The prospects of targeting DUX4 in facioscapulohumeral muscular dystrophy

Bouwman, L.F.; Maarel, S.M. van der; Greef, J.C. de

REVIEW

OPEN

The prospects of targeting DUX4 in facioscapulohumeral muscular dystrophy

Linde F. Bouwman, Silvère M. van der Maarel, and Jessica C. de Greef

Purpose of review
Facioscapulohumeral muscular dystrophy (FSHD) is a neuromuscular disorder, which is caused by incomplete repression of the transcription factor double homeobox 4 (DUX4) in skeletal muscle. To date, there is no DUX4-targeting treatment to prevent or delay disease progression. In the present review, we summarize developments in therapeutic strategies with the focus on inhibiting DUX4 and DUX4 target gene expression.

Recent findings
Different studies show that DUX4 and its target genes can be repressed with genetic therapies using diverse strategies. Additionally, different small compounds can reduce DUX4 and its target genes *in vitro* and *in vivo*.

Summary
Most studies that show DUX4 repression by genetic therapies have only been tested *in vitro*. More efforts should be made to test them *in vivo* for clinical translation. Several compounds have been shown to prevent DUX4 and target gene expression *in vitro* and *in vivo*. However, their efficiency and specificity has not yet been shown. With emerging clinical trials, the clinical benefit from DUX4 repression in FSHD will likely soon become apparent.

Keywords
double homeobox 4, facioscapulohumeral muscular dystrophy, gene therapy, therapeutics

INTRODUCTION

Double homeobox 4 (DUX4) is a transcription factor implicated in zygotic genome activation (ZGA) during the four-cell stage in human embryos where it acts as an activator of repetitive elements and cleavage-specific genes [1,2]. DUX4 is considered to be epigenetically repressed in most somatic tissues, including in skeletal muscles. In patients with facioscapulohumeral muscular dystrophy (FSHD; MIM 158900), a progressive neuromuscular disorder characterized by asymmetric weakness and wasting of the facial, scapular, and humeral muscles [3], epigenetic repression of the D4Z4 macrosatellite repeat is lost. This results in transcriptional activity from the *DUX4* locus, which is encoded within each D4Z4 repeat unit [4,5]. DUX4 activates genes that are generally not expressed in nonaffected skeletal muscles, including genes that are activated during ZGA and genes of the immune system [6,7]. DUX4 overexpression in myogenic cells induces different toxic cascades including an increase in oxidative stress, nonsense-mediated decay inhibition, and inhibition of myogenesis. These changes ultimately lead to the death of myogenic cells [8–11]. In most patients (FSHD1), the disease is caused by a contraction of the D4Z4 repeat to 1–10 units whereas nonaffected individuals carry 8–100 units [12]. FSHD can only occur when the contracted repeat is located on a permissive 4qA allele that contains a *DUX4* polyadenylation signal (PAS) adjacent to the most distal D4Z4 unit. Nonpermissive 4qB alleles lack this PAS, consequently DUX4 is not stably expressed [5]. Approximately 5% of patients (FSHD2) carry a permissive 4qA allele of 8–20 D4Z4 units together with

Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

Correspondence to Jessica C. de Greef, Department of Human Genetics, Leiden University Medical Center, Leiden, 2333 ZA Leiden, The Netherlands. Tel: +31 715269482; e-mail: J.C.de_Greef@lumc.nl

Curr Opin Neurol 2020, 33:635–640

DOI:10.1097/WCO.0000000000000849

KEY POINTS

- As there is no molecular therapy that prevents or delays disease progression in patients with FSHD, there is a high clinical need for new therapeutic strategies.
- Inappropriate expression of DUX4 in skeletal muscles causes FSHD, therefore preventing DUX4 or target gene expression should block all toxic downstream pathways.
- Using AONs and PMOs could be a promising therapeutic strategy for FSHD as they only target the disease gene.
- Different small compounds that have been tested for other diseases show promising results *in vitro* and *in vivo*, and can be tested in clinical trials in the short term.

a mutation in an epigenetic repressor of *DUX4*, namely SMCHD1, DNMT3B, or LRIF1 [13–15]. FSHD2 disease genes also act as modifiers in FSHD1, suggesting that FSHD1 and FSHD2 form a disease continuum resulting from the loss of epigenetic repression of *DUX4* in skeletal muscle with shared clinical phenotypes [16–18].

