Evaluating DUX4 Activity in a Phase 2, Randomized, Double-Blind, Placebo-Controlled, 48-Week Study of the Efficacy and Safety of Losmapimod in Subjects with FSHD

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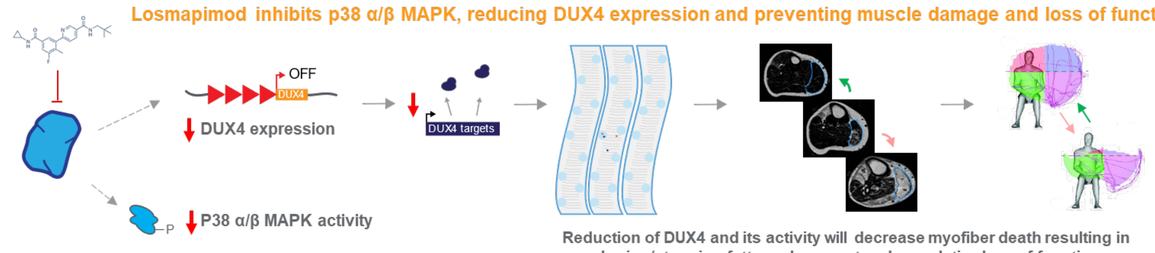
1. Rationale & Methods

Therapeutic Hypothesis in ReDUX4

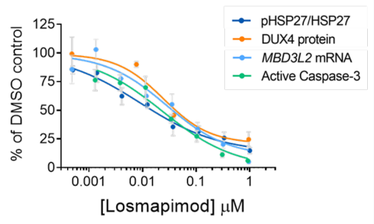
Losmapimod inhibits p38 α/β MAPK, reducing DUX4 expression and preventing muscle damage and loss of function



Reduction of DUX4 and its activity will decrease myofiber death resulting in slowing/stopping fatty replacement and cumulative loss of function

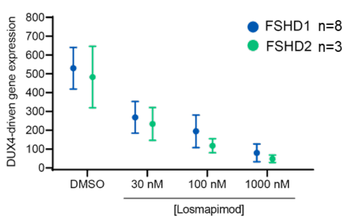
Losmapimod Reduces DUX4 Expression in Preclinical Models of FSHD

Losmapimod reduces DUX4 in patient-derived FSHD myotubes

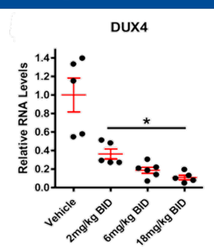


- **HSP27** is a substrate of p38 MAP kinase pathway
- ***MBD3L2*** is a DUX4-target gene
- **Caspase 3** is an indicator of cell death

Clinical-trial in a dish across multiple patient-derived FSHD1/2 myotubes



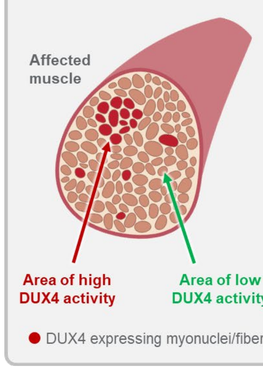
Losmapimod reduces DUX4 in vivo in a xenograft model of FSHD



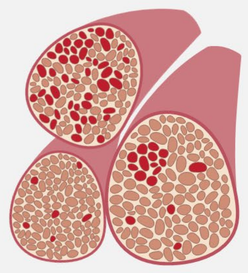
Oliva et al, JPET, 2019

Heterogeneity of Muscle Composition in FSHD

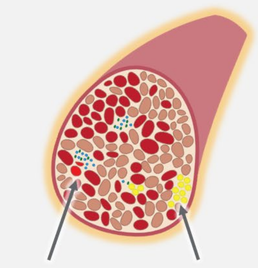
DUX4 expression happens in a subset of myonuclei and myofibers in the FSHD muscle



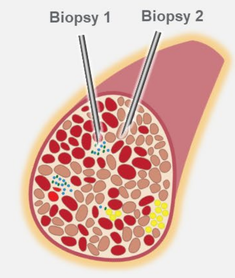
Different muscles in an individual with FSHD have different DUX4 expression levels and patterns

