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

**Ziqing “Leo” Qian, PhD**
**Co-Founder & Vice President, Discovery Research**

**Philly Cell and Gene Therapy Annual Conference**
**June 24, 2023**

# DISCLAIMER

This presentation includes express and implied “forward-looking statements. Forward looking statements include all statements that are not historical facts, and in some cases, can be identified by terms such as “may,” “might,” “will,” “could,” “would,” “should,” “expect,” “intend,” “plan,” “objective,” “anticipate,” “believe,” “estimate,” “predict,” “potential,” “continue,” “ongoing,” or the negative of these terms, or other comparable terminology intended to identify statements about the future. Forward-looking statements contained in this presentation include, but are not limited to, statements about our product development activities and clinical trials, our regulatory filings and approvals, our ability to develop and advance our current and future product candidates and discovery programs, our ability to establish and maintain collaborations or strategic relationships or obtain additional funding, the rate and degree of market acceptance and clinical utility of our product candidates, the ability and willingness of our third-party collaborators to continue research and development activities relating to our product candidates, our and our collaborators’ ability to protect our intellectual property for our products. By their nature, these statements are subject to numerous risks and uncertainties, including factors beyond our control, that could cause actual results, performance or achievement to differ materially and adversely from those anticipated or implied in the statements. You should not rely upon forward-looking statements as predictions of future events. Although our management believes that the expectations reflected in our statements are reasonable, we cannot guarantee that the future results, performance or events and circumstances described in the forward-looking statements will be achieved or occur. Recipients are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date such statements are made and should not be construed as statements of fact.

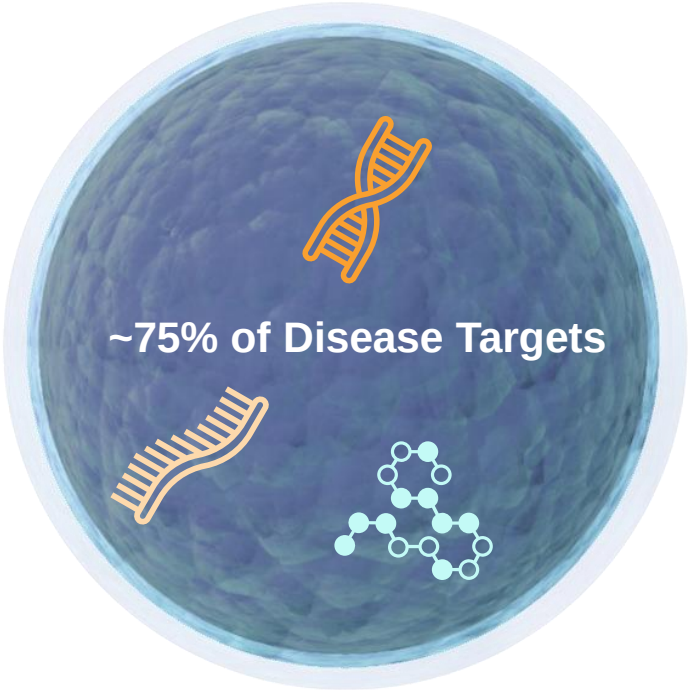
Certain information contained in this presentation and statements made orally during this presentation relate to or are based on studies, publications, surveys and other data obtained from third-party sources and our own internal estimates and research. While we believe these third-party studies, publications, surveys and other data to be reliable as of the date of this presentation, it has not independently verified, and makes no representations as to the adequacy, fairness, accuracy or completeness of, any information obtained from third-party sources. In addition, no independent source has evaluated the reasonableness or accuracy of our internal estimates or research and no reliance should be made on any information or statements made in this presentation relating to or based on such internal estimates and research.

