Therapeutic Potential of Endosomal Escape Vehicle (EEV™)-Oligonucleotide Conjugates in Preclinical Models of Neuromuscular Diseases

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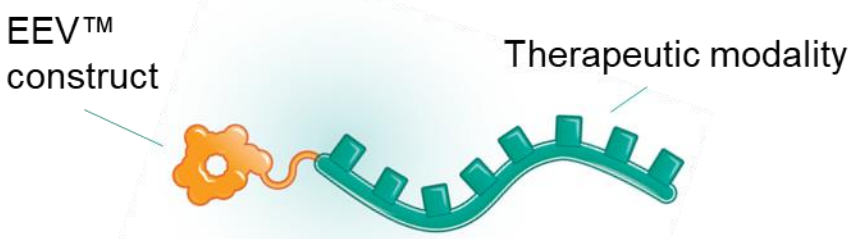
This presentation includes express and implied "forward-looking statements." Forward looking statements include all statements that are not historical facts, and in some cases, can be identified by terms such as "may," "might," "will," "could," "would," "should," "expect," "intend," "plan," "objective," "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 initiate and recruit for a healthy volunteer trial for ENTR-601-44 in the United Kingdom with first subject dosed in September 2023, expectations regarding the timing of data from our Phase 1 trial for ENTR-601-44 in the second half of 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, 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, 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 ENTR-701, our collaborators' ability to protect our intellectual property for our products, and the sufficiency of our cash resources through 2025. 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.

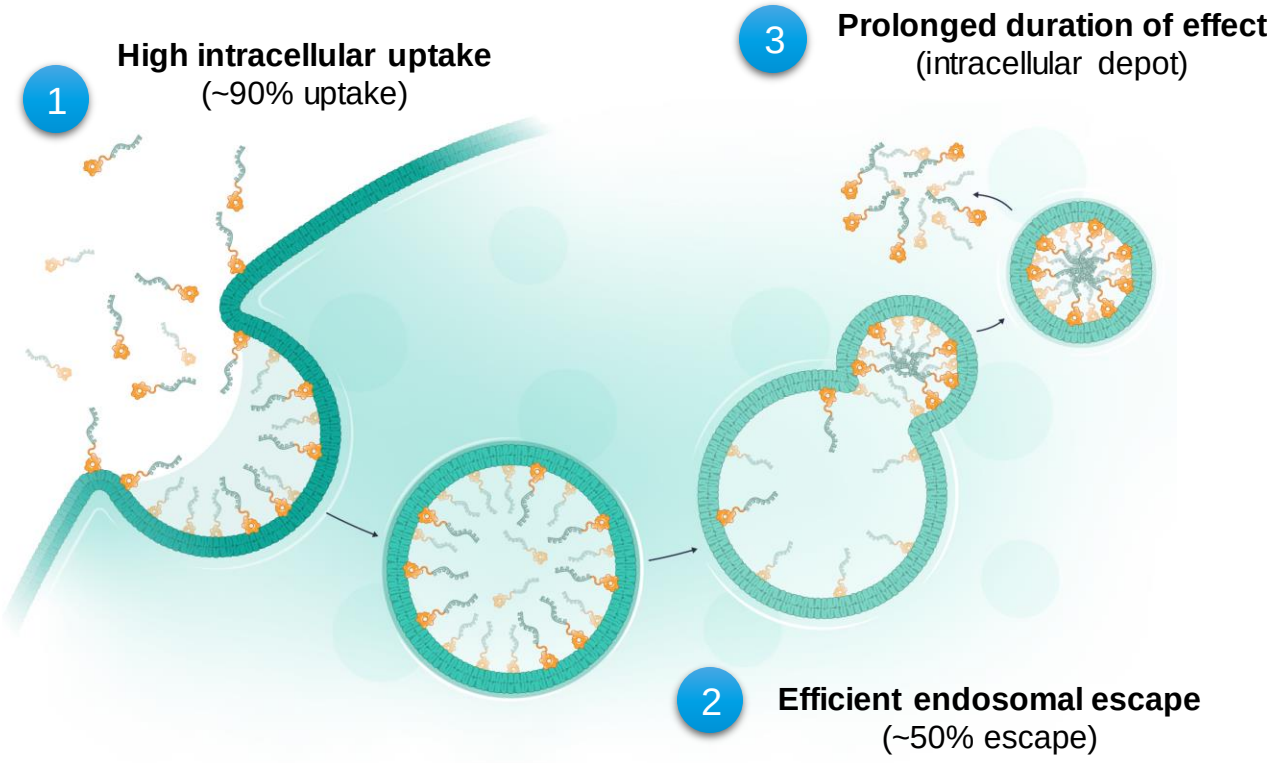
Certain information contained in this presentation and statements made orally during this presentation relate to or are based on studies, publications, surveys and other data obtained from third-party sources and our own internal estimates and research. While we believe these third-party studies, publications, 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.

