Losmapimod, a p38α/β MAPK Inhibitor for the Potential Treatment of Patients with FSHD

New Directions Annual Scientific Meeting

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Fulcrum Therapeutics




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Our Mission is to Treat Root Cause of Rare Genetic Diseases

We aim to

Deliver disease-modifying therapies that improve the lives of people with rare genetic diseases

Two Clinical-Stage Programs

FSHD: Phase 3

Sickle cell disease: Phase 1b patient study

Preclinical programs

Advancing high-value, de-risked targets for rare genetic diseases in both the muscle and benign hematology space

Losmapimod for Facioscapulohumeral Muscular Dystrophy (FSHD)

Currently, There Are No Treatment Options for People Living With FSHD

FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD) is caused by the aberrant expression of DUX4 in skeletal muscle

STOCHASTIC DUX4 EXPRESSION contributes to disease heterogeneity, asymmetry, and variability in disease phenotype

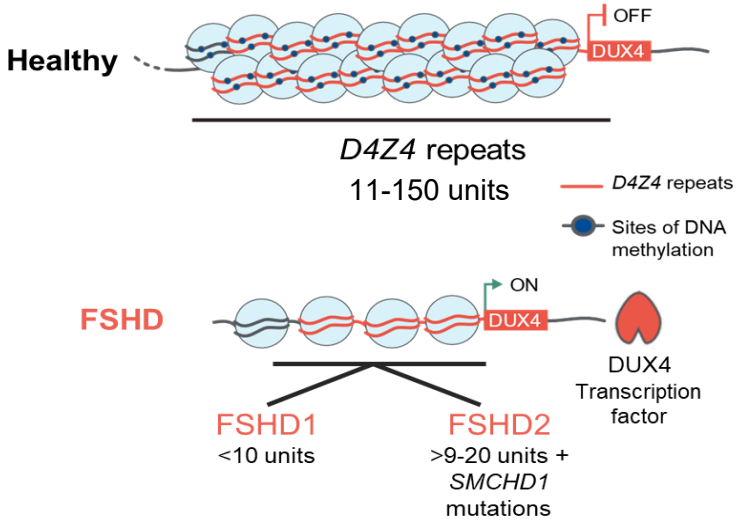
PATHOLOGICAL ACTIVITY AND MALADAPTIVE REMODELING lead to muscle fiber death and immune and fat infiltration

PROGRESSIVE MUSCLE LOSS AND FATTY REPLACEMENT can cause a chronic progressive descending weakness and loss of function in those affected

MUSCLE PATHOLOGY leads to accumulation of disability

Currently, there are no approved medication treatment options for people living with FSHD that prevent and/or slow muscle wasting and weakness

Aberrant DUX4 Expression is the Root Cause of FSHD

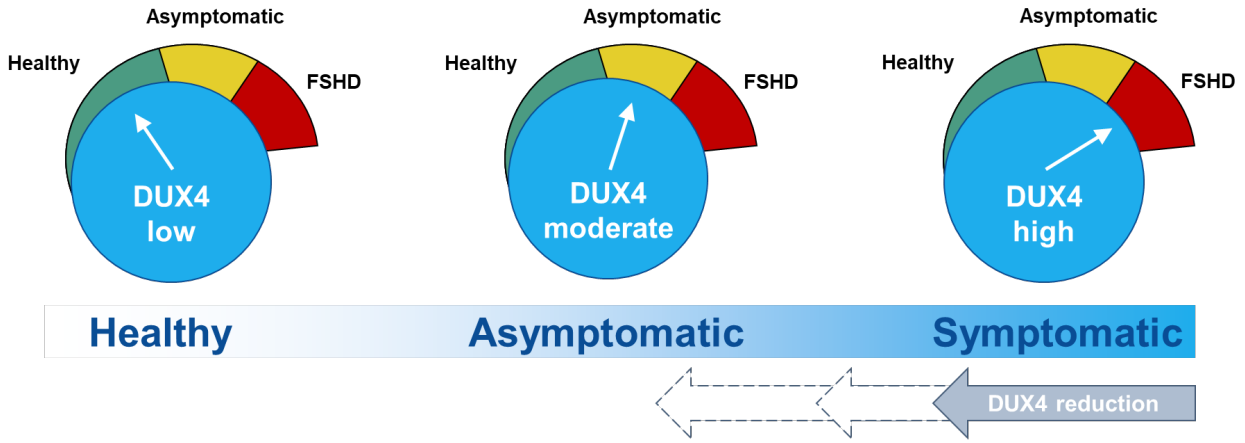


A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy

Richard J. L. F. Lemmers,[1] Patrick J. van der Vliet,[1] Rinse Klooster,[1] Sabrina Sacconi,[2] Pilar Camaño,[3,4] Johannes G. Dauwerse,[5] Lauren Snider,[6] Kirsten R. Straasheijm,[1] Gert Jan van Ommen,[1] George W. Padberg,[7] Daniel G. Miller,[8] Stephen J. Tapscott,[6] Rabi Tawil,[9] Rune R. Frants,[1] Silvère M. van der Maarel[1]*

Facioscapulohumeral muscular dystrophy (FSHD) is a common form of muscular dystrophy in adults that is foremost characterized by progressive wasting of muscles in the upper body. FSHD is associated with contraction of D4Z4 macrosatellite repeats on chromosome 4q35, but this contraction is pathogenic only in certain "permissive" chromosomal backgrounds. Here, we show that FSHD patients carry specific single-nucleotide polymorphisms in the chromosomal region distal to the last D4Z4 repeat. This FSHD-predisposing configuration creates a canonical polyadenylation signal for transcripts derived from *DUX4*, a double homeobox gene of unknown function that straddles the last repeat unit and the adjacent sequence. Transfection studies revealed that *DUX4* transcripts are efficiently polyadenylated and are more stable when expressed from permissive chromosomes. These findings suggest that FSHD arises through a toxic gain of function attributable to the stabilized distal *DUX4* transcript.

