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EPI-321: A Promising Gene Therapy for Facioscapulohumeral Muscular Dystrophy (FSHD) Targeting D4Z4 Epigenome

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ABSTRACT

Facioscapulohumeral muscular dystrophy (FSHD) affects 800,000 globally with no cure available, current therapies only manage symptoms. Disease-causing DUX4 protein expression in muscle leads to progressive muscle wasting through activation of apoptotic and other pathways. *DUX4* is encoded in the distal region of *4q35* chromosome from D4Z4 microsatellite array, which is hypomethylated in FSHD leading to *DUX4* expression.

At Epic Bio, we leveraged our proprietary GEMS platform to develop EPI-321, a treatment for FSHD that targets the D4Z4 epigenome and suppresses DUX4 expression permanently. EPI-321 is an AAV serotype rh74 vector with a catalytically inactive Cas protein fused to gene-suppressing modulators and a gRNA targeting D4Z4. EPI-321 showed no off-target to any known human protein coding gene *in silico*.

We showed that EPI-321 robustly suppress DUX4 and downstream genes in patient derived myoblasts *in vitro,* irrespective D4Z4 repeat length. Functionally, *in vitro* treatment of FSHD myoblasts with EPI-321 decreased rate of apoptotic nuclei to the level of normal sibling control. Further, we showed robust delivery and expression of EPI-321 in the humanized muscle tissue *in vivo* following a single intravenous dose in mice. In addition to decreasing the DUX4 pathway, EPI-321 was able to rescue FSHD muscle cell survival by 55% after 4 weeks of treatment. Importantly, EPI-321 in mice and NHP demonstrated no signs of toxicity, with no abnormal clinical, histopathological, or blood chemistry responses, indicating the safety of the treatment.

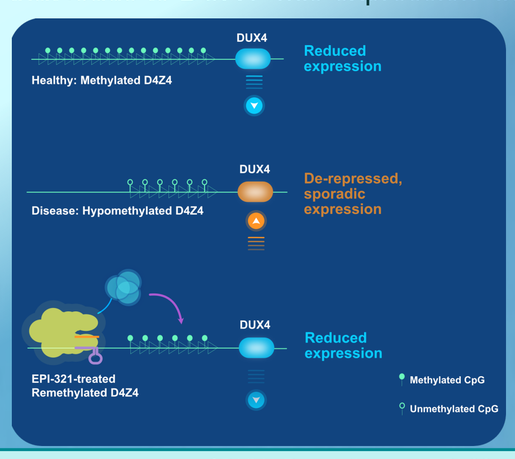
Our findings support EPI-321 as a potential gene therapy for FSHD, with IND submission planned for 2023 and first-in-human trials in 2024.

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

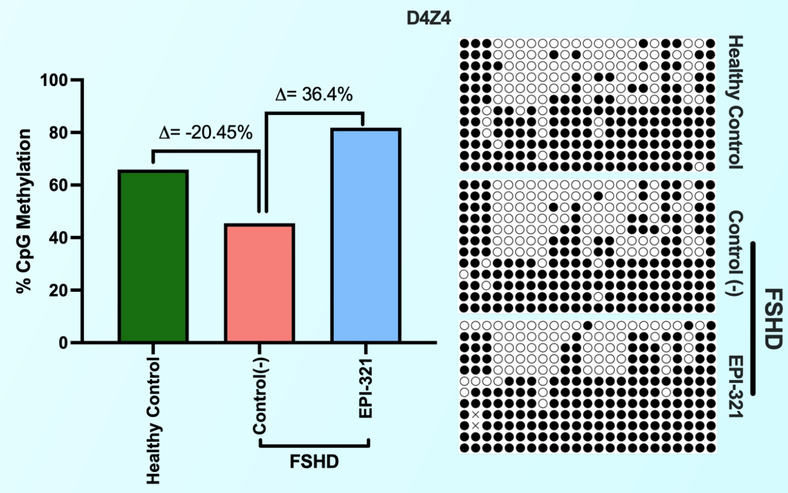
Epidemiology
- US Population: 16,000-38,000
- Global Population: 300,000-780,000
- One of the Most Common Adult Muscular Dystrophy

Standard-of-Care
- No disease-modifying drug available
- Exercise has been shown to reduce chronic fatigue and decelerate fatty infiltration of muscle in FSHD
- Surgery to treat scapulothoracic fusion



