A Biomarker of Aberrant DUX4 Activity to Evaluate Losmapimod Treatment Effect in FSHD Phase 2 Trials

P.231

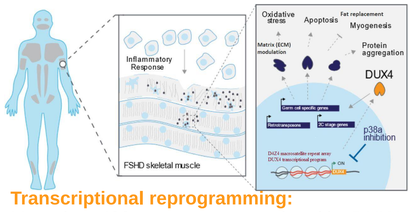
Lucienne Ronco[1], Kathryn Wagner[2], Diego Cadavid[1], Jayashree Chadchankar[1], Aaron Chang[1], Michelle Mellion[1], Adefowope Odueyungbo[1] Shane Raines[1], Anugraha Raman[1], Alan Robertson[1], Alejandro Rojas[1], Ning Shen[1], Perry Shieh[3], Jeffrey Statland[4], Rabi Tawil[5], Stephen Tapscott[6], Baziel van Engelen[7], Leo Wang[8], Nick Johnson[9], Owen Wallace[1]

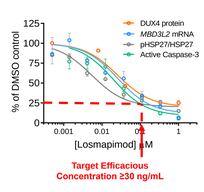
[1]Fulcrum Therapeutics, Cambridge, MA; [2]Kennedy Krieger Institute, Baltimore; [3]University of California, Los Angeles; [4]University of Kansas Medical Center; [5]University of Rochester Medical Center; [6]Fred Hutchinson Research Center, Seattle; [7]Radboud University Medical Centre, Netherlands; [8]University of Washington, [9]Virginia Commonwealth University

Background

- Facioscapulohumeral dystrophy (FSHD) is a rare inherited muscular disease caused by the pathological overexpression of the homeobox transcription factor DUX4 in affected skeletal muscle.
- Aberrant DUX4 expression results in transcriptional reprogramming and expression of a characteristic transcriptional array causing myofiber death resulting in muscle weakness and accumulation of disability.
- While detection of pharmacodynamic (PD) modulation of DUX4 protein and mRNA is challenging, DUX4-regulated gene transcripts are readily detected in affected muscles.
- DUX4-regulated transcripts may provide a surrogate marker of DUX4 activity.
- Fulcrum Therapeutics is developing losmapimod, a selective small molecule inhibitor of p38α/β, to reduce aberrant DUX4 expression in both FSHD1 & 2.
- This preparatory biomarker study (SRA-002-2018) was performed to confirm a set of stable (detected in longitudinal biopsies) DUX4-regulated gene transcripts that will provide a PD biomarker endpoint to measure treatment effect on DUX4 the root cause of FSHD.

1. Approach: DUX4 as Molecular Target





Losmapimod treatment of FSHD1 & 2 myotubes in culture results in reduction of: DUX4 and its transcriptional activity (MBD3L2), p38 activity (pHSP27) and cell death (caspase 3).

Transcriptional reprogramming:
More than 400 genes are upregulated by DUX4 expression compared to healthy muscle

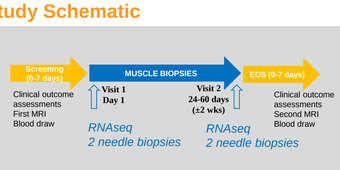
Chromosome 4q35
- **Healthy:** 11 to 100 D4Z4 repeat arrays > Represses DUX4 locus
- **FSHD1:** 1 to 9 D4Z4 repeat arrays (contraction) > Loss of repression of DUX4 locus
- **FSHD2:** DUX4 expression due to mutations in epigenetic control genes.

2. Primary Objective

Identification of a panel of DUX4-regulated gene transcripts to measure DUX4 activity in skeletal muscle needle biopsies from FSHD1 patients over 4-8 weeks

3. Study Design: MRI-guided Skeletal Muscle Biopsy

Study Schematic



Inclusion Criteria

- Age 18-65 years.
- Informed consent.
- Confirmed FSHD1 with 1-9 repeats via assessment of size of the D4Z4 array (4q35).
- Clinical Severity Score between 2 to 4 (Ricci scale; range 0 to 5).
- Willingness to receive whole body-MRI and to provide multiple biopsies.
- Presence of STIR positive signal at screening MRI in at least one leg skeletal muscle eligible for needle biopsy.

