Targeting DUX4 for Silencing with AOC for the Treatment of FSHD

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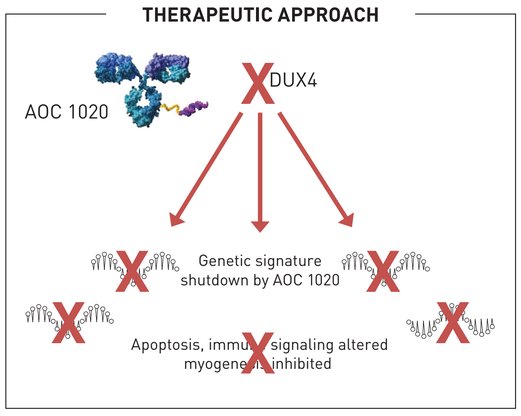
Background

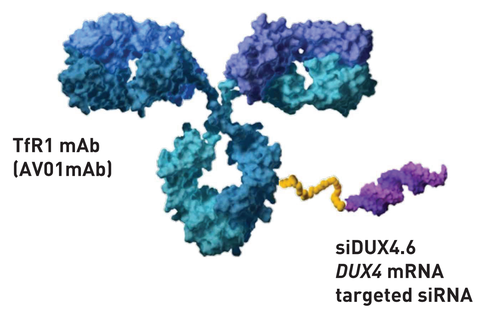
- Facioscapulohumeral dystrophy (FSHD) is a rare genetic muscular disorder, usually presenting with slow-progressing and asymmetric muscle weakness.[1]
- The cause of FSHD is aberrant expression of the transcription factor DUX4 in skeletal muscle, leading to a series of downstream events that result in skeletal muscle degeneration and wasting. Strategies aimed at reducing *DUX4* expression in the skeletal muscle of FSHD patients are promising therapeutic approaches.[2–7]
- Clinical development of oligonucleotide therapeutics for muscle diseases has been limited due to difficulty delivering oligonucleotides into muscle.[5] Avidity's antibody oligonucleotide conjugates (AOC™) platform combines the specificity of transferrin receptor 1 (TfR1)-directed monoclonal antibodies for muscle delivery with the potency and precision of small interfering RNA (siRNA) in reducing target RNA expression.
- Avidity has conducted a comprehensive *in vitro* screening of a DUX4 siRNA library in a variety of FSHD patient-derived muscle cells, which allowed selection of highly potent siRNA sequences with minimal off-target profile. The selected siDUX4.6 siRNA was conjugated to the murine TfR1 antibody to generate DUX4 AOC. A robust, dose-dependent response was observed for 8 weeks following a single intravenous (IV) dose of DUX4 AOC, with 75% or higher reduction of DUX4-regulated genes in skeletal muscle of the ACTA1-MCM;FLExDUX4 mouse model of FSHD.
- Data presented herein provide rationale and support for entering the clinic with AOC 1020 for the treatment of FSHD.

Avidity's Approach

Reducing *DUX4* Expression in the Muscle of FSHD Patients May Limit Downstream Expression of Toxic Genes

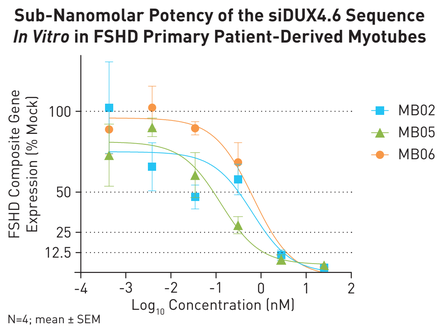


Avidity's AOC 1020 Targets *DUX4* mRNA for Degradation, Thus Reducing the Expression of the Disease–Causing Protein



Results

Figure 1: Lead siRNA Sequence siDUX4.6 Inhibits DUX4–Regulated Genes in FSHD Patient–Derived Muscle Cells



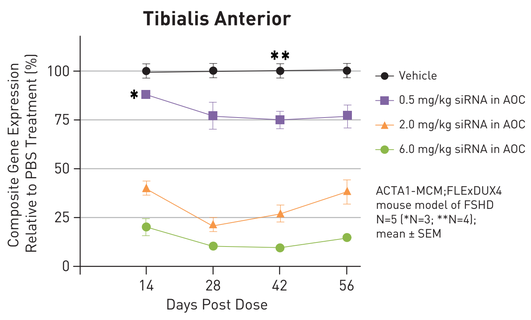
N=4; mean ± SEM

	IC_{50} (nM)	E_{max} (%)
MB02	0.639	100
MB05	0.127	95
MB06	0.665	100

- Robust reduction of DUX4-regulated genes was observed with the lead siDUX4.6 siRNAs in FSHD donor myotubes *in vitro*
- FSHD Composite is a mean expression of DUX4-regulated genes *KHDC1L*, *LEUTX*, *MBD3L2*, *ZSCAN4*[8]