Relationship Between DUX4 Expression and FSHD Disease Presentation

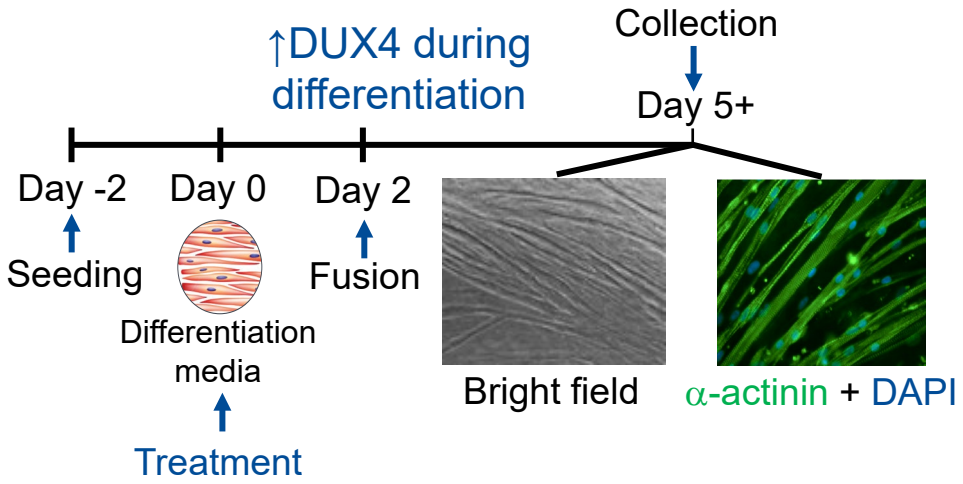


Any reduction in DUX4 may provide a functional benefit in FSHD patients

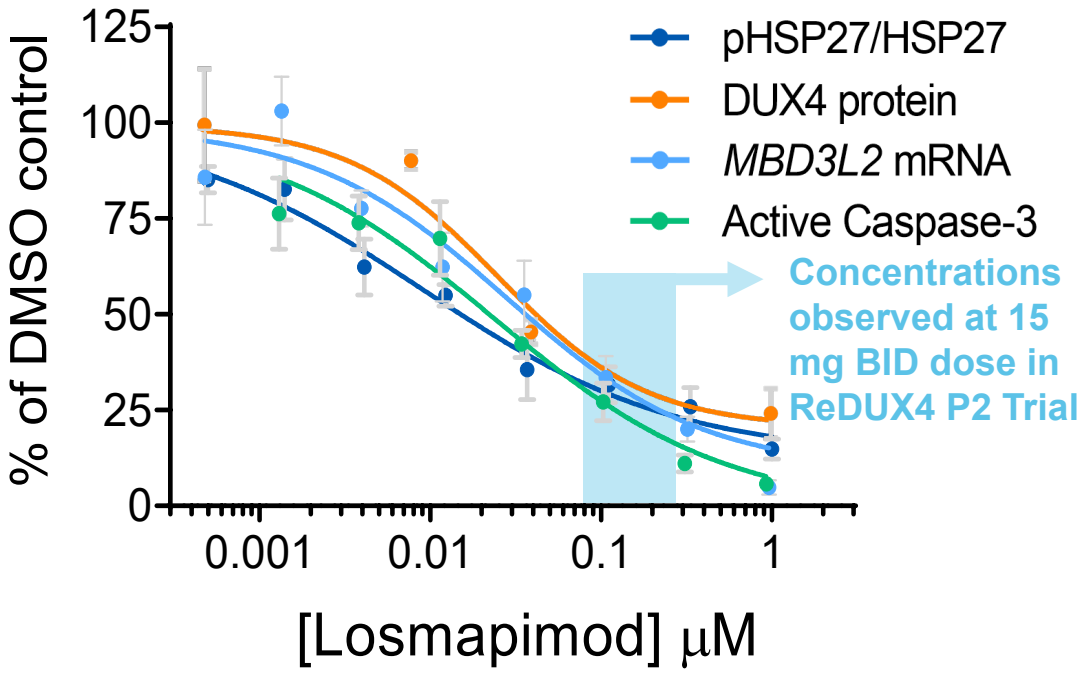
Fulcrum FSHD KOL Breakfast 2019; Lemmers, RJLF. Science. 2010

Discovery of Role of p38α/β in DUX4 Transcription

Screening assay using FSHD patient-derived myotubes



p38α/β inhibition decreases DUX4 expression & DUX4 program

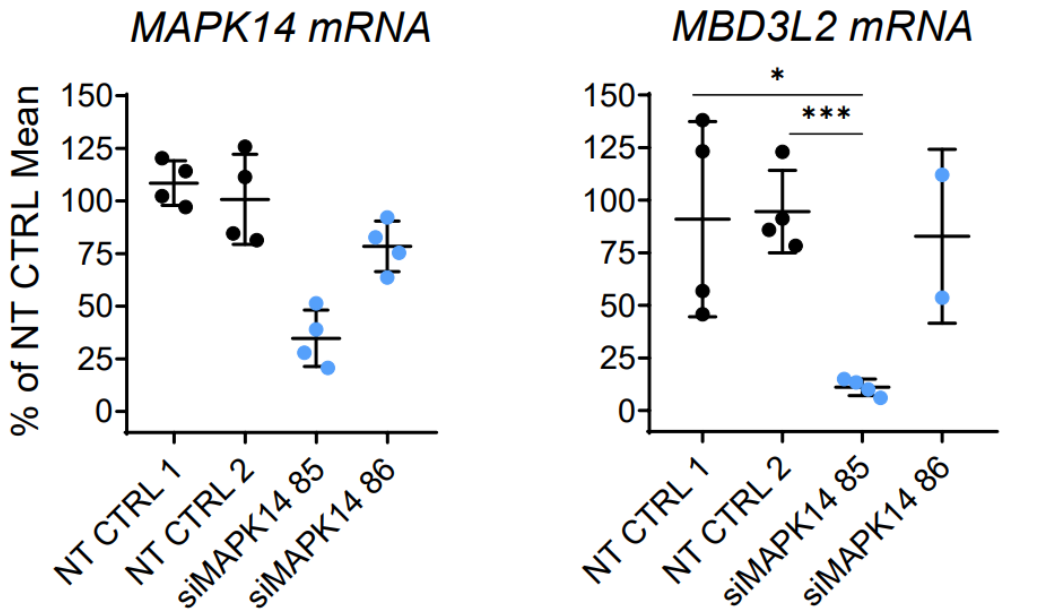


- Compounds applied during differentiation of patient-derived myoblasts to myotubes
- Modulation of MBD3L2—a DUX4 target gene—used to assess hits
- Inhibitors of p38α/β MAPK robustly inhibited DUX4, the DUX4 program, and muscle cell death

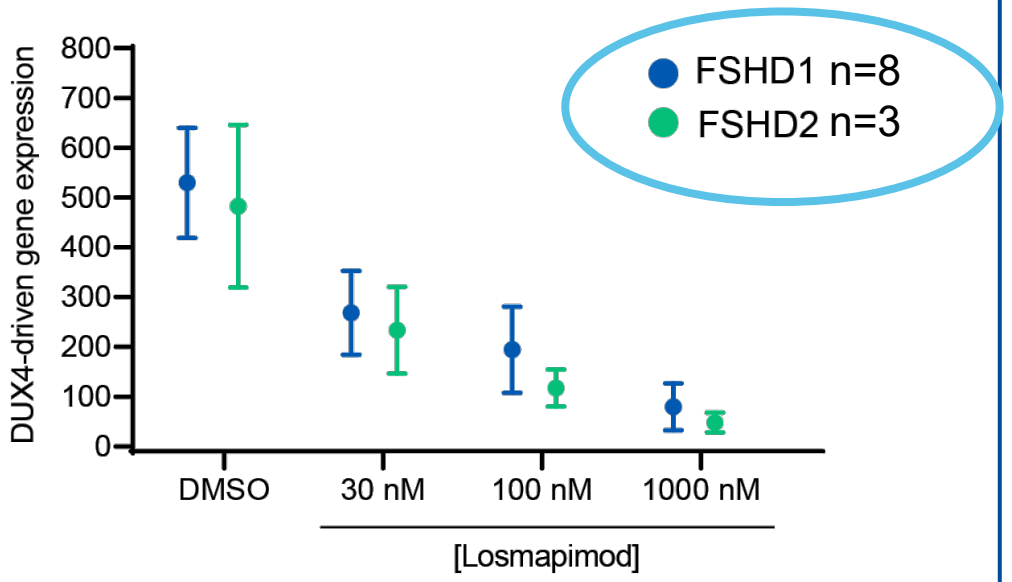
Rojas LA, et al. J Pharmacol Exp Ther 2020;374:489–498

Proof of Mechanism: Losmapimod, a Selective p38α/β MAPK Inhibitor, Reduces DUX4 Expression in FSHD Myotubes