ENDOSOMAL ESCAPE VEHICLE (EEV™) PLATFORM

The EEV Platform was developed to enhance the intracellular delivery of therapeutic cargo such as antisense oligonucleotides



- 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**

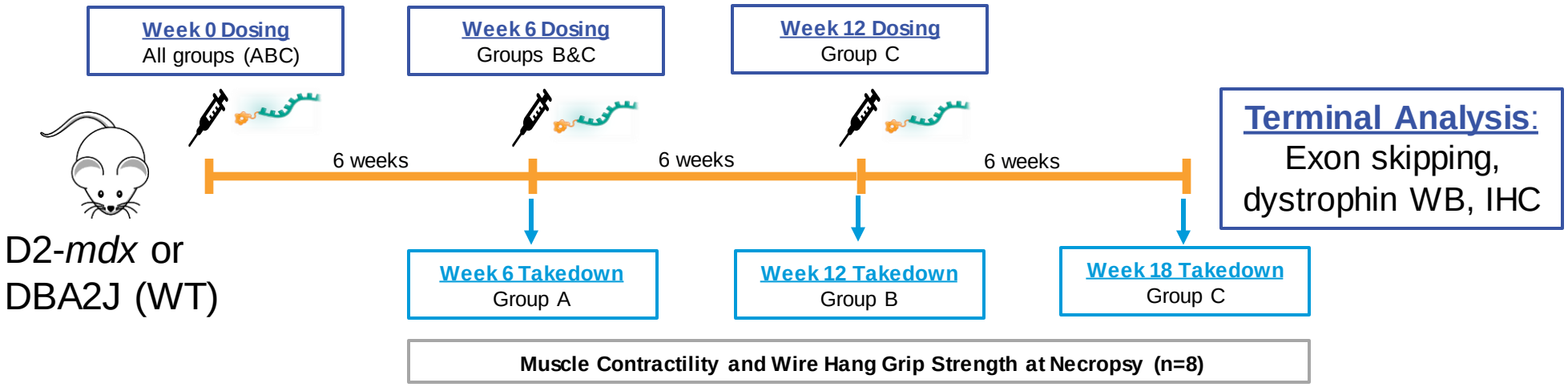


Qian, Z. et al. *ACS Chem. Biol.* 2013; Qian, Z. et al. *Biochemistry* 2014; Qian, Z. et al. *Biochemistry* 2016; Sahni, A. et al. *ACS Chem. Biol.* 2020; Pei, D. *Acc. Chem. Res.* 2022. **EEV**, endosomal escape vehicle. **PMO**, phosphorodiamidate morpholino oligomer.

EEV-PMO IN DMD PRECLINICAL MODELS

REPEAT Q6W DOSE EFFICACY OF PMO-EEV-23 IN D2-*mdx* MICE

Objective: Determine the efficacy of EEV-PMO-23 in ameliorating the pathology and improving muscle function with 3 dose regimen

