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

Barbora Malecova[1], David Sala[1], Garineh Melikian[1], Gulin Erdogan[1], Rachel Johns[1], Joanne Young[2], Erwann Ventre[2], Sole Gatto[3], Matthew Onorato[3]; Adam Pavlicek[3], Orsolya Kiraly[4], Martin Koegler[5], Philipp Hadwiger[5], Arthur Levin[1], Michael Flanagan[1], Mark Stahl[1]

[1]Avidity Biosciences, Inc. United States; [2]CYTOO SA, France; [3]Monoceros Biosystems LLC, United States; [4]The Jackson Laboratory, United States; [5]LGC Axolabs GmbH, Germany.

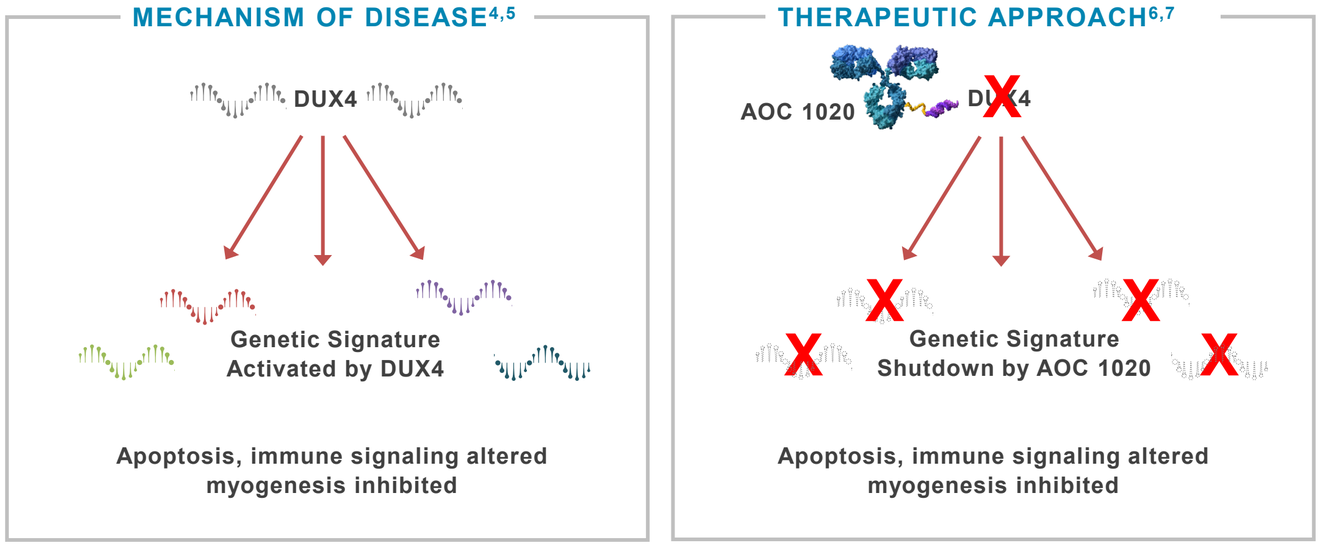
All authors have met the authorship criteria.

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]
- Clinical development of oligonucleotide therapeutics for muscle diseases has been limited due to difficulty delivering oligonucleotides into muscle cells.[3] Avidity's AOC™ platform combines the specificity of transferrin receptor 1 (TfR1)-directed monoclonal antibodies for muscle cells with the potency and precision of small interfering RNA (siRNA) in downregulating target RNA.
- 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-responsive activity was observed for 8 weeks following a single intravenous (IV) dose of DUX4 AOC, with 75% or higher downregulation 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 by the end of 2022.

Avidity's Approach to Treat FSHD

FSHD is caused by aberrant expression of DUX4 in muscle.
***DUX4* activates genes that are toxic to muscle cells**



Sponsorship and Disclosures
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Abbreviations
AOC, antibody oligonucleotide conjugate; FSHD, facioscapulohumeral dystrophy; IV, intravenous; mRNA, messenger RNA; RNA, ribonucleic acid; siRNA, small interfering RNA; TfR1, transferrin receptor 1.

References
[1]Tawil R, et al. *Neuromuscul Disord*. 2010;20(7):471–5; [2]Bouwmann LF, et al. *Curr Opin Neurol*. 2020;33(5):635–40; [3]Roberts TC, et al. *Nat Rev Drug Discov*. 2020;19(10):673–94. [4]Lemmers RJLF, et al. *Science*. 2010;329(5999):1650–3; [5]Snider L, et al. *PLoS Genet*. 2010;6(10):e1001181; [6]Ansseau E, et al. *Genes (Basel)*. 2017;8(3):93; [7]Jiang S, et al. *PLoS Genet*. 2020;16(5):e1008754.