Despite our increased understanding of the different genetic and epigenetic factors that contribute to FSHD development, there is no treatment that prevents or delays disease progression; only moderate exercise and cognitive behavioral therapy have shown some clinical benefit [19,20]. Thus far, most clinical trials focused on blocking one of the downstream pathways of DUX4. Short-term treatment with the corticosteroid immunosuppressant prednisone did not significantly improve muscle strength or mass [21]. Treating patients with different antioxidants to reduce oxidative stress in the muscles only slightly improved physical performance [22]. As DUX4 activates many pathways, blocking one of them may not be sufficient. In the present review, we will highlight recent progress made in developing new therapeutic strategies for FSHD, with a focus on approaches that prevent DUX4 expression, thereby affecting all downstream pathways.

TARGETING THE DOUBLE HOMEOBOX 4 TRANSCRIPT OR THE D4Z4 REPEAT WITH GENETIC THERAPIES

FSHD is caused by a gain of function mechanism. DUX4 suppression is therefore a promising treatment strategy that should block all effects consequent to DUX4 activity in skeletal muscle. However, numerous highly homologous copies of *DUX4* can be found in the human genome, and the D4Z4 repeat is extremely GC-rich, making it difficult to target. So far, most studies focused on blocking the DUX4 transcript as genomic editing has only recently become an exploitable alternative (Fig. 1, Table 1). Marsollier *et al.* [23] and Chen *et al.* [24] tested the efficiency of different antisense phosphorodiamidate morpholino oligomers (PMOs) to target the DUX4 transcript. Both studies identified two PMOs that efficiently repressed DUX4 and target gene expression in FSHD myotube cultures. Chen *et al.* [24,25] also tested the efficiency of a PMO that targets the *DUX4* PAS in a xenograft mouse model containing an engrafted muscle biopsy of a patient with FSHD and confirmed the reduction of DUX4 and target gene expression. Another study tested different antisense oligonucleotides (AONs) designed to interfere with DUX4 splicing or to target the *DUX4* PAS [26]. All six AONs reduced the percentage of DUX4-positive nuclei and atrophic myotubes. As an alternative for PMOs and AONs, DNA aptamers with specific secondary structural elements that target the DUX4 protein can be

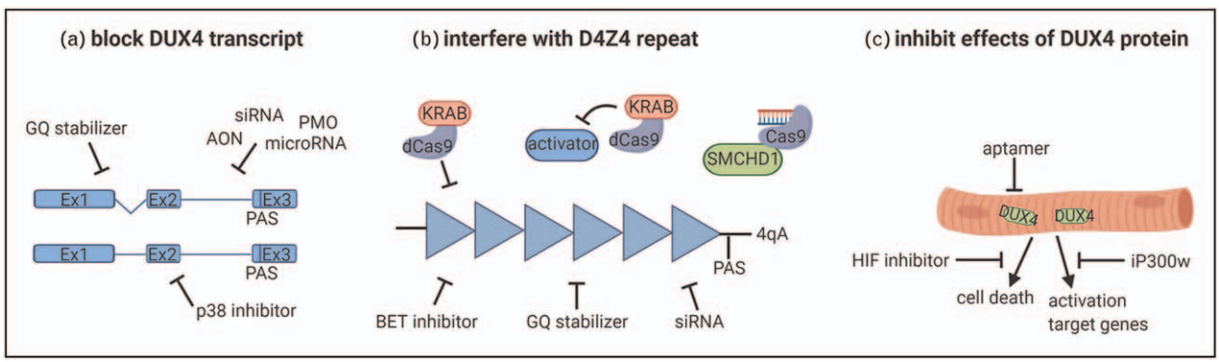

FIGURE 1. Overview of described therapeutics and where they target. (a) Therapies that repress the DUX4 transcript. (b) Therapies that restore epigenetic repression of the D4Z4 repeat. Each triangle represents a D4Z4 repeat unit in euchromatic state. (c) Therapeutics that block the DUX4 protein or prevent muscle damage caused by the DUX4 protein.