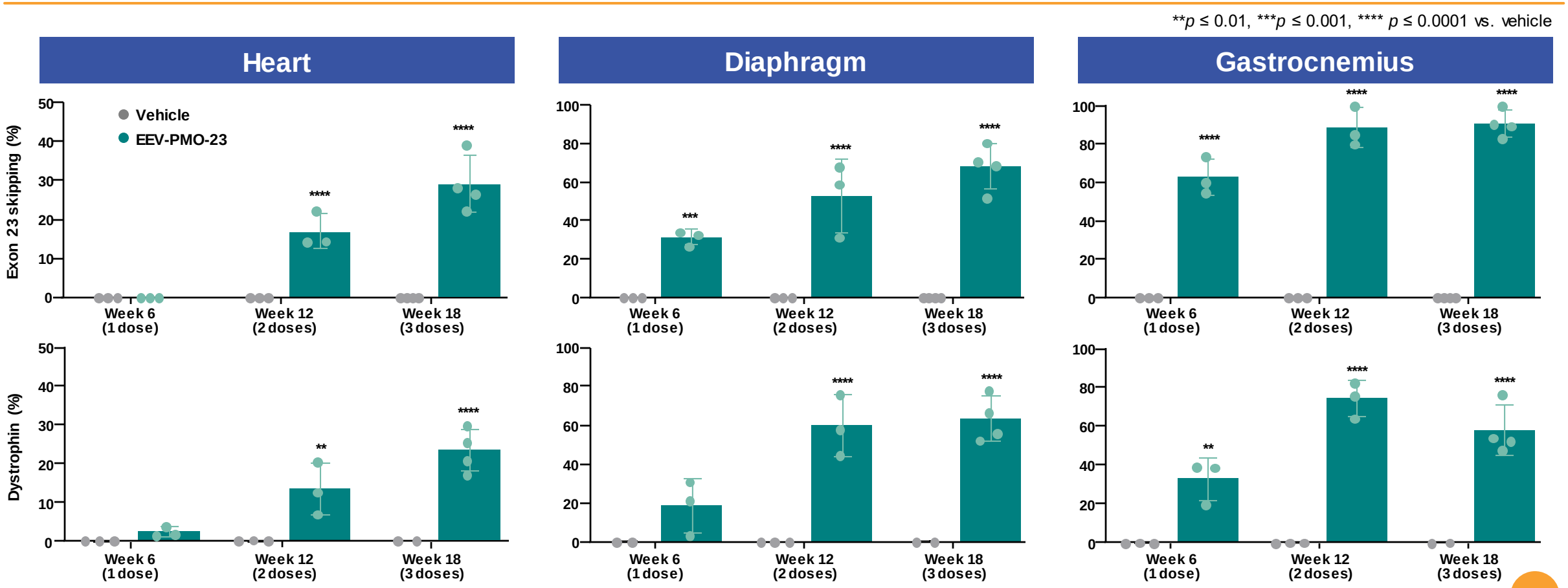


- D2-*mdx* (n=8) mice received one, two, or three injections of vehicle or 80 mg/kg EEV-PMO-23 once every six weeks.
- Muscle contractility, grip strength, and wire hang time were determined at 6, 12, and 18 weeks.
- Animals were euthanized 6 weeks after the last dose.
- Exon skipping, dystrophin protein, contractile function, and histological analysis were performed.

EEV-PMO-23 consists of an EEV construct conjugated to a mouse DMD exon 23 skipping PMO. **WT**, wild type; **WB**, western blot, **IHC**, immunohistochemistry.

EXON SKIPPING AND DYSTROPHIN PRODUCTION WITH Q6W EEV-PMO-23 IN D2-*mdx* MICE

Exon 23 skipping and dystrophin protein expression in cardiac and skeletal muscle following each dose of EEV-PMO-23 administered every six weeks