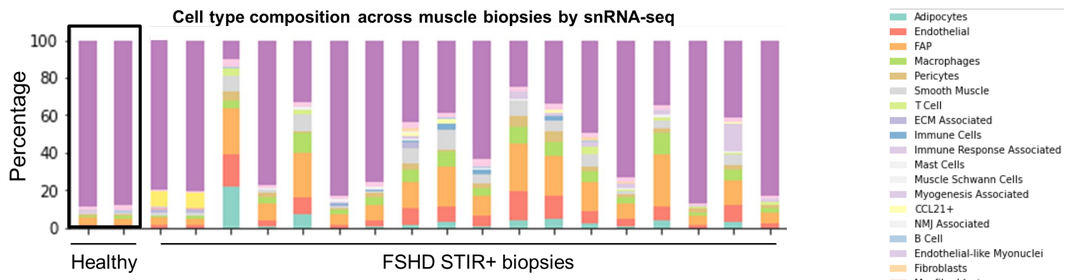


STIR signal in MRI helps to identify muscles more likely to express DUX4



Biopsy procedure was designed to sample proximal regions in repeated biopsies



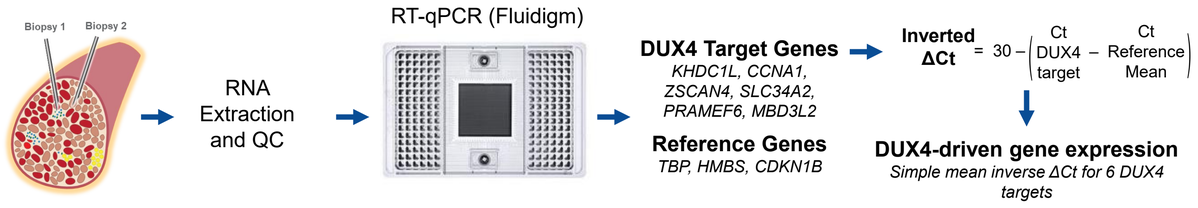


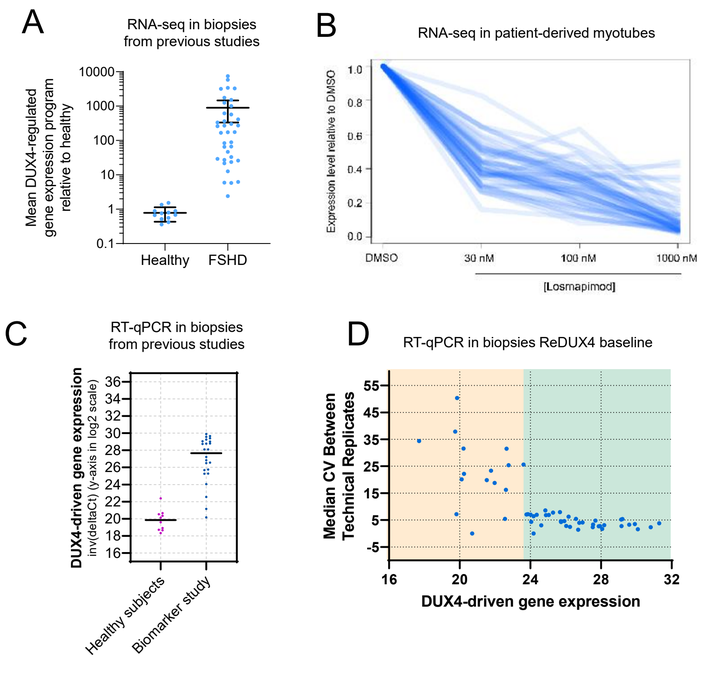
snRNA-seq analysis of muscle biopsies from previous Fulcrum studies showed:

- Large heterogeneity in cellular composition in FSHD muscle biopsies
- DUX4 activity is detected only in 1 in 3,000 myonuclei

*For more details: "Use of snRNA-seq to characterize the skeletal muscle microenvironment during pathogenesis in FSHD", Anu Raman Ph.D., FSHD Society IRC 2021