Table 1. Overview of studies and their main outcomes using different therapeutic strategies to repress DUX4

Reference	Treatment	Model	Main results
Genetic therapies			
Marsollier *et al.* [23]	PMO targeting DUX4	Immortalized FSHD myotubes	↓DUX4 transcript, ↓target genes
Chen *et al.* [24]	PMO targeting DUX4	Primary FSHD muscle cells FSHD xenograft mouse model	*In vitro*: ↓DUX4 protein, ↓target genes *In vivo*: ↓DUX4 transcript, ↓target genes
Ansseau *et al.* [26]	AON targeting DUX4	Primary FSHD myoblast and myotubes	↓DUX4 protein, ↓atrophic myotubes
Klingler *et al.* [27▪]	Aptamers targeting DUX4	–	Aptamers can bind to DUX4 protein
Wallace *et al.* [28,29▪]	miDUX4.405	Mice overexpressing DUX4 by AAV	↓skeletal muscle pathology, ↓DUX4 transcript, ↑grip strength, low toxicity
Lim *et al.* [30]	siRNAs targeting coding and noncoding regions	Primary FSHD muscle cells	↓DUX4 transcript, ↓target genes,
Himeda *et al.* [31]	CRISPR/dCas9 targeting D4Z4 locus	Primary FSHD myocytes	↓DUX4 transcript, ↓target genes
Himeda *et al.* [32▪]	CRISPR/dCas9 targeting D4Z4 activators	Primary FSHD myocytes	↓DUX4 transcript
Goossens *et al.* [33▪▪]	Restoring SMCHD1 with CRISPR/Cas9	Gene edited FSHD monoclonal myotubes	↓DUX4 transcript, ↓target genes, ↑wild-type SMCHD1 transcript
Small compounds			
Bosnakovski *et al.* [35▪]	iP300w	Myotubes from FSHD myoblast clonal cell lines iDUX4pA mice	*In vitro*: ↓target genes *In vivo*: ↓target genes, ↓fibrosis genes
Campbell *et al.* [38]	BET inhibitors	Immortalized FSHD myogenic cells	↓DUX4 transcript, ↓target genes
Ciszewski *et al.* [39▪]	Berberine	Immortalized FSHD myoblasts/myotubes Mice overexpressing DUX4 by AAV	*In vitro*: ↓DUX4 transcript, ↓target genes, ↑fusion index *In vivo*: ↓DUX4 protein, ↑muscle specific force
Oliva *et al.* [41▪▪]	p38 inhibitors	Immortalized FSHD myotubes/myoblasts FSHD xenograft mouse model	*In vitro*: ↓DUX4 transcript, ↓target genes *In vivo*: ↓DUX4 transcript, ↓target genes
Lek *et al.* [43▪▪]	Hypoxia signaling inhibitors	Immortalized myoblasts FSHD primary myotubes	Immortalized myoblasts: ↓DUX4-induced cell death, ↓DUX4 protein FSHD primary myotubes: ↓target genes

designed that may improve specificity and affinity [27▪]. Their efficiency in reducing DUX4 and target genes in myogenic cells has not yet been shown. Wallace *et al.* [28] tested the use of RNA interference to inhibit the DUX4 transcript. MicroRNA miDUX4.405 that targets one of the homeodomain-encoding sequences in the DUX4 open reading frame (ORF) reduced DUX4 expression and DUX4-induced muscle pathology in mice intramuscularly injected with AAV6.DUX4 and AAV6.miDUX4.405. The safety and toxicity of miDUX4.405 was assessed by injecting different concentrations intramuscularly or intravenously in wild-type mice. Although miDUX4.405 was well tolerated, another DUX4 microRNA showed high toxicity in skeletal muscles [29▪]. Finally, another study tested novel designed siRNAs and siRNAs that mimic the endogenously generated small RNAs targeting the *DUX4* coding region and regions upstream of the coding region [30]. Both siRNAs targeting the coding and noncoding region reduced DUX4 and target gene expression in FSHD myotubes, indicating that the endogenous

RNAi pathway is involved in maintaining the repressive state of D4Z4 and could be exploited to restore epigenetic repression.