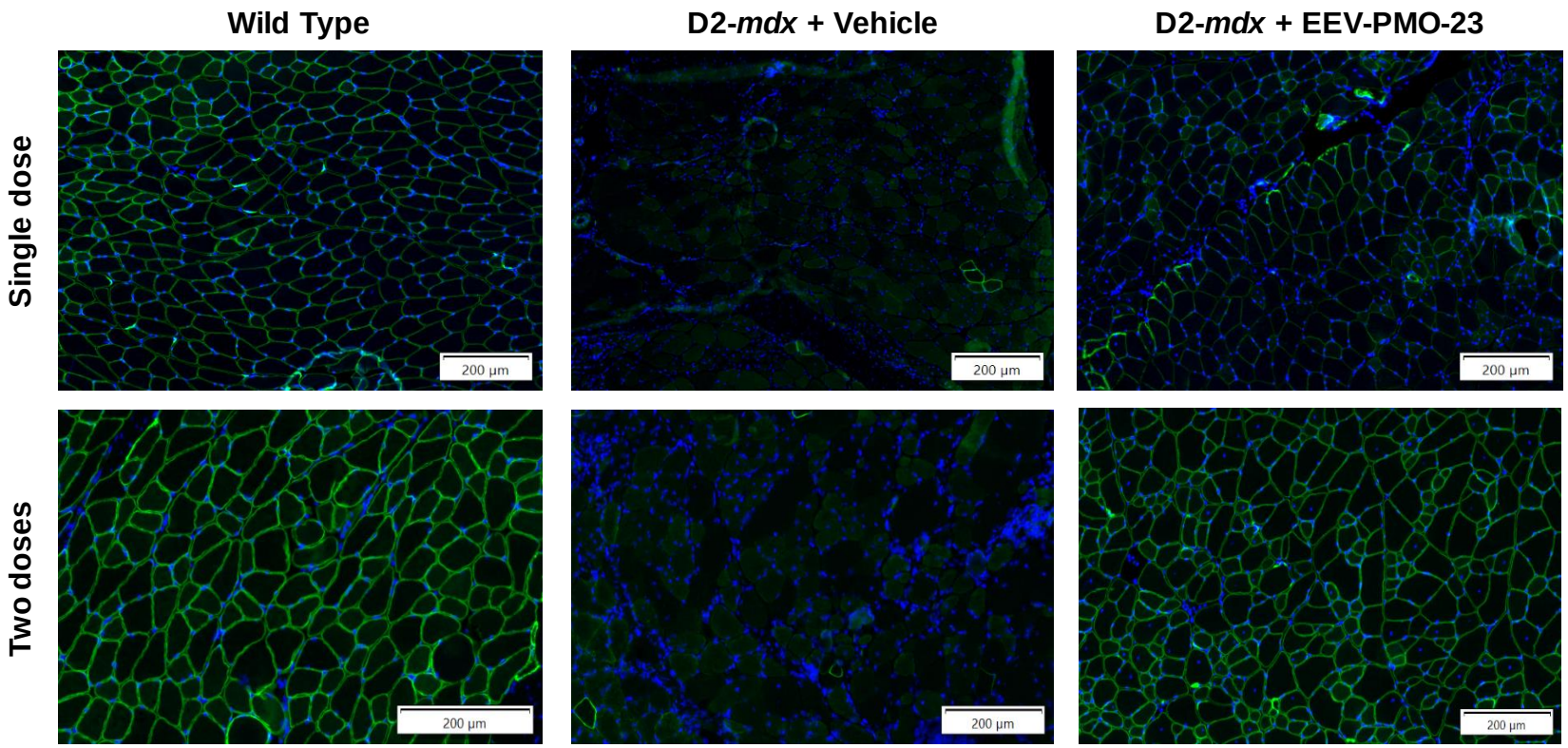


D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Exon skipping and dystrophin production were analyzed 6 weeks after each dose. Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; **ANOVA**, analysis of variance; **EEV**, endosomal escape vehicle; **PMO**, phosphorodiamidate morpholino oligomer; **Q6W**, every 6 weeks; **SD**, standard deviation.