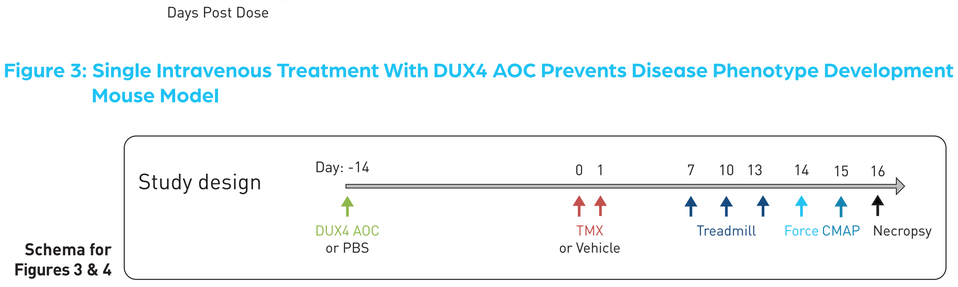
Figure 2: siDUX4.6 Shows Potent Inhibition of DUX4–Regulated Genes in Transgenic Mouse Model of FSHD for 8 Weeks After Single Dose

Dose-dependent inhibition of DUX4–regulated genes in skeletal muscles



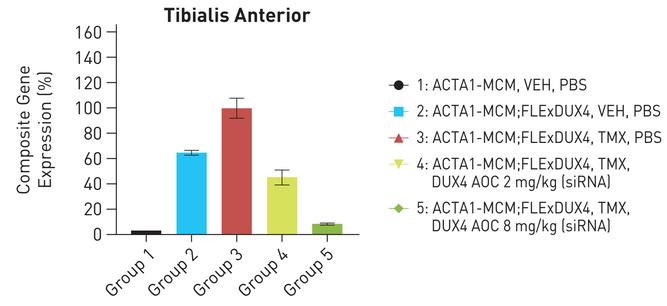
- The siRNA clinical candidate siDUX4.6 demonstrated activity *in vivo* towards the human *DUX4* mRNA, measured by downregulation of DUX4-regulated mouse genes *Wfdc3*, *Ilvbl*, *Slc15a2*, *Sord*[9,10]
- Approximately a 75% reduction in DUX4 responsive genes was induced after a single systemic IV administration of 6 mg/kg of siRNA within the AOC (mTfR1-siDUX4.6)

Figure 3: Single Intravenous Treatment With DUX4 AOC Prevents Disease Phenotype Development in FSHD Mouse Model



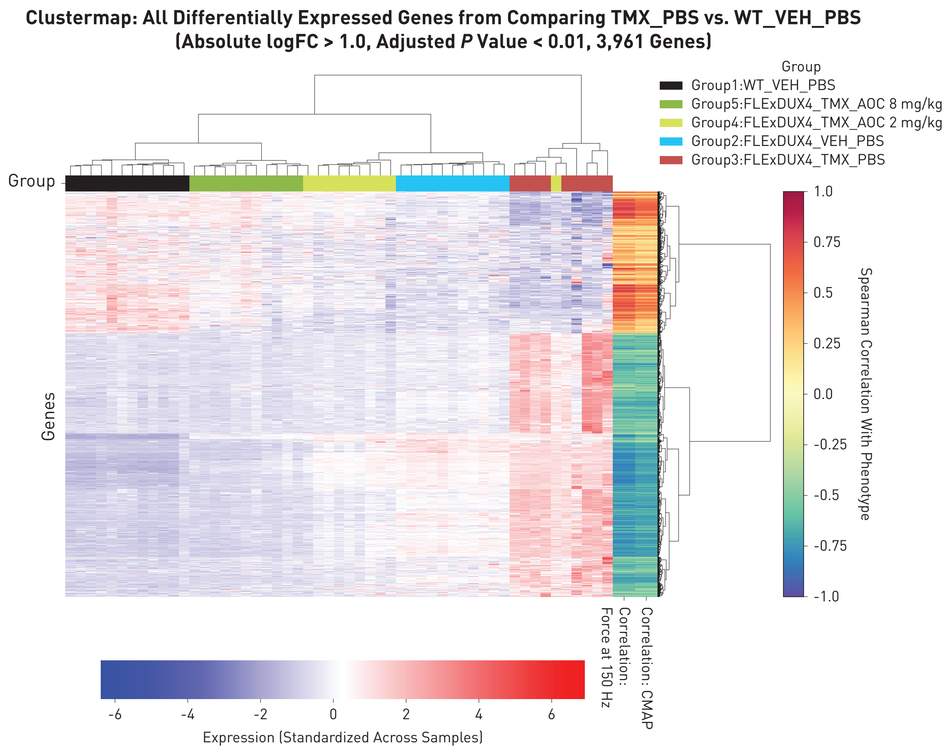
Results (Continued)