P38α knockdown reduces activity of DUX4 in FSHD myotubes



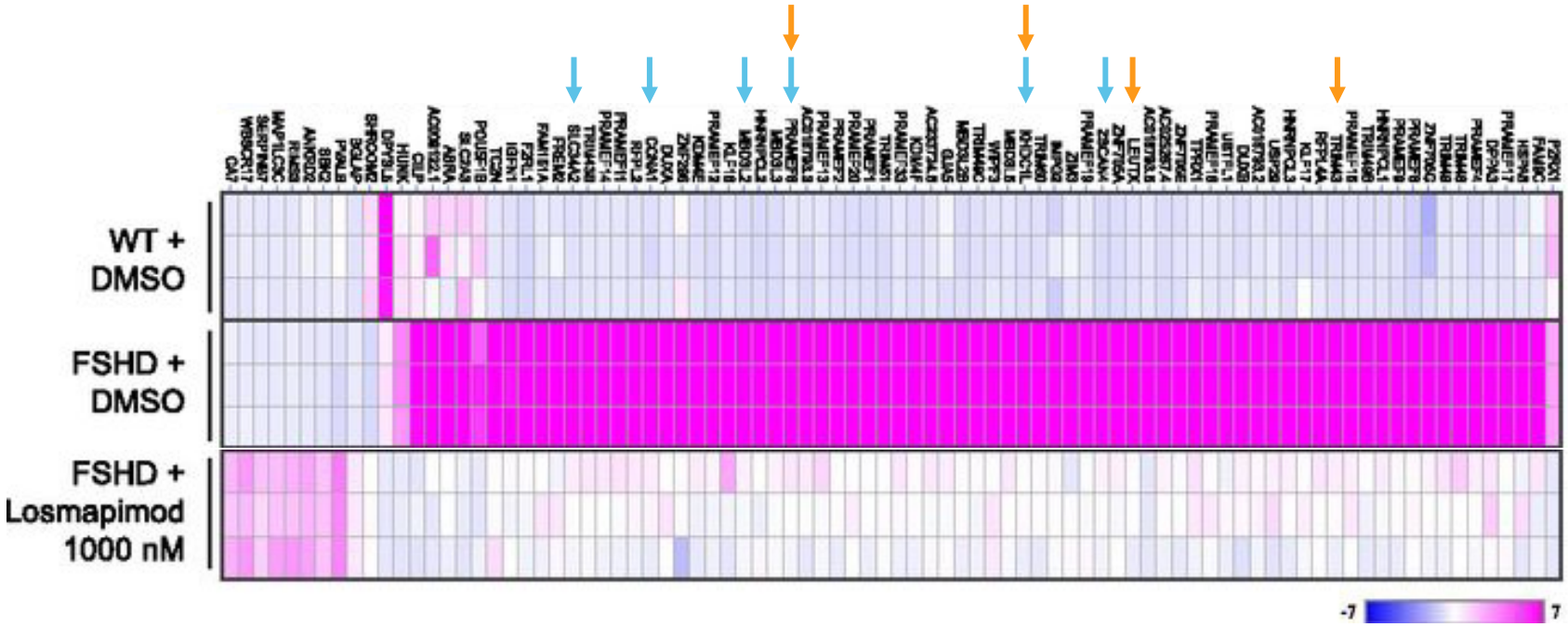
Losmapimod demonstrates reduction of DUX4 activity across multiple patient-derived FSHD1/2 myotubes



Rojas LA, et al. J Pharmacol Exp Ther 2020;374:489–498

Informing Biomarker Discovery: Losmapimod Reduces Expression of DUX4 and All Commonly Studied Downstream Genes (Preclinical Data)

RNAseq analysis demonstrate that DUX4 downstream gene expression is reduced to almost wildtype levels and targets selected for clinical biomarker assay represent changes across the entire DUX4 program



Fulcrum clinical biomarker set: ***CCNA1, KHDC1L, MBD3L2, ZSCAN4, SLC34A2, PRAMEF6***

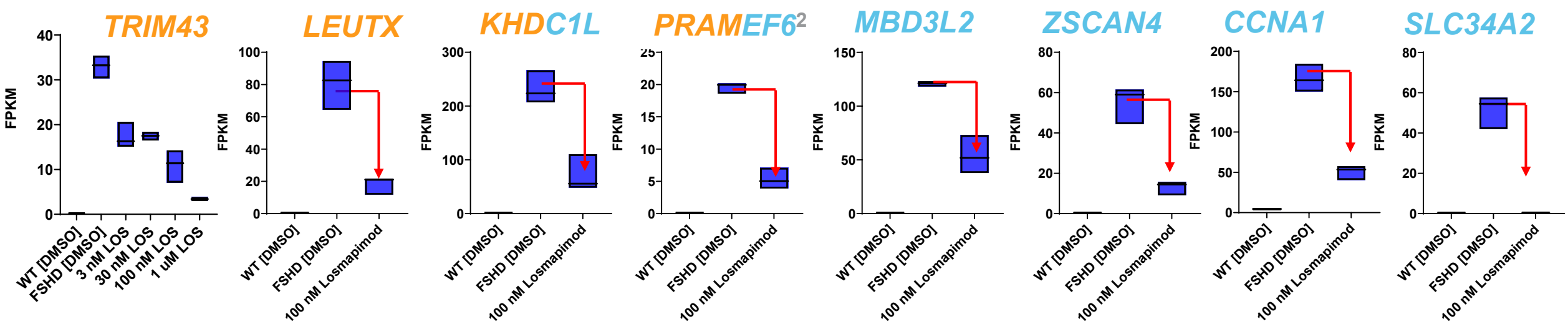
Wellstone biomarker set: ***KHDC1L, PRAMEF2, TRIM43, LEUTX***

Rojas LA, et al. J Pharmacol Exp Ther 2020;374:489–498; Yao, et al Human Molecular Genetics 2014, 23(20), 5342–5352 (Yao, et al proposed PRAMEF2)

Informing Biomarker Discovery: Fulcrum[1] and Wellstone[2] (Preclinical Data) – 100 nM

Fulcrum Markers

Wellstone Markers



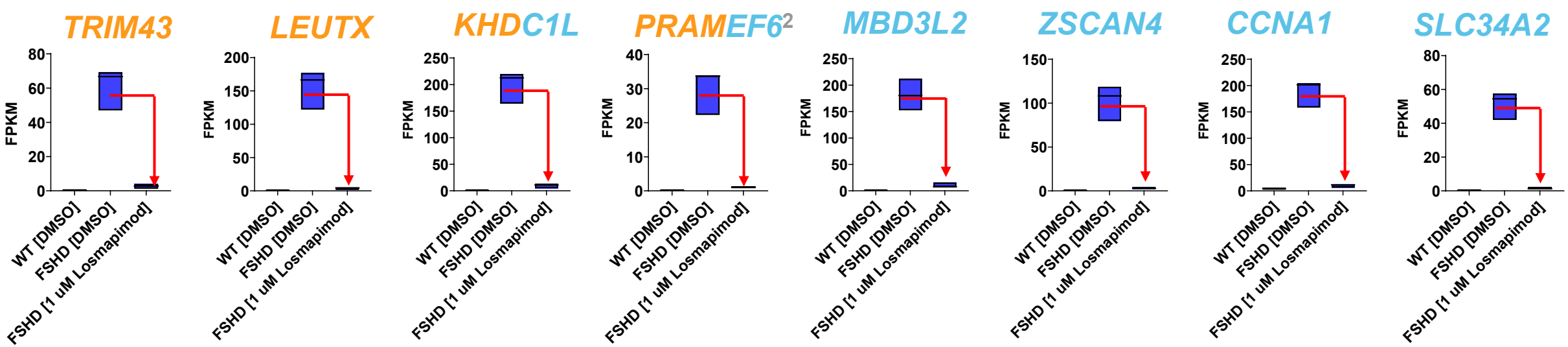
Treatment of FSHD cells with 100 nM losmapimod causes expression levels of key DUX4-driven biomarkers to return to near-WT levels

[1] Rojas, et al 2020 & Ronco, et al A Biomarker of Aberrant DUX4 Activity to Evaluate Losmapimod Treatment Effect in FSHD Phase 2 Trials; [2] Yao, et al Human Molecular Genetics 2014, 23(20), 5342–5352;
[2] Yao, et al proposed PRAMEF2

Informing Biomarker Discovery: Fulcrum[1] and Wellstone[2] (Preclinical Data) – 1000 nM

Fulcrum Markers

Wellstone Markers

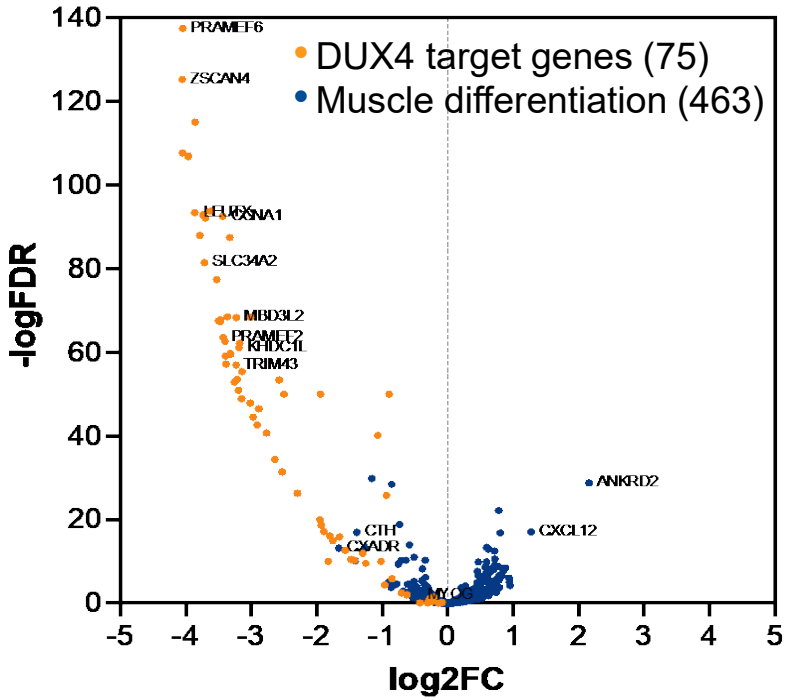


Treatment of FSHD cells with 1 µM losmapimod causes expression levels of key DUX4-driven biomarkers to return to near-WT levels