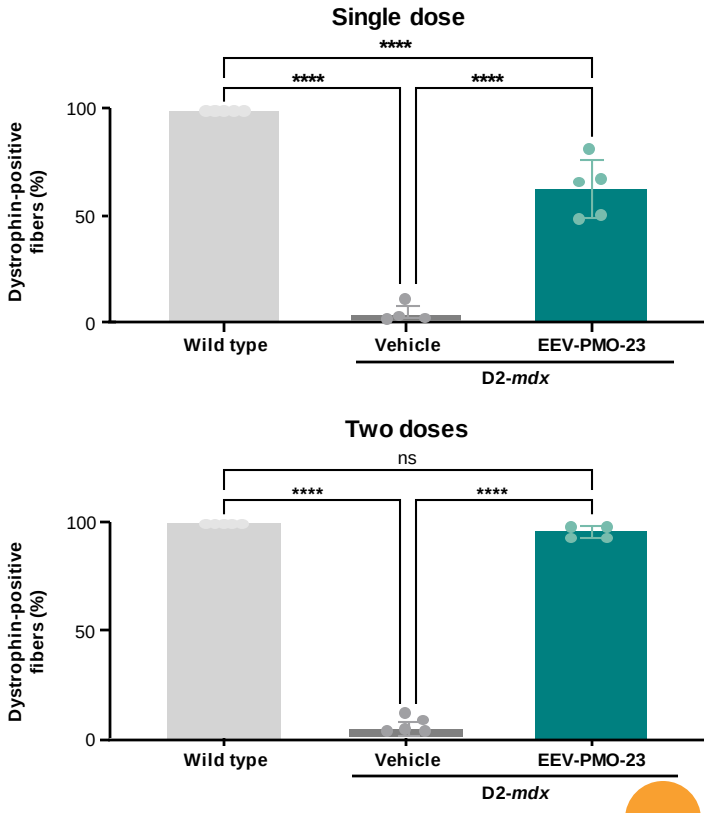
DYSTROPHIN RESTORATION IN SKELETAL MUSCLE WITH Q6W EEV-PMO-23 IN D2-*mdx* MICE

Two Q6W doses of EEV-PMO-23 significantly increased dystrophin expression in skeletal muscle of D2-*mdx* mice to similar levels observed in wild type mice

