

# Optimization and Application of the Endosomal Escape Vehicle (EEV™) Platform for Enhanced Delivery of Oligonucleotides to Skeletal and Cardiac Muscle

Mah Kheirabadi, PhD

Director, Discovery Chemistry

2024 CPP Next Generation Delivery Systems Conference



# 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, statements related to our ability to continue to recruit for and complete its healthy volunteer trial, ENTR-601-44-101, in the United Kingdom, expectations regarding the timing of data from our Phase 1 trial for ENTR-601-44 in October 2024, the ability to resolve the clinical hold for ENTR-601-44 and subsequent activities, expectations regarding the timing or content of any update regarding our regulatory filings including for a Phase 2 clinical trial in the fourth quarter of 2024, expectations regarding the safety and therapeutic benefits of ENTR-601-44, our ability to develop and advance our current and future product candidates and discovery programs, expectations regarding the results of preclinical studies predicting the results of later preclinical studies or any clinical trials of our therapeutic candidates, our ability to establish and maintain collaborations or strategic relationships, our ability to raise additional funding, the rate and degree of market acceptance and clinical utility of our product candidates, the potential of our EEV product candidates and EEV platform, the ability and willingness of our third-party collaborators to continue research and development activities relating to our product candidates, including our Vertex partnership for VX-670, expectations regarding the expected timing, progress and success of our collaboration with Vertex, including the ability to recruit for and complete its Phase 1/2 trial and any future payments we may receive under our collaboration and license agreements, our collaborators’ ability to protect our intellectual property for our products, expectations regarding the timing of preclinical data results and planned CTA/IND submissions for ENTR-601-45 and ENTR-601-50, the continued development and advancement of ENTR-601-44, ENTR-601-45 and ENTR-601-50 for the treatment of DMD, and VX-670 for the treatment of DM1, and the sufficiency of our cash resources through the second quarter of 2026. 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.

OUR MISSION

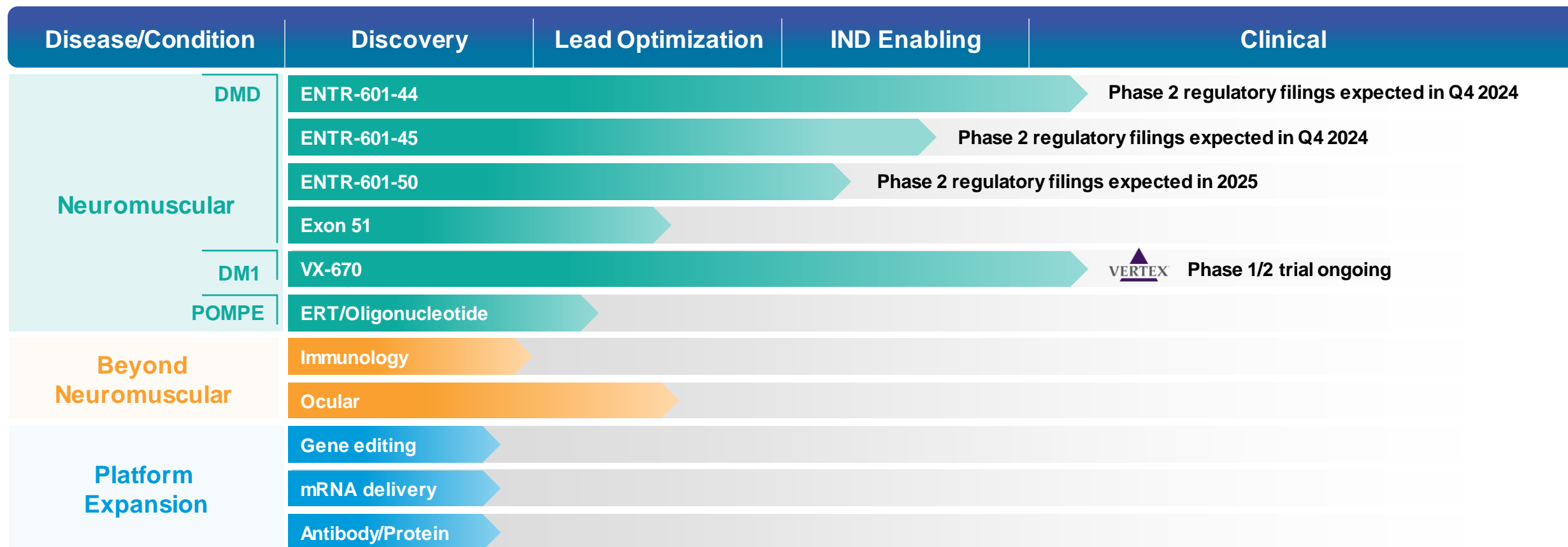
To Treat Devastating  
Diseases with  
Intracellular Therapeutics



# A DIFFERENTIATED AND EXPANDING PIPELINE



Entrada's pipeline includes a diverse array of high potential and high value assets; Each disease has a substantial patient population with a significant unmet medical need