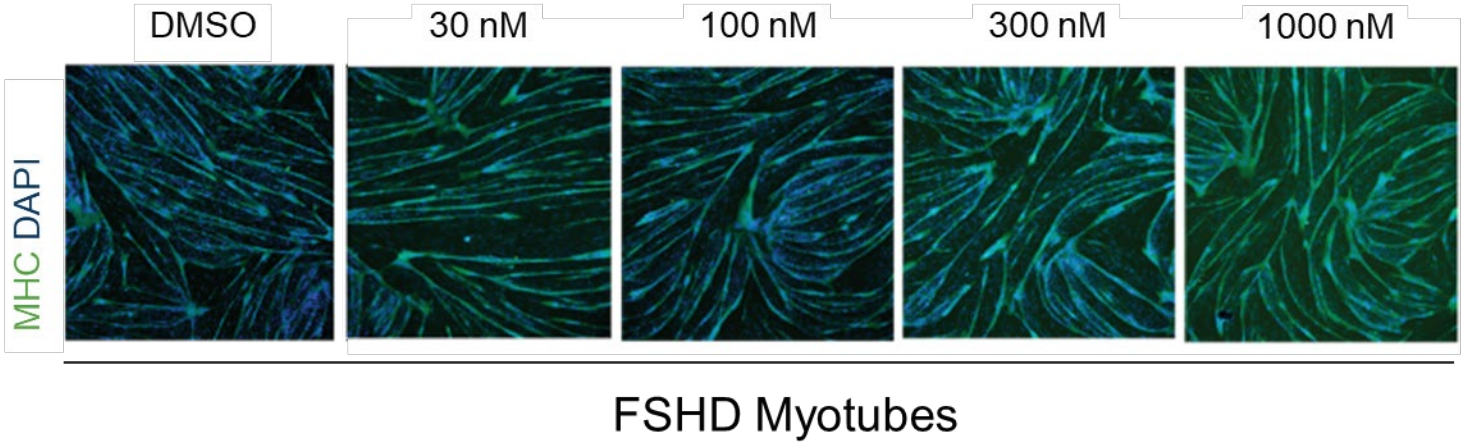
[1] Rojas, et al 2020 & Ronco, et al A Biomarker of Aberrant DUX4 Activity to Evaluate Losmapimod Treatment Effect in FSHD Phase 2 Trials; [2] Yao, et al Human Molecular Genetics 2014, 23(20), 5342–5352;
[2] Yao, et al proposed PRAMEF2

Effect of Losmapimod on Patient-Derived FSHD Myotube Differentiation (Preclinical Data)

C6 FSHD cells; DMSO vs. 1 µM Los



Dose-Response for Losmapimod in Patient-Derived FSHD Myotubes

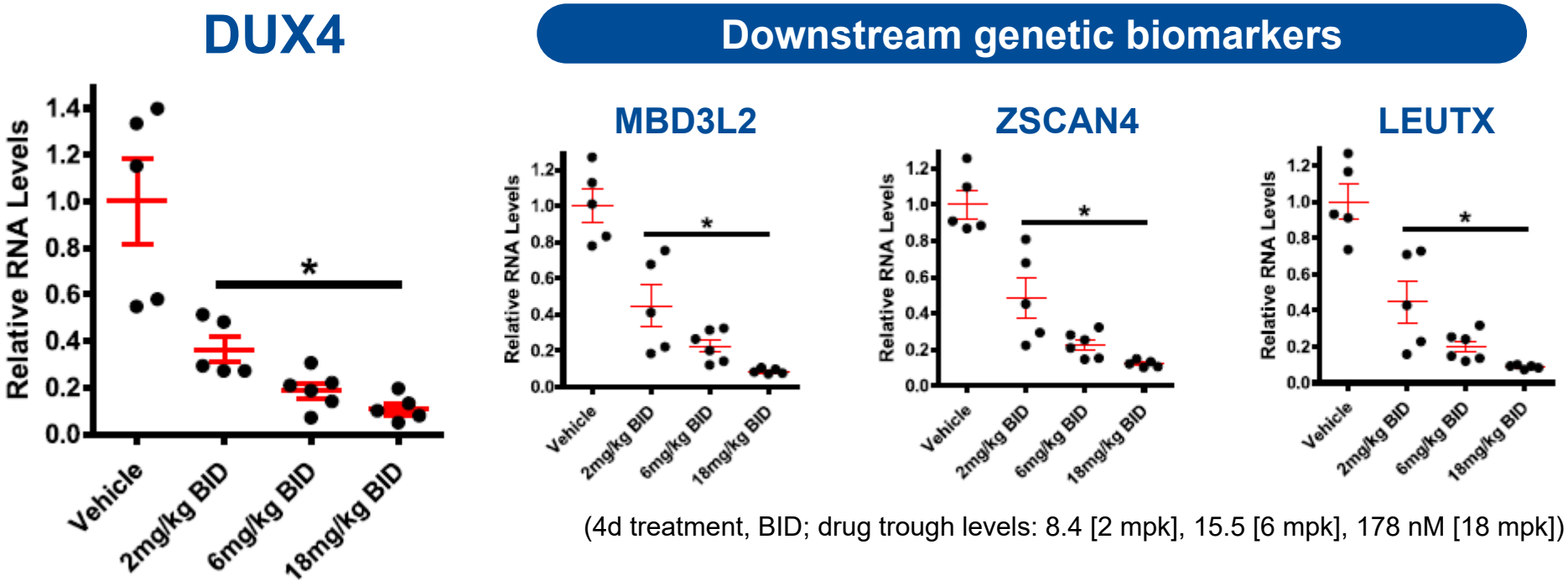


- RNA-sequencing shows no dysregulation of key drivers of myogenic programming, comparing FHSD DMSO vs. losmapimod treatment
- Treatment with losmapimod does not impact differentiation index quantified by ICC

Rojas LA, et al. J Pharmacol Exp Ther 2020;374:489–498

DUX4 target genes defined by Mitsuhashi, et al *Human Molecular Genetics* **2021**, 30(7), 552-563;
Muscle cell differentiation gene set GO:0042692

Losmapimod Significantly Reduces DUX4 Gene Expression in a Mouse Xenograft Model of FSHD



(4d treatment, BID; drug trough levels: 8.4 [2 mpk], 15.5 [6 mpk], 178 nM [18 mpk])

- Immunodeficient (NOD-Rag) mouse; no T, B or NK cells
- Significant reduction in DUX4 mRNA and in key downstream biomarkers
- Human myoblast differentiation and content of human cells in engrafted tissue was not affected (data not shown)

Xenograft: Olivia, et al, *J Pharmacol Exp Ther* **2019**, 370, 219–230; Biomarkers: Yao, et al *Human Molecular Genetics* **2014**, 23(20), 5342–5352

Interim Summary: Losmapimod Preclinical Program

- Losmapimod Inhibits DUX4 Driven Gene Expression and Muscle Cell Death in FSHD Patient Cells
- Losmapimod demonstrates reversal of FSHD disease signature (RNAseq)
- Losmapimod impacts gene expression changes across the entire DUX4 program
- Losmapimod is highly selective against p38α, p38β, and MK2 (MAPKAPK2), a kinase downstream within the same pathway
- In vitro studies using primary and immortalized myoblast differentiation have demonstrated no detrimental effects on myogenesis at clinically relevant concentrations, as evidenced by transcriptomic and imaging analyses
- Preclinical in vivo studies have not identified any negative effects on muscle tissue regeneration or function