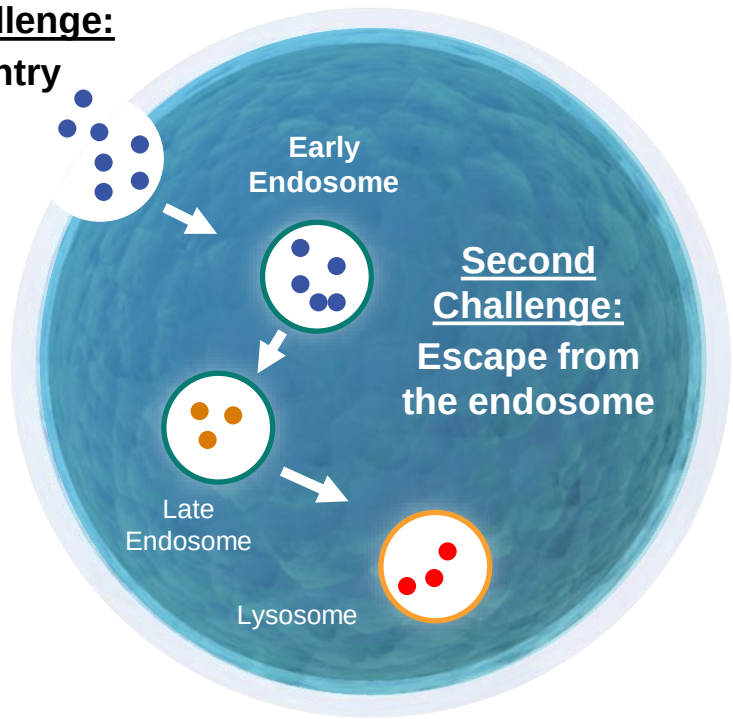
# ENTRADA'S MISSION

***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



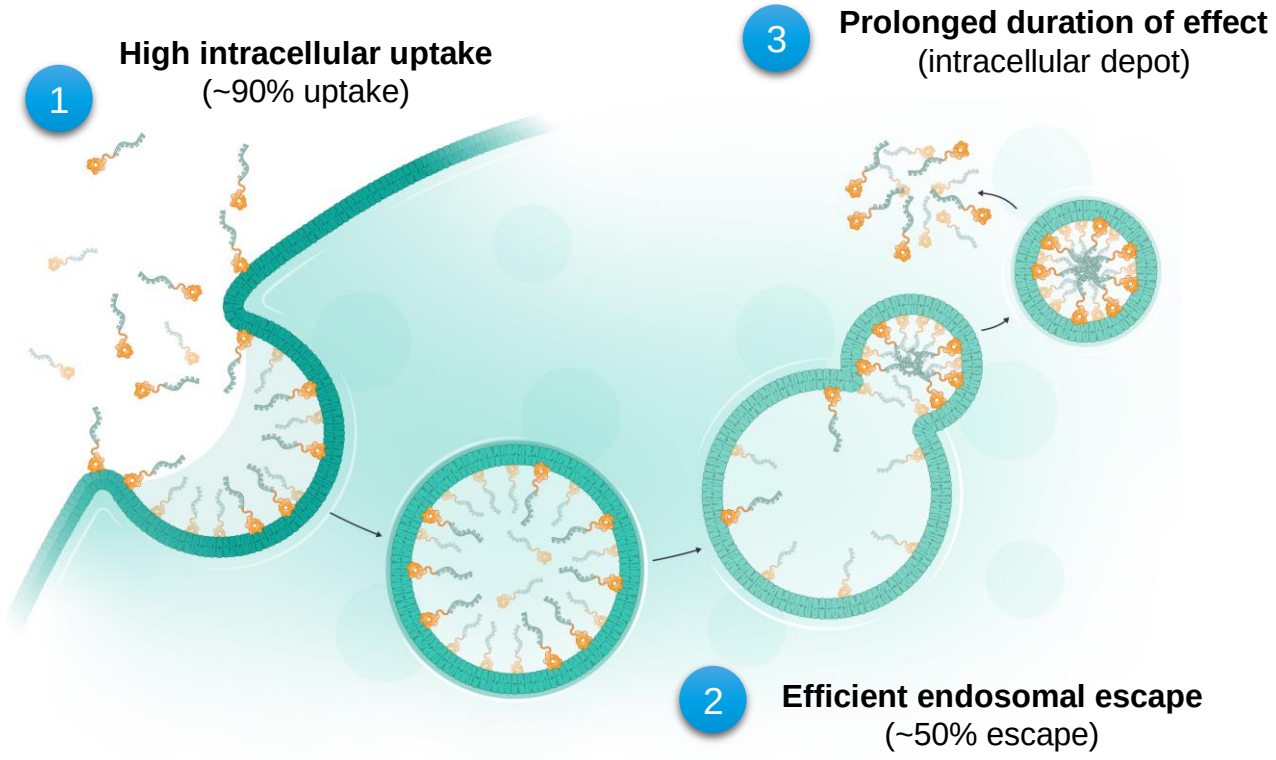




**The Endosomal Escape Vehicle (EEV™) Platform aims to solve the fundamental problem: Lack of efficient cellular uptake and escape from the endosome**

# ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

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**



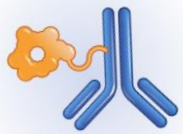



Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022.

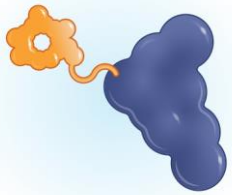
# A BROADLY APPLICABLE PLATFORM

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

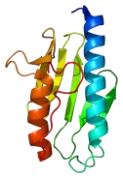
## Antibodies



## Enzymes

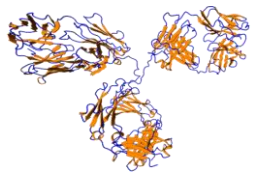


## Oligonucleotides



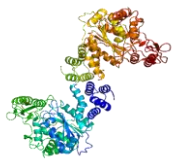
**550-600 KDa**

Hybrid frataxin



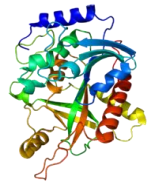
**150 KDa**

Antibody



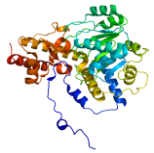
**98 KDa**

Thymidine phosphorylase



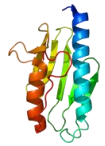
**96 KDa**

Purine nucleoside phosphorylase



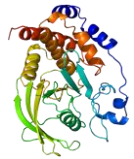
**86 KDa**

Alanine-glyoxylate aminotransferase



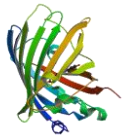
**46 KDa**

Human frataxin



**37 KDa**

PTP1B catalytic domain



**32 KDa**

EGFP

**16 KDa**

Nanobody

**6 KDa**

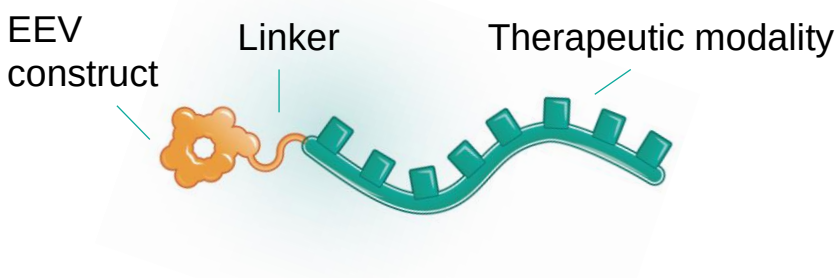
Oligonucleotide

**1-3 KDa**

Various peptide cargos

# 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 **linker & conjugation chemistry** for desired therapeutic modality

## Screening Cascade for EEV Candidates

*Chemically-diverse >500 member EEV library*

*EEV constructs with robust target cell uptake and efficacy*

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

*Well-tolerated EEV constructs with desired tissue functional delivery*

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

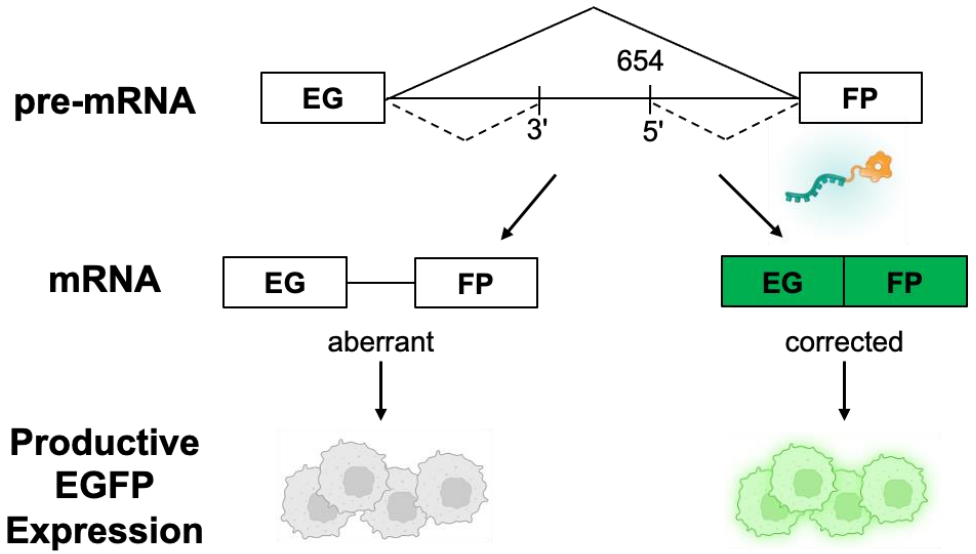
*Fit-for-purpose EEV candidate for target indication*