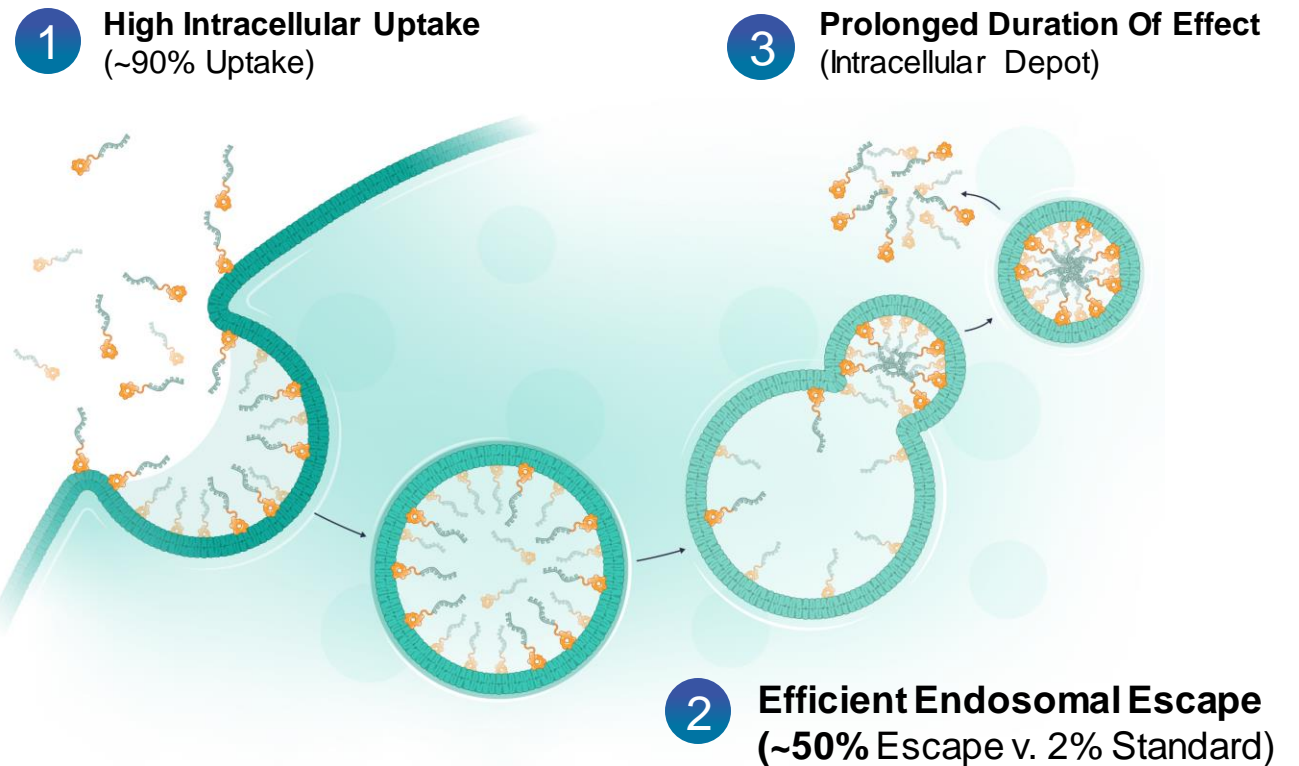


# EEV™ PLATFORM DEVELOPMENT AND OPTIMIZATION

# Endosomal Escape Vehicle (EEV™) Therapeutics

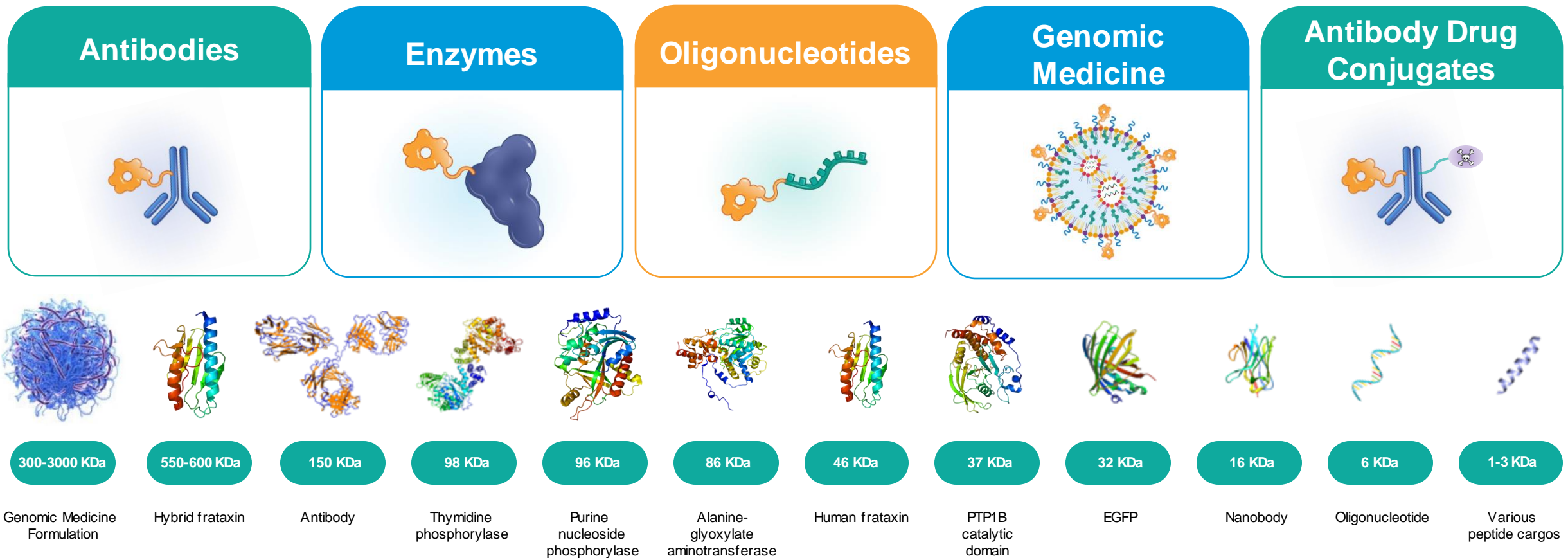
- Unique chemistry results in **improved uptake and endosomal escape**
- Cyclic structure designed to **extend half life and increase stability**
- Phospholipid binding potentially **enables broad biodistribution to all cells**
- Mechanism of **internalization conserved across species**

