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CRISPR-Cas9 mediated removal of an intronic SMCHD1 mutation supresses DUX4 expression in FSHD myocytes in vitro

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Facioscapulohumeral dystrophy (FSHD) is one of the most prevalent muscular dystrophies, with an estimated prevalence of 1:8500. FSHD patients suffer from progressive, often asymmetric wasting of muscles of the face, shoulder and upper arms. The molecular hallmark of FSHD is the mis-expression of the transcription factor DUX4 in skeletal muscle. *DUX4* is located within the D4Z4 macrosatellite repeat array on chromosome 4, which can consist of 8-100 units in non-affected individuals. In somatic cells, D4Z4 is normally kept in a heterochromatic state, but in FSHD the D4Z4 chromatin is more relaxed, leading to DUX4 expression in myonuclei and subsequent muscle wasting. In the majority of FSHD patients D4Z4 chromatin relaxation can be explained by a contraction of one of the D4Z4 repeats to a size of 1-10 units. However, in a subset of patients the repeat is within the normal size range. In these cases the disease is caused by mutations in D4Z4 chromatin modifiers, most often Structural Maintenance of Chromosomes Hinge Domain containing 1 (*SMCHD1*). We have detected a mutation in intron 34 of *SMCHD1* in an FSHD family, which causes inclusion of a pseudo-exon in the transcript. This inclusion causes a premature stop codon, and a partial loss of SMCHD1 at D4Z4 leading to DUX4 expression. Removal of the intronic mutation by CRISPR-Cas9 mediated genome editing in cultured patient myocytes was effective in restoring expression of functional *SMCHD1* transcript from the affected allele. Importantly, we observed a significant reduction in *DUX4* expression in edited cultures, showing that restoring endogenous SMCHD1 expression levels can efficiently supress *DUX4* in somatic FSHD cells. This study shows that a potential future treatment aimed at increasing SMCHD1 expression levels in muscle can be of clinical benefit to FSHD patients. Furthermore, these results emphasize the necessity of sequencing *SMCHD1* intronic sequences for FSHD diagnostics.

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A Phase 2, randomized, placebo-controlled, 24-Week study of the efficacy and safety of losmapimod in treating subjects with FSHD: ReDUX4

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We evaluate the efficacy of losmapimod in inhibiting the aberrant expression of DUX4, the root cause of FSHD. Secondary objectives are to evaluate safety, tolerability, pharmacokinetic (PK), and target engagement (TE) in blood and muscle. FSHD is caused by aberrant expression of the transcription factor DUX4 in skeletal muscle. DUX4 activates a downstream transcriptional program resulting in skeletal muscle loss and motor disability. Losmapimod is a small molecule inhibitor of p38α/β. Pre-clinical studies demonstrated treatment with losmapimod resulted in dose-dependent reduction of DUX4 protein, DUX4 transcriptional program and skeletal muscle cell death in FSHD myotubes across all genotypes tested. Studies across other adult indications resulted in over 3,500 human exposures with adequate safety and tolerability. Eighty subjects age 18 to 65 with genetically confirmed FSHD1, clinical severity score of 2 to 4 (Ricci scale 0-5) and MRI identified skeletal muscle(s) for needle biopsy were randomized 1:1 to receive 15 mg losmapimod (n=40) or placebo (n=40) tablets PO BID for 24 weeks. Subjects participate in the study for approximately 29 weeks. All participants undergo muscle biopsies pre-treatment and at Week 16 to measure treatment effect on DUX4 activity. PK and TE are measured in blood and muscle. Musculoskeletal MRIs will be performed at screening, Week 12, and Week 24 for assessment of change in musculoskeletal parameters. Clinical outcome assessments include reachable workspace (RWS), FSHD-timed up and go (FSHD-TUG), dynamometry, and motor function measure domain 1 (MFM). Patient reported outcomes include FSHD-Health Index (FSHD-HI) and patient global impression of change (PGIC). Based on supportive pre-clinical and preliminary clinical data we have designed and launched a phase 2b clinical trial to assess the efficacy of losmapimod to treat the root cause of FSHD.

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Need for therapy and effect of symptomatic treatment in myotonic disorders: the Myotonia observation survey of patient access to therapy (MyoPath)

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Myotonic disorders are a family of conditions resulting from rare genetic mutations affecting skeletal muscle cells. They can be categorized as either dystrophic (DM) or non-dystrophic myotonia (NDM) and can have significant impact on patient quality of life (QoL), causing a range of symptoms including muscle stiffness. Mexiletine (MEX) is currently the only EU-approved antimyotonic therapy for adult patients with NDM. MyoPath aimed to obtain data on treatment access and impact on daily life for patients with myotonic disorders, as well as effectiveness of MEX therapy for these conditions. In total, 390 surveys were completed in 11 European countries, including 213 patients with DM type 1 (DM1) and 41 with NDM, of whom 37 had myotonia congenita (MC). Patients were asked if they felt that drug treatment could improve their QoL; those who were treatment naïve for MEX reported different motivations, depending on their condition. Those with DM1 (n=172) highlighted 'emotional well-being' and 'ability to exercise' (69% and 52% of respondents, respectively) as key reasons for wanting treatment; for patients with MC (n=12), 'allowing muscles to warm up more quickly before physical activity' was the most important reason (67% of respondents). For MEX treatment-experienced patients with MC (n=13), the largest benefits of the therapy were seen in reducing muscle stiffness, reducing time for muscles to warm up, and increasing mobility (>80% moderately to drastically improved); these were key motivations listed by patients with MC lacking treatment with MEX. Access to MEX treatment remains variable. While 78% of patients with MC had heard of MEX prior to the survey, there are currently no approved therapies for DM, including MEX. The most common reason given for never taking MEX in patients with DM1 was lack of a prescription (54%). Further research is needed to assess MEX efficacy in patients with DM to address this medical need.