Identify EEV candidate with desired **therapeutic profile**

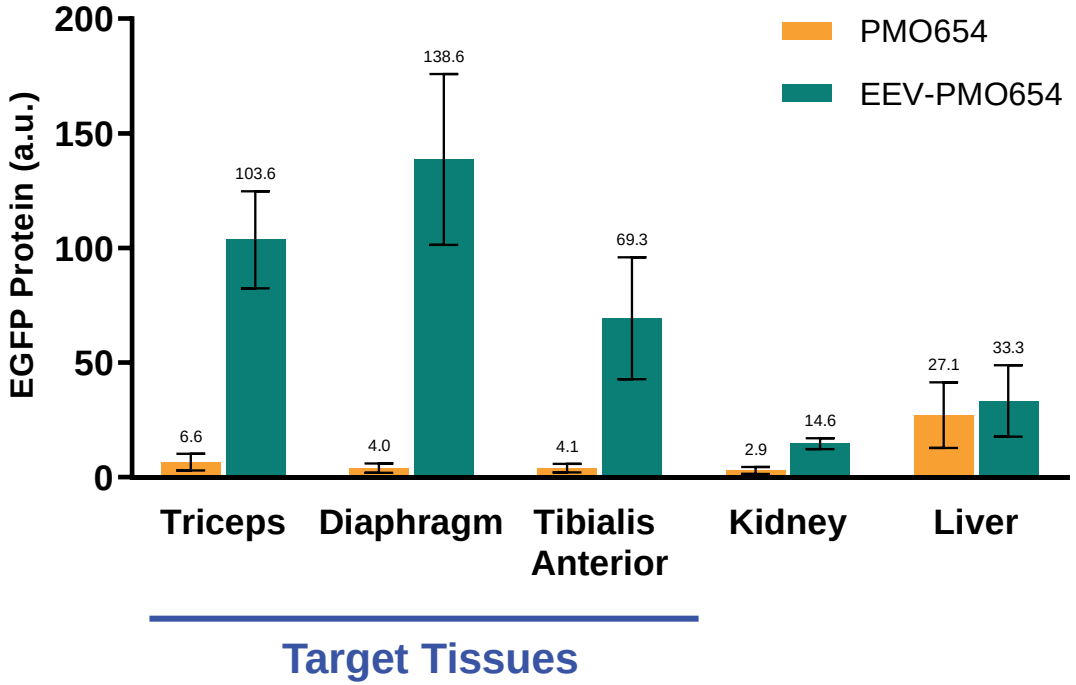
# FUNCTIONAL DELIVERY FOR TARGET TISSUES

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

## EGFP-654 Transgenic Mice




## Functional Delivery to Target Tissues








**PMO**, phosphorodiamidate morpholino oligomer (Summerton, J. et al. *Antisense Nucleic Acid Drug Dev.* 1997); **EGFP-654** transgenic mouse model contains an EGFP gene interrupted by human beta-globin intron 2 with mutated nt654 (Sazani, P. et al. *Nature Biotech.* 2002); **PMO654**, splicing switching PMO targeting nt654; shown as mean ± standard deviation.

# 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

+

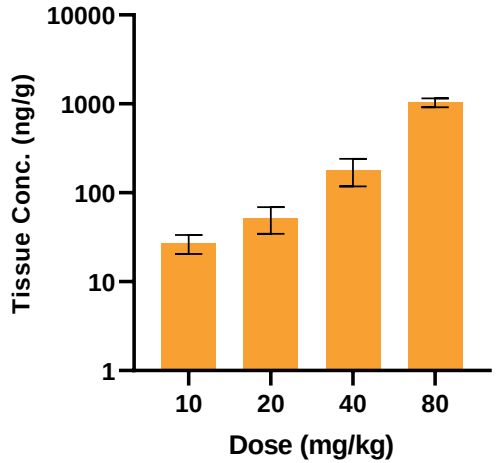
## Intracellular Delivery

- ✓ Endosomal escape
- ✓ Nuclear localization

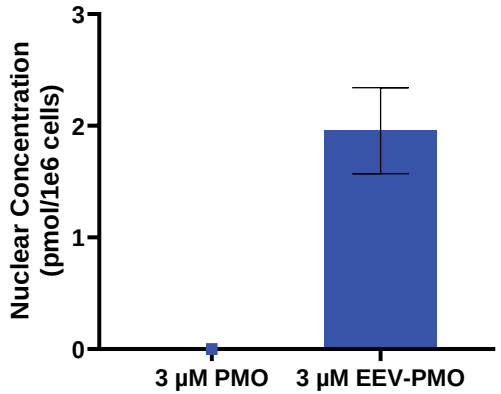
=

## Pharmacodynamic Outcome

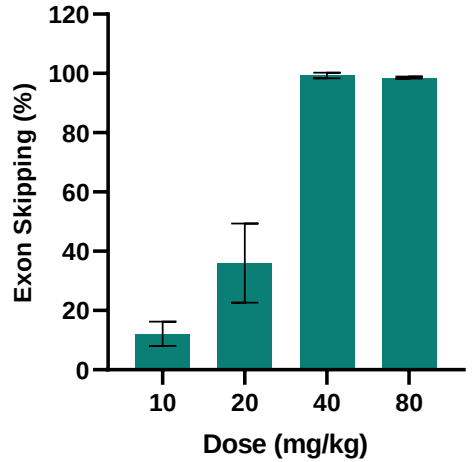
- ✓ Rapid, dose-dependent efficacy
- ✓ Duration of at least 12 weeks




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

**EEV-PMO Therapeutic**

**EEV-PMO to upregulate target gene expression**

**EEV-PMO to down-regulate target gene expression**

## 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

**PMO**, phosphorodiamidate morpholino oligomer

# DUCHENNE MUSCULAR DYSTROPHY (DMD)

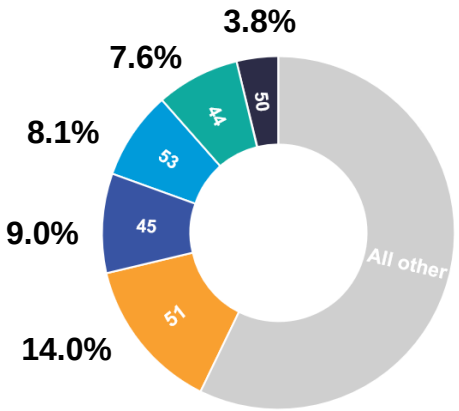
# SIGNIFICANT THERAPEUTIC NEED EXISTS WITHIN A VALIDATED DUCHENNE MARKET

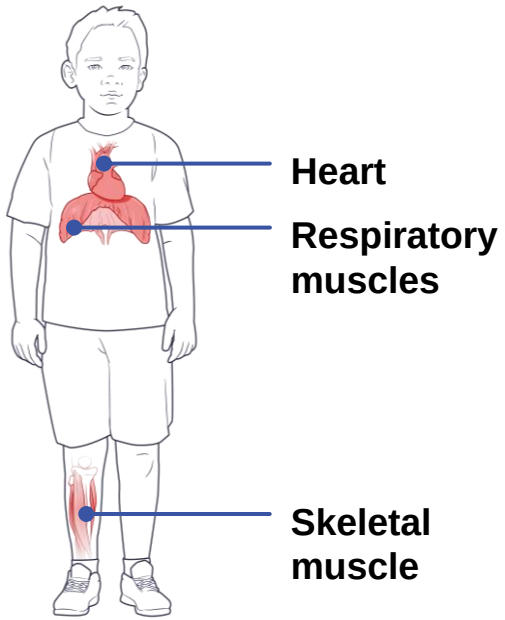
Duchenne is caused by **mutations in the DMD gene, which lead to a lack of functional dystrophin**, causing progressive loss of muscle function throughout the body

