DUX4 siRNA Optimization for the Development of an Antibody-Oligonucleotide Conjugate (AOC™) for the Treatment of FSHD

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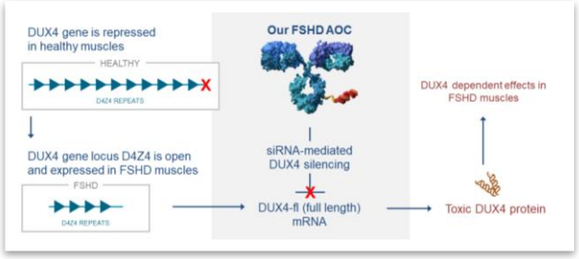
BACKGROUND

- Facioscapulohumeral Muscular Dystrophy (FSHD) is one of the most common forms of muscular dystrophy
- FSHD is caused by aberrant expression of the double homeobox 4 (DUX4) transcription factor in skeletal muscle, leading to a gene deregulation cascade and progressive skeletal muscle wasting
- Currently there are no approved disease modifying treatments available
- Strategies aimed at reducing *DUX4* expression in the skeletal muscle of FSHD patients offer a promising therapeutic approach
- The difficulty to deliver oligonucleotides into muscle cells has limited the clinical development of oligonucleotide therapeutics for neuromuscular diseases
- To solve this problem, Avidity is developing a novel Antibody-Oligonucleotide-Conjugate (AOC™) that combines a monoclonal antibody directed towards the human Transferrin Receptor 1 (TfR1) with an siRNA targeted to *DUX4* mRNA
- The antibody component binds to the TfR1 on the cell surface and results in internalization of the AOC through transferrin-receptor-mediated endocytosis, while the siRNA component provides the pharmacological mechanism of action (i.e. siRNA-mediated reduction of the *DUX4* mRNA)

AVIDITY'S APPROACH TO TREAT FSHD

- We performed a series of in vitro DUX4 siRNA screening studies in a variety of FSHD patient-derived myotubes, informing the selection of the most potent siRNA sequences with minimal off-target profile
- Ultimately, the lead DUX4 siRNA will be conjugated to the TfR1 antibody to generate the therapeutic AOC™ for FSHD that will be further characterized in vivo

FIGURE 1. Molecular Basis of FSHD Disease and AOC Approach Targeting *DUX4*



Disclosures

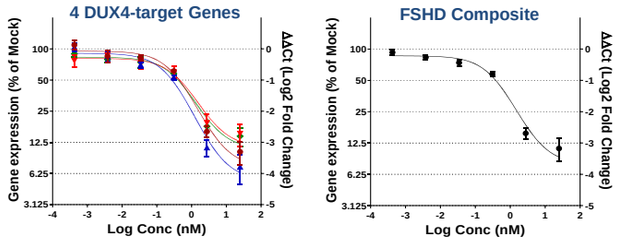
- This poster is sponsored by Avidity Biosciences
- Authors are or were employees of Avidity Biosciences and may have stock options

RESULTS

Potent DUX4 siRNA-mediated Reduction in Gene Expression of 4 Known FSHD Biomarkers in FSHD Primary Myotubes *in vitro*

- Primary FSHD myoblasts (MB06) were transfected with a DUX4 siRNA. After inducing differentiation, myotubes were harvested and gene expression analyzed using qPCR
- *DUX4* expression in FSHD muscles and in FSHD cultured myotubes is low and sporadic, imposing a challenge in detecting *DUX4* expression directly
- Instead, expression levels of 4 well-established DUX4-target genes were evaluated: *MBD3L2, ZSCAN4, LEUTX*, and *KHDC1L*
- DUX4-target gene expression was normalized to two reference genes: *AHSA1* and *RPL27*

FIGURE 2. DUX4-target Genes Showed Concentration-dependent Response to a DUX4 siRNA in FSHD Primary Myotubes

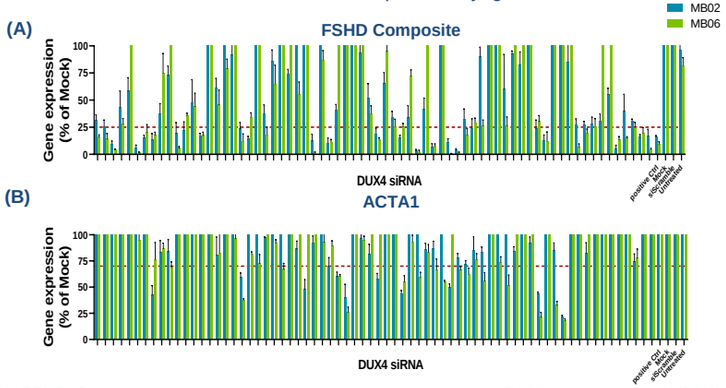


** The FSHD Composite score integrates the expression levels of the 4 DUX4-target genes. Data represented as Mean ± SEM (N=4).*

