EPI-321: A Novel Epigenetic Gene Therapy for FSHD Targeting D4Z4 Epigenome

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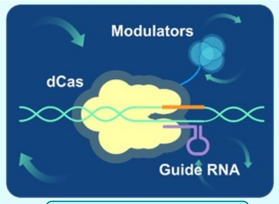
Epic Bio - Who We Are?

- 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 most common types of adult muscular dystrophies with an annual incidence rate of about 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.

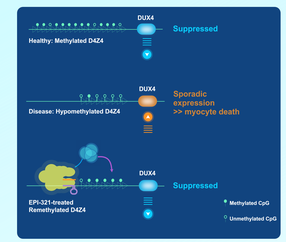
At Epic-Bio, we leverage our proprietary Gene Expression Modulation System (GEMS) platform to develop a treatment for FSHD that targets the D4Z4 epigenome and permanently suppress *DUX4* expression. Our product, EPI-321 is a single vector AAV serotype *rh74* encoding an ultracompact, catalytically inactive Cas protein (effector) fused to gene-suppressing modulators, under the expression of the muscle specific promoter, *CK8e*, and a guide RNA targeting *D4Z4* locus.

Our preclinical studies showed that EPI-321 administration leads to robust and dose-dependent 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 antiapoptotic activity as measured by Caspase 3/7 staining. Mechanistically, EPI-321 showed re-methylation of the D4Z4 target locus leading to suppression of *DUX4* expression. Further, *in vivo* evaluation of EPI-321 in humanized FSHD mouse model showed a dose-dependent suppression of DUX4-pathway at the mRNA and protein level, and antiapoptotic activity in muscle tissues. Additionally, 3D engineered human muscle tissue (3D EMT) using FSHD patient-derived immortalized myoblasts transduced by EPI-321 resulted in efficient suppression of *DUX4* and DUX4-pathway genes up to 46 days and demonstrated significant dose dependent improvement in muscle contractility, shown by increased twitch and tetanic force post-treatment.

Taken together, our findings provide robust evidence for EPI-321 as a potential single-administration gene therapy for treating FSHD by permanently suppressing the pathogenic *DUX4* gene through epigenetic silencing. We intend to submit an Investigational New Drug (IND) application this year and are looking forward to commencing first-in-human trials in 2024.

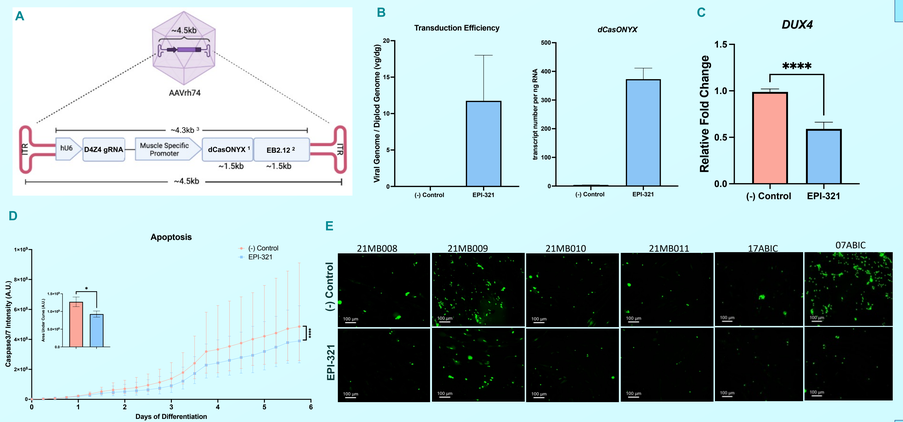
BACKGROUND

- 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.



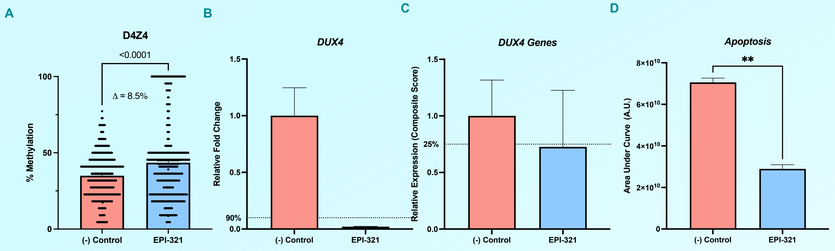
Molecular Mechanism of *DUX4* Regulation and EPI-321 Approach to Treat FSHD