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



# A BROADLY APPLICABLE PLATFORM

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



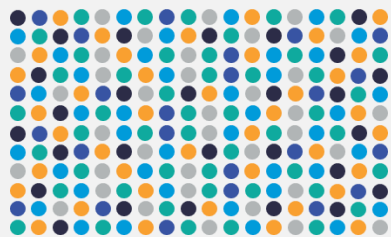
# DISCOVERY ENGINE FOR EEV THERAPEUTICS

## EEV-OLIGO EXAMPLE

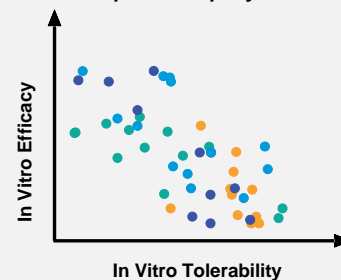
Fit-for-purpose EEVs can be designed for target indications and modalities via iterative optimizations of EEV peptides through medicinal chemistry, *in vitro* and *in vivo* screenings



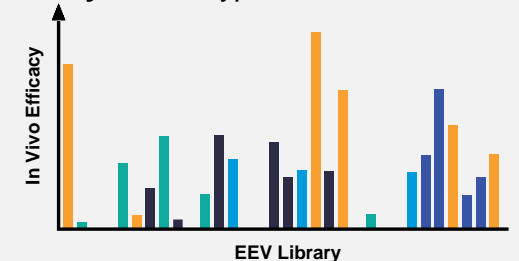
Chemically diverse macrocyclic **EEV library** generated through medicinal and combinatorial chemistry



**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



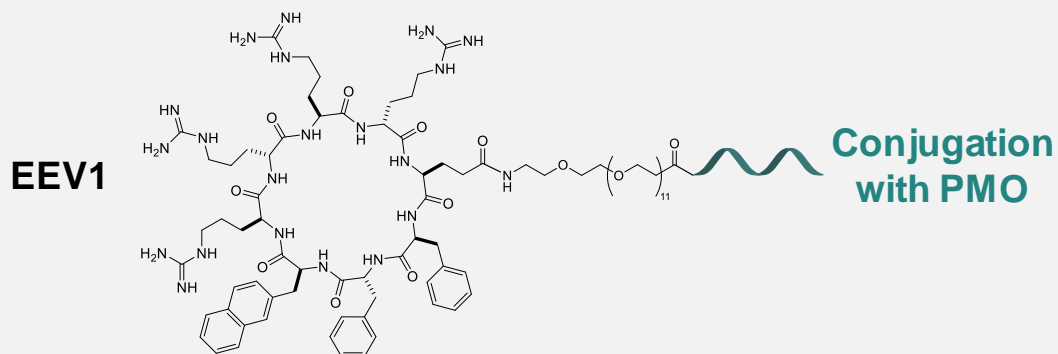


# OLIGO DELIVERY WITH FIRST GENERATION EEV

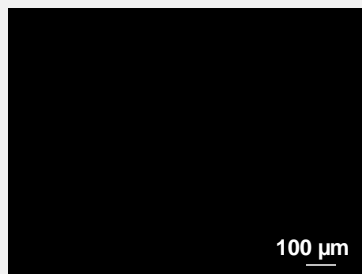
## EEV1 EXAMPLE

A first-generation EEV1 peptide-PMO construct enhanced splice correction *in vitro* and after local injection, demonstrated functional delivery of oligonucleotides

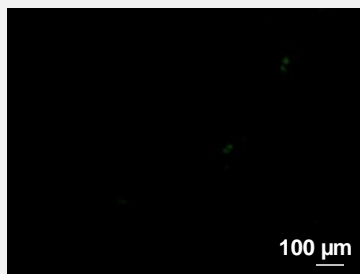
### Splicing Correction in HeLa EGFP-654 Cells