Several studies used different approaches to reestablish epigenetic repression at the FSHD locus by targeting D4Z4 modifiers (Fig. 1B). Himeda *et al.* [31] tested the recruitment of the transcriptional repressor KRAB to the D4Z4 repeat using catalytically inactive dCas9 in FSHD myocytes. As dCas9 is unable to make double-strand breaks in the genome, it should not induce permanent DNA damage at other sites, avoiding one of the main concerns for CRISPR-based therapeutics. The recruitment of KRAB promoted the repressive regulators KAP1, HP1α, and HP1β at the D4Z4 repeat and as a result the expression of DUX4 and target genes was reduced. The same approach was used to repress the DUX4 activators BAZ1A, BRD2, KDM4C, and SMARCA5, which were identified in a targeted knockdown screen in FSHD myocytes [32▪]. By using the CRISPR/dCas9-KRAB approach, they confirmed that recruitment of KRAB to D4Z4 activators reduced DUX4 expression in FSHD myocytes. Another strategy is to genetically manipulate D4Z4 modifiers, which most often have a single copy locus. Goossens *et al.* [33▪▪] identified a SMCHD1 variant in a FSHD2 family leading to the inclusion of a pseudo-exon that disrupts the SMCHD1 ORF. Removal of the pseudo-exon by genome editing increased wild-type SMCHD1 transcript levels and reduced DUX4 and target gene expression in myotubes derived from the affected family. Furthermore, it has been shown that moderate SMCHD1 overexpression in FSHD1 and FSHD2 myotubes results in reduced DUX4 and target gene levels [34]. Thus, restoring SMCHD1 by gene editing could treat patients with FSHD2 with a loss-of-function mutation in SMCHD1. For other patients with FSHD, increasing SMCHD1-mediated repression of DUX4 could be an alternative strategy.

Blocking the DUX4 transcript or restoring epigenetic repression at the FSHD locus may prevent muscle damage in patients with FSHD. However, most DUX4 therapeutics have only been tested in myocytes and are not yet designed to target skeletal muscles *in vivo*. To accelerate the availability of a therapy for patients, more efforts should be made to test novel therapeutics in animal models.

THERAPEUTIC STRATEGIES USING SMALL COMPOUNDS

As an alternative approach, compounds can be used to suppress DUX4 in a direct or indirect manner (Fig. 1, Table 1). An advantage is that some of these compounds are already tested for other diseases, therefore more is known about their safety. One study used a histone acetyltransferase p300 inhibitor (iP300w) to inhibit the DUX4 protein from activating its target genes as DUX4 utilizes p300 for this [35▪,36]. As expected, iP300w barely affected DUX4 expression in FSHD myotubes, but target gene expression was severely reduced. *In vivo*, iP300w administration prevented muscle mass loss and reduced the expression of target and fibrosis genes in iDUX4pA mice carrying a doxycycline-inducible DUX4 transgene [35▪,37]. Campbell *et al.* [38] performed a screen in FSHD myogenic cells to identify novel compounds that reduce DUX4 expression. Different BET bromodomain inhibitors that have already been tested in clinical trials for other diseases reduced DUX4 and target gene expression by inhibiting the BET protein BRD4. BET proteins enhance gene transcription by recruiting transcriptional elements to acetylated chromatin. Another study tested berberine, a compound that binds and stabilizes certain secondary nucleic acid structures including G-quadruplexes [39▪]. Multiple G-quadruplexes were identified within the enhancer and promoter regions of DUX4 and in the DUX4 transcript itself. In FSHD myoblasts, berberine treatment reduced DUX4 and target gene expression. To test the effect of berberine *in vivo*, mice injected intramuscularly with DUX4 AAVs received an intraperitoneally injection with berberine. Berberine reduced DUX4 protein expression and some DUX4-mediated muscle pathology, but overall the effect was mild [39▪].

Two studies reported that DUX4 can be repressed by enhancing cyclic adenosine monophosphate levels using β2 adrenergic receptor agonists or phosphodiesterase inhibitors [38,40]. Recently, both Fulcrum Therapeutics and Oliva *et al.* reported DUX4 suppression by p38α/β mitogen-activated protein kinase (MAPK) inhibition [41▪▪,42▪]. p38 MAPK is activated by β2 adrenergic signaling and responds to different stress stimuli. Several commercially available p38 inhibitors reduced DUX4 and target gene expression in FSHD myotubes and myoblasts [41▪▪]. To study the effect of p38 inhibitors *in vivo*, losmapimod and PH-797804 were tested in a FSHD xenograft mouse model containing transplanted FSHD myoblasts after barium chloride-induced muscle damage. Both inhibitors reduced DUX4 and target gene expression in the xenografted tibialis anterior muscle, however the exact mechanism of DUX4 suppression by p38 inhibitors is unknown. Fulcrum Therapeutics is currently performing a clinical trial testing losmapimod in patients with FSHD. The first results are expected mid-2020. Finally, a genome-wide CRISPR-Cas9 screening approach was recently performed in a myoblast cell line containing a doxycycline-inducible

