# Efficacy and Safety of EPI-321, an Investigational Single Dose Epigenome Editing Therapy Targeting D4Z4 in Facioscapulohumeral Muscular Dystrophy (FSHD)

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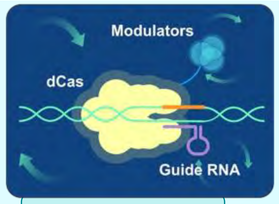
## Epic Bio - Who We Are?

- 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 common types of adult muscular dystrophies with an annual incidence rate of ~ 1 in 10,000 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 skeletal muscle leads to muscle loss through increased apoptosis. DUX4 gene is encoded in sub-telomeric D4Z4 macrosatellite array in chromosome 4. Irrespective of the FSHD disease subtype, the D4Z4 macrosatellite array is hypomethylated that leads to stochastic and transient DUX4 expression that further makes the development of cure challenging.

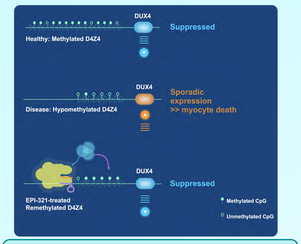
EPI-321 is an investigational drug product for the treatment of FSHD. It is a single vector AAVrh74 encoding an ultracompact, dead Cas protein fused to gene-suppressing modulators, and a gRNA targeting D4Z4 locus to permanently suppress DUX4 expression through re-methylation of the D4Z4 locus.

Our preclinical studies showed that EPI-321 leads to dose-dependent suppression of DUX4 (>50%) and DUX4-downstream genes expression in 10 different FSHD patient-derived myoblasts in vitro, irrespective of the number of D4Z4 repeats, and showed significantly decreased apoptosis. EPI-321 showed re-methylation of the D4Z4 target locus leading to suppression of DUX4. In vivo evaluation of EPI-321 in humanized FSHD mice showed a dose-dependent suppression of DUX4-pathway, and anti-apoptotic activity in muscle at 4 weeks endpoint. Additionally, twitch and tetanic forces were significantly improved in 3D engineered FSHD muscle tissues upon EPI-321 treatment assayed over 46 days. The safety of EPI-321 was assessed in both mice and non-human primate (NHP) for 3- and 6-months. This comprehensive examination encompassed in-life observations, clinical and anatomic pathological examinations, immunogenic response, biodistribution studies, spatial distribution in the gonads, vector shedding and off-target effects. These analyses underscored the favorable safety profile of EPI-321.

Our findings provide robust evidence for EPI-321 as a “one-and-done” gene therapy for treating FSHD by permanently suppressing DUX4. We intend to submit an IND application and are looking forward to commencing first-in-human trials in 2025.

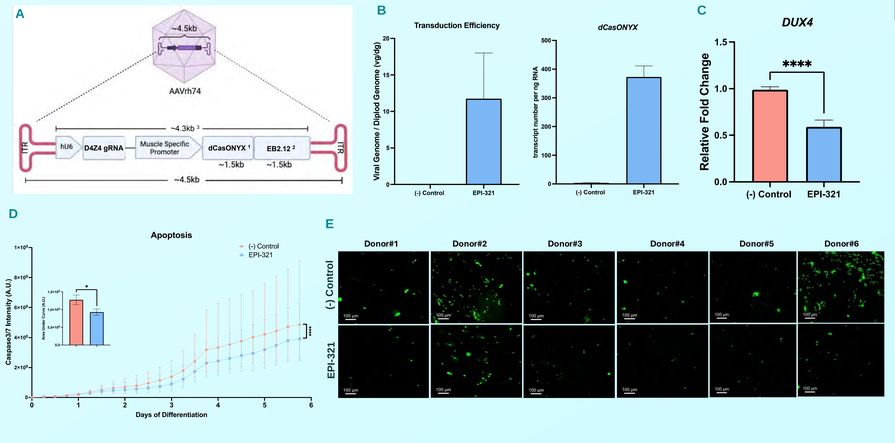
## BACKGROUND

- 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.