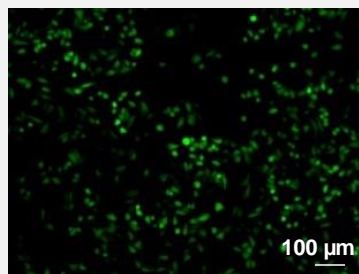
Control



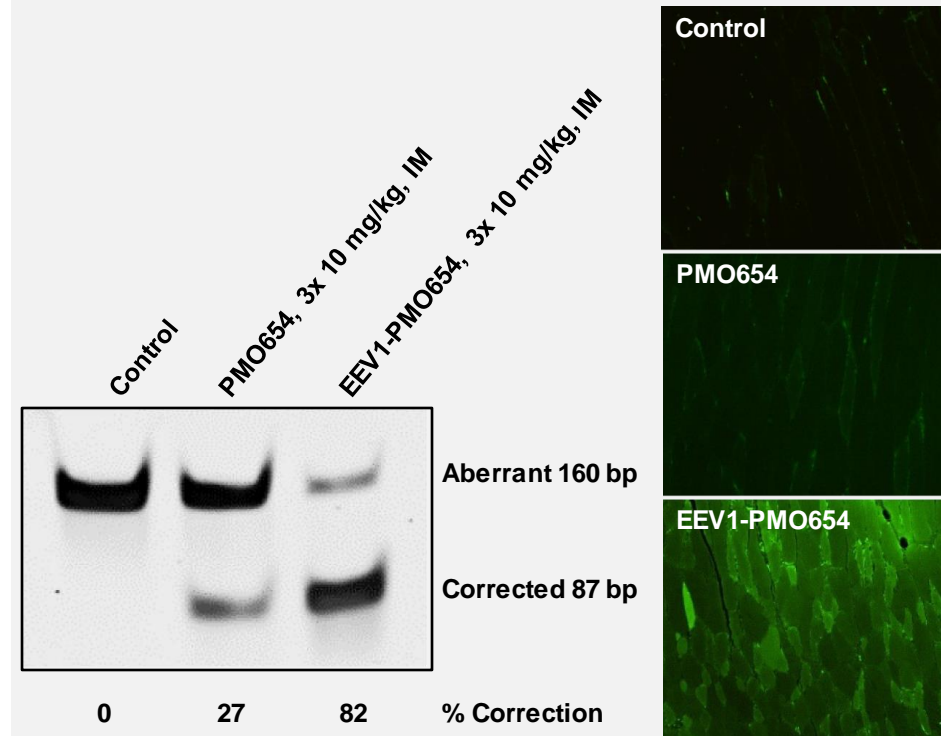
PMO654



EEV1-PMO654



### Three Daily IM Doses of EEV1-PMO654



<sup>a</sup>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.