Demographics and Disease Characteristics of 17 FSHD1 patients enrolled in the Biomarker Preparatory Study

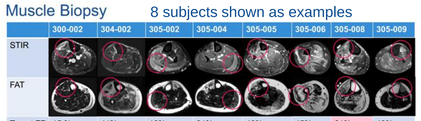
Demographics	Values
Age [mean (SD);range]	49.4 (13); 23-65
% Males	70%

Disease Characteristics	Values
D4Z4 repeat [mean (SD); range]	5.2 (1.46); 3-7
Clinical Severity Score [mean (SD); range]	3 (0.7); 2-4

SRA-002-2018 Muscle Biopsy Summary

- An MRI-eligible muscle for biopsy (STIR+ and MFF 10-40% in a leg muscle safe to biopsy), as determined by a central reader was identified at baseline
- Initial MRI criteria identified STIR+ regions containing Muscle Fat Fractions (MFF) >10 and <50%.
- Biopsies were conducted according to a clinical manual using either a UCH needle or vacuum assisted Vacora System; 20 to 100 mg of tissue was collected per biopsy and 4-6 biopsies were taken per time point
- After an interim assessment, upper MFF was changed from <50% to <40% as fat interfered with RNA quality

Muscle Biopsy — 8 subjects shown as examples



	300-002	304-002	305-002	305-004	305-005	305-006	305-008	305-009
Target FF	15 %	44%	16%	21%	46%	45%	61%	48%
MFF	13 %	62%	19%	12%	42%	66%	61%	39%
RIN	7.8	2.6 / 3.8	8.6 / 7.3	9.1 / 7.8	7.2 / 6.7	5.1 / 6	5.4 / 5.8	6.3 / 6.4
RICCI	2.5	4	2.5	2.5	3	4	2.5	4

Biopsy Characterization and Results

- Tibialis Anterior was most frequently biopsied muscle
- RNA extracted from a majority of the biopsies was of good quality (RIN 5-9)
- RNA quality score (RIN) did not correlate with strength of DUX4 signature
- The type of muscle biopsied did not affect DUX4 gene signature detection
- Good quality RNA sequence was collected from 16 subjects; fat levels very high in one subject which precluded analysis
- To determine inter-biopsy variability, 2 tissue samples collected at the same time from each patient were analyzed by RNA sequencing (RNAseq) at each visit. Correlation between paired samples was very high (average ~0.987).

4. SRA-002-2018 Samples Cluster With FSHD Muscle Biopsies from the Seattle Wellstone Cohort

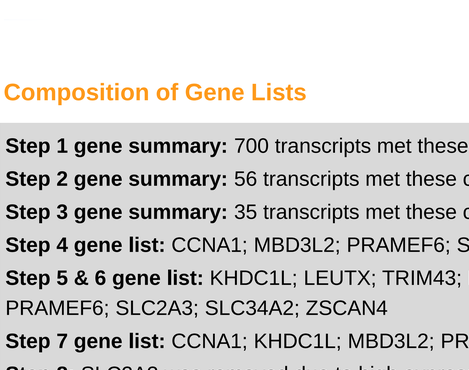
Seattle Wellstone
- 20 FSH subjects
- 9 Healthy Controls

Cohort 1 -Test Set

Fulcrum Study SRA-002-2018
- 16 FSH subjects
- 10 Healthy Controls

Cohort 2 -Confirmation Set

The Seattle Wellstone Cohort was a one-year follow up study including lower extremity MRI and repeat biopsy of the same muscle. RNAseq was performed. (Wong et al, 2020)