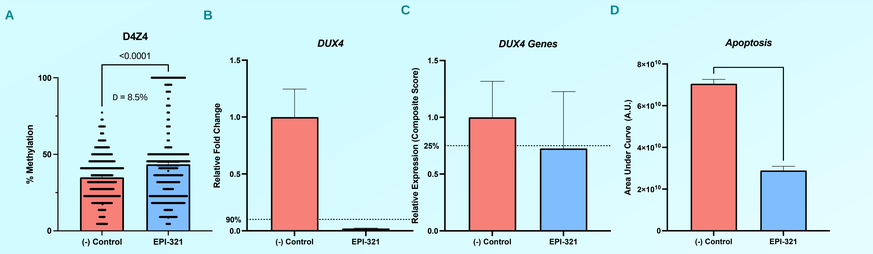

Molecular Mechanism of *DUX4* Regulation and EPI-321 Approach to Treat FSHD

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



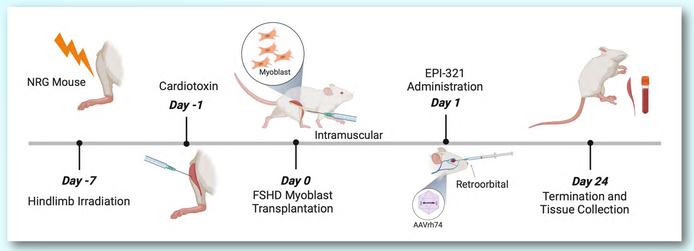

**A**. EPI-321 AAV Design. [1]**Safety:** EPI-321 utilizes a proprietary library of compact nuclease-dead versions of CRISPR (dCas), resulting in **NO DNA cuts.** [2]**Precision:** EPI-321 controls expression of the endogenous gene through methylation of the target sequence. [3]**Delivery:** EPI-321 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 Primary Myoblasts

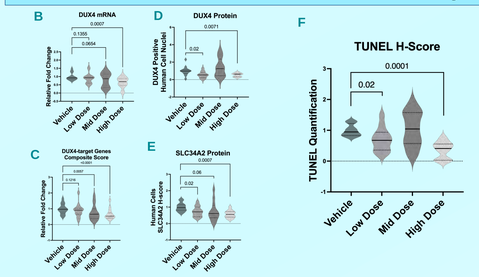



**EPI-321 restores D4Z4 epigenome through DNA re-methylation in patient derived FSHD myoblasts**. **A.** FSHD patient-derived primary myoblasts treated with EPI-321 shows increased methylation compared to (-) Control test article treated myoblasts. The genomic DNA extracted at the assay endpoint of day 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 Shows Dose-Dependent Suppression of *DUX4 &* DUX4-genes With Improvement in FSHD Muscle Cell Survival In Humanized Mice In Three Genetically Different FSHD Patients

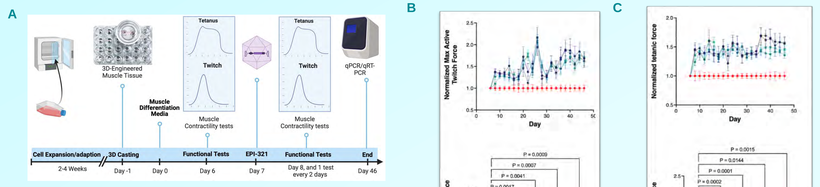



## EPI-321 Shows Dose-Dependent Suppression of *DUX4 &* DUX4-genes With Improvement in FSHD Muscle Cell Survival In Humanized Mice Using 3 Different Patient-Derived Myoblasts (Continued..)



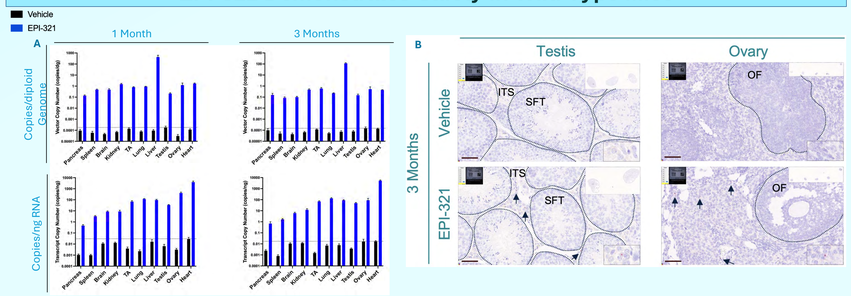

**EPI-321 represses DUX4 and improves DUX4-associated phenotypes in humanized mice. A**. Schematic showing *in vivo* experimental outline using humanized mice model. **B.** mRNA expression of *DUX4* in humanized TA muscle tissue of mice. **C**. Animal tissue as in B were assayed for DUX4 pathway genes expression were plotted as composite score. **D-E**. Tissues in B. were stained for DUX4 protein (D), SLC34A2 protein (E) - a biomarker for DUX4 activity by IHC. Images were quantified and plotted . **F.** Tissues in B. were stained for TUNEL- a marker for DNA damage and apoptosis. Images were quantified and plotted.

## 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.

## EPI-321 Demonstrates Safety in Wild Type Mice




**EPI-321 safety profile in wild type mice. A.** Mouse administered with EPI-321 were terminated at 1 month and 3 months post dosing. DNA and RNA isolated is probed for EPI-321 transgene and transcript by qPCR and RT-qPCR respectively. **B.** 4mm slices of fixed tissues were hybridized by RNAscope probe targeting EPI-321 trasgene. Left panel, Testis., Right panel. Ovary. ITS. Intertubular space, SFT. seminiferous tubule, OF. ovarian follicle. Scale. 100mm

## 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 both *in vitro* FSHD patient derived myoblasts and humanized *in vivo* mice model.
- 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 survival in humanized mice model *in vivo*.
- EPI-321 demonstrates a safety profile in preclinical safety studies indicating the readiness for first in human studies