DUX4 transgene with the aim to identify new pathways involved in DUX4-induced cell death [43▪▪]. Doxycycline-induced myoblasts usually die within 48 h, therefore edited cells that survive were hypothesized to carry loss-off-function mutations in genes required for DUX4-induced cell death. In DUX4-resistant cells, loss-off-function mutations were identified in multiple hypoxia signaling pathway genes including in HIF1A and ARNT, subunits of the transcription factor HIF-1. Treatment with HIF signaling inhibitors reduced the amount of DUX4 protein, target gene expression and cell death (Fig. 1C). Different FDA-approved hypoxia signaling inhibitors are available, which could lead to a rapid clinical translation. Also, the other unexplored genes that were identified in this screen may reveal new pathways involved in DUX4-induced cell death.

The above described therapeutics can be tested in the short term in clinical trials as most compounds have already been studied in FSHD mouse models or tested in clinical trials for other diseases. However, some of these therapeutics target factors, like p300 and p38 that are involved in many other pathways, may give significant side effects in patients [44,45].

CONCLUSION

At present, there is no therapy that prevents or delays disease progression in patients with FSHD. Most studies targeting DUX4 have only performed experiments in myoblast and myotube cultures derived from patients. Because of the complex disease mechanism, the restriction of the D4Z4 repeat to primates, the heterogeneity of DUX4 expression, and the toxicity of DUX4 during development, animal models to test new therapeutic strategies were scarce [7,46,47]. Recently, different mouse models with controllable DUX4 expression in skeletal muscles have been developed [37,48–50]. These models open a new window for the development and safety assessment of new therapeutics.

As skeletal muscles compromise a large part of the body, the delivery of the therapeutics may not be efficient and administration of high doses can be toxic. For example, the life time of AONs are short and PMOs show difficulties in penetrating the cell membrane. Adjustments in their backbone can be made to increase their stability and delivery to the skeletal muscles [51]. As not all skeletal muscles are equally affected in patients with FSHD, local delivery to a limited number of muscles that are the most affected or the most important for maintaining independence may be considered. Also, it is unknown to what level DUX4 needs to be suppressed in skeletal muscles. DUX4 expression has been reported in myogenic cells and skeletal muscle biopsies in unaffected individuals, suggesting that low levels of DUX4 may be tolerated [52]. Finally, DUX4 was considered to be silenced in all somatic tissues, but recently DUX4 has been detected in the thymus and epidermis of healthy controls [53,54]. More research is needed to determine whether DUX4 has a function in somatic tissues and what the consequences of DUX4 suppression are.

Acknowledgements

We apologize to the many investigators whose work we could not cite because of space limitations and we thank all patients and family members for their participation in our studies. The authors are members of the European Reference Network for Rare Neuromuscular Diseases [ERN EURO-NMD].

Financial support and sponsorship

Our FSHD research is supported by grants from the US National Institutes of Health (National Institute of Neurological Disorders and Stroke P01NS069539; National Institute of Arthritis and Musculoskeletal and Skin Diseases R01AR066248), the Prinses Beatrix Spierfonds (W.OP14–01; W.OR15–26; W.OR17–04; W.OR19–06), the FSHD Society (Sylvia & Leonard Marx Foundation Fellowship FSHS-82017-02), the European Union Horizon 2020 Research and Innovation Program (Marie Skłodowska-Curie Individual Fellowship 795655), and Spieren voor Spieren.