**~30,000**

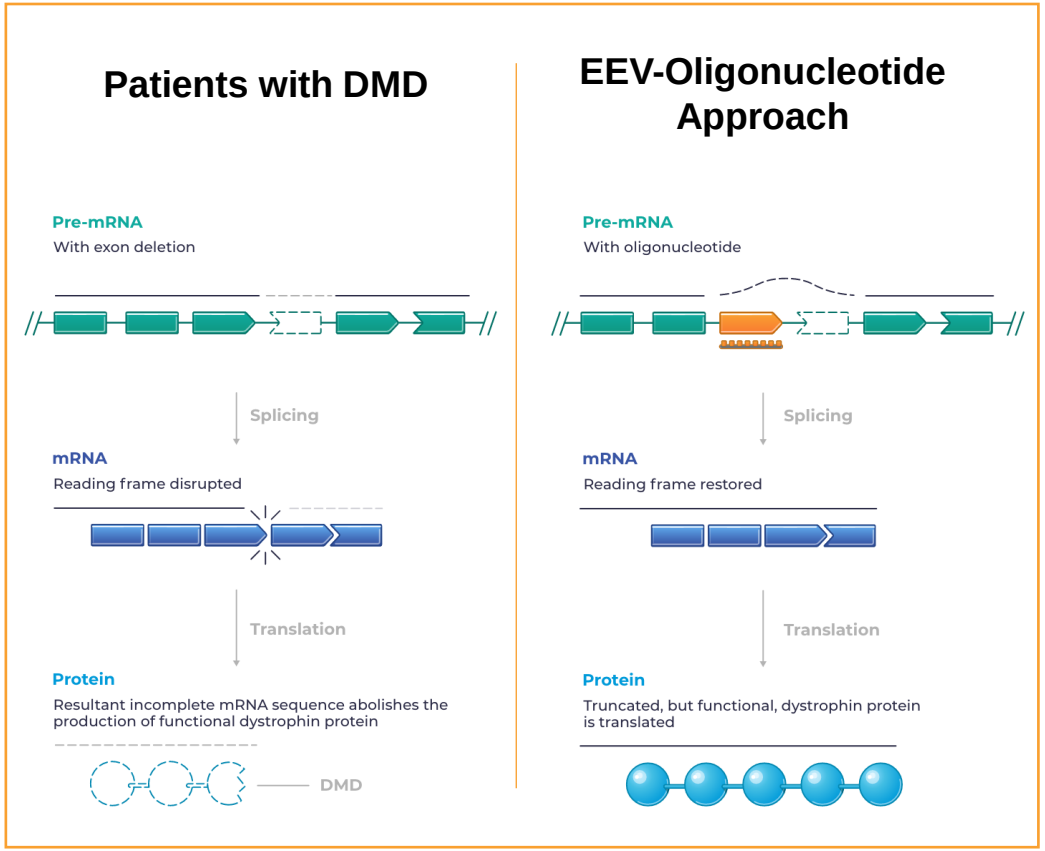
people in the **U.S. and Europe** have Duchenne[1]

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



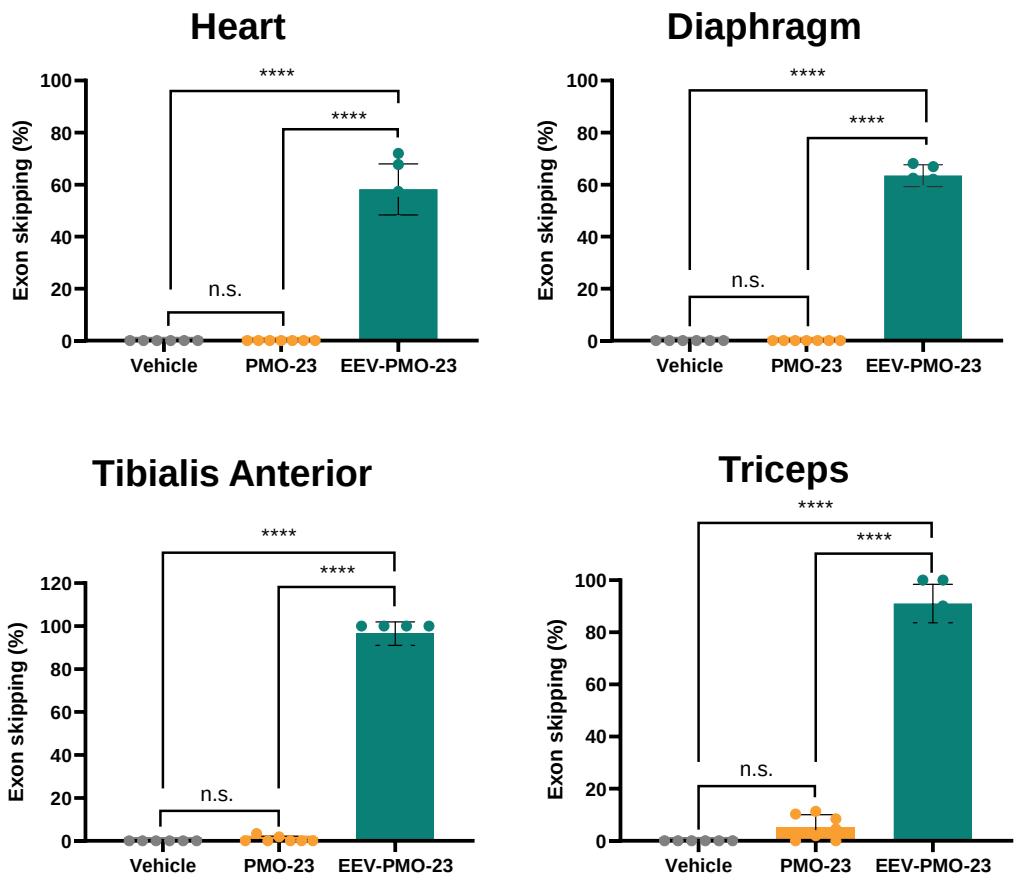

Exon skipping therapeutics have been approved based on **modest improvement in dystrophin levels ranging from ~1 to 6%**



