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

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ABSTRACT

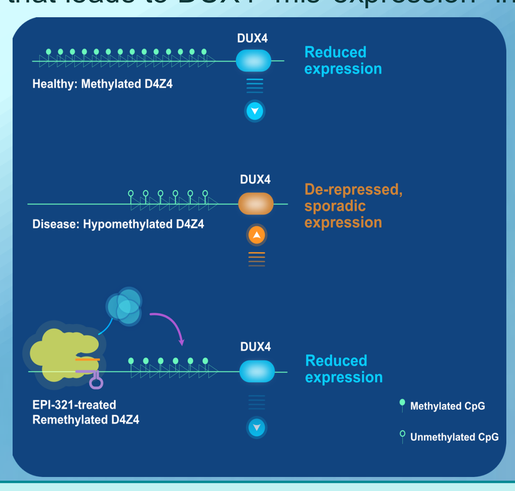
EPI-321 is an investigational drug product for the treatment of FSHD. It is a single vector AAVrh74 encoding an ultracompact, dead Cas protein fused to gene-suppressing modulators expressed from a muscle specific promoter, and a gRNA targeting D4Z4 locus to permanently suppress DUX4 expression through re-methylation of the D4Z4 locus. In the efficacy study, a humanized-muscle mouse model transplanted with patient-derived myoblasts was employed. Dose range-finding involved intravenous administration of low, mid, and high doses of EPI-321 in 3 unique patient-derived humanized-muscle mouse models, with an endpoint set at 23 days post-administration. For the Toxicology and Biodistribution study, male and female C57BL/6 mice aged 8-10 weeks received high-dose EPI-321, followed by in-life examinations throughout the study duration. Tissues for clinical pathology, anatomic pathology, biodistribution, and germline-spatial distribution were collected at endpoints of one- and three-months post-EPI-321 administration. Robust delivery and expression of EPI-321 in skeletal muscle tissue in a dose-dependent manner were confirmed via qPCR and RT-qPCR. Systemic administration significantly reduced DUX4 transcript and protein levels in FSHD muscle in vivo. Additionally, the expression of DUX4 target genes was markedly reduced at the mRNA and protein level, consistent with a lowered rate of cellular apoptosis, demonstrating the therapeutic efficacy of EPI-321. Dose-ranging studies identified the low dose as the minimum efficacious dose, balancing efficacy and potential dose-related side effects. Safety evaluation revealed no abnormalities in in-life examinations or liver blood chemistries. Tissue and organ histopathology assessments showed no notable changes at one- and three-months post-administration. Biodistribution analysis confirmed robust and stable transgene delivery across multiple tissues, with specific target tissue transgene expression. Spatial distribution analysis in testes and ovaries confirmed the absence of genetic material in germline cells, ensuring genetic safety. These preclinical studies provide compelling evidence of the efficacy and safety of EPI-321 as a promising therapeutic candidate for FSHD. Further development and clinical evaluation are warranted to address the unmet medical needs of FSHD patients.

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

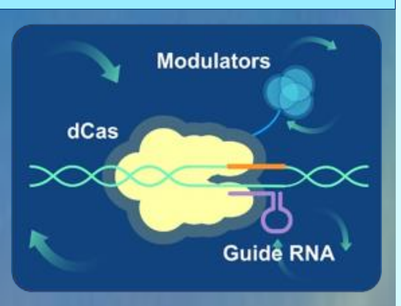
Standard-of-Care
- No disease-modifying drug available
- Exercise has been shown to reduce chronic fatigue and decelerate fatty infiltration of muscle in FSHD
- Surgery to treat scapulothoracic fusion



