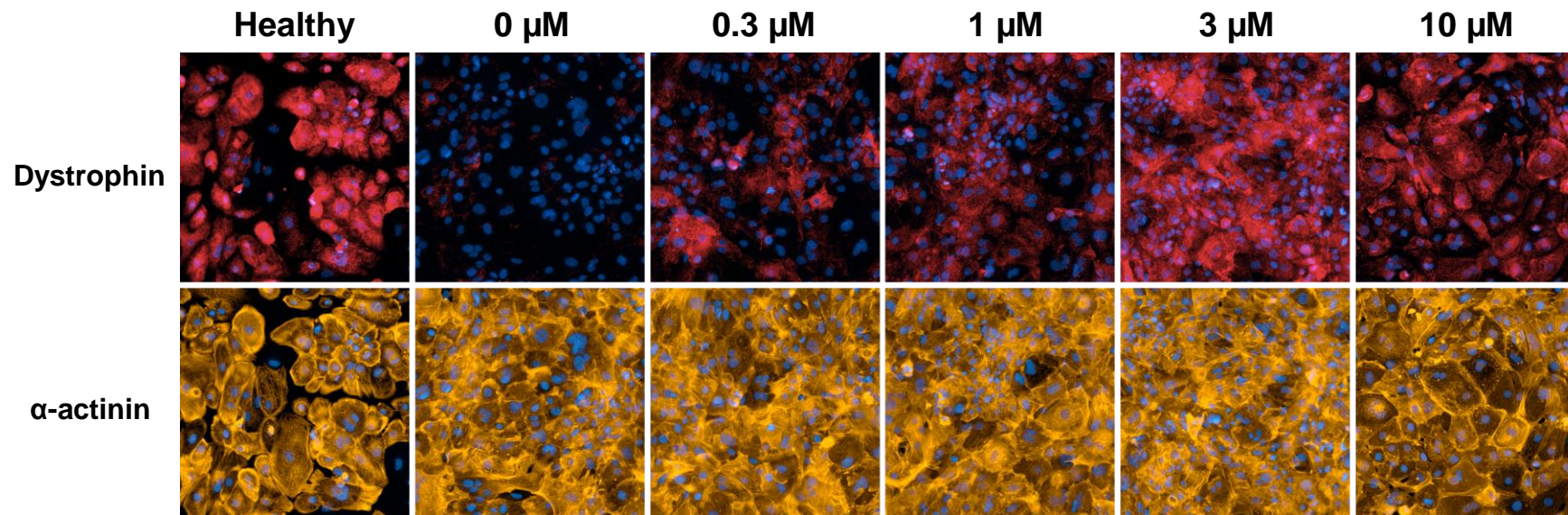


- DMD patient-derived skeletal muscle cells (DMD Δ 46-48, n=6) were treated with ENTR-601-45 for 24 hours and analyzed 5 days later.

ENTR-601-45 IN CARDIAC MUSCLE CELLS

ENTR-601-45 produced dose-dependent dystrophin restoration in patient-derived cardiac muscle cells

DMD Δ 46-48 Cardiac Muscle Cells + ENTR-601-45



- DMD patient-derived cardiac muscle cells (DMD Δ 46-48, n=4) were treated with ENTR-601-45 for 24 hours and analyzed 48 hours later.

INTERFERON REGULATORY FACTOR 5 (IRF5)

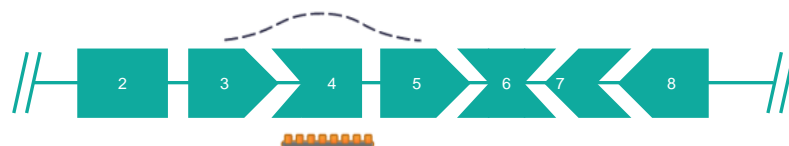
EEV-PMO TREATMENT REDUCED IRF5 EXPRESSION IN VITRO

Interferon regulatory factor 5 (IRF5) overexpression has been implicated in several autoimmune and inflammatory diseases

EEV-PMO Approach to Knockdown IRF5

IRF5 pre-mRNA

With EEV-PMO inducing exon skipping

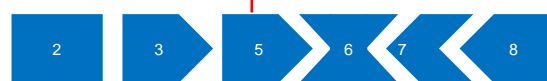


Splicing



IRF5 mRNA

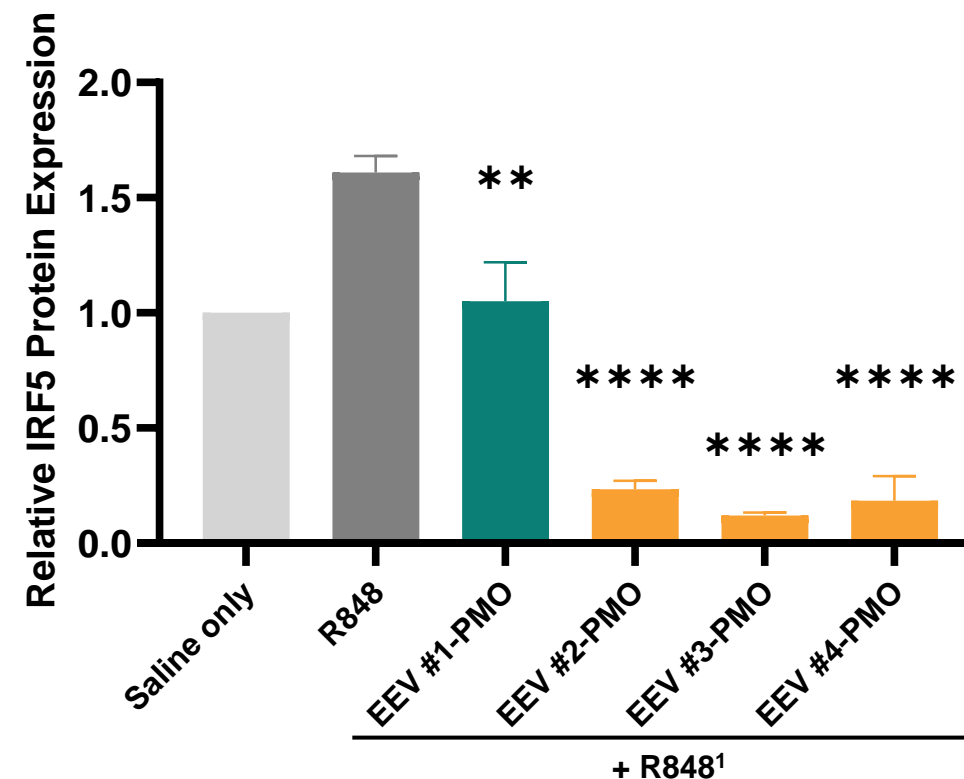
Out of frame shift and premature stop codon