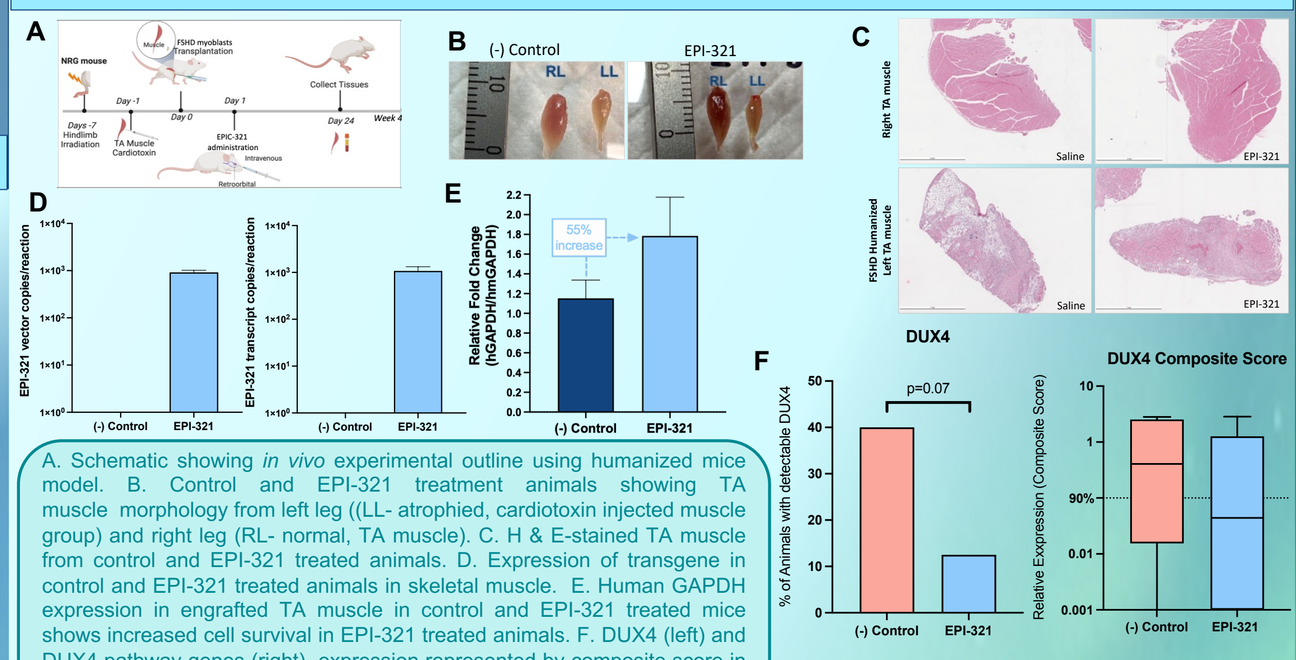
Molecular Mechanism of DUX4 Regulation and EPI-321 Approach to Treat FSHD

EPI-321 Represses DUX4 Expression through Re-methylation of D4Z4



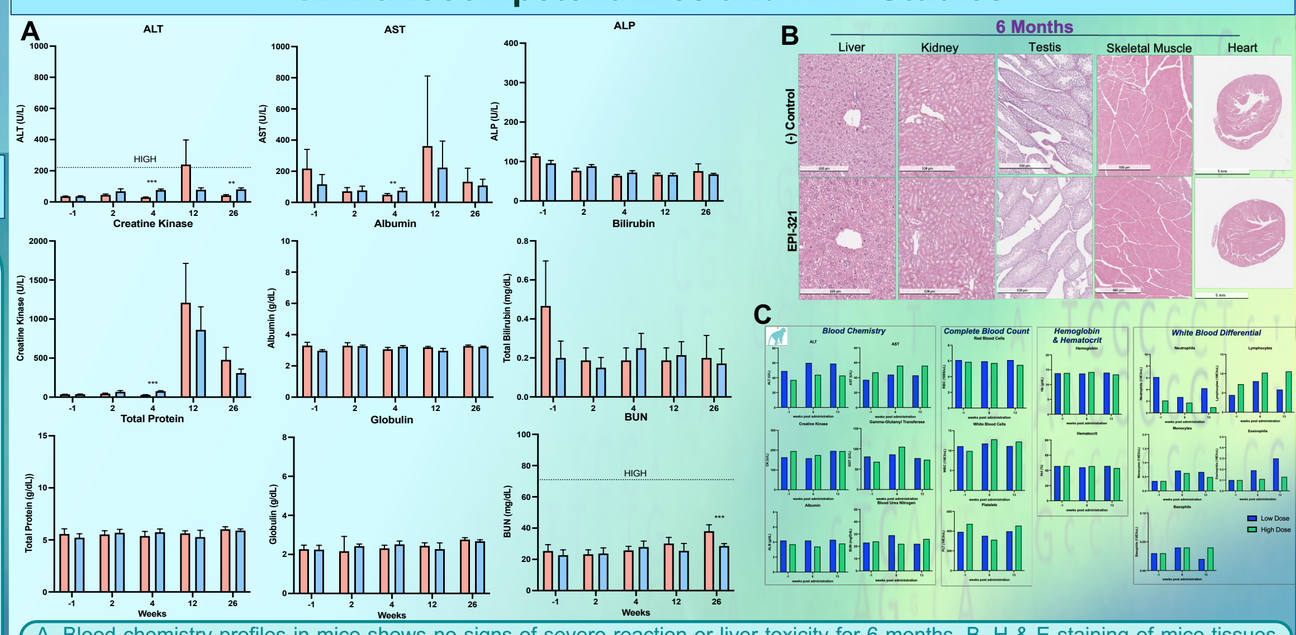
EPI-321 restores D4Z4 epigenome through DNA re-methylation in patient derived FSHD myoblasts. Percent of CpG methylation at EPI-321 targeting D4Z4 locus in healthy sibling control, FSHD patient derived myoblasts treated with control AAV and EPI-321 (Left panel). Median % CpG methylation assayed by Enzymatic conversion of DNA followed by sanger sequencing of the target loci. Sequences are analyzed using QUMA tool with default parameters. A representative lollipops plot is shown (Right panel).

