Preclinical In Vitro and Ex Vivo Evaluation of EPI-321, an Investigational Single Dose Epigenome Editing Gene Therapy, Efficacy for Facioscapulohumeral Muscular Dystrophy (FSHD) Treatment

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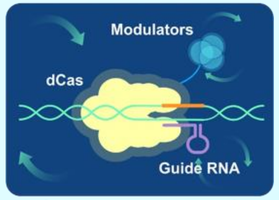
Epicrispr Biotechnologies - Who We Are?

- CRISPR3.0 Epigenome Engineering Platform Biotech
- Proprietary Gene Expression Modulation System (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



Overview of GEMS Platform

ABSTRACT

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common types of adult muscular dystrophies with an annual incidence rate of ~ 1 in 10,000, affecting approximately 1 million people globally. With no cure available, current therapeutic strategies only involve managing symptoms to improve overall quality of life. Misexpression of disease-causing protein, DUX4, in muscle leads to slow and progressive muscle degeneration through activation of apoptotic and other downstream pathways. DUX4 gene is encoded at the distal region 4q35 chromosome from D4Z4 macrosatellite array. In FSHD patients, the D4Z4 macrosatellite array is hypomethylated, leading to stochastic and transient DUX4 expression, which makes the development of cure challenging.

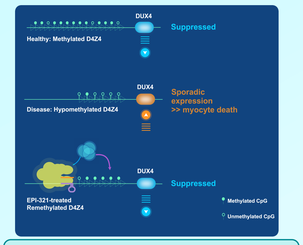
EPI-321 is an investigational drug product for the treatment of FSHD. It is a single vector AAVrh74 encoding an ultracompact, dead Cas protein fused to gene-suppressing modulators expressed from a muscle specific promoter, and a gRNA targeting D4Z4 locus to permanently suppress DUX4 expression through re-methylation of the D4Z4 locus.

Our pre-clinical studies showed that EPI-321 administration leads to robust and dose-dependent transduction and expression of AAV cargo, consequential suppression of DUX4 and DUX4-downstream gene expression in ten different FSHD patient-derived immortalized and primary myoblasts in vitro, irrespective of the number of D4Z4 repeats, and showed significant anti-apoptotic activity. Mechanistically, EPI-321 showed re-methylation of the D4Z4 target locus leading to suppression of DUX4 expression. Further, 3D engineered human muscle tissue (3D EMT) using FSHD patient-derived immortalized myoblasts transduced by EPI-321 resulted in efficient suppression of DUX4 up to 46 days and demonstrated significant dose dependent improvement in muscle contractility and strength, shown by increased twitch and tetanic force post-treatment.

Taken together, our findings provide robust evidence for EPI-321 as a potential single-administration, “one-and-done”, gene therapy for treating FSHD by permanently suppressing the pathogenic DUX4 gene through epigenetic silencing. We intend to submit an IND application and are looking forward to commencing first-in-human trials in 2025.

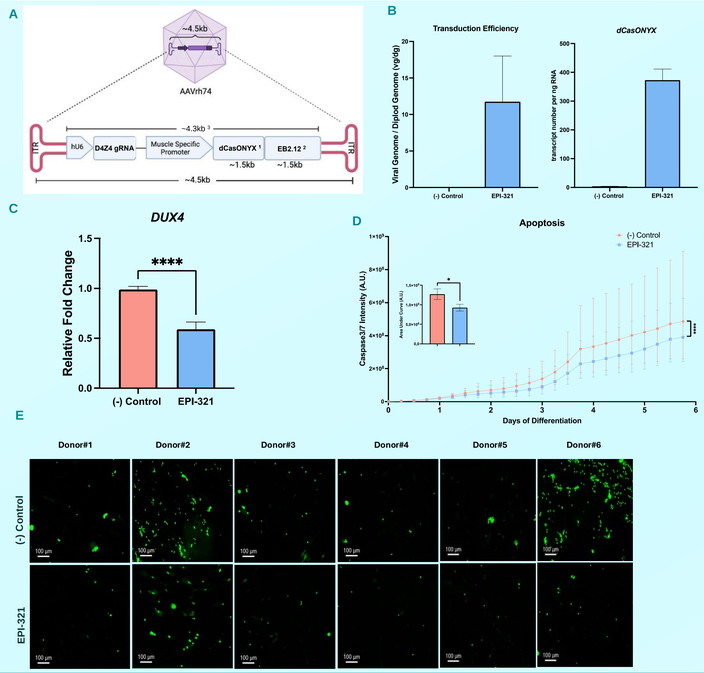
BACKGROUND

- Facioscapulohumeral Muscular Dystrophy (FSHD) is a debilitating genetic disorder leading to progressive muscle degeneration.
- Progressive weakness resulting in loss of movement of the face and loss of extremity function and mobility.
- Muscle degeneration pathology due to increased muscle cell death.
- Epigenetic rare disease due to loss of methylation that leads to DUX4 “mis-expression” in skeletal muscle.



