EPI-331: A CRISPR-based epigenome editing as a mutation-agnostic treatment of Duchenne Muscular Dystrophy (DMD)

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ABSTRACT

Duchenne muscular dystrophy (DMD) is the most common X-linked neuromuscular disease, affecting approximately 1 in 3,500–5,000 live male births worldwide. Mutations in the DMD gene lead to loss of functional dystrophin, a protein essential for maintaining muscle fiber integrity. Without dystrophin, muscle cells are highly vulnerable to contraction-induced damage, leading to repeated cycles of degeneration and regeneration. Over time, this results in inflammation, fibrosis, progressive muscle weakness, loss of ambulation, and premature death due to cardiac or respiratory failure.

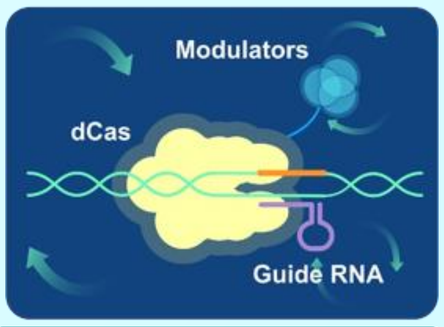
Utrophin (UTRN), a closely related paralog of dystrophin, shares key structural and functional features with dystrophin that can compensate for it's loss. In multiple preclinical animal models, increasing utrophin expression has been shown to reduce muscle pathology and improve function, supporting utrophin upregulation as a broadly applicable therapeutic strategy for DMD. Utrophin upregulation is expected to overcome the challenges that are restrictive with truncated micro-dystrophin constructs that often provide only partial functional benefit or immune response in some patients, and exon-skipping approaches that provides modest effectiveness for selective mutations.

Using the GEMS (**G**ene **E**xpression **M**odulation **S**ystem) platform of Epicrispr Biotechnologies- a clinical stage company, we developed EPI-331, a CRISPR-based epigenetic editing gene therapy product designed to selectively activate endogenous *UTRN*. EPI-331 uses a proprietary ultracompact, catalytically inactive Cas protein fused to a durable transcriptional activator and guided to the *UTRN* promoter by a sequence-specific guide RNA. The compact design allows delivery of the full system in a single AAV vector - the only currently approved delivery system for systemic delivery in muscular dystrophy. Utrophin activation was evaluated in human skeletal myoblasts at mRNA and protein using dPCR and/or qPCR and western blot, respectively. Further evaluations are ongoing in ex vivo DMD iPSC derived myoblasts in 3D engineered muscle tissue (3D EMTs) to assess utrophin upregulation and muscle strength. EPI-331 showed robust and durable activation of the utrophin gene (>10-fold) in human skeletal myoblasts. We are now advancing preclinical studies in vitro in DMD patient-derived myoblasts, and ex vivo in DMD (3D EMTs) to assess utrophin upregulation & localization, improvements in muscle function, membrane stability, and resistance to contraction-induced injury..

Taken together, our approach represents a single dose, mutation-independent gene therapy with the potential to benefit DMD patient population in a mutation independent manner by safely harnessing the natural compensatory capacity of utrophin without the risks associated with neo-antigenic dystrophin expression.

Epicrispr Biotechnologies - Who Are We?

- Clinical-stage CRISPR3.0 Epigenome Engineering Platform Biotech
- Proprietary **G**ene **E**xpression **M**odulation **S**ystem (GEMS) Platform - can modulate single or multiple genes persistently or transiently facilitating broad pipelines
- Compact modular system compatible with *in vivo* (AAV or LNP) and *ex vivo* (Lentivirus and Retrovirus) deliveries
- Exclusive License to CasMINI- smallest functional Cas effector in human cells
- Developed **EPI-321**, a next-generation epigenetic editing gene therapy for **Facioscapulohumeral Muscular Dystrophy (FSHD)**. Currently in Phase-I/II clinical trial (*NCT06907875*).