EPI-321 Improves FSHD Muscle Cell Survival and Suppresses DUX4 In Humanized Mice



A. Schematic showing *in vivo* experimental outline using humanized mice model. B. Control and EPI-321 treatment animals showing TA muscle morphology from left leg ((LL- atrophied, cardiotoxin injected muscle group) and right leg (RL- normal, TA muscle). C. H & E-stained TA muscle from control and EPI-321 treated animals. D. Expression of transgene in control and EPI-321 treated animals in skeletal muscle. E. Human GAPDH expression in engrafted TA muscle in control and EPI-321 treated mice shows increased cell survival in EPI-321 treated animals. F. DUX4 (left) and DUX4 pathway genes (right) expression represented by composite score in control and EPI-321 treated humanized mice model of FSHD.

EPI-321 Shows No Signs of Toxicity or Adverse Events in Non-GLP Immunocompetent Mice and NHP Studies

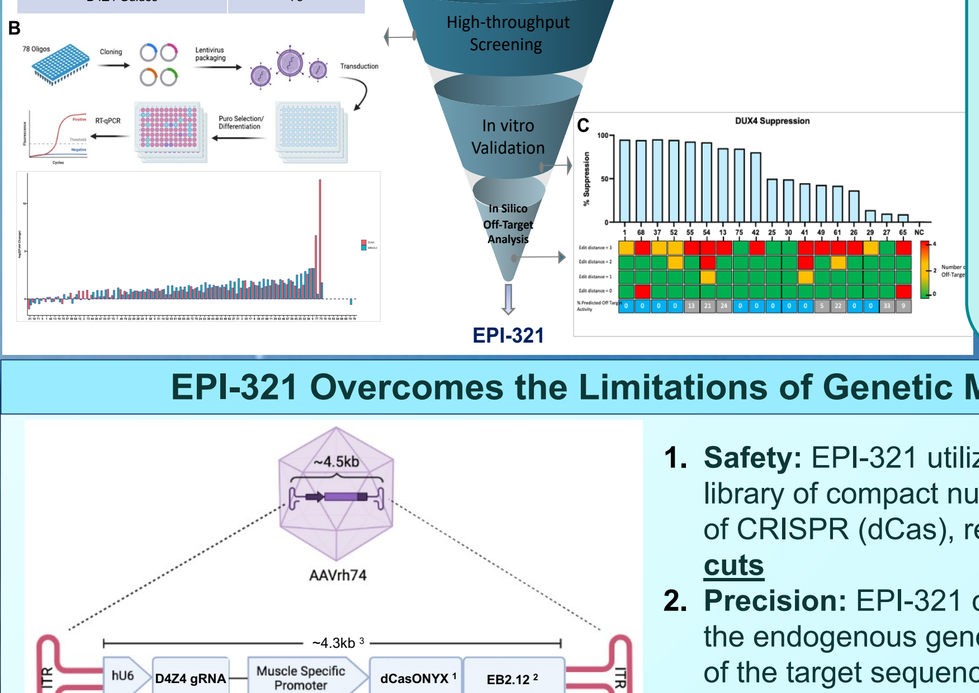


A. Blood chemistry profiles in mice shows no signs of severe reaction or liver toxicity for 6-months. B. H & E staining of mice tissues from saline and EPI-321 injected mice after 6-months. C. Blood chemistry and profile in low and high dose EPI-321 injected NHP shows no adverse events, liver toxicity or severe immune response for 3 months post injection with EPI-321 indicating the safety profile of EPI-321.