Conflicts of interest

S.M.v.d.M. is co-inventor on several FSHD-related patent applications and consultant for Fulcrum Therapeutics.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. De Iaco A, Planet E, Coluccio A, *et al.* DUX-family transcription factors regulate zygotic genome activation in placental mammals. Nat Genet 2017; 49:941–945.
2. Hendrickson PG, Dorais JA, Grow EJ, *et al.* Conserved roles of mouse DUX and human DUX4 in activating cleavage-stage genes and MERVL/HERVL retrotransposons. Nat Genet 2017; 49:925–934.
3. Deenen JC, Arnts H, van der Maarel SM, *et al.* Population-based incidence and prevalence of facioscapulohumeral dystrophy. Neurology 2014; 83:1056–1059.
4. Daxinger L, Tapscott SJ, van der Maarel SM. Genetic and epigenetic contributors to FSHD. Curr Opin Genet Dev 2015; 33:56–61.
5. Lemmers RJ, van der Vliet PJ, Klooster R, *et al.* A unifying genetic model for facioscapulohumeral muscular dystrophy. Science (New York, NY) 2010; 329:1650–1653.
6. Geng LN, Yao Z, Snider L, *et al.* DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy. Dev Cell 2012; 22:38–51.
7. Wong CJ, Wang LH, Friedman SD, *et al.* Longitudinal measures of RNA expression and disease activity in FSHD muscle biopsies. Hum Mol Genet 2020; 29:1030–1043.
8. Feng Q, Snider L, Jagannathan S, *et al.* A feedback loop between nonsense-mediated decay and the retrogene DUX4 in facioscapulohumeral muscular dystrophy. eLife 2015; 4:e04996.

9. Turki A, Hayot M, Carnac G, *et al.* Functional muscle impairment in facioscapulohumeral muscular dystrophy is correlated with oxidative stress and mitochondrial dysfunction. Free Radic Biol Med 2012; 53:1068–1079.
10. Banerji CRS, Panamarova M, Hebaishi H, *et al.* PAX7 target genes are globally repressed in facioscapulohumeral muscular dystrophy skeletal muscle. Nat Commun 2017; 8:2152.
11. Bosnakovski D, Toso EA, Hartweck LM, *et al.* The DUX4 homeodomains mediate inhibition of myogenesis and are functionally exchangeable with the Pax7 homeodomain. J Cell Sci 2017; 130:3685–3697.
12. Tawil R, van der Maarel SM, Tapscott SJ. Facioscapulohumeral dystrophy: the path to consensus on pathophysiology. Skelet Muscle 2014; 4:12.
13. Lemmers RJ, Tawil R, Petek LM, *et al.* Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. Nat Genet 2012; 44:1370–1374.
14. van den Boogaard ML, Lemmers R, Balog J, *et al.* Mutations in DNMT3B modify epigenetic repression of the D4Z4 repeat and the penetrance of facioscapulohumeral dystrophy. Am J Hum Genet 2016; 98:1020–1029.
15. Hamanaka K, Šikrová D, Mitsuhashi S, *et al.* A homozygous nonsense variant in LRIF1 associated with facioscapulohumeral muscu-lar dystrophy. Neurology 2020; 94:e2441–e2447.
16. de Greef JC, Lemmers RJ, Camano P, *et al.* Clinical features of facioscapulohumeral muscular dystrophy 2. Neurology 2010; 75:1548–1554.
17. de Greef JC, Lemmers RJ, van Engelen BG, *et al.* Common epigenetic changes of D4Z4 in contraction-dependent and contraction-independent FSHD. Hum Mutat 2009; 30:1449–1459.
18. Sacconi S, Briand-Suleau A, Gros M, *et al.* FSHD1 and FSHD2 form a disease continuum. Neurology 2019; 92:e2273–e2285.
19. Voet N, Bleijenberg G, Hendriks J, *et al.* Both aerobic exercise and cognitive-behavioral therapy reduce chronic fatigue in FSHD: an RCT. Neurology 2014; 83:1914–1922.
20. Andersen G, Prahm KP, Dahlqvist JR, *et al.* Aerobic training and postexercise protein in facioscapulohumeral muscular dystrophy: RCT study. Neurology 2015; 85:396–403.
21. Tawil R, McDermott MP, Pandya S, *et al.* A pilot trial of prednisone in facioscapulohumeral muscular dystrophy. FSH-DY Group. Neurology 1997; 48:46–49.
22. Passerieux E, Hayot M, Jaussent A, *et al.* Effects of vitamin C, vitamin E, zinc gluconate, and selenomethionine supplementation on muscle function and oxidative stress biomarkers in patients with facioscapulohumeral dystrophy: a double-blind randomized controlled clinical trial. Free Radic Biol Med 2015; 81:158–169.
23. Marsollier AC, Ciszewski L, Mariot V, *et al.* Antisense targeting of 3' end elements involved in DUX4 mRNA processing is an efficient therapeutic strategy for facioscapulohumeral dystrophy: a new gene-silencing approach. Hum Mol Genet 2016; 25:1468–1478.
24. Chen JC, King OD, Zhang Y, *et al.* Morpholino-mediated knockdown of DUX4 toward facioscapulohumeral muscular dystrophy therapeutics. Mol Ther 2016; 24:1405–1411.
25. Zhang Y, King OD, Rahimov F, *et al.* Human skeletal muscle xenograft as a new preclinical model for muscle disorders. Hum Mol Genet 2014; 23:3180–3188.
26. Ansseau E, Vanderplanck C, Wauters A, *et al.* Antisense oligonucleotides used to target the DUX4 mRNA as therapeutic approaches in faciosscapulohumeral muscular dystrophy (FSHD). Genes 2017; 8:93.
27. ■ Klingler C, Ashley J, Shi K, *et al.* DNA aptamers against the DUX4 protein reveal novel therapeutic implications for FSHD. FASEB J 2020; 34:4573–4590.