Nonsense Mediated Decay machinery

IRF5 Knockdown

IRF5 Production



The EEV-PMO approach has broad applicability to upregulate and downregulate target gene expression through distinct mechanisms of action

- **Increased Dystrophin Production in DMD Models via Exon Skipping**

- ENTR-601-44 produced robust exon skipping and dystrophin production in several preclinical models of DMD^a
- ENTR-601-45 showed robust exon skipping and dystrophin protein production in vitro and in vivo
 - IND filing is planned for H2 2024

- **IRF5 Knockdown in vitro via Exon Skipping-Induced Decay**

- An exon-skipping EEV-PMO reduced IRF5 production in mouse macrophages/monocytes

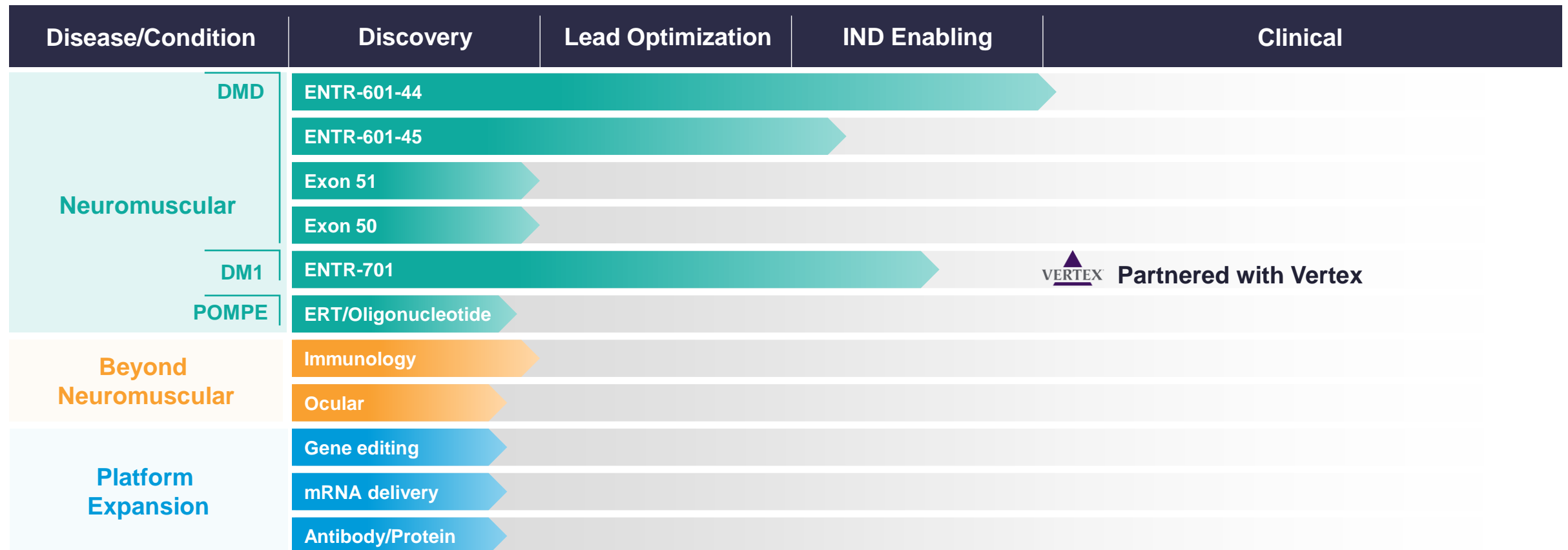
These results demonstrate that the EEV platform efficiently delivers oligonucleotides to several cell and tissue types

PIPELINE & DISCOVERY PROGRAMS

OUR DIFFERENTIATED AND EXPANDING PIPELINE


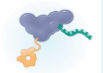

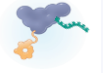




Entrada's pipeline includes a diverse array of high potential and high value assets



ADDITIONAL PLATFORM OPPORTUNITIES

Entrada continues to invest in and build upon our EEV Platform to extend our efforts in developing novel EEV-therapeutic candidates

Target		Platform Approach		Goal
	DNA		Gene editing	Deliver CRISPR enzyme and repair gene function with guide RNA
	RNA		RNA editing	Deliver oligonucleotide therapeutics for RNA editing
			RNA splicing	Modify RNA via exon/intron splicing to activate protein expression
			RNA blocking	Block trinucleotide repeats in RNA to inhibit adverse binding
			RNA silencing	Silence or knockdown RNA to prevent protein expression
	Protein		Protein replacement	Replace proteins and enzymes
			Protein inhibition	Inhibit protein signaling pathways
			Protein degradation	Degrade disease-causing proteins

Thank you!



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