Robust GEMS Screening Platform Identifies EPI-321

A

Components	# Screened/Tested
Effectors (Cas molecules)	2
Modulators (KRAB etc.)	4
D4Z4 Guides	78



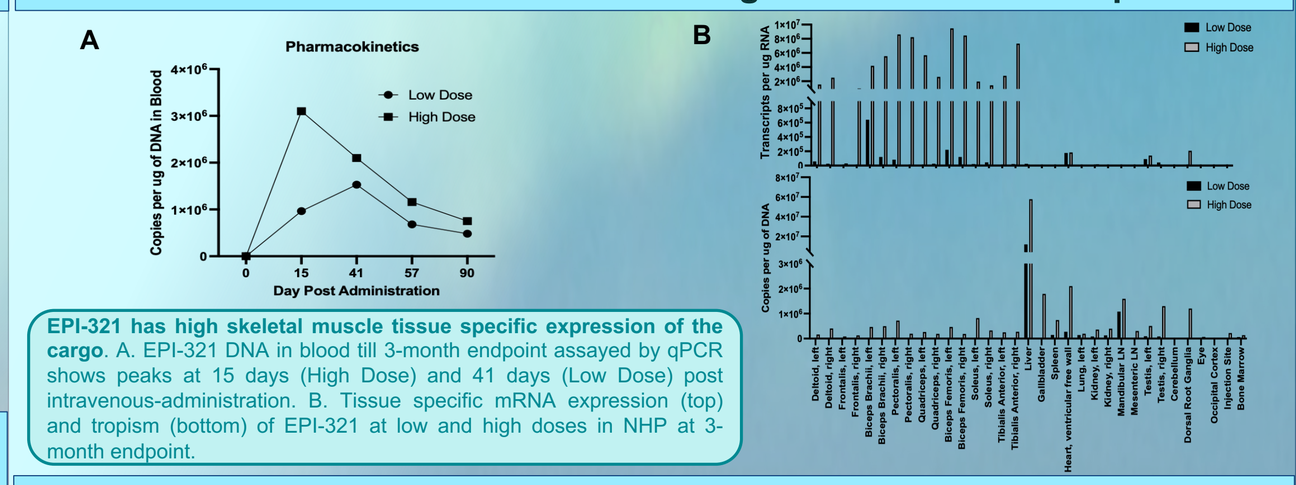
A. Table illustrating number of components screened and tested. B. Schematic outline of the experimental design of the screening assay (Top), and qPCR expression of DUX4 and MBD3L2 - a DUX4 target gene (bottom). C. Validation of Top-18 guides from (B) (Top), and Heatmap showing number of predicted Off-targets *In Silico*(Bottom).