Losmapimod Clinical Development Program To Date

Complete	**Fulcrum Preparatory Studies**	▪ Refined clinical endpoints: DUX4, MRI, Muscle Function, PROs
	Phase 1	▪ Generally well-tolerated in FSHD subjects ▪ Target engagement demonstrated ▪ Losmapimod penetrates FSHD muscle
	Phase 2b ReDUX4 *48-week analysis* *(n = 80; 1:1)*	▪ Losmapimod demonstrated disease modifying properties as evidenced by treatment benefit on structural and functional measures of FSHD disease progression
	Phase 2 Open Label Study (OLS) *52-week analysis (n = 14)*	▪ Provides supporting evidence of losmapimod demonstrating disease modifying properties as observed in ReDUX4

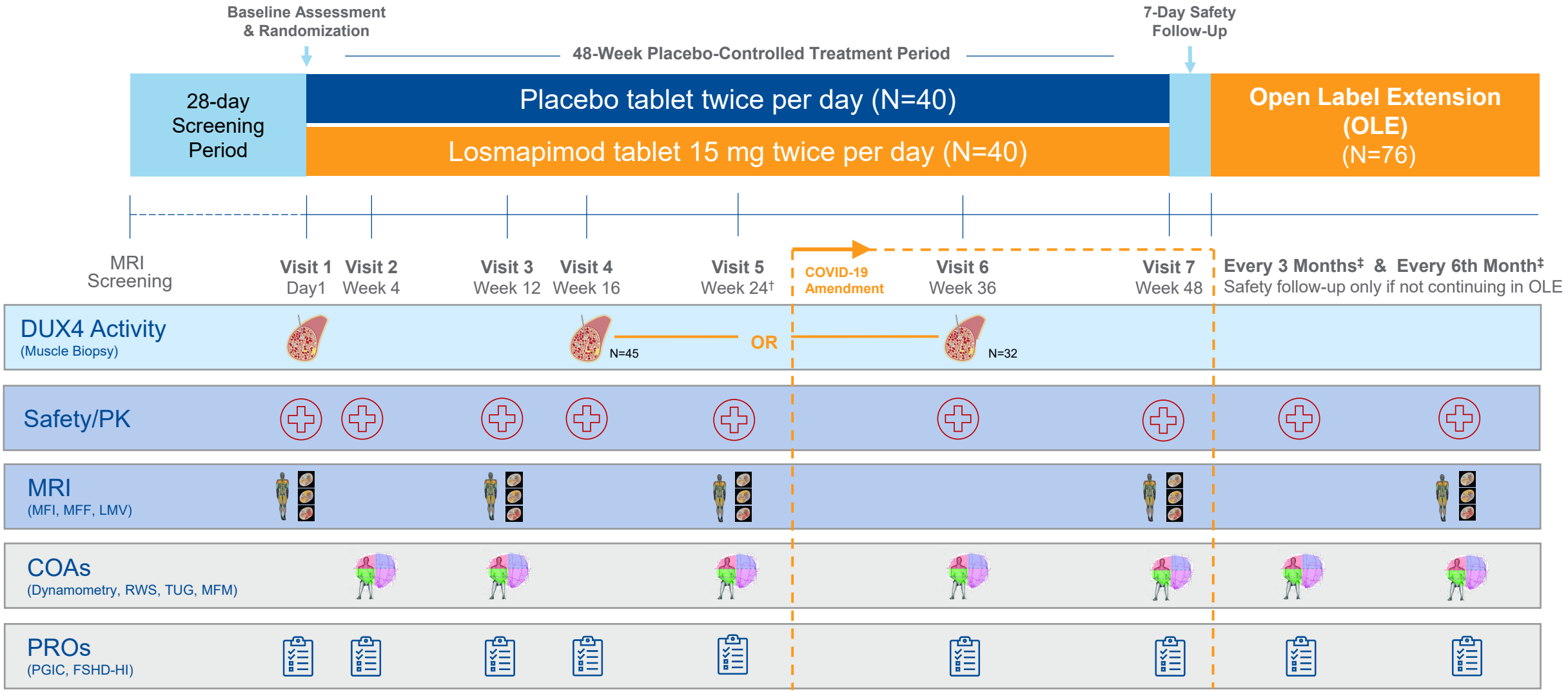
Ongoing Studies	**Phase 2b ReDUX4***
	Long Term Open Label Extension (OLE)
		▪ Continued long term evaluation of
		  ▪ Clinical assessments of upper extremity function and mobility
		  ▪ Quantitative whole body musculoskeletal MRI
		  ▪ Patient reported outcomes

	Phase 2 Open Label Study (OLS)* Extension	
	Phase 3 REACH Trial *Pivotal 48-week study* *(n = 260; 1:1)*	▪ Global, randomized, double-blind, placebo-controlled, 48-week, parallel-group study of the efficacy and safety of losmapimod

*Main studies complete, long term open-label extensions ongoing

Building on >3,600 Patient Safety Data Base (AEs Generally Mild; Drug is Well Tolerated)

ReDUX4 Trial Design*



*All analyses were pre-specified in the statistical analysis plan, with the exception of dynamometry, which is now presented as percent change from baseline. †Protocol amended due to COVID-19 to allow collection of data to inform study endpoints. 16 subjects had completed the Wk24 visit and had already rolled over to the OLE at the time of amendment approval. ‡PK measurements will not be assessed in OLE study. COAs=clinical outcome assessments; FSHD-HI=facioscapulohumeral muscular dystrophy health index; MFF=muscle fat fraction; MFI=muscle fat infiltration; MFM=motor function measure; MRI=magnetic resonance imaging; LMV=lean muscle volume; PGIC=patients' global impression of change; PK=pharmacokinetics; PROs=patient reported outcomes; RWS=reachable workspace; TUG=timed up and go.

ReDUX4 Study Participant Randomization Was Well Balanced

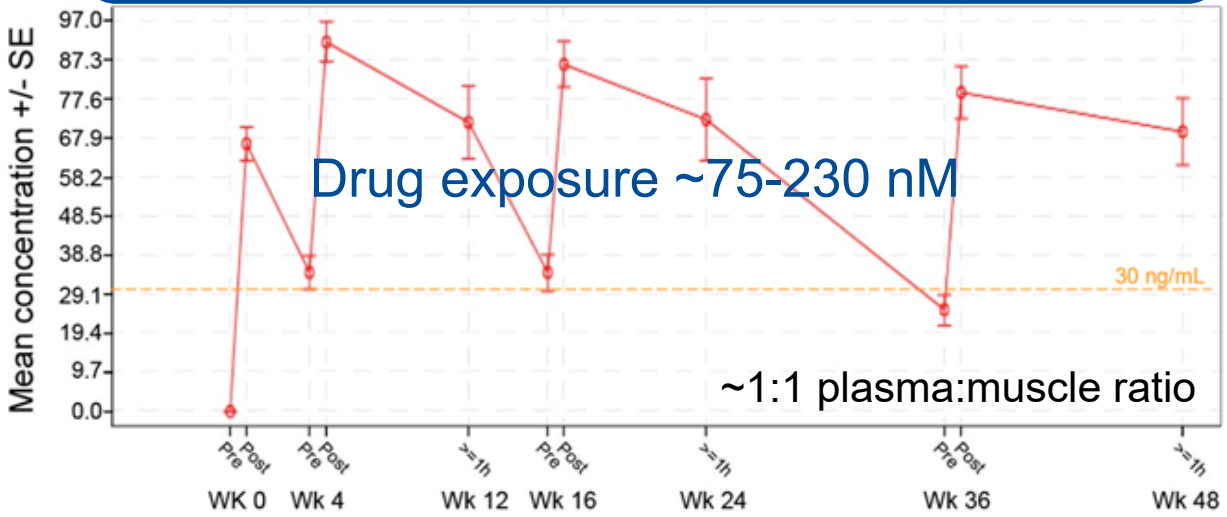
		Placebo BID (N=40)	Losmapimod 15 mg BID (N=40)
Completed, n (%)		38 (95)	39 (97.5)
Discontinued*, n (%)		2 (5.0)	1 (2.5)
DEMOGRAPHICS			
Age (years)	N	40	40
	Mean (SD)	45.7 (+/- 12.7)	45.7 (+/- 12.4)
Race, n (%)	White	39 (97.5)	31 (77.5)
	Asian	0	5 (12.5)
	Other	0	1 (2.5)
	Not Applicable	1 (2.5)	3 (7.5)
Ethnicity, n (%)	Hispanic or Latino	3 (7.5)	0
	Not Hispanic or Latino	36 (90.0)	37 (92.5)
	Not Applicable	1 (2.5)	3 (7.5)
Body Mass Index (BMI) (kg/m^2)	N	39	40
	Mean (SD)	26.2 (+/- 4.9)	25.7 (+/- 5.4)
D4Z4 Repeat Category, n (%)	1-3 Repeats	6 (15.0)	7 (17.5)
	4-9 Repeats	34 (85.0)	33 (83.5)
Ricci Score n (%)	2	0	0
	2.5	7 (17.5)	5 (12.5)
	3	18 (45.0)	19 (47.5)
	3.5	7 (17.5)	11 (27.5)
	4	8 (20.0)	5 (12.5)

*Discontinuations were not related to study drug.