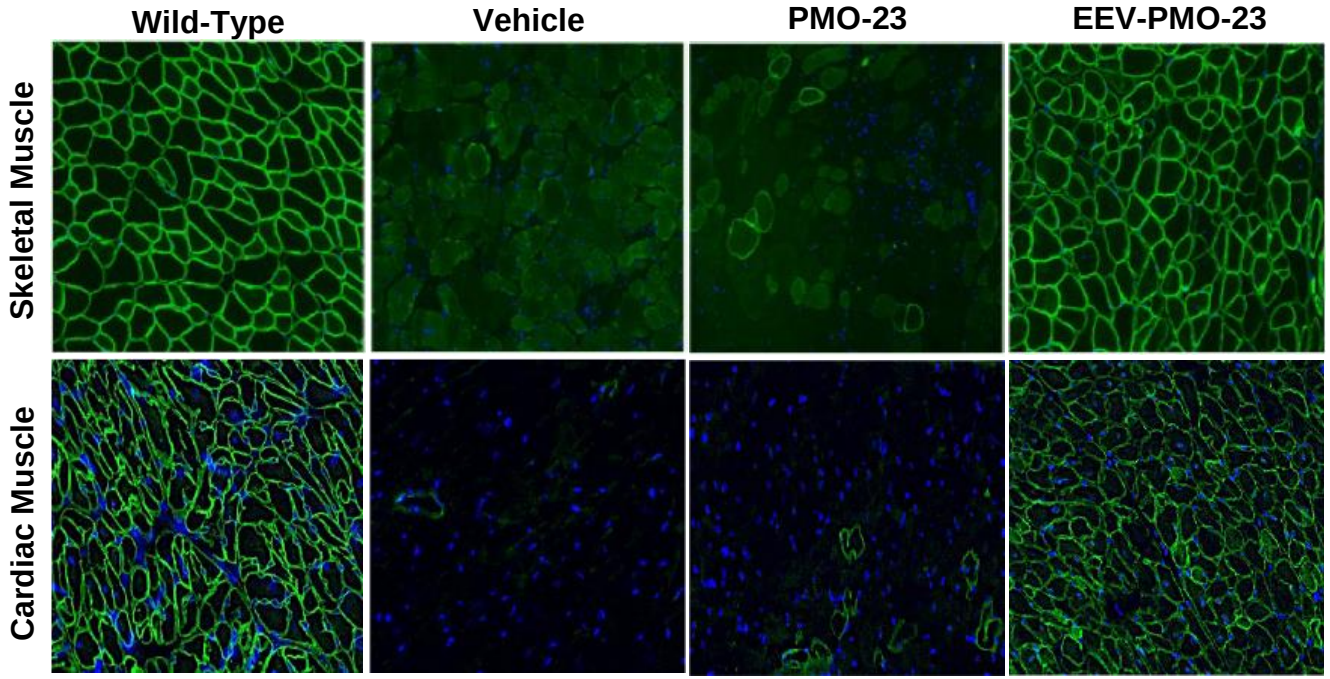

[1]Crispi V, Matsakas A. Duchenne muscular dystrophy: genome editing gives new hope for treatment. Postgraduate Medical Journal. 2018;94(1111):296-304.

# 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.



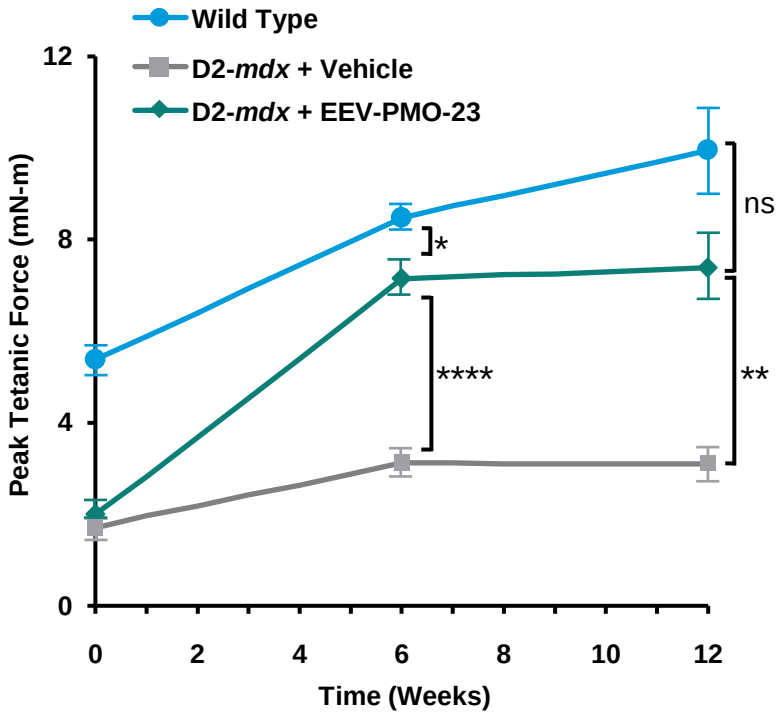


**D2-*mdx*** is a DMD mouse model with a nonsense mutation in *DMD* exon 23 (Coley et al. Hum. Mol. Genet. 2016). **EEV**, Endosomal Escape Vehicle; **PMO-23**, mouse *DMD* exon 23 skipping phosphorodiamidate morpholino oligomer. ****p<0.0001; **n.s.**, not significant; shown as mean ± standard deviation.