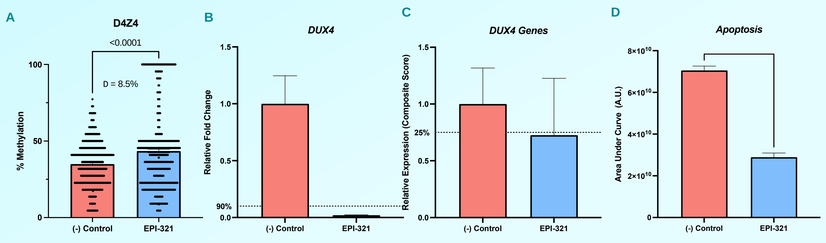
Molecular Mechanism of *DUX4* Regulation and EPI-321 Approach to Treat FSHD

EPI-321 Represses DUX4 and Rescues Apoptosis In FSHD Patients-Derived Myoblasts



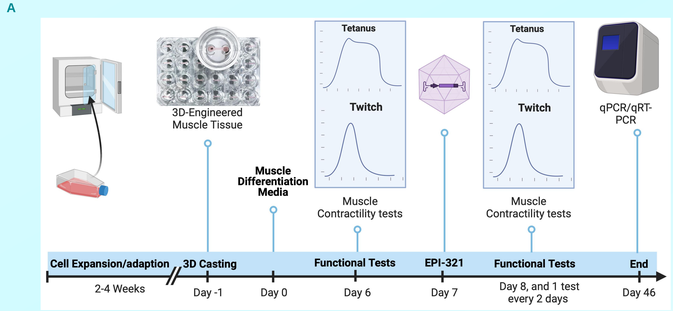
A. EPI-321 AAV Design. [1]**Safety:** EPI-321 utilizes a proprietary library of compact nuclease-dead versions of CRISPR (dCas), resulting in **NO DNA cuts.** [2]**Precision:** EPI-321 controls expression of the endogenous gene through methylation of the target sequence. [3]**Delivery:** EPI-321 is ultracompact, allowing it to be packaged it into AAVrh74. B. Transduction efficiency of EPI-321 estimating AAV genome copy using dPCR (left panel) & mRNA expression of cargo, dCasONYX (right panel) in 6 primary FSHD myoblasts. **C.** mRNA expression of DUX4 in EPI-321 and control treated myoblasts. **D.** Apoptosis analysis over the course of differentiation assayed in 6-different primary myoblasts using live cell imaging. Geometric means of Caspase 3/7 signal each myoblasts are plotted. Areas under the curve is shown in inset. **E.** A representative images of the cells assayed in C. at the experimental endpoint. Caspase 3/7$^+$ cells stained in green.*p<0.05, ****p<0.0001

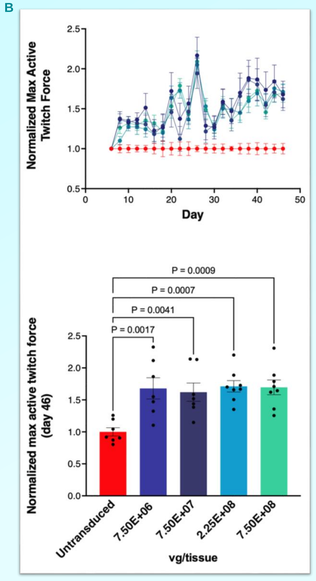
EPI-321 Rescues D4Z4 Epigenome through DNA Re-Methylation in FSHD Patient-derived Myoblasts

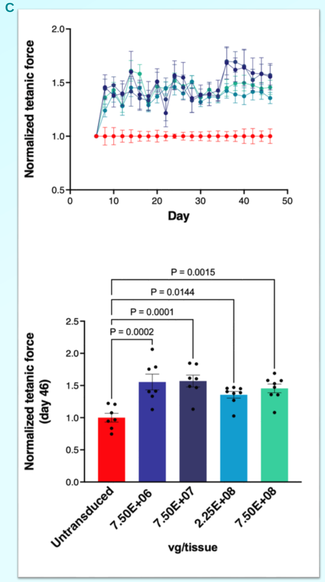


EPI-321 restores D4Z4 epigenome through DNA re-methylation in patient derived FSHD myoblasts. **A.** FSHD patient-derived primary myoblasts treated with EPI-321 shows increased methylation compared to (-) Control test article treated myoblasts. The genomic DNA extracted at the assay endpoint of day 7 of differentiation were analyzed using an NGS-method for targeted methylation for higher depth and sensitivity. **B-C.** mRNA expression of *DUX4* (B) and 6 DUX4 genes namely MBD3L2, ZSCAN4, LEUTX, TRIM43, TRIM48 & RFPL2 (C) in sample from A. Geometric means of relative expressions of 6 DUX4-genes expression are plotted in C. **D.** Primary myoblasts from A assayed for apoptosis over the course of differentiation by imaging Caspase3/7 apoptotic signal. Area Under Curve (AUC) were plotted that shows decreased apoptosis in cells treated with EPI-321. **p<0.01

EPI-321 Improves Contractility in Patient-Derived Engineered Muscle Tissue (EMT)







EPI-321 treatment of FSHD 3D organoid tissues improves functional twitch and tetanus forces at multiple doses. A. Experimental outline of the functional *ex vivo* 3D organoid tissue assay. **B**. Normalized max active twitch force was plotted over time (above) and at the end point at day 46 (below) in 3D organoid tissue. **C**. Normalized tetanic force was plotted over time (above) and at the end point at day 46 (below) in 3D organoid tissue.

CONCLUSION

- Epic Bio’s GEMS screening platform identifies highly efficient effector-modulator combination suitable for treating genetic disease with unmet need like FSHD.
- EPI-321 is a compact AAV product that utilizes hypercompact nuclease-dead Cas molecule and modulates endogenous gene through methylation of target sequence.
- EPI-321 represses DUX4 target locus and decreases expression of downstream DUX4-pathway genes expression *in vitro* FSHD patient derived primary and immortalized myoblasts.
- EPI-321 improves functional twitch and tetanus forces in *ex vivo* 3D organoid tissues from FSHD myoblast.
- EPI-321 also rescues the apoptosis level *in vitro* in patient derived myoblast and improve FSHD myoblasts.
- EPI-321 demonstrates a safety profile in preclinical safety studies indicating the readiness for first in human studies