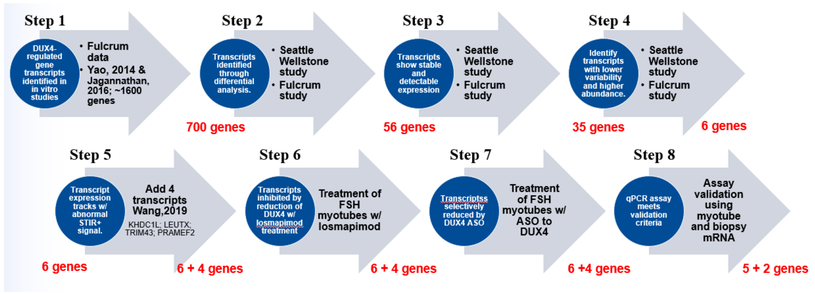
Principal component Analysis of RNAseq
Healthy control samples:
- 22 (Lindholm et al., 2014)
- 6 (Gift of N.E Johnson, VCU)
- 9 (Wong et al, 2020)

FSHD Samples:
- 34 FSHD Seattle Wellstone
- 47 FSHD Fulcrum SRA-002-2018

- Fulcrum 002-2018
- Johnson Healthy Controls
- Lindholm Healthy Controls
- Seattle Wellstone FSHD
- Seattle Wellstone Healthy Controls

Results:
- SRA-002-2018 samples all cluster with FSHD and due to stricter inclusion criteria, less heterogeneity is observed compared to Seattle Wellstone samples
- Two Seattle Wellstone biopsy samples clustered with controls

5. Methodology to identify DUX-4 regulated gene transcript subset



Composition of Gene Lists

Step 1 gene summary: 700 transcripts met these criteria.

Step 2 gene summary: 56 transcripts met these criteria.

Step 3 gene summary: 35 transcripts met these criteria.

Step 4 gene list: CCNA1; MBD3L2; PRAMEF6; SLC2A3; SLC34A2; ZSCAN4

Step 5 & 6 gene list: KHDC1L; LEUTX; TRIM43; PRAMEF2; CCNA1; MBD3L2; PRAMEF6; SLC2A3; SLC34A2; ZSCAN4

Step 7 gene list: CCNA1; KHDC1L; MBD3L2; PRAMEF6; SLC2A3; ZSCAN4

Step 8: SLC2A3 was removed due to high expression in healthy control tissue

Reference gene list: HBS; CDKN1B; TBP

6. Feasibility and Validation of Real Time qPCR by Fluidigm

Transcript DUX4 Composite	Assay Efficiency at 375 ng/rx	Range of Ct per rx in biopsy samples (n=8) at 375 ng/rx
CCNA1	88.4%	15-23
KHDC1L	89.3%	14-22
MBD3L2	93.2%	12-24
PRAMEF6	87.9%	14-25
SLC34A2	91.9%	14-25
ZSCAN4	94.0%	14-22
CDKN1B (reference)	103.5%	11-13
HMBS (reference)	106.8%	14-17
TBP (reference)	93.0%	15-17

rx= reaction

Gene	Group	Visit	n	Reported Value Mean	SD	Median	Min	Max	n	Change from Baseline Value [1] Mean	SD	Median	Min	Max
CCNA1	Overall (N=17)	Visit 1	12	26.544	1.535	26.700	17.61	30.10						
		Visit 2	12	27.049	2.760	28.300	21.10	30.19	12	0.505	3.209	0.695	-4.87	7.16
KHDC1L	Overall (N=17)	Visit 1	12	27.932	3.020	28.025	21.47	30.84						
		Visit 2	12	27.858	3.251	29.105	20.00	31.20	12	0.327	3.133	0.370	-6.05	4.64
MBD3L2	Overall (N=17)	Visit 1	12	26.681	3.993	26.980	16.77	30.87						
		Visit 2	12	27.013	3.260	28.115	20.63	30.52	12	0.332	4.228	0.865	-6.24	10.22
PRAMEF6	Overall (N=17)	Visit 1	12	25.837	4.602	27.530	16.77	30.41						
		Visit 2	12	26.700	4.197	27.985	17.13	30.91	12	0.863	4.325	0.935	-5.89	9.56
SLC2A3	Overall (N=17)	Visit 1	12	28.217	0.881	28.500	26.10	29.23						
		Visit 2	12	28.760	1.049	28.985	27.11	30.46	12	0.543	1.108	0.780	-1.42	1.79
SLC34A2	Overall (N=17)	Visit 1	12	25.793	3.247	26.005	16.77	29.15						
		Visit 2	12	25.593	4.263	27.220	12.96	28.45	12	-0.358	4.817	0.980	-12.00	8.26
ZSCAN4	Overall (N=17)	Visit 1	12	26.513	1.687	26.510	23.38	28.73						
		Visit 2	12	26.658	2.305	27.235	22.23	29.54	12	0.626	1.573	0.570	-2.68	2.26