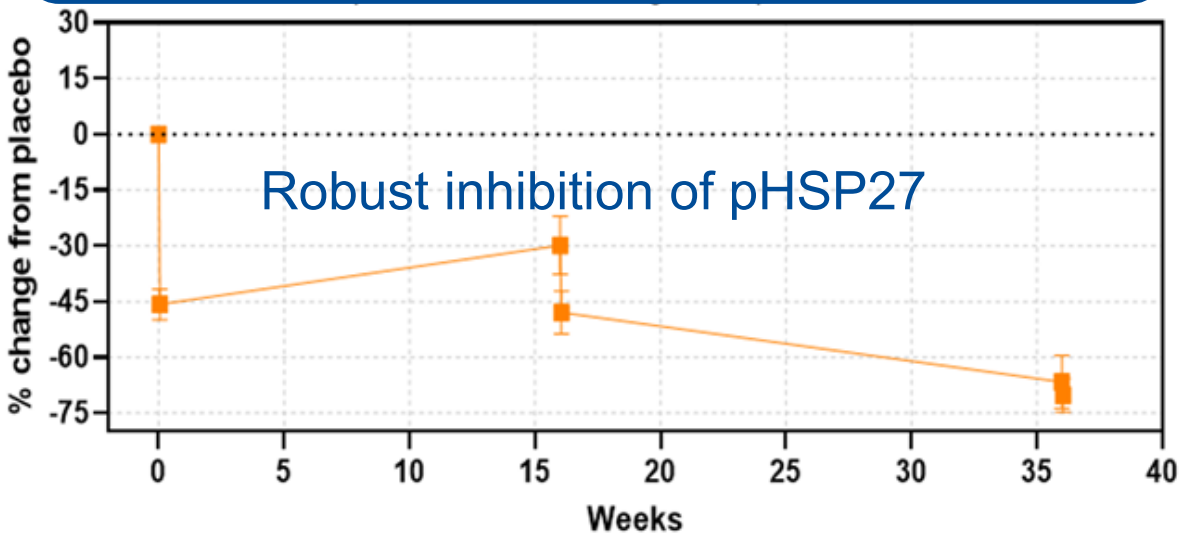
ReDUX4 Data: Losmapimod Has Expected Exposure in Blood and Muscle and Shows Target Engagement Through Week 36

- Plasma and muscle concentrations of drug are well above key EC50's
- Drug exposure is well below where effects on myogenesis are observed preclinically
- Robust target engagement through 36 weeks (last assessment) as assessed by pHSP27 levels
  - (Levels of pHSP27/tHSP27 in blood after sorbitol stimulation ex vivo show a reduction of ~35% to 65% at C_{max})

Losmapimod concentration in plasma by visit

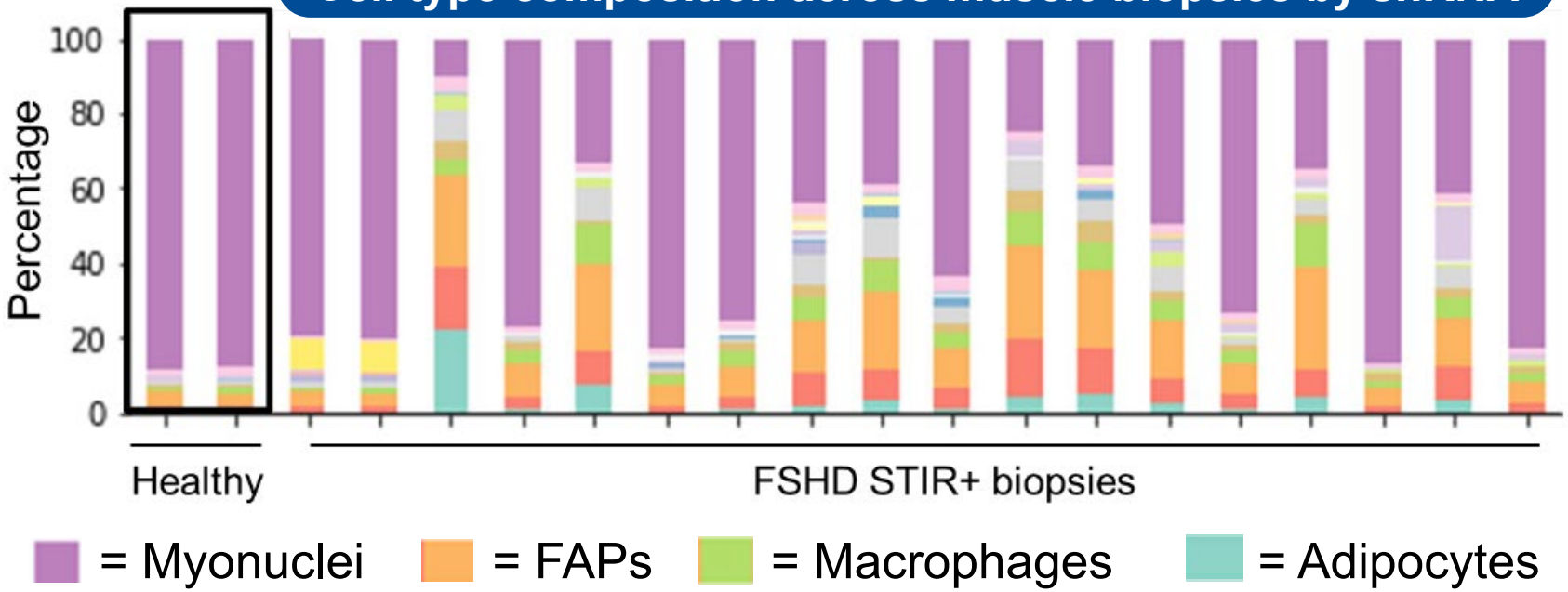


pHSP27/tHSP27 change from placebo

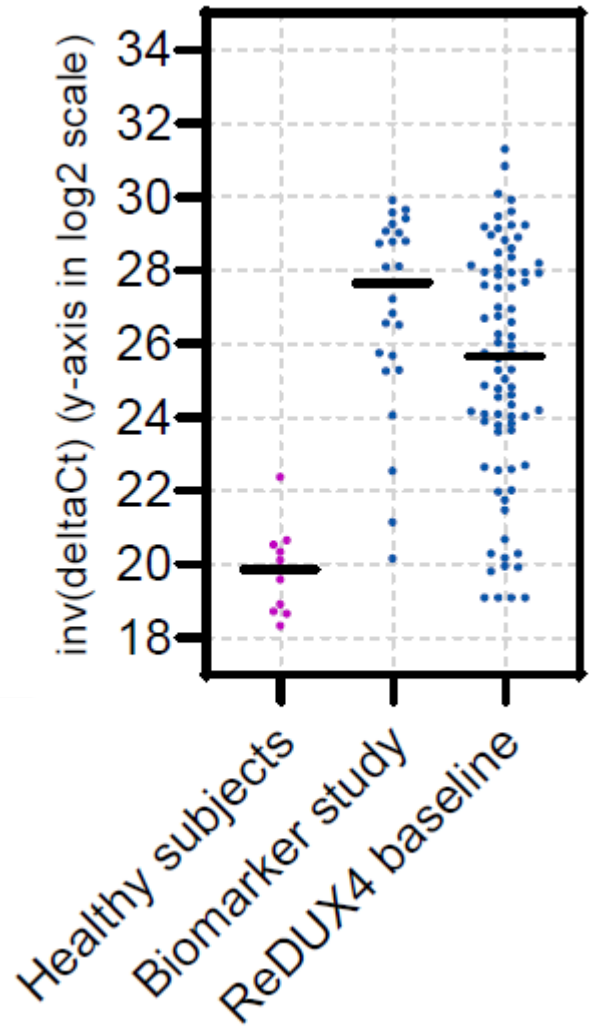


DUX4 Activity in Biopsies in ReDUX4 were Highly Heterogenous

Cell type composition across muscle biopsies by snRNA



DUX4-driven gene expression



- Heterogeneity in Biopsy Composition and in DUX4 Activity
- Cellular composition of biopsy samples is highly diverse
- >1,000-fold differences in baseline DUX4-driven gene expression