Dystrophin Restoration in D2-*mdx* Skeletal Muscle



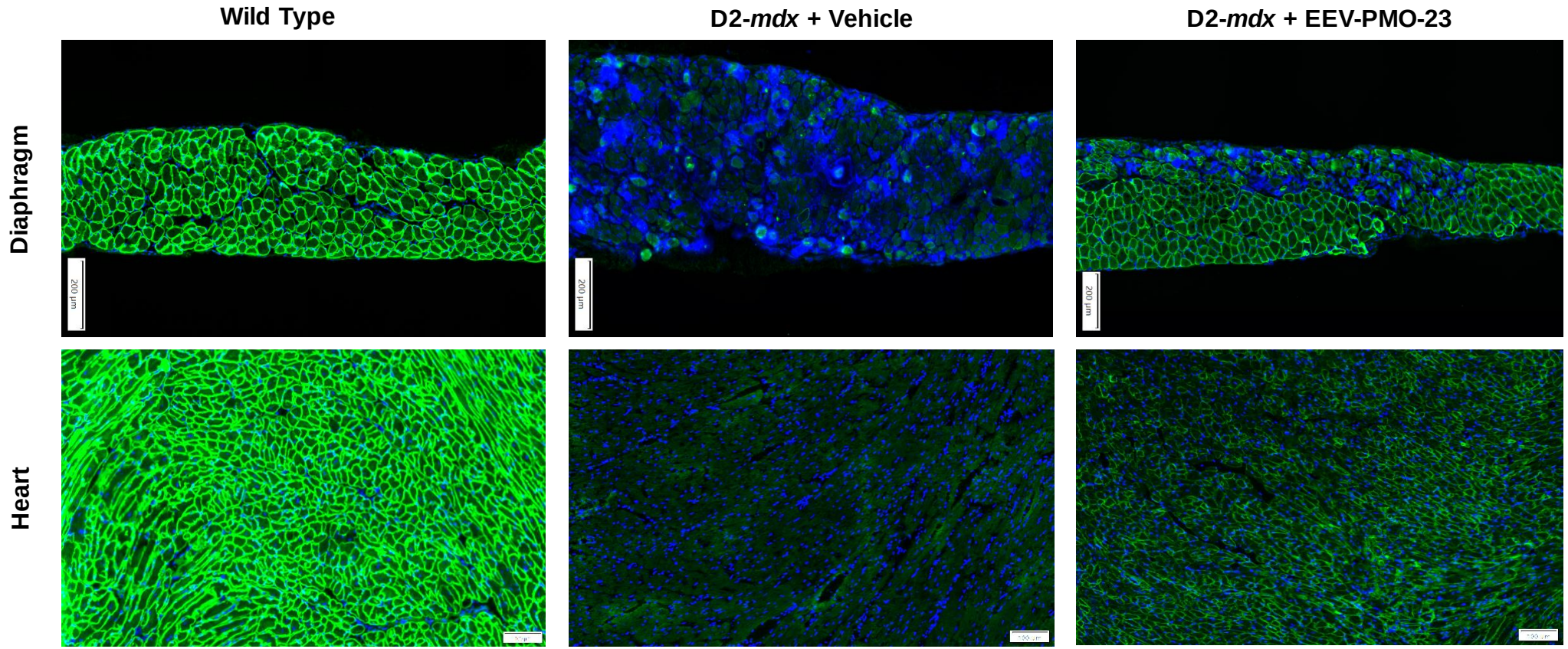
Dystrophin-positive Fibers



D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Gastrocnemius muscle was assessed for dystrophin expression 6 weeks after each dose. Immunofluorescence showing dystrophin in green (left). Quantification of dystrophin positive fibers (right). Data shown as mean ± SD. One-way ANOVA was used for statistical comparison; ****$p<0.0001$ vs. vehicle. **ANOVA**, analysis of variance; **EEV**, endosomal escape vehicle; **ns**, not significant; **PMO**, phosphorodiamidate morpholino oligomer; **Q6W**, every 6 weeks; **SD**, standard deviation.

DYSTROPHIN RESTORATION IN DIAPHRAGM AND HEART WITH TWO Q6W EEV-PMO-23 DOSES

Two Q6W doses of EEV-PMO-23 significantly increased dystrophin expression in the diaphragm of D2-*mdx* mice



D2-*mdx* mice were treated with 80 mg/kg EEV-PMO-23 or vehicle Q6W. Diaphragm and heart were assessed for dystrophin expression 6 weeks after the second dose. Immunofluorescence showing dystrophin in green. **EEV**, endosomal escape vehicle; **PMO**, phosphorodiamidate morpholino oligomer; **Q6W**, every 6 weeks; **SEM**, standard error of the mean.

EEV-PMO CLINICAL CANDIDATES FOR DMD

Preclinical efficacy of EEV-PMO conjugates led to the development of two clinical candidates for the treatment of exon 44 and exon 45 skip amenable DMD

ENTR-601-44

- 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 NHP

ENTR-601-45

- Robust exon skipping and dystrophin protein production in patient-derived cardiac and skeletal muscle cells
- High levels of exon skipping were measured in hDMD mouse heart and skeletal muscle tissue
- Exon Skipping resulted in dystrophin protein production in the disease model amenable for exon 45 skipping

ENTR-601-44-101 Phase 1 Clinical Trial in Healthy Subjects is Ongoing

- Completed dosing of 3 cohorts
- Data anticipated in H2 2024

Entrada is planning for regulatory submission in Q4 2024

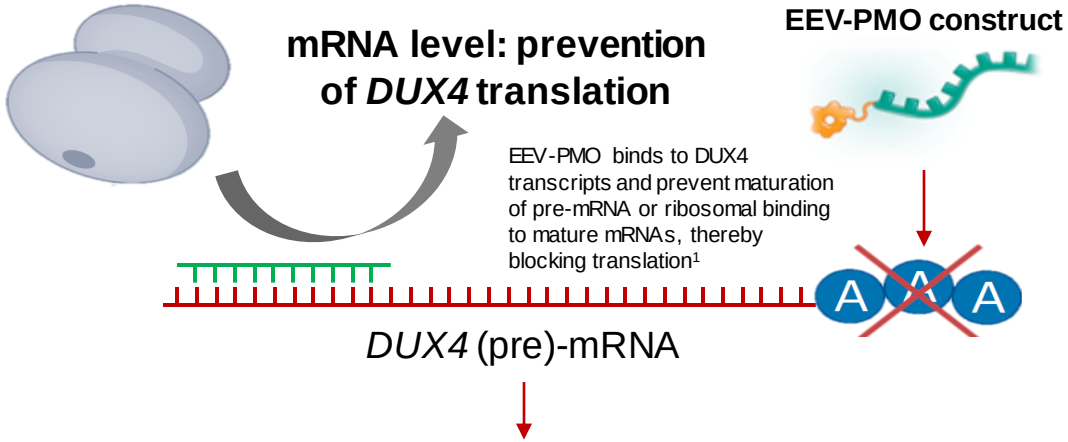
- Planning for a direct to MAD trial in Duchenne patients for ENTR-601-45