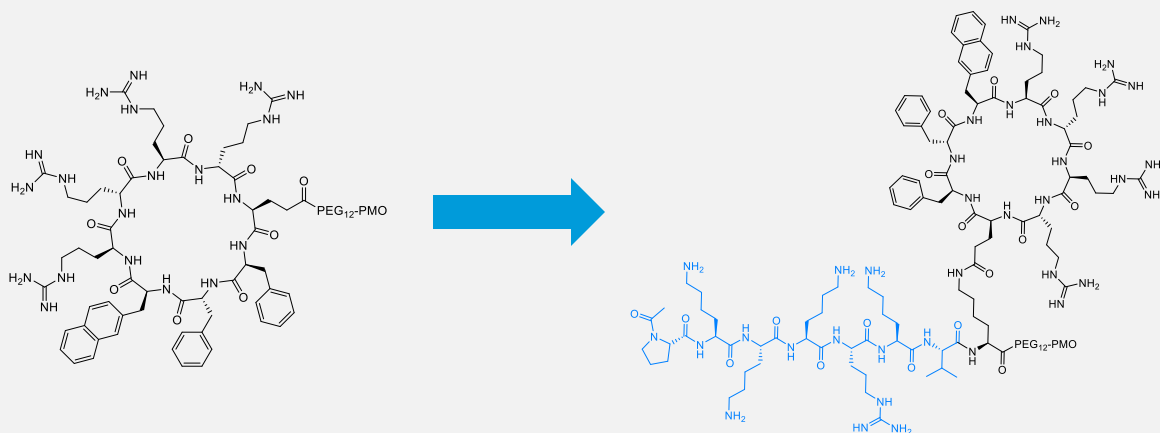
# ENHANCED OLIGONUCLEOTIDE DELIVERY

## 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

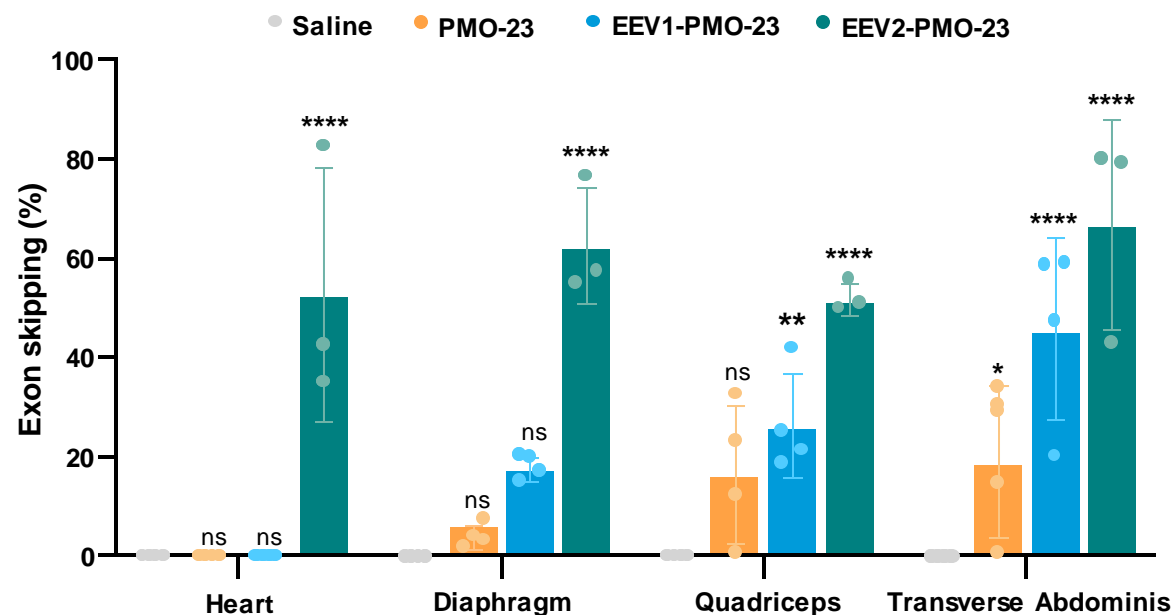
### Structure of EEV2 Construct

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

# OPTIMIZATION OF EEV FOR MUSCLE DELIVERY

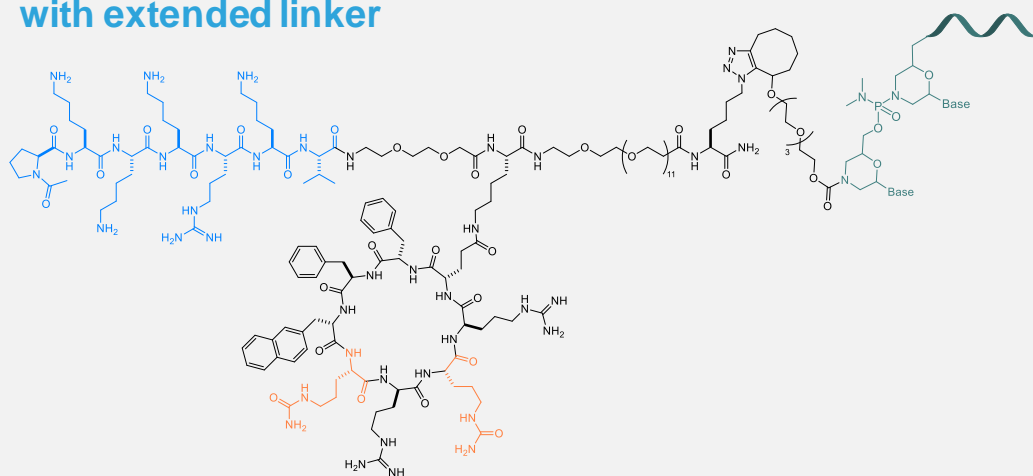
## EEV3 EXAMPLE

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

### EEV3-PMO654 Structure and Medicinal Chemistry

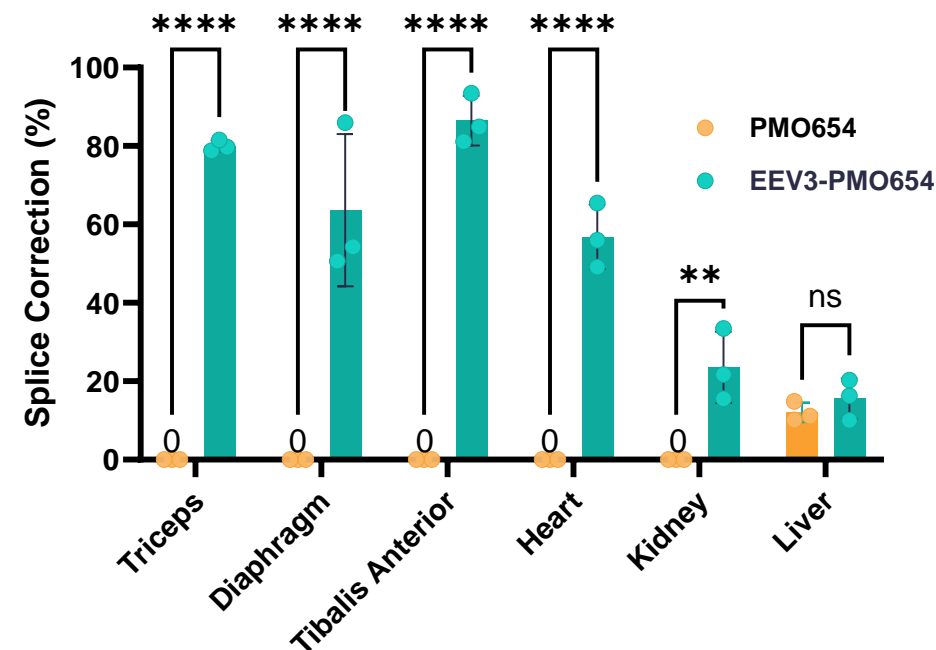
Exocyclic peptide sequence with extended linker