Overview of GEMS Platform

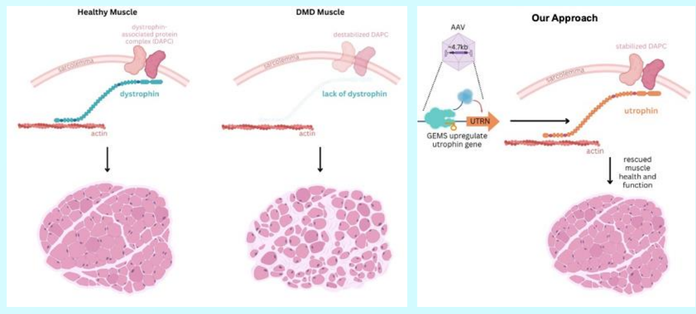
Background

(Source: www.CureDuchenne.org)

- Duchenne Muscular Dystrophy (DMD) is the most common muscular with ~17,000 individuals currently living with the disease in the US.
- It is caused by the loss of functional dystrophin (DMD) protein - a key structural protein that maintains muscle fiber integrity during contraction and relaxation.
- *DMD* is the largest known human gene (~2.4Mb; 79 exons).
- Over 7000 disease causing mutations are reported: ~65-70% deletions, ~10% duplications and ~20% point mutations/indels.
- High unmet need remains as there are no curative therapies. Current treatments (e.g., corticosteroids, exon-skipping, gene replacement) slow progression but do not fully restore dystrophin function.