Determining DUX4 Activity in Muscle Needle Biopsies





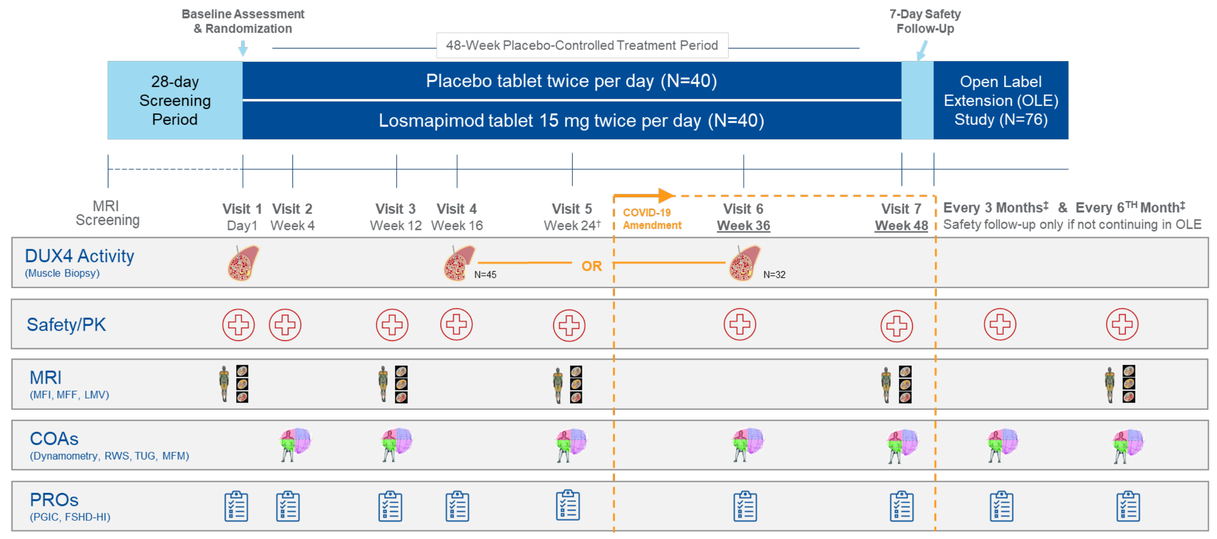
(A) Expression of DUX4 target genes is detected by RNA-seq in FSHD muscle biopsies from previous biomarker study and datasets obtained by the Seattle Wellstone group

(B) DUX4 target genes are coordinately downregulated by losmapimod in patient-derived myotubes. Indicating they represent DUX4 activity as a transcription factor

(C) Fluidigm RT-qPCR assay using 6 DUX4 target genes, recapitulate previous findings by RNA-seq and analysis of healthy muscle biopsies serve to establish biologically relevant bottom of the assay.

(D) RT-qPCR is a highly sensitive tool for detecting DUX4 target gene transcripts, however at inverseΔCt levels <24 we observed a reduction in the precision of the measurement (CV>30% between technical replicates)