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

# OPTIMIZATION OF EEV FOR MUSCLE DELIVERY

## UPTAKE AND OUTCOMES IN A MURINE MODEL

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



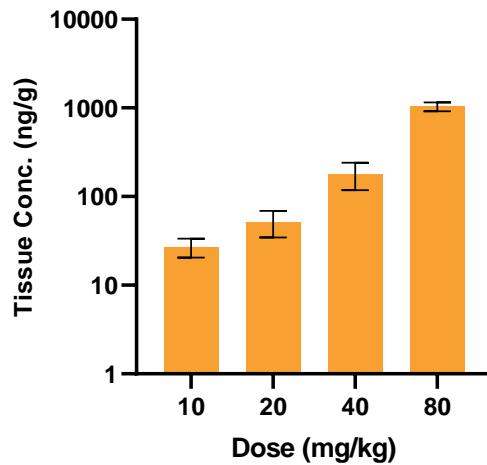
- ✓ Endosomal escape
- ✓ Nuclear localization

=

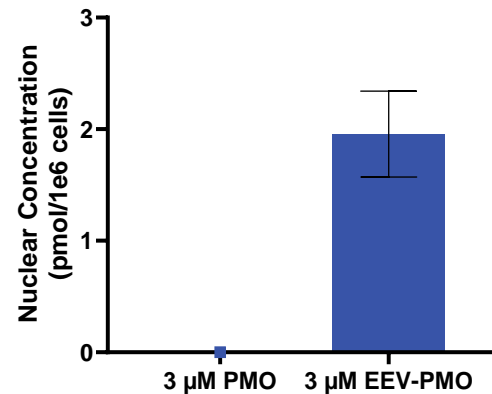
### Pharmacodynamic Outcome



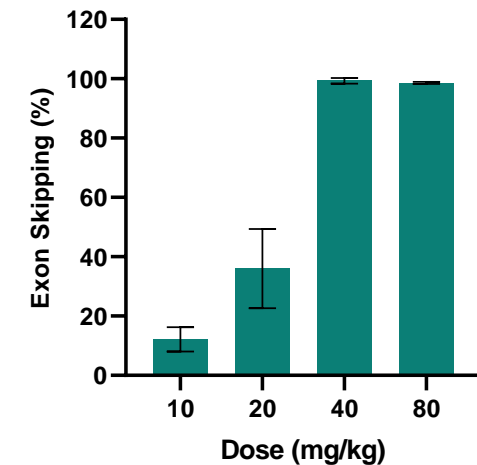
- ✓ Rapid, dose-dependent response
- ✓ Duration of at least 12 weeks



