CRISPR-Based Epigenetic Upregulation of Endogenous Utrophin as a Mutation-Agnostic Therapeutic Strategy for Duchenne Muscular Dystrophy

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ABSTRACT

Duchenne muscular dystrophy (DMD) is the most common, progressive neuromuscular disorder, with an X-linked genetic inheritance affecting approximately 1 in 3,500 to 5,000 live male births worldwide. It is caused by mutations in the DMD gene leading to the absence of functional dystrophin protein. Loss of dystrophin destabilizes sarcolemma, rendering myofibers highly susceptible to contraction-induced damage and resulting in chronic cycles of degeneration and regeneration. This pathological cascade leads to inflammation, necrosis, fibrosis, and progressive muscle wasting, culminating in loss of ambulation and premature mortality due to cardiopulmonary failure, typically by the third decade of life.

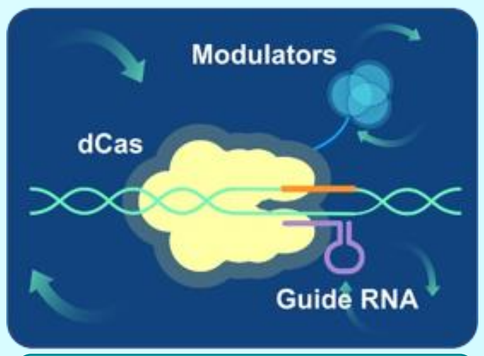
While recent advances in dystrophin-restoration therapies have shown promise, these approaches have faced challenges. Most mutation-independent gene therapies focus on replacing dystrophin. However, due to exceptionally large size of the gene and packaging limitations in promising delivery vectors, only engineered truncated versions called micro-dystrophin are widely being explored. While these engineered truncated dystrophins provide partial protection, they are inherently limited in restoring full protein function. In addition, exon skipping strategies are largely mutation-specific and applicable only to a subset of patients. An attractive alternative is the upregulation of utrophin, an autosomal dystrophin paralog with high structural and functional homology to full-length dystrophin. Utrophin can compensate for mutation-independent dystrophin deficiency, and its overexpression has been shown in preclinical models (mice and dogs) to mitigate muscle pathology and improve functional outcomes.

At Epicrispr Biotechnologies, we leveraged our proprietary, novel gene regulation platform-Gene Expression Modulation System (GEMS), to develop a drug candidate that drives robust upregulation of endogenous UTRN expression in disease relevant cell types through targeted epigenetic editing. Our system employs an ultracompact, catalytically inactive Cas protein (effector) fused to a persistent transcriptional activator (modulator), guided to the UTRN promoter-specific guide RNA. This compact design enables packaging of the full therapeutic cassette into a single AAV vector suitable for in vivo delivery. We have shown successful activation of utrophin in human cells and are currently advancing preclinical studies to evaluate the efficacy of our utrophin-targeting therapy in dystrophic mouse models and in DMD patient-derived iPSC 3D muscle organoids. These studies aim to assess phenotypic rescue including muscle contractility and resistance to contraction-induced injury.

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

Epicrispr Biotechnologies - Who Are We?

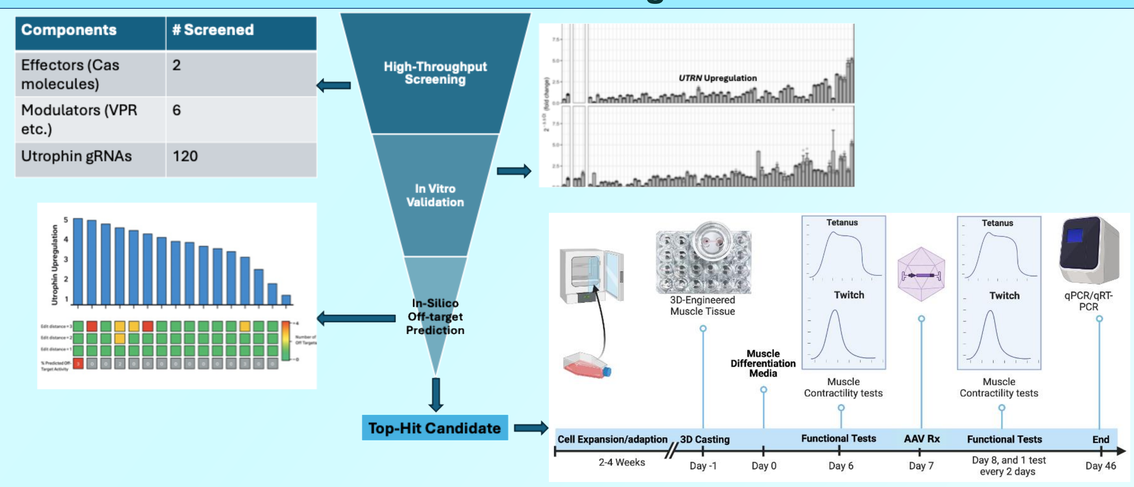
- Clinical Stage CRISPR3.0 Epigenome Engineering Platform Biotech
- Proprietary **G**ene **E**xpression **M**odulation **S**ystem (GEMS) Platform
- GEMS can modulate single or multiple genes persistently or transiently facilitating broad pipelines
- Compact and interchangeable components that can support regulation of single and multiple genes *in vivo* (AAV or LNP) and *ex vivo* (Lentivirus and Retrovirus)
- Exclusive License to CasMINI- smallest known Cas effector shown to function 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