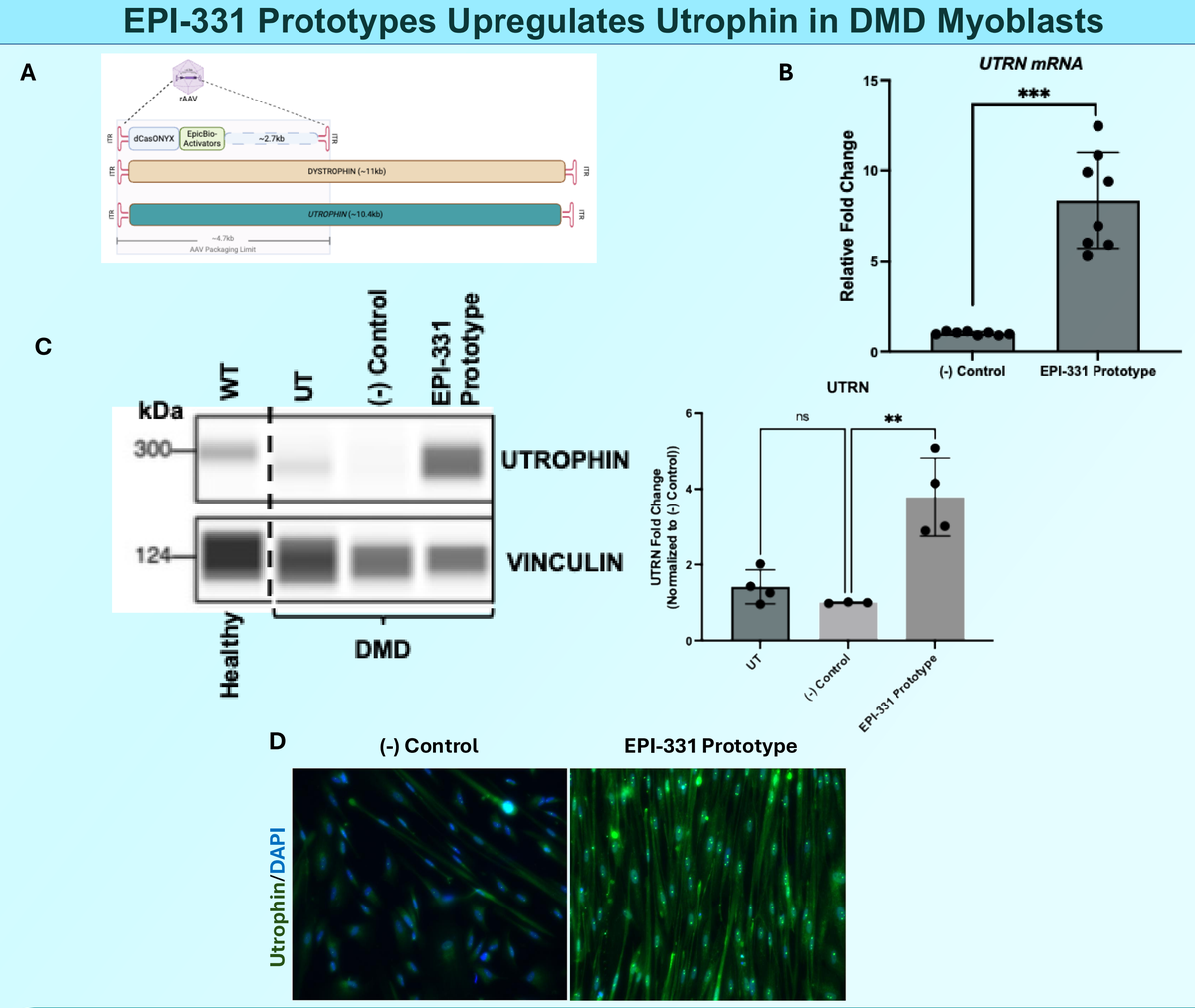
Rationale

- Utrophin is an autosomal paralog of dystrophin with high sequence and functional homology.
- Fetal utrophin is replaced by dystrophin at sarcolemma perinatally highlighting the high functional homology.
- No immune response against endogenous utrophin is expected as it is already expressed.
- Our approach of persistent epigenetic upregulation of utrophin by Epicripsr Biotechnologies' proprietary GEMS platform.
- Our activator has shown persistence activation of target gene over 40 days.[$]



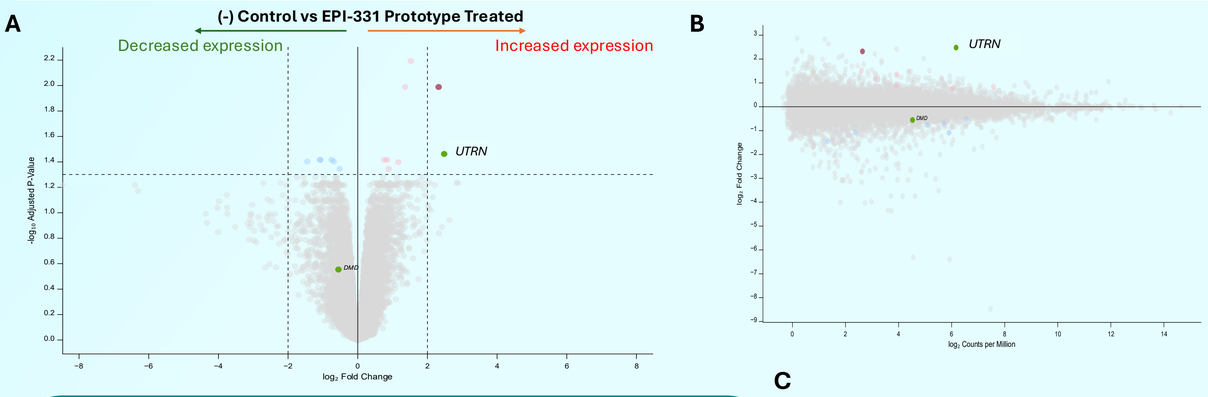
An illustration depicting the dystrophin protein role in muscle, and how Epicrispr's approach can address the loss of dystrophin protein by upregulation of Utrophin gene.