This study provides a new therapeutic strategy to block the DUX4 protein instead of the DUX4 transcript, however its efficiency has not been shown in FSHD myogenic cells or mice.

28. Wallace LM, Liu J, Domire JS, *et al.* RNA interference inhibits DUX4-induced muscle toxicity in vivo: implications for a targeted FSHD therapy. Mol Ther 2012; 20:1417–1423.
29. ■ Wallace LM, Saad NY, Pyne NK, *et al.* Preclinical safety and off-target studies to support translation of AAV-mediated RNAi therapy for FSHD. Mol Ther Methods Clin Dev 2018; 8:121–130.

This study emphasizes the importance of testing the safety of therapeutics in animal models as one of the two tested microRNA's induced skeletal muscle damage in wild-type mice.

30. Lim JW, Snider L, Yao Z, *et al.* DICER/AGO-dependent epigenetic silencing of D4Z4 repeats enhanced by exogenous siRNA suggests mechanisms and therapies for FSHD. Hum Mol Genet 2015; 24:4817–4828.
31. Himeda CL, Jones TI, Jones PL. CRISPR/dCas9-mediated transcriptional inhibition ameliorates the epigenetic dysregulation at D4Z4 and represses DUX4-fl in FSH muscular dystrophy. Mol Ther 2016; 24:527–535.
32. ■ Himeda CL, Jones TI, Virbasius CM, *et al.* Identification of epigenetic regulators of DUX4-fl for targeted therapy of facioscapulohumeral muscular dystrophy. Mol Ther 2018; 26:1797–1807.

This research group shows that the dCas9 system can be used to repress DUX4 by recruiting KRAB to the D4Z4 repeat or to DUX4 enhancers. dCas9 is catalytically inactive and should not be able to induce permanent DNA damage.

33. ■■ Goossens R, van den Boogaard ML, Lemmers R, *et al.* Intronic SMCHD1 variants in FSHD: testing the potential for CRISPR-Cas9 genome editing. J Med Genet 2019; 56:828–837.

This is the first study that shows that restoring SMCHD1 by gene editing can repress DUX4 in FSHD2 myotubes.

34. Balog J, Thijssen PE, Shadle S, *et al.* Increased DUX4 expression during muscle differentiation correlates with decreased SMCHD1 protein levels at D4Z4. Epigenetics 2015; 10:1133–1142.
35. ■ Bosnakovski D, da Silva MT, Sunny ST, *et al.* A novel P300 inhibitor reverses DUX4-mediated global histone H3 hyperacetylation, target gene expression, and cell death. Sci Adv 2019; 5:eaaw7781.

This study shows that preventing DUX4 from activating its target genes can reduce skeletal muscle damage in FSHD mice.