Overview of GEMS Platform

Robust GEMS Screening Platform

Components	# Screened
Effectors (Cas molecules)	2
Modulators (VPR etc.)	6
Utrophin gRNAs	120



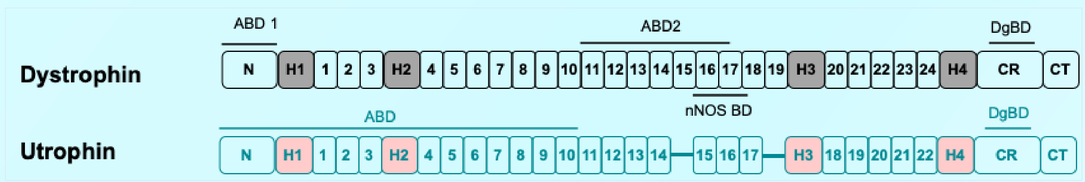
Schematic outline of the experimental design of GEMS screening Platform. (Top Left) Table illustrating number of components are screened and tested during high-throughput screening stage for the program. (Middle Right) A representative qPCR expression of Utrophin illustrating an *in vitro* validation of top candidates in relevant cell types. (Bottom Left) Top candidates from in vitro validation assay are further analyzed for potential off-targets *in silico* to identify "Top-Hit" candidate with maximum efficacy and no off-target. The top-hit is assayed for both utrophin upregulation and functional rescue in 3D EMT using appropriate dystrophic human cells.

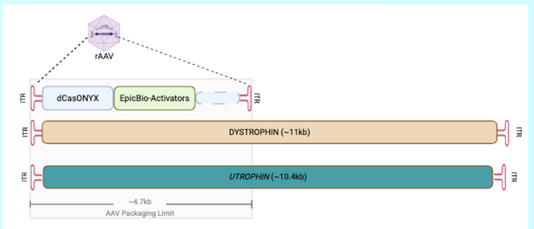
Background

(Source: www.CureDuchenne.org)

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

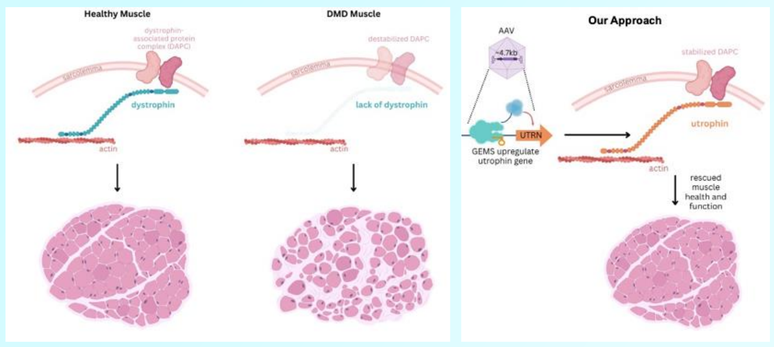
Rationale





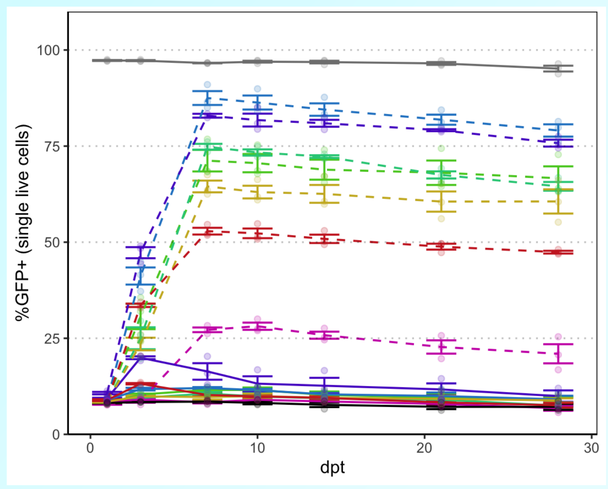
A schematic showing Epicripsr's proprietary activators are compact enough for a single AAV delivery.

- Utrophin is autosomal paralog of Dystrophin protein that shares high sequence 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(https://doi.org/10.1101/2023.06.02.543492)



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.

GEMS Identifies Potent Activators of Utrophin in Human Cell Line



A. A persistent activation of reporter gene expression with GEMS in TREG3 cells. The cells were assayed till 28 days post-transient transfection (dpt). **B.** Preliminary screening with two modulators identifies more potent GEMS (activators) of Utrophin expression in HEK293 cells than previously identified. qRT-PCR assay of Utrophin expression in HEK293 cells 3 days post-transfection of GEMS. (-) 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's GEMS screening platform identifies highly efficient effector-modulator combination suitable for treating genetic disease with unmet need like DMD, FSHD.
- Current dystrophin-restoration strategies (micro-dystrophin, exon skipping) face significant limitations, leaving many patients without effective treatment.
- Utrophin, a natural dystrophin paralog, offers mutation-independent compensation and has demonstrated functional benefits in preclinical models (PMID: 40064877).
- In preliminary analysis, we have identified GEMS that are potent activators of Utrophin gene comparable or better than previously published.
- Epicrispr's GEMS platform enables targeted, durable upregulation of endogenous utrophin via compact, even AAV-deliverable one-time epigenetic editing therapy that has the potential to broadly benefit DMD patients by safely leveraging utrophin's inherent compensatory function.