Figure 4: Single Dose of DUX4 AOC Inhibits DUX4–Regulated Gene Expression in Muscle of Tamoxifen–Induced FSHD Mouse Model



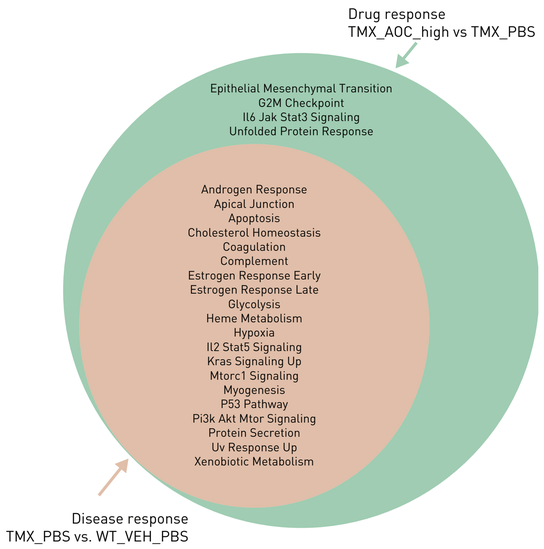
- The siRNA clinical candidate siDUX4.6 robustly inhibits expression of DUX4-regulated mouse genes (*Wfdc3*, *Ilvbl*, *Slc15a2*, *Sord*)[9,10] in skeletal muscle 1 month after single IV administration at therapeutically relevant doses

Figure 5A: DUX4 AOC Treatment Prevents Global FSHD Disease–Related Gene Expression Response in Muscle of FSHD Mouse Model



- RNA-seq analysis of TA muscle identifies 3,961 genes with significant responses in a tamoxifen-induced FSHD mouse model, many of which correlate with functional outcomes (force and CMAP measurements)
- Treatment with DUX4 AOC prevents this disease gene expression response, reflected by treated samples clustering with normal controls

Figure 5B: RNA–Seq Analysis of TA Muscle Identifies Hallmark Gene Sets[8] With Significant Enrichment Responses in a Tamoxifen–Induced FSHD Mouse Model



Jones et al. 2020	Avidity's Pathway Analysis				
		Disease Response		DUX4 AOC Treatment Response	
GO Superterms	Hallmark gene set	NES	FWER (*P*-val)	NES	FWER (*P*-val)
Apoptosis	Apoptosis	2.013	0.006	-1.990	0.009
Muscle	Myogenesis	2.064	0.003	-2.105	0.001
Immune	Inflammatory Response	1.820	0.045	-1.866	0.034
Cell cycle	G2m Checkpoint	1.896	0.025	-1.977	0.009
Cell cycle	Mitotic Spindle	1.823	0.044	-1.780	0.075

- Treatment with DUX4 AOC prevents this disease gene set response, reflected by significant enrichment responses in the opposite direction from tamoxifen induction
- Four pathways with significant enrichment responses with DUX4 AOC treatment show a dysregulation in disease in the opposite direction, but their significance in disease response does not match the stringent FWER *P* value threshold of 0.01
- This mouse model recapitulates some DUX4-activated pathways observed in FSHD disease. The myopathic, apoptotic and immune infiltration pathways consistent with FSHD[12] and the effect of DUX4 AOC treatment on these pathways are depicted in the adjacent table

Conclusion

- siDUX4.6:
  - Was selected as clinical candidate siRNA targeting *DUX4* mRNA, having an activity across all tested 11 FSHD patient-derived muscle cell lines, with a sub-nanomolar potency *in vitro*
  - Demonstrates efficacy *in vitro* by downregulating a panel of known DUX4-regulated genes in FSHD patient-derived myotubes
  - Demonstrates a dose-dependent activity and long duration of action (8 weeks) after single systemic IV dose *in vivo* in FSHD mouse model expressing human DUX4
  - Prevents a muscle weakness development after 2 and 8 mg/kg (siRNA within AOC) single systemic IV dose in FSHD mouse model
  - Has minimal seed-mediated off-target profile in human muscle cells
- Avidity is evaluating AOC 1020 in the Phase 1/2 FORTITUDE™ clinical trial in adults with FSHD

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Abbreviations

AOC, antibody oligonucleotide conjugate; CMAP, compound muscle action potential; FSHD, facioscapulohumeral dystrophy; FWER, family-wise error rate; IV, intravenous; mRNA, messenger RNA; NES, normalized enrichment score; PBS, phosphate-buffered saline; RNA, ribonucleic acid; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SEM, standard error of the mean; siRNA, small interfering RNA; TA, tibialis anterior; TfR1, transferrin receptor 1; TMX, tamoxifen; VEH, vehicle.

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