FAPs: Fibroadipogenic Progenitor Cells

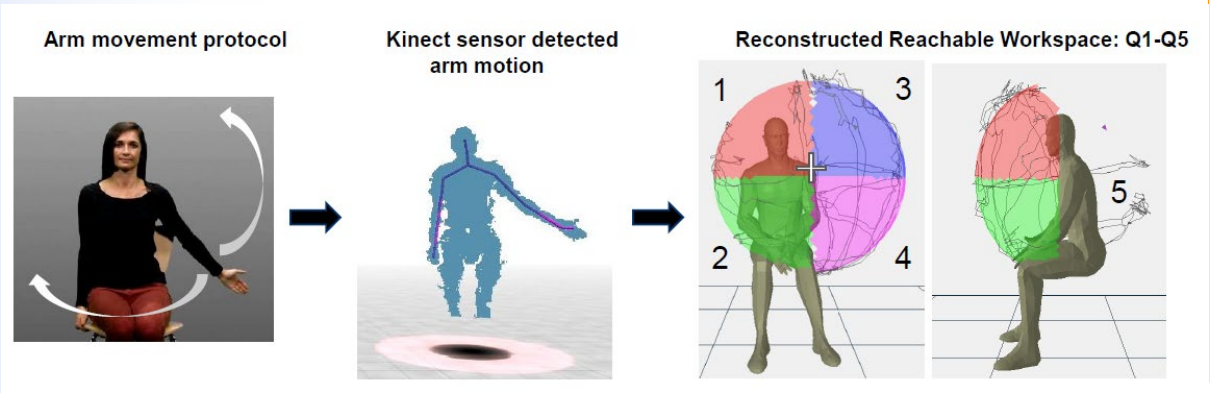
ReDUX4 Showed Clinical Benefits at Week 48

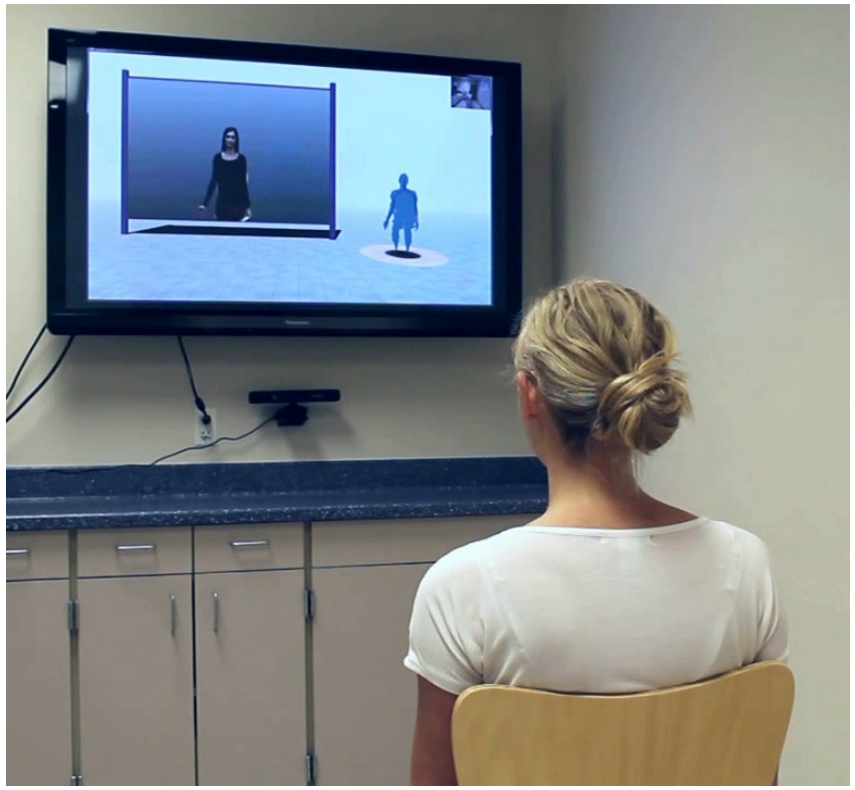
RWS: reachable workspace; MFI: muscle fat infiltration; PGIC: patients' global impression of change

Reachable Work Space (RWS)

Evaluating Upper Arm and Shoulder Function in FSHD subjects

- ***Centrally-read*** evaluation of individual global upper extremity function, including shoulder and proximal arm
- Subjects sit in front of Microsoft Kinect sensor and undergo standardized upper extremity movement protocol
- Reliable and sensitive to change
- Evaluation performed with and without weights

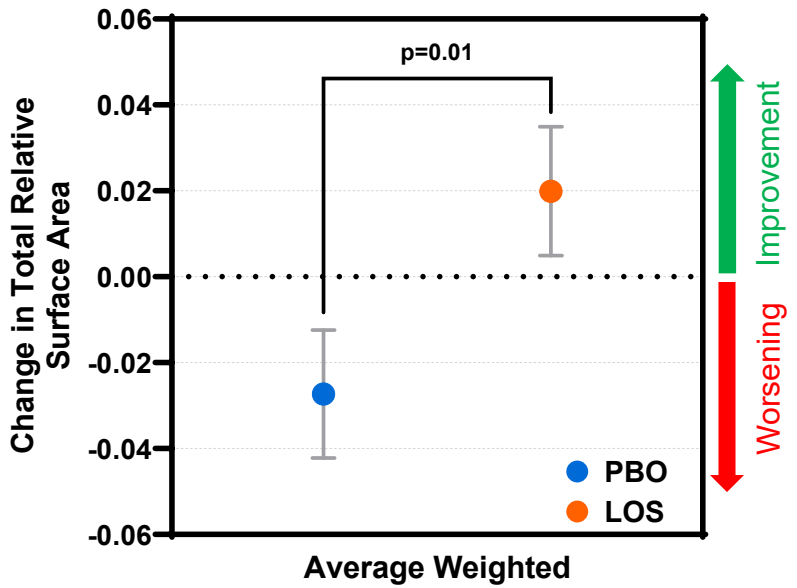




Han et al., 2015 "Reachable workspace reflects dynamometer-measured upper extremity strength in facioscapulohumeral muscular dystrophy"

Losmapimod Demonstrated Significant Improvement in RWS Relative to Placebo with a Durability of Effect in Open Label Extension