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



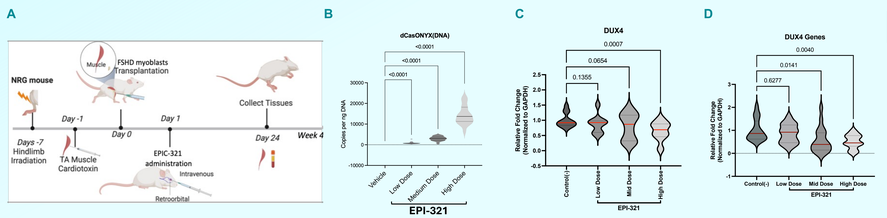
A. EPI-321 AAV Design. [1]**Safety:** EPI-321 utilizes a proprietary library of compact nuclease-dead versions of CRISPR (dCas), resulting in **NO DNA cuts.** [2]**Precision:** EPI-321 controls expression of the endogenous gene through methylation of the target sequence. [3]**Delivery:** EPI-321 is ultracompact,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

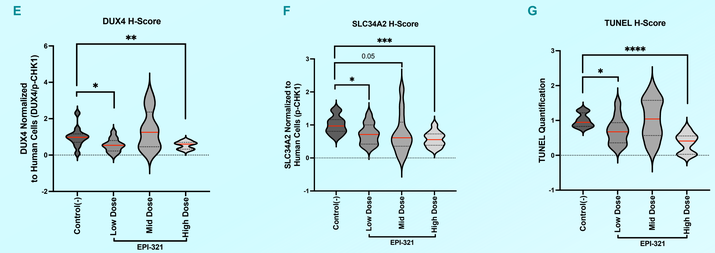


EPI-321 restores D4Z4 epigenome through DNA re-methylation in patient derived FSHD myoblasts. **A**. FSHD patient-derived primary myoblasts treated with EPI-321 shows increased methylation compared to (-) Control test article treated myoblasts. The genomic DNA extracted at the assay endpoint of day 7 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 With Three Genetically Different FSHD Patients

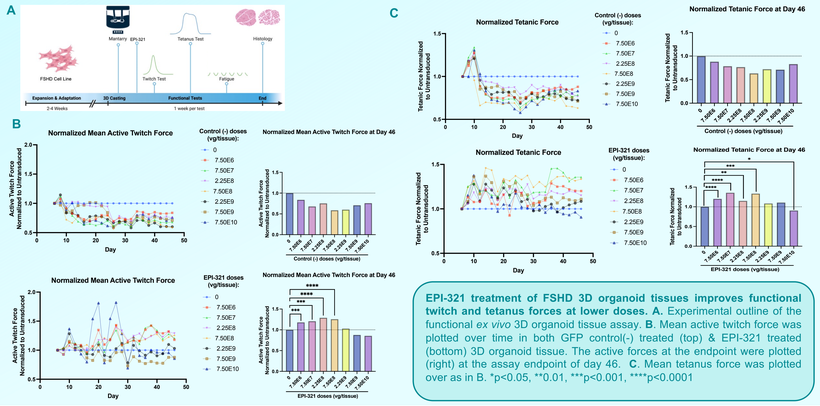


EPI-321 Shows Dose-Dependent Suppression of *DUX4* & DUX4-genes With Improvement in FSHD Muscle Cell Survival In Humanized Mice Using 3 Different Patient-Derived Myoblasts (Continued..)



EPI-321 represses DUX4 and improves DUX4-associated phenotypes in humanized mice. A. Schematic showing *in vivo* experimental outline using humanized mice model. **B.** Biodistribution of EPI-321 assayed by qPCR estimate of EPI-321 viral genome in TA muscle tissues of mice that were treated at three different doses of EPI-321. **C.** mRNA expression of *DUX4* in humanized TA muscle tissue of mice from B. **C**. Animal tissue as in B were assayed for DUX4 pathway genes *ZSCAN4, MBD3L2, LEUTX, TRIM43*. Pathway genes expression were plotted as composite score. **D**. . **E-F**. Tissues in B. were stained for DUX4 protein (E), SLC34A2 protein (F) - a biomarker for DUX4 activity. Images were quantified and plotted . **G**. Tissues in B. were stained for TUNEL- a marker for DNA damage and apoptosis. Images were quantified and plotted. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

EPI-321 Improves Twitch and Tetanus Forces In 3D *ex vivo* FSHD Organoid



EPI-321 treatment of FSHD 3D organoid tissues improves functional twitch and tetanus forces at lower doses. A. Experimental outline of the functional *ex vivo* 3D organoid tissue assay. **B**. Mean active twitch force was plotted over time in both GFP control(-) treated (top) & EPI-321 treated (bottom) 3D organoid tissue. The active forces at the endpoint were plotted (right) at the assay endpoint of day 46. **C**. Mean tetanus force was plotted over as in B. *p<0.05, **0.01, ***p<0.001, ****p<0.0001

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*.

ACKNOWLEDGEMENT

SOLVE FSHD

UMass Chan MEDICAL SCHOOL
Wellstone Center of FSHD

University of Rochester Medical Center
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