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

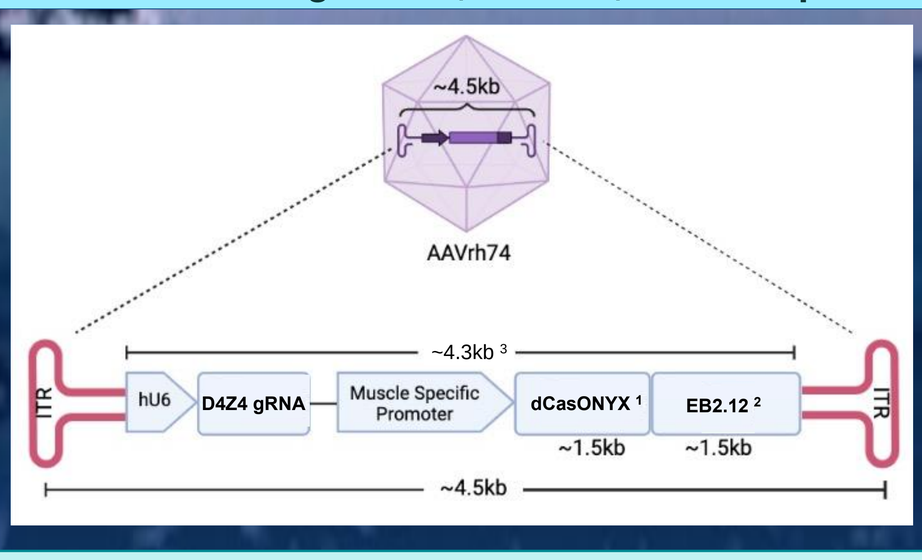
Epic Bio – Who We Are

- 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



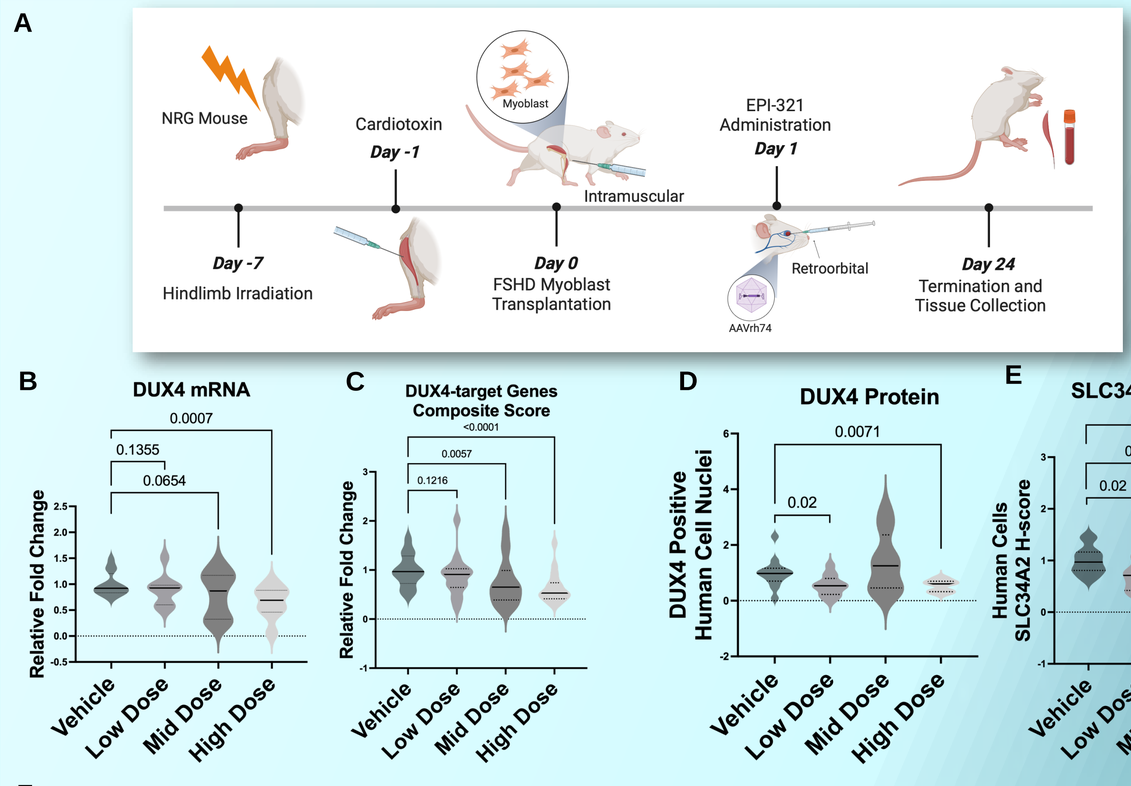
Overview of GEMS Platform

EPI-321 Design: Safe, Precise, and Compact



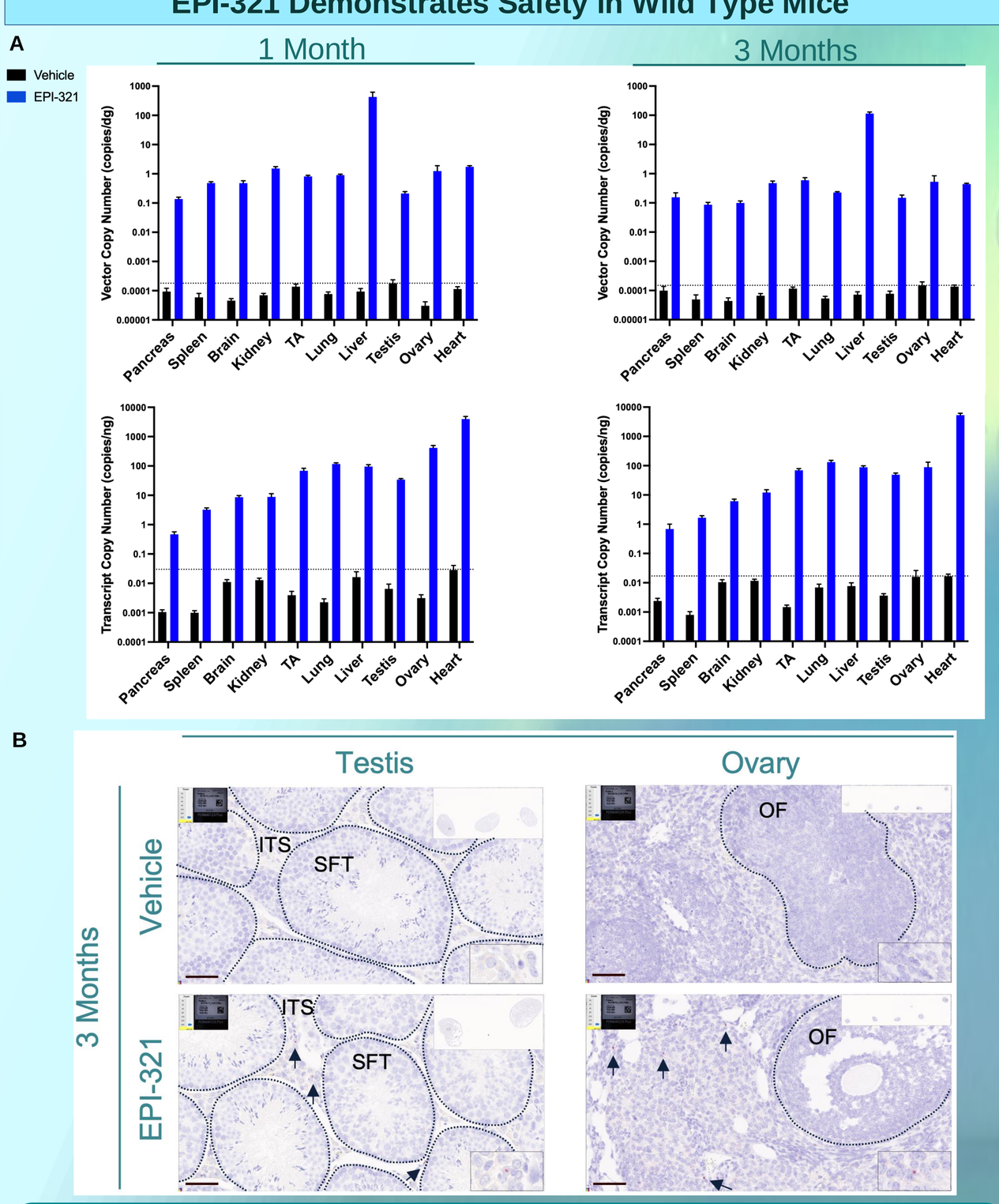
EPI-321 AAV Design. [1]**Safety:** EPI-321 utilizes a proprietary library of compact nuclease-dead versions of CRISPR (dCas), resulting in **NO DNA cuts.** [2]**Precision:** EPI-321 controls expression of the endogenous gene through methylation of the target sequence. [3]**Delivery:** EPI-321 is ultracompact,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

EPI-321 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 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 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 in humanized *in vivo* mice model.
- EPI-321 also improves FSHD myoblast xenograft survival *in vivo*.
- EPI-321 demonstrates a safety profile in preclinical safety studies indicating the readiness for first in human studies