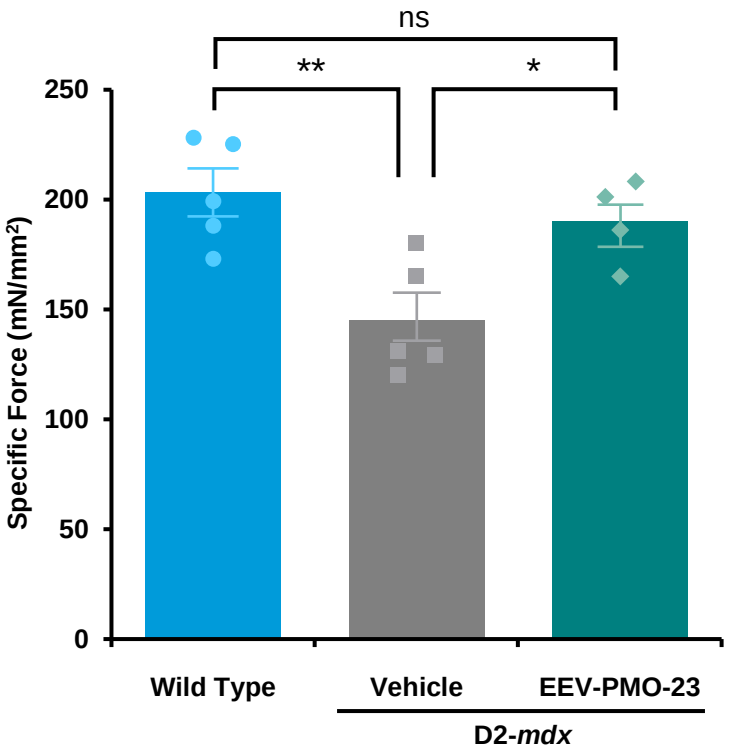
# REPEAT EEV-PMO TREATMENT RESULTS IN IMPROVED MUSCLE CONTRACTILITY

## 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

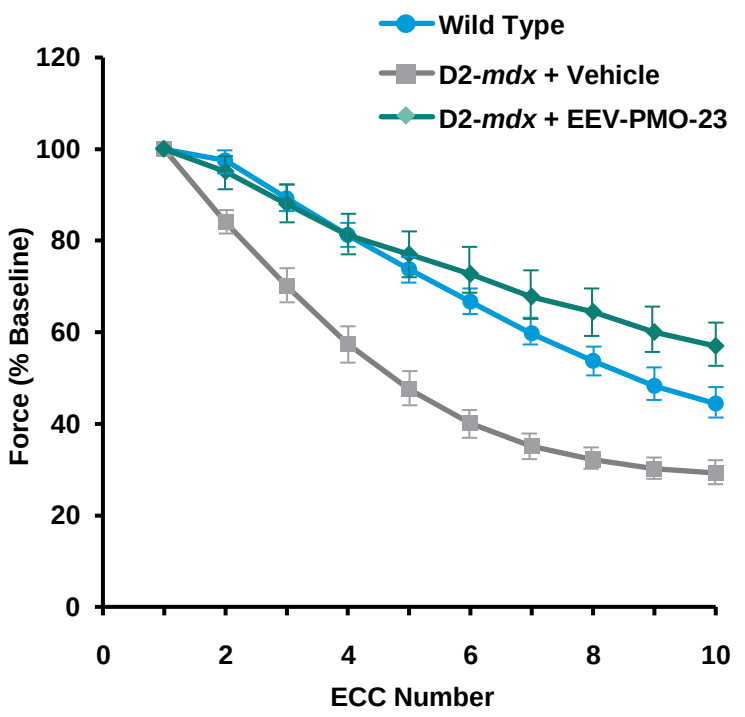
### Longitudinal Tetanic Force




### Week 12 Specific Force




### Repeated Muscle Contraction





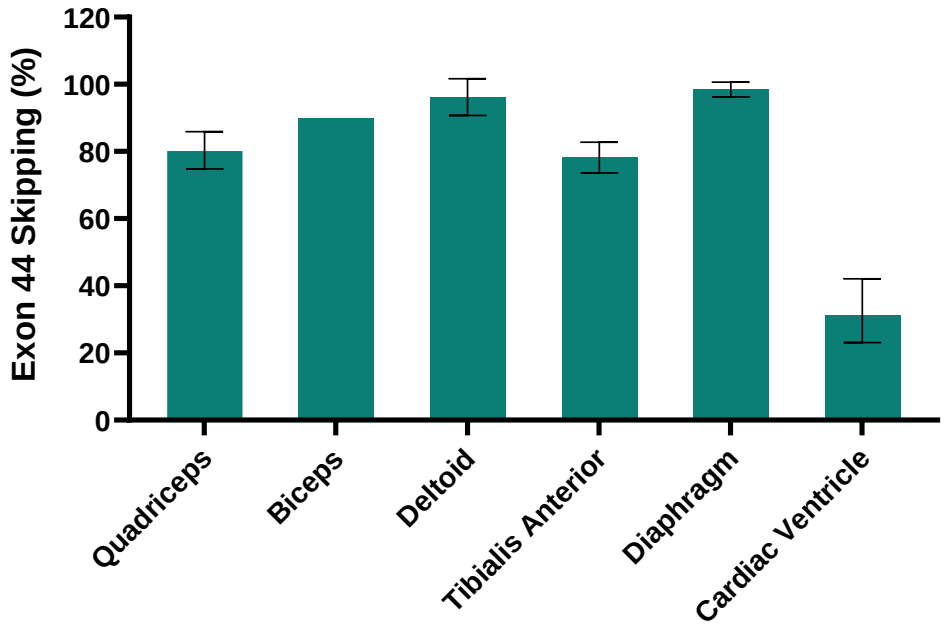



D2-*mdx* mice were treated with vehicle or 80 mg/kg (Q2W) EEV-PMO-23. Muscle contractility was assessed at Weeks 6 and 12 via isometric force generated by tetanic contraction of plantar flexor muscle group and eccentric force (ECC) generated by repeated tetanic contraction of TA muscle. *p<0.05, **p<0.01, ****p<0.0001; **n.s.**, not significant; shown as mean ± SEM.

# ENTR-601-44 FOR EXON 44 SKIP AMENABLE DMD

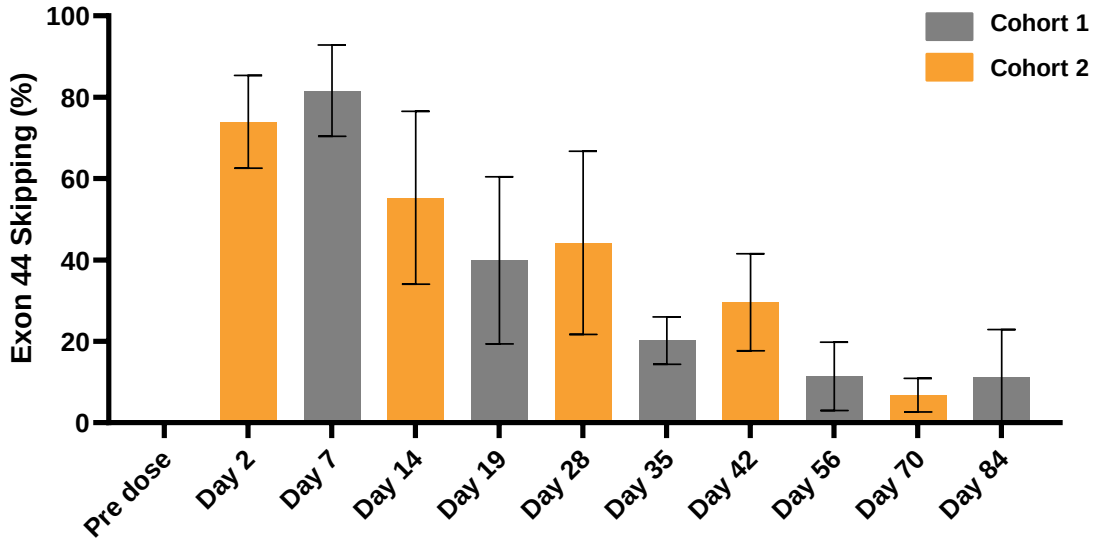
A single IV dose of ENTR-601-44 resulted in robust exon skipping in both skeletal and cardiac muscles in NHP, as well as prolonged duration of effect for at least 12 weeks