IV, hDMD mice, 5-day post injection



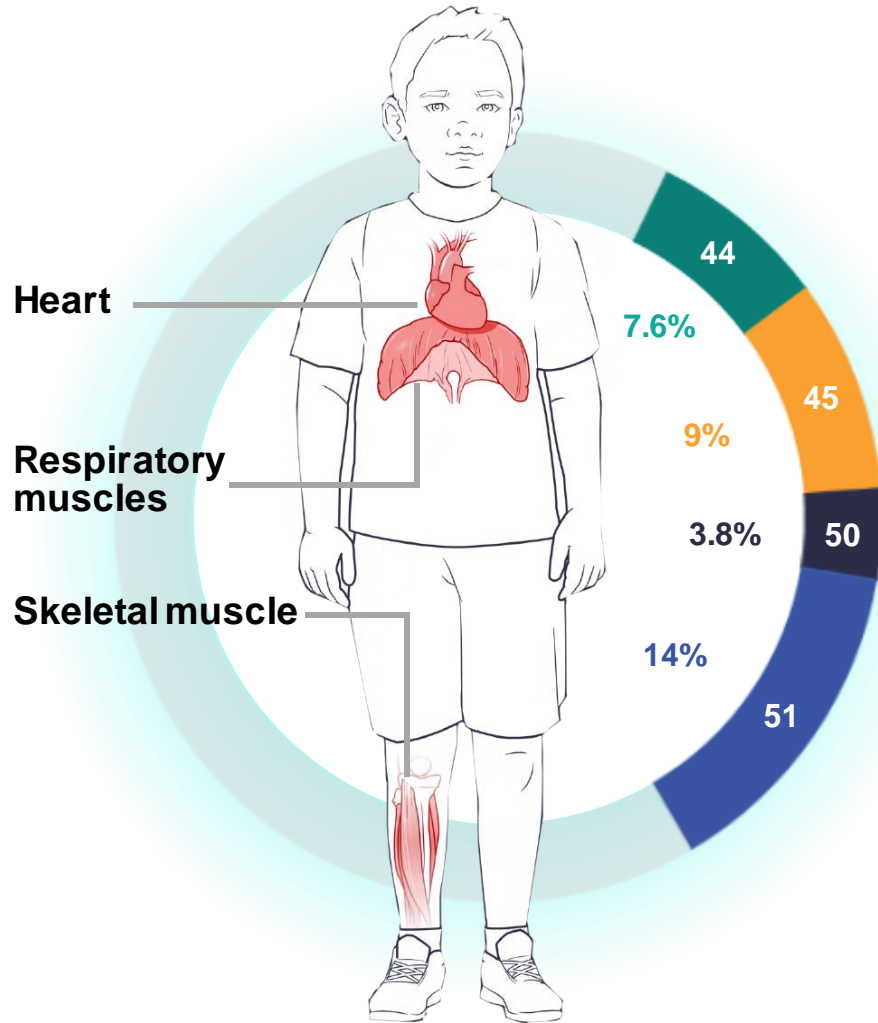
24-hour incubation



IV, hDMD mice, 5-day post injection

# DUCHENNE MUSCULAR DYSTROPHY: DELIVERY OF OLIGONUCLEOTIDES

# DMD: SIGNIFICANT UNMET NEED



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

## ~40,000

people in the **US and Europe** have Duchenne<sup>1</sup>

## Duchenne Franchise

### ENTR-601-44 Phase 1

Phase 1 data expected October 2024  
Phase 2 regulatory filings expected Q4 2024

### ENTR-601-45 IND Enabling

Phase 2 regulatory filings expected Q4 2024

### ENTR-601-50 IND Enabling

Phase 2 regulatory filings expected 2025

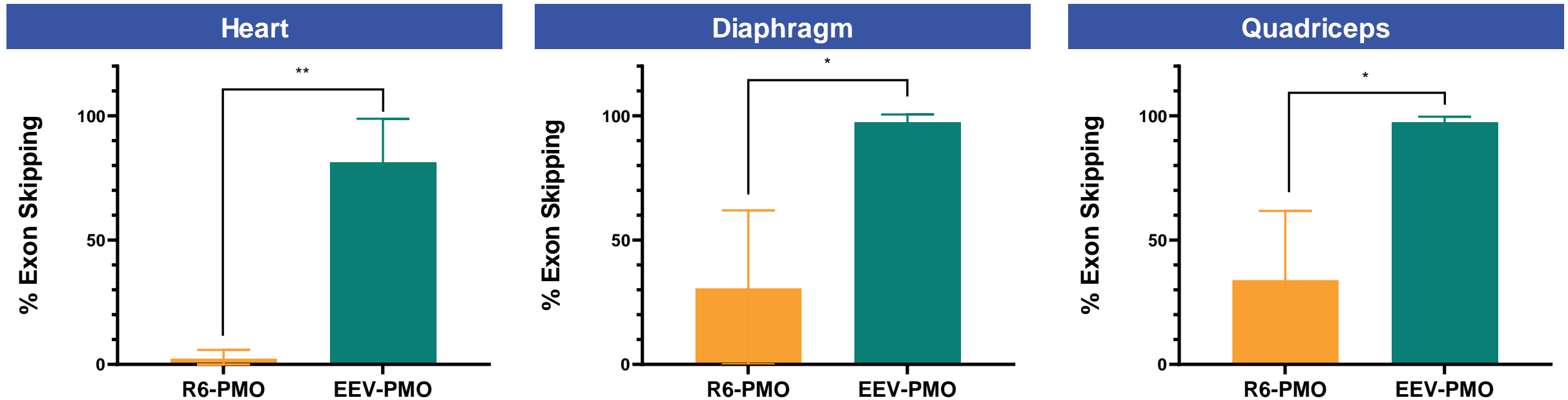
### Exon 51 Lead Optimization

Candidate selection expected in 2024

# SUPERIOR TO ALTERNATIVE PEPTIDES

## R6-PMO Example

EEV-PMO significantly improved exon 23 skipping after 3 days in *mdx* mice as compared to competitive R6-PMO

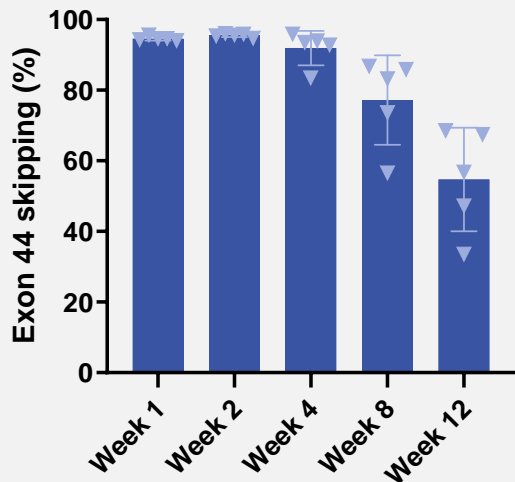


- EEV-PMO-23 demonstrates significantly improved PD effects after single 40 mg/kg IV dose in *mdx* mice