EEV-PMO IN FSHD PATIENT CELLS

EEV-PMO-PAS DOWNREGULATES GENE EXPRESSION IN FSHD PATIENT-DERIVED CELLS

EEV-PMO-PAS reduces *DUX4* transcript and several of its downstream target genes, indicating that the EEV-PMO approach can be utilized to downregulate target genes

Exon Skipping in FSHD[1]



Altered expression of downstream genes

MBD3L2

MBD3L2 amplifies *DUX4* expression, and potentially facilitates DUX4 spread to neighboring nuclei within individual myofibers[2]

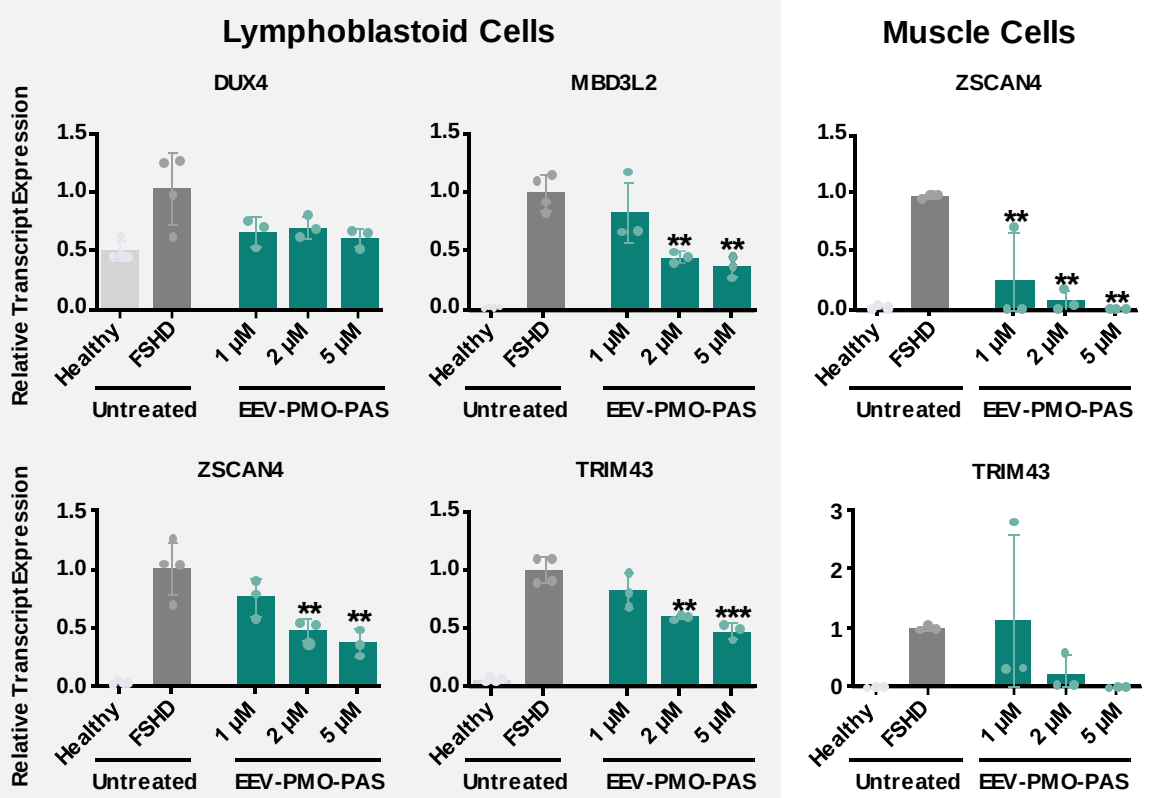
ZSCAN4

ZSCAN4 is a transcription factor involved in amplification of the DUX4 signaling cascade[3]