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Open-Label study of losmapimod evaluating safety, tolerability, and changes in biomarker and clinical outcome assessments in subjects with FSHD1

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by loss of repression at the D4Z4 locus resulting in aberrant expression of the homeobox transcription factor DUX4. DUX4 expression activates its downstream transcriptional program resulting in cell death, skeletal muscle loss and progressive motor disability. Fulcrum Therapeutics is developing losmapimod to treat FSHD at its root cause. Losmapimod is a potent and highly selective small molecule inhibitor of p38α/β that in preclinical studies reduced DUX4 activity and its downstream transcriptional program in FSHD myotubes resulting in the prevention of cell death without impacting myogenesis. Losmapimod has been tested across many adult indications resulting in over 3,500 human exposures and has shown satisfactory safety and tolerability. The hypothesis is that treatment of FSHD with losmapimod will slow or arrest disease progression by reducing aberrant DUX4 expression via inhibition of p38α/β MAP kinase. Fourteen genetically confirmed FSHD1 adult subjects were enrolled at Radboudumc. Subjects will participate for up to 64-weeks including an 8-week pre-treatment period followed by a 52-week treatment period. This study will investigate the safety, tolerability, pharmacokinetics, and target engagement; and explore the treatment effects on molecular and imaging biomarkers, objective and subjective clinical outcomes, and real-world mobility assessments with wearables. The design and baseline characteristics of this study will be presented, including the adaptions required by the COVID-19 pandemic.

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New insights from post-hoc analyses of the OPTIMISTIC trial into the relation of the DM1-Activ-c questionnaire with other commonly used outcome measures

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The clinical trial OPTIMISTIC demonstrated positive effects of cognitive behavioral therapy (CBT) on participation, activity and exercise capacity of myotonic dystrophy type 1 (DM1) patients. A total of 28 different patient reported outcome measures, clinical tests and accelerometry measures were used in the trial. Here, we performed further post-hoc analyses to study the correlations among the outcome measurements, to potentially limit the number of outcome measures in future DM1 trials. The primary outcome measure, the DM1-Activ-c questionnaire, significantly correlated with the majority of other measurements at baseline, and may make other measurements such as the Six Minute Walk Test (6MWT), Myotonic Dystrophy Health Index (MDHI), Muscle Impairment Rating Scale (MIRS), Individualized Neuromuscular Quality of Life Questionnaire (INQoL) and accelerometry measurements redundant if used as a cross-sectional instrument. Longitudinal measurements, assessed by CBT-induced differences over the course of 10 months, correlated less and revealed a discordance among the physical 6MWT assessment and the DM1-Activ-c assessment on participation. This highlights the heterogeneity in CBT response observed in the OPTIMISTIC trial. To better understand this heterogeneity, we implemented a machine learning based regression approach to select variables associated with CBT response. Nine variables have been identified as potentially significant predictors of CBT response, one of which hints towards a more favorable CBT response if patients have a higher number of the various CBT module indications at baseline, which is compatible with a higher disease burden at baseline. The combined results can guide the design of prospective trials in DM1 and other neuromuscular disorders.

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A biomarker of DUX4 activity to evaluate losmapimod treatment effect in FSHD Phase 2 trials

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To identify a panel of DUX4-regulated gene transcripts pathologically expressed in FSHD muscle biopsies to measure treatment effect of losmapimod on the root cause of FSHD. Both FSHD1 and 2 are caused by the pathogenic expression of the *homeobox transcription factor* DUX4. Aberrant DUX4 expression in skeletal muscle results in profound transcriptional dysregulation and a characteristic DUX4-regulated transcriptional signature leading to myofiber death and the replacement of muscle with fat. Clinical evaluation of the p38a/b inhibitor losmapimod is ongoing in FSHD including assessment of efficacy for inhibition of DUX4 expression in affected skeletal muscle. While pharmacodynamic detection of DUX4 protein and *DUX4* mRNA in FSHD muscle is challenging, this is not the case for DUX4-regulated gene transcripts which are readily detected at sites of active disease (Wang, 2018). To support the development of a molecular efficacy endpoint for the treatment of the root cause of FSHD, we completed a preparatory biomarker study (FIS-002-2018) that included examination of repeated muscle biopsies from affected muscle tissue for evidence of DUX4 activity. 17 subjects were enrolled and 16 completed the FIS-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 RNA sequencing data from a previous study (Wang, 2018) 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. DUX4-regulated gene transcripts provide a pharmacodynamic biomarker endpoint to measure treatment effect for the root cause of FSHD in therapeutic clinical trials. We have identified a panel of such transcripts that will be used to measure DUX4 activity in affected skeletal muscles from FSHD patients.

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Single-cell transcriptomes in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) causes progressive muscle wasting triggered by aberrant de-repression of DUX4, a pro-apoptotic transcription factor, in <1:200 muscle cells. A single-cell approach may be informative to gain mechanistic insights regarding FSHD from these rare cells. We are using single-cell analyses to characterize i) infrequent cells expressing DUX4 during distinct stages of myogenic reprogramming, ii) cellular heterogeneity among FSHD models and acutely isolated cells from affected muscle, and iii) immune cell phenotypes in FSHD muscle. We aim