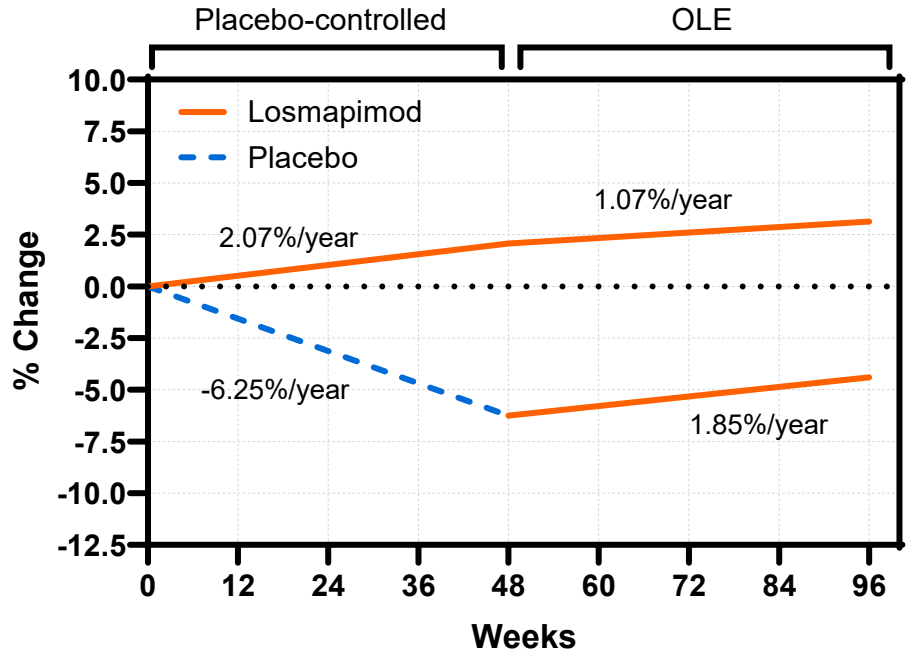
Change from Baseline in Average Total RSA (Q1-5) + Weight at 48 Weeks



Absolute Baseline Average Total RSA + Weight

	PBO	LOS
Baseline RSA (SE)	0.540 (± 0.038)	0.532 (± 0.036)

Annualized % Change of Average Total RSA (Q1-5) + Weight



	RCT (48 Weeks) LOS vs. PBO	OLE (96 Weeks) LOS vs. LOS
P-value*	0.04	0.80

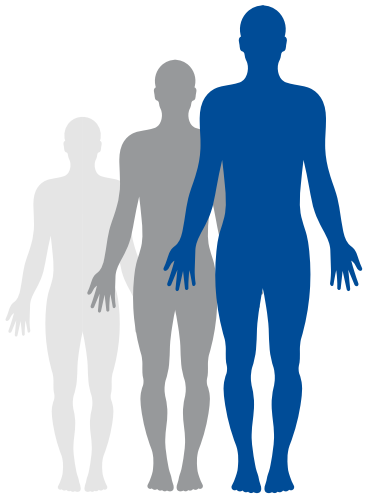
Data from ReDUX4 trial and the ReDUX4 OLE trial; RSA: Relative surface area; OLE: Open label extension; PBO: placebo; LOS: losmapimod

*P-value of the difference in rate of change

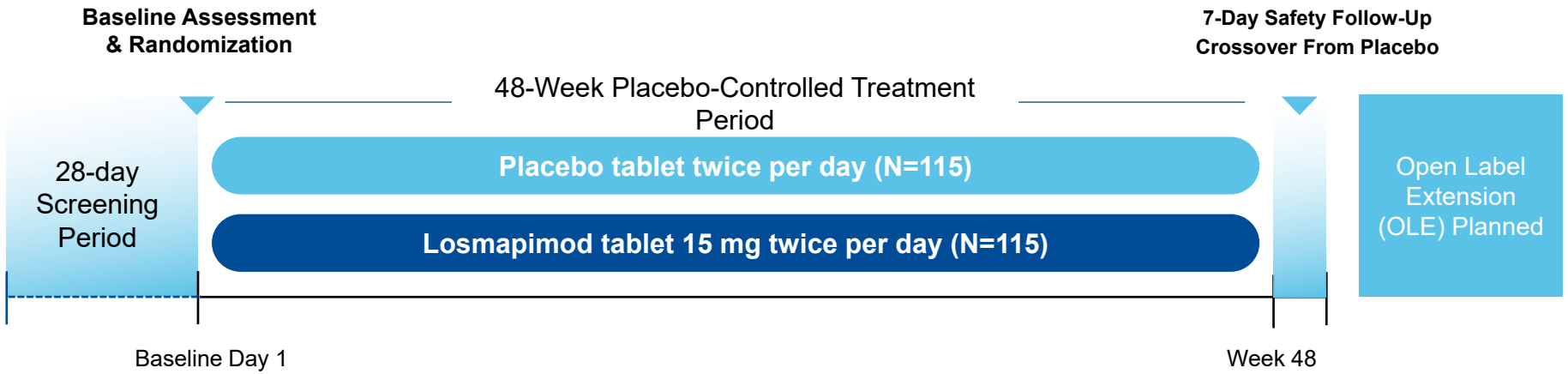
REACH: Global Phase 3 Trial of Losmapimod in FSHD

Study Population

Enrollment completed:
260 patients*, 18-65 years old



Study Design



Study Endpoints

Primary

Average RWS quantification of total relative surface area with 500g wrist weight in dominant arm and non-dominant arms

Secondary

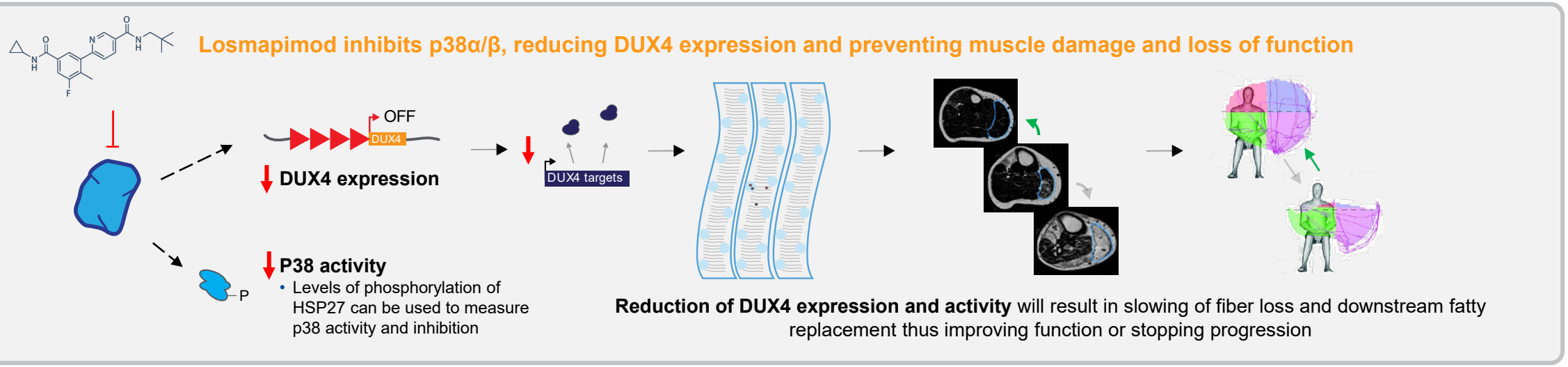
- Neuro-QoL Upper Extremity
- PGIC
- MFI
- Shoulder Dynamometry
- Safety and tolerability

Exploratory

- Healthcare utilization questionnaire
- EQ-5D questionnaire

RWS: Reachable Workspace; MFI: Muscle Fat Infiltration; PGIC: Patients' Global Impression of Change
*Trial was oversubscribed

Summary



- Losmapimod inhibits p38α/β, reducing DUX4 transcription and preventing muscle damage and loss of function
- Preclinical data show a profound effect of losmapimod on DUX4-driven gene expression in FSHD patient-derived myoblasts
- Clinical data demonstrate benefits in upper limb movement, muscle fat infiltration, and patient-reported outcomes
- The pivotal REACH clinical trial is on track for read-out in Q4 of this year

Acknowledgements

People Living With FSHD Participating in This Study

ReDUX4 & REACH Study Sites

Physical Therapists

Study Coordinators

Clinical and Scientific Advisors

- Baziel van Engelen, MD, PhD Radboud UMC
- Jeffrey Statland, MD. KUMC
- Lee Sweeney, PhD. UFL
- Leslie Leinwand, PhD. UC Boulder
- Peter Jones, PhD. UNR
- Rabi Tawil, MD. URMC
- Silvère van der Maarel, PhD. LUMC
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ReDUX4 Assessing losmapimod in FSHD

Reach

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PPD

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VIRTUALSCOPICS Imaging for Clinical Trials

IMMUNOLOGIX LABORATORIES

BIONIKS

Q² Solutions a Quintiles Quest Joint Venture

Patient Groups

FSHD SOCIETY

MDA Muscular Dystrophy Association

NORD National Organization for Rare Disorders

friends of FSH research