EPI-321 Overcomes the Limitations of Genetic Medicine

EPI-321 AAV Design

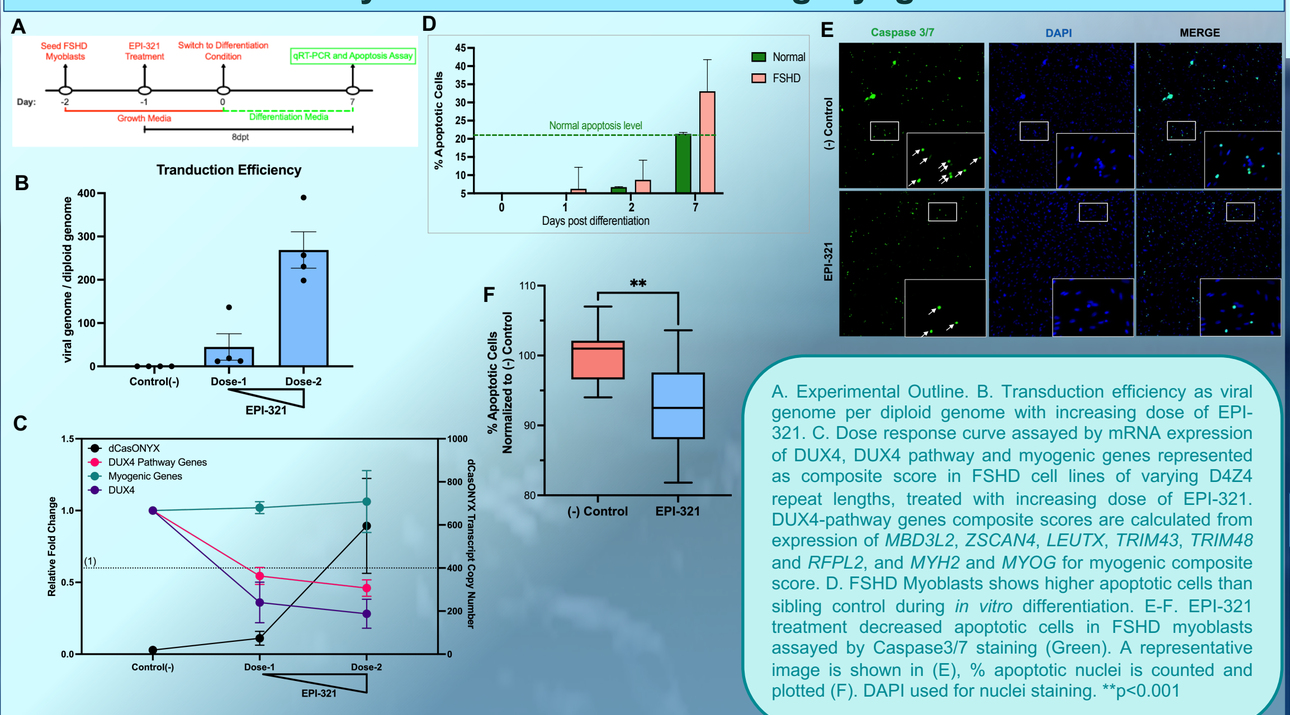
1. **Safety:** EPI-321 utilizes a proprietary library of compact nuclease-dead versions of CRISPR (dCas), resulting in **NO DNA cuts**
2. **Precision:** EPI-321 controls expression of the endogenous gene through methylation of the target sequence
3. **Delivery:** EPI-321 is ultracompact, allowing it to be packaged it into AAVrh74

EPI-321 Biodistribution in NHP Shows High Skeletal Muscle Expression



EPI-321 has high skeletal muscle tissue specific expression of the cargo. A. EPI-321 DNA in blood till 3-month endpoint assayed by qPCR shows peaks at 15 days (High Dose) and 41 days (Low Dose) post intravenous-administration. B. Tissue specific mRNA expression (top) and tropism (bottom) of EPI-321 at low and high doses in NHP at 3-month endpoint.

EPI-321 Represses DUX4 & Downstream Genes, and Rescues Apoptosis in FSHD Myoblasts Without Affecting Myogenic Genes



A. Experimental Outline. B. Transduction efficiency as viral genome per diploid genome with increasing dose of EPI-321. C. Dose response curve assayed by mRNA expression of DUX4, DUX4 pathway and myogenic genes represented as composite score in FSHD cell lines of varying D4Z4 repeat lengths, treated with increasing dose of EPI-321. DUX4-pathway genes composite scores are calculated from expression of *MBD3L2*, *ZSCAN4*, *LEUTX*, *TRIM43*, *TRIM48* and *RFPL2*, and *MYH2* and *MYOG* for myogenic composite score. D. FSHD Myoblasts shows higher apoptotic cells than sibling control during *in vitro* differentiation. E-F. EPI-321 treatment decreased apoptotic cells in FSHD myoblasts assayed by Caspase3/7 staining (Green). A representative image is shown in (E), % apoptotic nuclei is counted and plotted (F). DAPI used for nuclei staining. **p<0.001

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 also rescues the apoptosis level *in vitro* in patient derived myoblast and improve FSHD myoblasts survival in humanized mice model *in vivo*.
- EPI-321 has clean safety profiles in both immunocompetent mice and NHP that shows no signs of toxicity or severe immune response to EPI-321.
- EPI-321 has high skeletal muscle specific expression in NHP and shows peak blood DNA concentrations at 15 days (High Dose) and 41 days (Low Dose) following IV administration.

ACKNOWLEDGEMENT

SOLVE FSHD

UMass Chan MEDICAL SCHOOL
Wellstone Center of FSHD

UNIVERSITY of ROCHESTER MEDICAL CENTER
FSHD Research Center

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