Max = maximum; Min = minimum; n = number of subjects; SD = standard deviation.

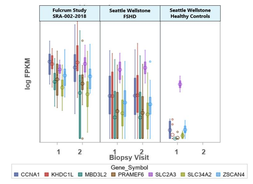
DUX-4 Regulated Gene Transcript Expression Values and Composite Score (Delta-CT) and Changes from Baseline by Visit – Descriptive Statistics by qPCR

Score	Group	Visit	Reported Value n	Mean	SD	Median	Min	Max	Change from Baseline Value [1] n	Mean	SD	Median	Min	Max
DUX4 Score 1: Simple Mean	Overall (N=17)	Visit 1	12	26.725	2.701	27.026	21.153	29.653						
		Visit 2	12	27.059	2.880	28.097	20.163	29.904	12	0.334	2.931	0.975	-5.099	5.360

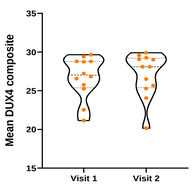
Max= maximum; Min=minimum; n=number of subjects; SD= standard deviation
*qPCR results reported for n=12 subjects where biopsy RNA material was sufficient to conduct the validated assay. Remaining 5 of 17 subjects had insufficient RNA after RNAseq analysis.

7. DUX4-regulated Gene Subset Analysis presented as individual genes and composite

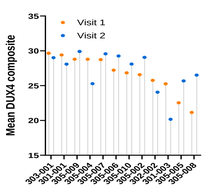
FPKM Individual genes
SRA-002 and Seattle Wellstone Cohorts



qPCR Mean Composite
Study Population
SRA-002-2018



qPCR Mean Composite
By Subject From Biopsy Visits 1 & 2
SRA-002-2018



Results:
Data summarized by 2 methods:
Individual genes using logarithmic transformation of normalized counts (log (FPKM).
Mean Composite score combining all 6 genes into a single DUX4 Score. Composite Score is the Mean C_t of 6 genes CCNA1; KHDC1L; MBD3L2; PRAMEF6; ZSCAN4 normalized to 3 reference genes HBS; CDKN1B; TBP.
Mean qPCR Composite score for each subject (n=12) by qPCR – Mean 26.48 C_t (SD 3.12)

8. Summary

17 subjects were enrolled and 16 completed the SRA-002-2018 study. The mean (SD) age was 49 (13) and 70% were males with a mean severity score of 3. Muscle needle biopsies were well tolerated. Using published RNAseq data from previous studies (Wang, 2018; Wong, 2020) and new RNAseq data from this study, a subset of DUX4-regulated gene transcripts was identified based on consistent expression in repeated skeletal muscle needle biopsies of affected muscles identified by MRI.

The DUX4-gene expression assay is robust and showed good repeatability (ICC ~0.5) in the preparatory biomarker study in FSHD.

9. Conclusion

DUX4-regulated gene transcripts may provide a pharmacodynamic biomarker endpoint of DUX4 activity to measure treatment effect for the root cause of FSHD in losmapimod therapeutic clinical trials.
Fulcrum has identified a panel with 9 transcripts (6 DUX4 and 3 housekeeping) that is being used to measure DUX4 activity in affected skeletal muscles from FSHD patients.