TRIM43

TRIM43 is elevated in myocytes of patients with FSH and is a biomarker of DUX4 activation[4]

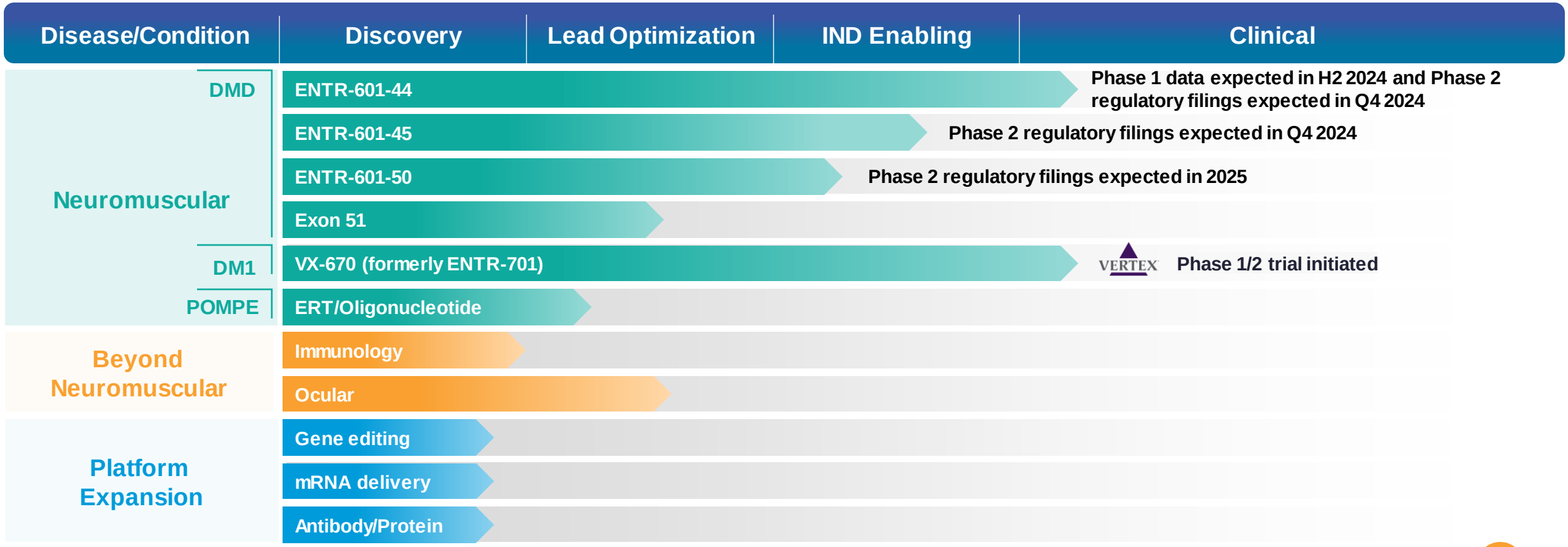
EEV-PMO-PAS In FSHD Patient-Derived Cells[5]



p<0.01, ****p<0.0001 vs. Vehicle. Values are shown as mean ± standard deviation. **1. Tihaya, M.S. et al. *Nat Rev Neurol*. 2023. **2.** Campbell, A.E. et al. eLife. 2018. **3.** Moccario, E. et al. *Cells*. 2021. **4.** Ferreboeuf M. et al. *Hum Mol Gen*. 2014. **5.** Li, X. et al. *Mol. Ther. Nucleic Acids* 2023. **EEV**, endosomal escape vehicle; **FSHD,** facioscapulohumeral muscular dystrophy; ***MBD3L2***, (methyl-CpG-binding domain protein 3 like 2; **PAS,** polyadenylation signal; **PMO**, phosphorodiamidate morpholino oligomer; ***ZSCAN4***, zinc finger and scan domain containing 4; **TRIM43**, tripartite motif containing 43.

ENTRADA THERAPEUTICS 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



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