## 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

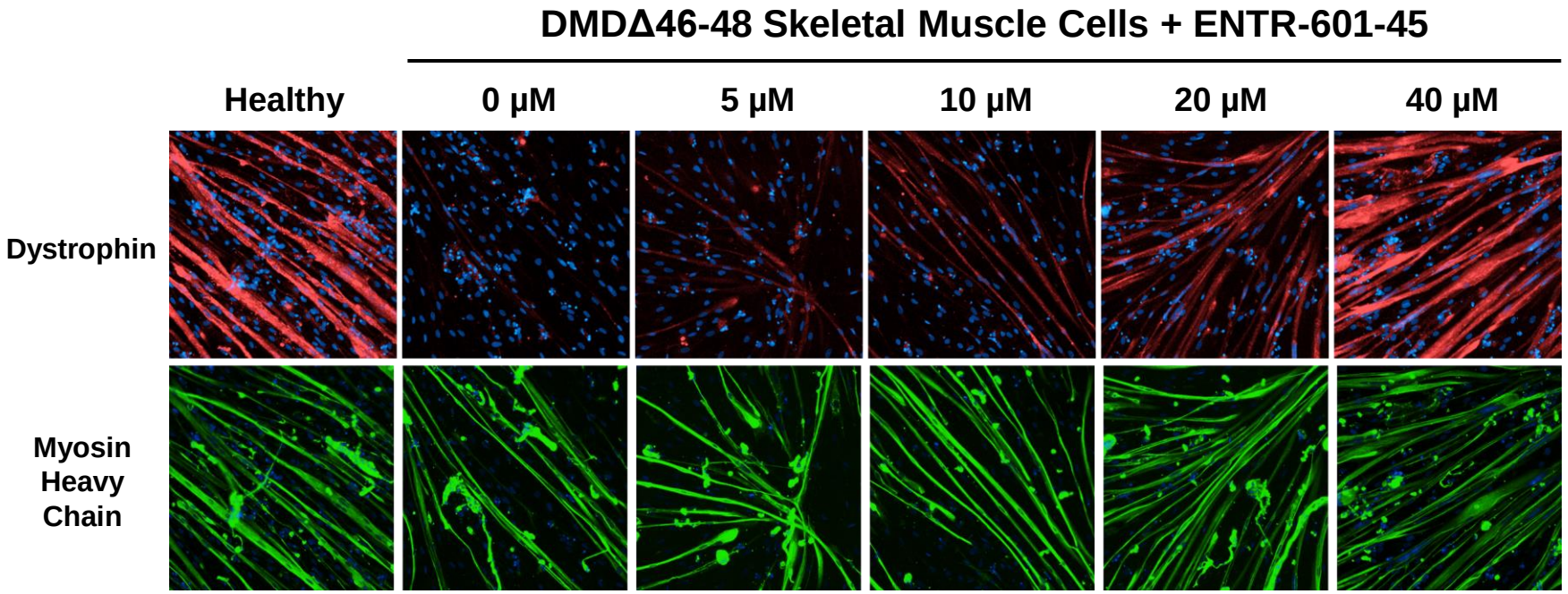


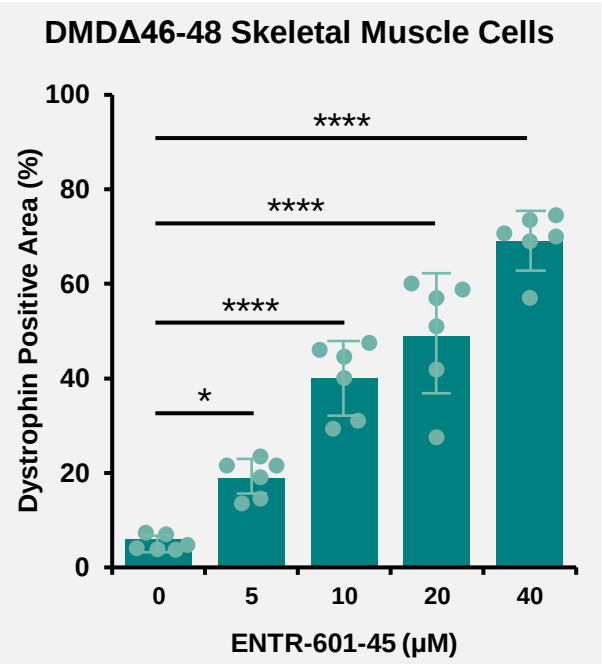

- Post IV infusion of 35 mg/kg (PMO equivalent), robust exon 44 skipping observed in biceps in the ENTR-601-44 treated NHP (n=3 per cohort) for at least 12 weeks

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

# ENTR-601-45 IN SKELETAL MUSCLE CELLS

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




- DMD patient-derived skeletal muscle cells (DMDΔ46-48, n=6) were treated with ENTR-601-45 for 24 hours and analyzed 5 days later.

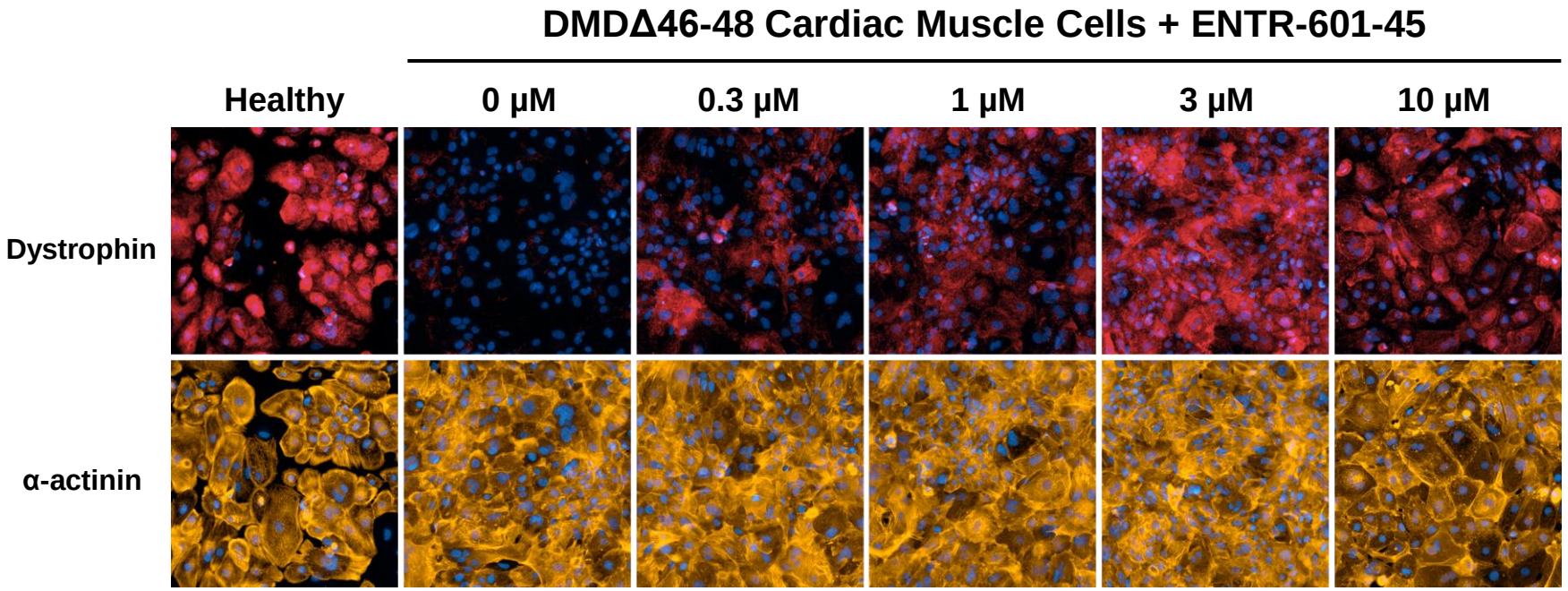


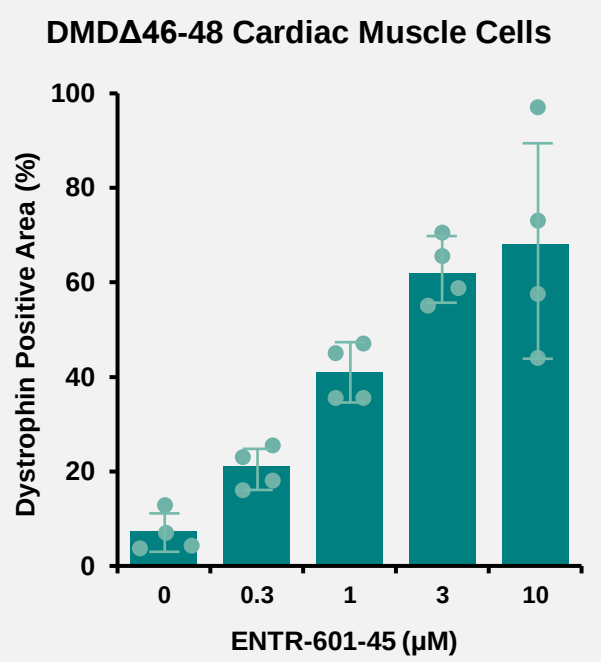


DMDΔ46-48 iPSC-derived skeletal muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation were treated for 24 hours and analyzed after 5 days of differentiation. Data are shown as mean ± SD; one-way ANOVA and Dunnett's multiple comparison test *p<0.05, ****p<0.0001; relative to untreated DMDΔ46-48 cells.

# ENTR-601-45 IN CARDIAC MUSCLE CELLS

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




- DMD patient-derived cardiac muscle cells (DMDΔ46-48, n=4) were treated with ENTR-601-45 for 24 hours and analyzed 48 hours later.





DMDΔ46-48 iPSC-derived cardiac muscle cells from an exon 45 skip amenable DMD patient harboring an exon 46-48 deletion mutation were treated for 24 hours and analyzed 48 hours later. Data are shown as mean ± SD.