36. Choi SH, Gearhart MD, Cui Z, *et al.* DUX4 recruits p300/CBP through its C-terminus and induces global H3K27 acetylation changes. Nucl Acids Res 2016; 44:5161–5173.
37. Bosnakovski D, Chan SSK, Recht OO, *et al.* Muscle pathology from stochastic low level DUX4 expression in an FSHD mouse model. Nat Commun 2017; 8:550.
38. Campbell AE, Oliva J, Yates MP, *et al.* BET bromodomain inhibitors and agonists of the beta-2 adrenergic receptor identified in screens for compounds that inhibit DUX4 expression in FSHD muscle cells. Skelet Muscle 2017; 7:16.
39. ■ Ciszewski L, Lu-Nguyen N, Slater A, *et al.* G-quadruplex ligands mediate downregulation of DUX4 expression. Nucleic Acids Res 2020; 48:4179–4194.

This study identified G-quadruplex secondary structures in the DUX4 transcript and enhancer and promoter regions of DUX4. Stabilizing these secondary structures reduced DUX4 and target gene expression.

40. Cruz JM, Hupper N, Wilson LS, *et al.* Protein kinase A activation inhibits DUX4 gene expression in myotubes from patients with facioscapulohumeral muscular dystrophy. J Biol Chem 2018; 293:11837–11849.
41. ■■ Oliva J, Galasinski S, Richey A, *et al.* Clinically advanced p38 inhibitors suppress DUX4 expression in cellular and animal models of facioscapulohumeral muscular dystrophy. J Pharmacol Exp Ther 2019; 370:219–230.

Both Oliva *et al.* and Fulcrum Therapeutics identified p38 inhibitors as a new treatment for FSHD.

42. ■ Rojas AL, Valentine E, Accorsi A, *et al.* P38α regulates expression of DUX4 in a Model of facioscapulohumeral muscular dystrophy. J Pharmacol Exp Ther 2020.

Both Fulcrum Therapeutics and Oliva *et al.* identified losmapimod as a compound to prevent DUX4 expression. Fulcrum Therapeutics is currently testing losmapimod in patients with FSHD.

43. ■■ Lek A, Zhang Y, Woodman KG, *et al.* Applying genome-wide CRISPR-Cas9 screens for therapeutic discovery in facioscapulohumeral muscular dystrophy. Sci Transl Med 2020; 12:eaay0271.

This is the first published CRISPR-Cas9-based screen that identifies new compounds that may be used to treat FSHD. In contrast to other screens, it is aimed to find compounds that prevent DUX4-induced cell death instead of inhibiting DUX4 and target gene expression.

44. Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. Genes Dev 2000; 14:1553–1577.
45. Cuenda A, Rousseau S. p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim Biophys Acta 2007; 1773:1358–1375.
46. Leidenroth A, Hewitt JE. A family history of DUX4: phylogenetic analysis of DUXA, B, C and Duxbl reveals the ancestral DUX gene. BMC Evol Biol 2010; 10:364.
47. van den Heuvel A, Mahfouz A, Kloet SL, *et al.* Single-cell RNA sequencing in facioscapulohumeral muscular dystrophy disease etiology and development. Hum Mol Genet 2019; 28:1064–1075.
48. Giesige CR, Wallace LM, Heller KN, *et al.* AAV-mediated follistatin gene therapy improves functional outcomes in the TIC-DUX4 mouse model of FSHD. JCI Insight 2018; 3:123538.
49. Jones T, Jones PL. A cre-inducible DUX4 transgenic mouse model for investigating facioscapulohumeral muscular dystrophy. PloS One 2018; 13:e0192657.
50. Bosnakovski D, Shams AS, Yuan C, *et al.* Transcriptional and cytopathological hallmarks of FSHD in chronic DUX4-expressing mice. J Clin Invest 2020; 130:2465–2477.
51. Nguyen Q, Yokota T. Antisense oligonucleotides for the treatment of cardiomyopathy in Duchenne muscular dystrophy. Am J Transl Res 2019; 11:1202–1218.
52. Jones TI, Chen JC, Rahimov F, *et al.* Facioscapulohumeral muscular dystrophy family studies of DUX4 expression: evidence for disease modifiers and a quantitative model of pathogenesis. Hum Mol Genet 2012; 21:4419–4430.
53. Das S, Chadwick BP. Influence of repressive histone and DNA methylation upon D4Z4 transcription in non-myogenic cells. PLoS One 2016; 11:e0160022.
54. Gannon OM, Merida de Long L, Saunders NA. DUX4 is derepressed in late-differentiating keratinocytes in conjunction with loss of H3K9me3 epigenetic repression. J Invest Dermatol 2016; 136:1299–1302.