EPI-331 Prototypes Upregulates Utrophin in DMD Myoblasts



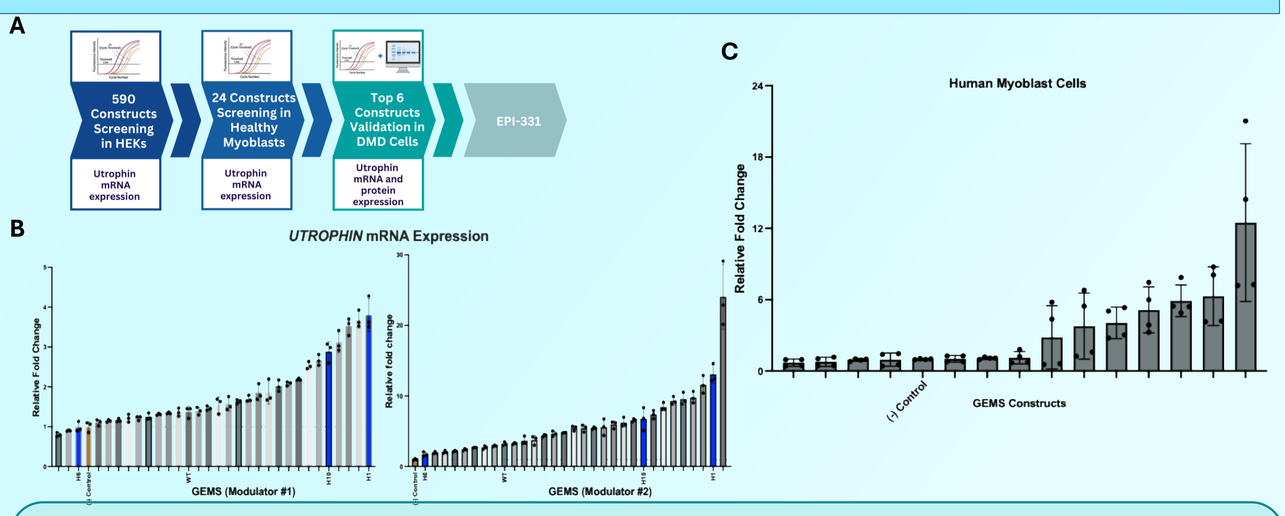
A. A schematic showing Epicripsr's proprietary activators are compact enough for a single AAV delivery. B. Utrophin mRNA expression in DMD patient–derived myoblasts ~10 days post-transduction measured by RT-qPCR. **C.** (Right panel) Representative Western blot showing utrophin protein upregulation following EPI-331 transduction in DMD myoblasts. (Left Panel) Quantification of utrophin protein levels from (B), normalized to non-targeting (–) control. Vinculin was used as a loading control. Data are presented as mean ± SD (n=4). **p<0.01, ***p<0.001 & ns-not significant. **D.** Cells in B were also stained for Utrophin (Green). DAPI (Blue) was used to stain the nuclei. A representative images were shown.

EPI-331 Prototype Shows Highly Specific Activation of Utrophin Without Affecting Dystrophin in DMD Myoblasts



A. Volcano plot of differentially expressed genes comparing EPI-331 prototype treated DMD cells to non-targeting (-) control (*adj. p-value* ≤0.05 and log2(fold-change)≥2). **B.** MA plot displaying log2 fold-change ((-)Control vs EPI-331 treated) vs transcript abundance demonstrating highly selective transcriptional modulation by EPI-331 prototype. **C.** Heatmap of selected differentially expressed genes demonstrating consistent transcriptional modulation across biological replicates upon EPI-331 prototype treatment.

GEMS Identifies Potent Activators of Utrophin in Human Cell Lines



A. Schematic outline of the experimental design of GEMS screening in human cell lines. **B.** GEMS screening in HEK293 cells identifies more potent GEMS of Utrophin expression. **C.** Top-15 GEMS from B. were further screened in human skeletal myoblasts. Cells were assayed by RT-qPCR for Utrophin expression in HEK293 cells (B) and human myoblasts (C) 3 days post-transfection and ~ 8 - 10 days post transduction (Day-6 of differentiation), respectively. (-) Control represents non-targeting guide control; WT represent no-transfection control. Bar graphs in blue are previously published activators of Utrophin (PMID: 40064877). The data plotted are means with SD.

Conclusion

- Epicrispr Biotechnologies is a clinical stages company with proprietary Gene Expression Modulation System (GEMS) platform that has identified EPI-321- first of its kind gene therapy to treat FSHD.
- The GEMS screening platform identifies highly efficient effector-modulator combination suitable for treating genetic disease with unmet need like DMD.
- EPI-331 potently activates endogenous *UTRN* expression, achieving comparable or superior activity to previously published approaches.
- RNA-seq analysis demonstrates highly selective transcriptional modulation, supporting a favorable specificity and safety profile for EPI-331.
- Preclinical studies in *ex vivo* 3D-EMTs are ongoing to assess functional rescue, membrane stability and resistance to contraction-induced injury.
- Epicrispr's GEMS platform enables a compact, single-AAV, one-time, mutation-independent epigenetic upregulation of endogenous utrophin for DMD.

[$](https://doi.org/10.1101/2023.06.02.543492)