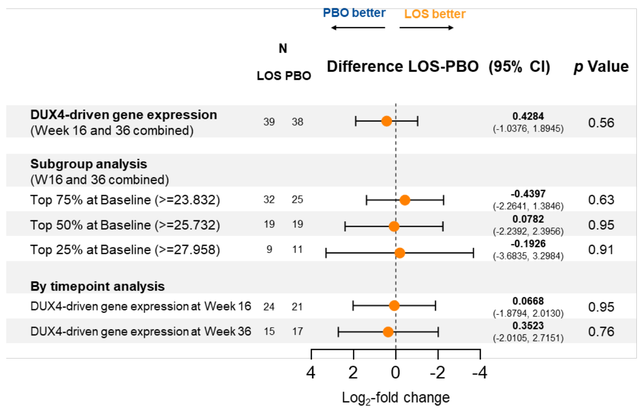
2. ReDUX4 Trial Design



3. Primary Endpoint

DUX4-Driven Gene Expression in Muscle Biopsies in ReDUX4

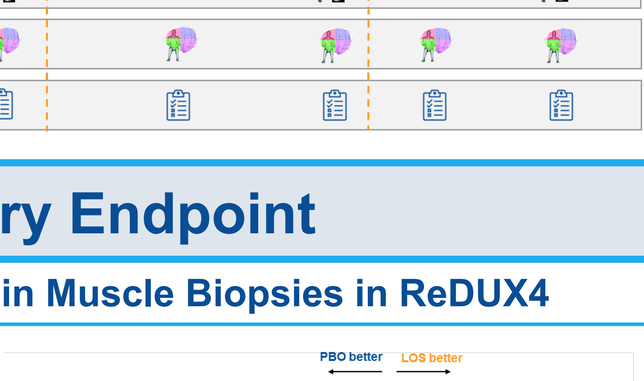
- Changes in either group were not observed in the treatment period.
- Subgroup analysis by quartile of DUX4-driven gene expression levels showed no differences between losmapimod and placebo.
- Results previously reported from the interim analysis of ReDUX4 were based on a smaller number of samples (losmapimod n=3, placebo n=5).
- DUX4-driven gene expression is highly heterogeneous in both groups.



4. Heterogeneity in DUX4 activity in ReDUX4

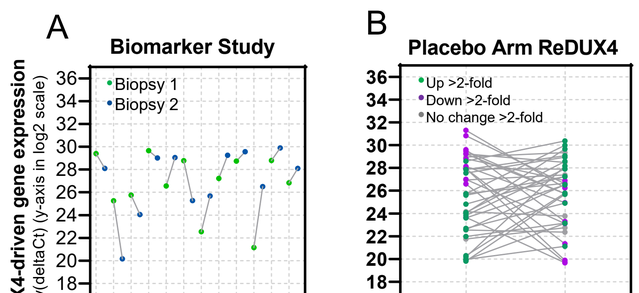
Variability Between Biopsies in Longitudinal Studies

- Preparatory biomarker study showed variability in DUX4 activity between repeated biopsies taken 4-12 weeks apart. **(A)**
- Placebo arm in ReDUX4, shows large variability in DUX4 activity between repeated biopsies. **(B)** (16 and 36 week combined)
- As a **population**, mean DUX4 activity between baseline and postbaseline appears stable but the levels observed in the first biopsy are not representative of levels at the second biopsy
- Change from baseline levels in placebo participants decreased more than two-fold in 30% of samples and increased by more than two-fold in 45%



DUX4 Activity in Biopsies in ReDUX4 is Highly Heterogenous

- In preclinical models in vitro and in vivo, clinically achievable concentrations of losmapimod resulted in >50% reduction in DUX4 expression and activity. Based on the reduction of p38 MAPK activity observed in ReDUX4, ~35-65%, we expected similar decreases in DUX4-driven gene expression in affected myofibers.
- ReDUX4 confirmed the large heterogeneity of DUX4 activity in the FSHD muscle resulting in CV>180% in the population sampled.
- Based on this biological variability, it is not possible to determine if losmapimod had an effect on DUX4 expression and activity in ReDUX4.



Conclusions

- In this study, prespecified population and subgroup analyses did not show differences in DUX4-driven gene expression at week 16 or week 36, thus the primary endpoint was not met.
- DUX4-driven gene expression proved to be highly variable at all time points in both groups. The baseline values spanned over 1000-fold differences, a greater variance than anticipated based on pilot work. This variability contributed to our inability to detect changes and differences in the study.
- Multiple sources of the variability include the stochastic nature of DUX4-driven gene expression as well as the relative imprecision in the biopsy procedure, which had to be performed consistently across 17 clinical trial sites.
- **Despite the challenges in quantifying losmapimod-mediated changes in DUX4-driven gene expression, changes in whole-body imaging, clinical and patient reported outcomes observed in ReDUX4 are consistent with our hypothesis that losmapimod reduces DUX4 expression/activity in muscle resulting in meaningful benefit to patients.**

*Please see "*A Phase 2, Randomized, Double-Blind, Placebo-Controlled, 48-Week, Parallel-Group Study of the Efficacy and Safety of Losmapimod in Treating Subjects with Facioscapulohumeral Muscular Dystrophy (FSHD) with Open Label Extension (OLE): ReDUX4"*, FSHD IRC, 2021.