# INTERFERON REGULATORY FACTOR 5 (IRF5)

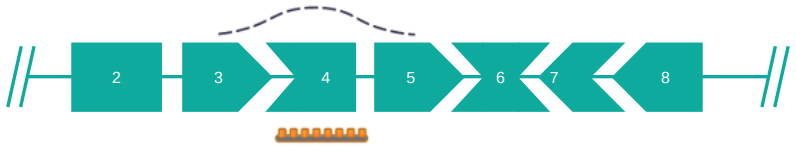
# EEV-PMO TREATMENT REDUCED IRF5 EXPRESSION IN VITRO

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

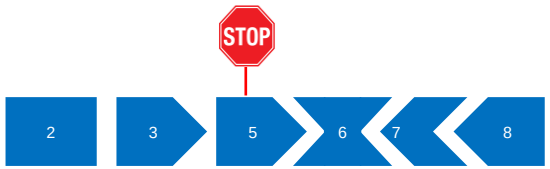
## EEV-PMO Approach to Knockdown IRF5

**IRF5 pre-mRNA**

With EEV-PMO inducing exon skipping




Splicing



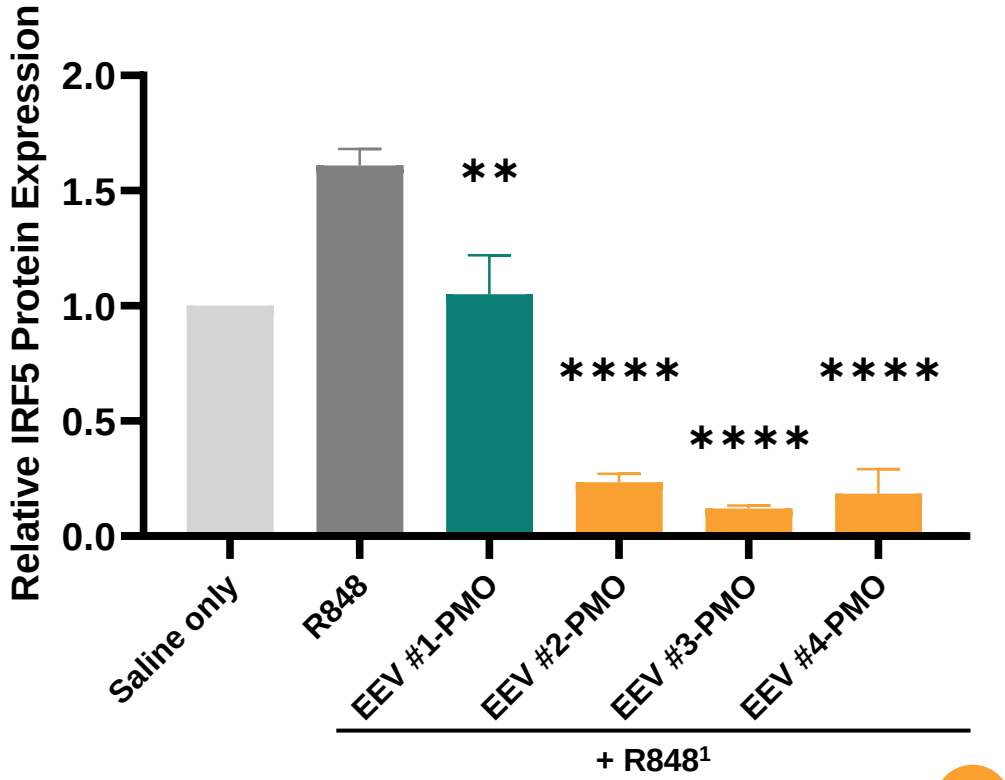

**IRF5 mRNA**

Out of frame shift and premature stop codon

Nonsense Mediated Decay machinery

**IRF5 Knockdown**

## IRF5 Production




[1]R848 is a TLR7/8 agonist that upregulates IRF5. Mouse macrophage cells were pre-treated with EEV-PMO (#1-4) for 4 hours, followed by stimulation with R848 overnight. At 24 hours post treatment, cells were harvested and evaluated by Western Blot. The lowest concentration (2 µM) is shown in the graph. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

# SUMMARY

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



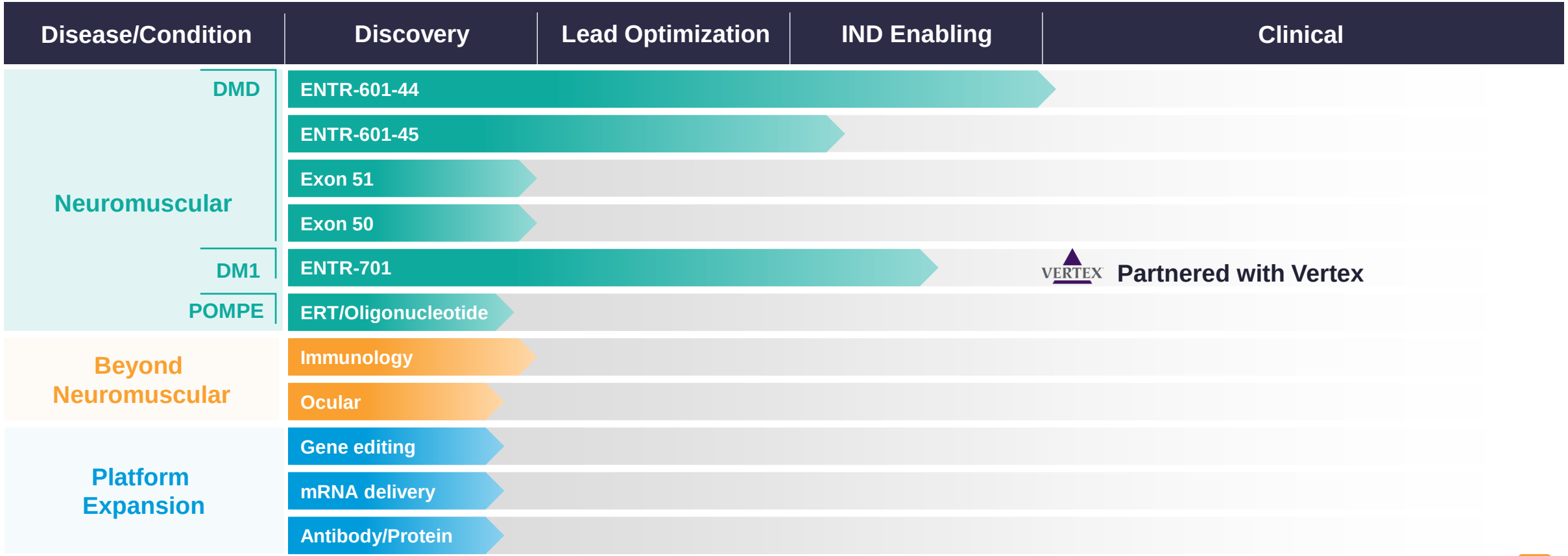


[a]Entrada received a clinical hold notice from the FDA regarding the IND for ENTR-601-44 in December 2022. Entrada plans to share additional updates pending further communications with the Agency.

# 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 |

# ACKNOWLEDGEMENTS

entrada THERAPEUTICS

## Thank you!

**WANT TO LEARN MORE?**

Scan the QR code for access to our recent publications and conference presentations.