Screening of a 70 DUX4 siRNA Library in FSHD Primary Myotubes

- Two different primary FSHD myoblast cell lines (MB02 and MB06) were transfected with the DUX4 siRNA library (10nM). After inducing differentiation, myotubes were harvested and gene expression analyzed using qPCR
- Top DUX4 siRNAs were selected based on: (1) best activity; (2) minimal impact on myogenic differentiation
- Top 14 DUX4 siRNAs moved forward for further *in vitro* characterization

FIGURE 3. Screening in FSHD Patient-derived Primary Myotubes Identified Several Active DUX4 siRNAs with Minimal Impact on Myogenic Differentiation



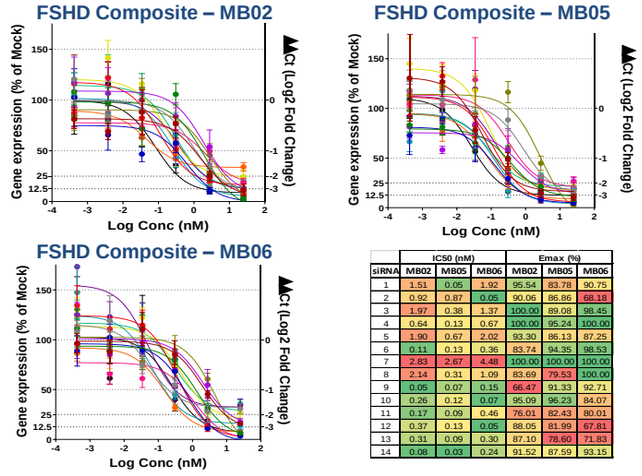
(A) FSHD Composite score integrating the expression levels of 4 DUX4-target genes (*MBD3L2, ZSCAN4, LEUTX,* and *KHDC1L*) normalized to two reference genes (*AHSA1* and *RPL27*).

(B) ACTA1 expression levels were normalized to *AHSA1* reference gene. Data represented as Mean ± SEM (N=4)

Potency and Efficacy of Top 14 DUX4 siRNAs *in vitro*

- MB02, MB05, and MB06 primary FSHD myoblast cell lines were transfected with increasing concentrations of top 14 DUX4 siRNAs
- After inducing differentiation, myotubes were harvested and changes in the FSHD Composite score analyzed by qPCR

FIGURE 4. Concentration-response of Top 14 DUX4 siRNAs in FSHD Patient-derived Myotubes

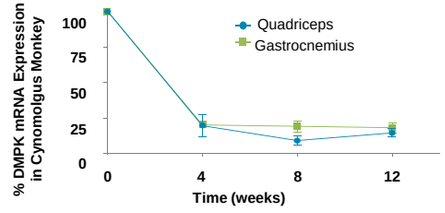


siRNA	IC50 (nM) MB02	IC50 (nM) MB05	IC50 (nM) MB06	Emax (%) MB02	Emax (%) MB05	Emax (%) MB06
1	1.51	0.05	1.92	95.54	83.78	90.75
2	0.92	0.87	0.05	90.06	86.86	68.18
3	1.97	0.38	1.37	100.00	89.08	98.45
4	0.64	0.13	0.67	100.00	95.24	100.00
5	1.90	0.67	2.02	93.30	86.13	87.25
6	0.11	0.13	0.36	83.74	94.35	98.53
7	2.83	2.67	4.48	100.00	100.00	100.00
8	2.14	0.31	1.09	83.69	79.53	100.00
9	0.05	0.07	0.15	66.47	91.33	92.71
10	0.26	0.12	0.07	95.09	96.23	84.07
11	0.17	0.09	0.46	76.01	82.43	80.01
12	0.37	0.13	0.05	88.05	81.99	67.81
13	0.31	0.09	0.30	87.10	78.60	71.83
14	0.08	0.03	0.24	91.52	87.59	93.15

Robust Long-term Activity with AOCs *In vivo* in Skeletal Muscles of Non-human Primates

- Single 2mg/kg i.v. dose of a DMPK siRNA conjugated to humanized TfR1 antibody into WT cynomolgus monkeys
- Tissues were collected and analyzed 4, 8, and 12 weeks post dosing

FIGURE 5. A hTfR1.Ab-siDMPK AOC downregulated *DMPK* for 12 weeks in monkeys dosed once at 2 mg/kg



SUMMARY

- FSHD Composite score integrating the expression of 4 known FSHD biomarkers (*MBD3L2, ZSCAN4, LEUTX, KHDC1L*) was used as a robust readout for screening
- DUX4 siRNAs showed robust activity in primary FSHD patient-derived myotubes in vitro
- Top DUX4 siRNAs showed activity across a range of FSHD patient-derived cell lines
- Several highly potent (IC50<1nM) and efficacious (Emax>85%) DUX4 siRNAs have been identified in vitro
- Best DUX4 siRNAs will be further characterized in vivo using AOCs