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

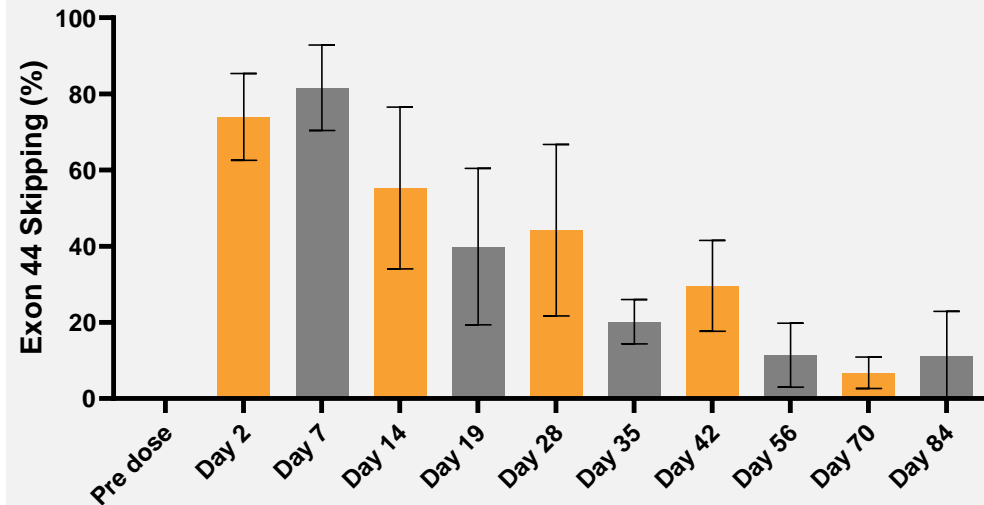
Significant patient benefit is implied by data in the mouse and the monkey at clinically relevant levels; *in vitro* data suggests much higher target engagement in patient cells

## Exon 44 Skipping in hDMD Mouse



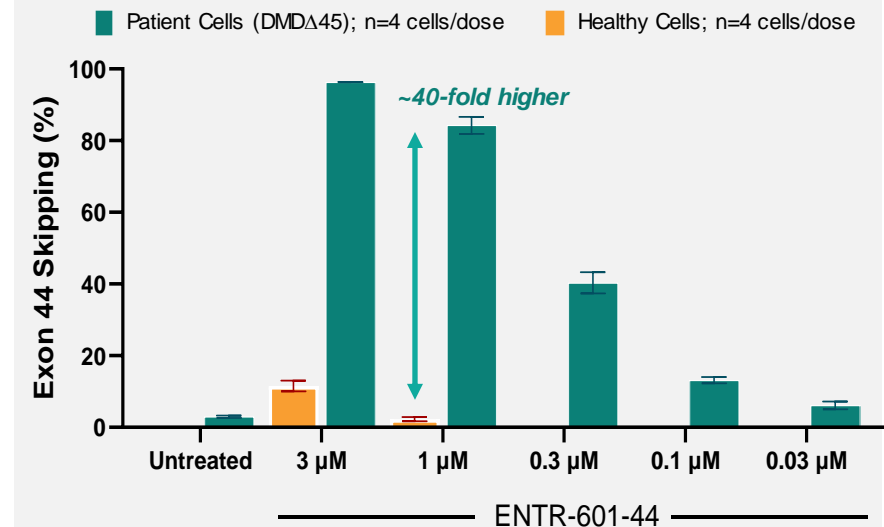
- Single IV 60 mg/kg dose of ENTR-601-44
- Tibialis Anterior

## Exon 44 Skipping in Monkey



- Post IV infusion of single 35 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

## 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



Significant patient benefit is implied by data in the mouse and the monkey at clinically relevant levels;  
*in vitro* data suggests much higher target engagement in patient cells

- ✓ High levels of exon skipping across *mdx*, D2-*mdx*, human dystrophin mouse and NHP studies
- ✓ Exon skipping translates to promising dystrophin production in heart and skeletal muscles
- ✓ Dystrophin production observed results in functional improvement in D2-*mdx* mouse
- ✓ Extended circulating half-life and durable exon skipping over 12+ weeks from a single injection of ENTR-601-44 was observed in the NHP

## **ENTR-601-44-101: Phase 1 clinical trial ongoing**


- First participant dosed in September 2023
- Initiated dosing of the fourth and final cohort
- Data readout anticipated in October 2024
- Phase 1 clinical data will support the global clinical trial in patients\*

\*MAD/Phase 2b study is subject to regulatory feedback and the outcome of the SAD study.

# PLATFORM OPPORTUNITIES

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

## TARGET



DNA



RNA



PROTEIN

## 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



**WANT TO LEARN MORE?**

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

The logo for Entrada Therapeutics is positioned in the lower right quadrant of the image. It features a stylized white icon on the left consisting of several curved lines that resemble a fan or a set of wings. To the right of this icon, the word "entrada" is written in a large, lowercase, white sans-serif font. Below "entrada", the word "THERAPEUTICS" is written in a smaller, uppercase, white sans-serif font. The entire logo is overlaid on a blue-tinted background of a young girl blowing bubbles in a park.