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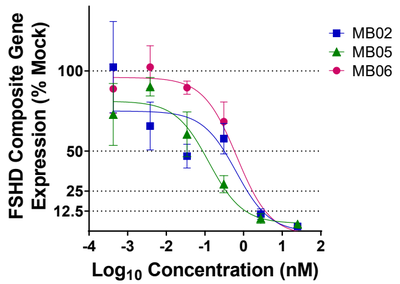
Results

1. Sub-Nanomolar Potency of the siDUX4.6 Sequence *In Vitro* in FSHD Primary Patient-Derived Myotubes

A

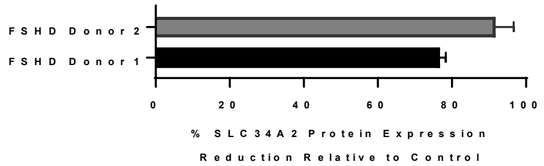


B

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

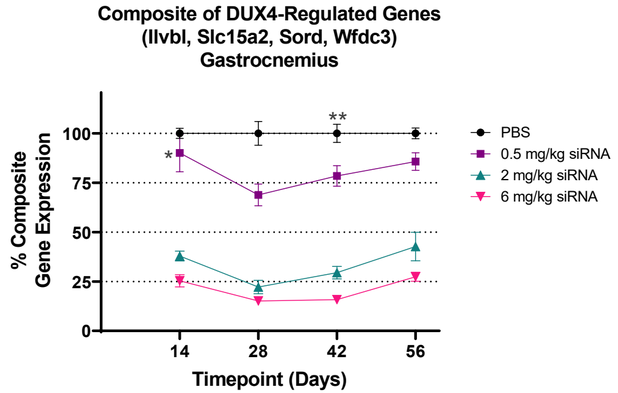
siDUX4.6 sequence was transfected in three FSHD1 patient-derived primary myoblasts named MB02, MB05, and MB06 at 25, 2.778, 0.309, 0.034, 0.004, and 0.0004 nM final concentrations and differentiated into myotubes. Isolated RNA was analyzed for gene expression by quantitative reverse transcription polymerase chain reaction (RT-qPCR) monitoring four DUX4 target genes: *MBD3L2*, *ZSCAN4*, *LEUTX*, and *KHDC1L*. The FSHD composite score was calculated as a mean expression of the four DUX4 regulated genes, normalized to average expression of two housekeeping genes. The FSHD composite gene expression data are represented as a percent of mock transfection control (mean ± standard error of the mean [SEM], N=4). A) Concentration–response graph. Log(inhibitor) versus response three parameters calculation was used to fit the concentration–response curve. B) The best-fit values for IC_{50} and E_{max} are reported in the table.

2. siDUX4.6 Sequence Inhibits SLC34A2 Protein Expression by >75% in FSHD Donor Myotubes



FSHD donor myotubes were transfected with final concentrations of 10 nM siRNA. Cultured myotubes were fixed 8 days after siRNA treatment and immune-labeled for myosin heavy chain (marker of differentiated myotubes) and SLC34A2, a potential biomarker of FSHD. siRNA activity was calculated in each condition as a percentage of reduction of SLC34A2-positive myotubes compared with treatment control (mean ± SEM, N=4).

3. Dose-Dependent Activity of mTfR1-siDUX4.6 AOC in Skeletal Muscle in ACTA1-MCM; FLExDUX4 Mouse Model of FSHD is Sustained for 8 Weeks Post Single IV Dose



siDUX4.6 was conjugated to the murine TfR1 antibody to generate mouse-specific mTfR1-siDUX4.6 AOCs. DUX4 AOC was administered intravenously in ACTA1-MCM; FLExDUX4 mouse model of FSHD disease that expresses the human DUX4 transgene.[2] Skeletal muscles were collected at indicated timepoints post single IV dose of AOCs. Gene expression was analyzed by nanostring, and the composite expression levels of four DUX4-regulated genes – *Wfdc3*, *Ilvbl*, *Slc15a2*, and *Sord4* – are shown.[1,2] DUX4-target gene composite expression was normalized to a composite of 10 reference genes. The level of DUX4-regulated gene downregulation was determined relative to phosphate-buffered saline (PBS) vehicle-treated animals (mean ± SEM, N=5) (*N=3; **N=4).

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Abbreviations

AOC, antibody oligonucleotide conjugate; FSHD, facioscapulohumeral dystrophy; IV, intravenous; 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; TfR1, transferrin receptor 1.

References

[1]Whiddon JL, et al. *Nat Genet.* 2017;49(6):935–40; [2]Jones T and Jones PL. *PLoS One.* 2018;13(2):20192657, [3]Yao Z, et al. *Hum Mol Genet.* 2014;23(20):5342–52; [4]Chen C, et al. *Nucleic Acids Res.* 2005;33(20):e179.

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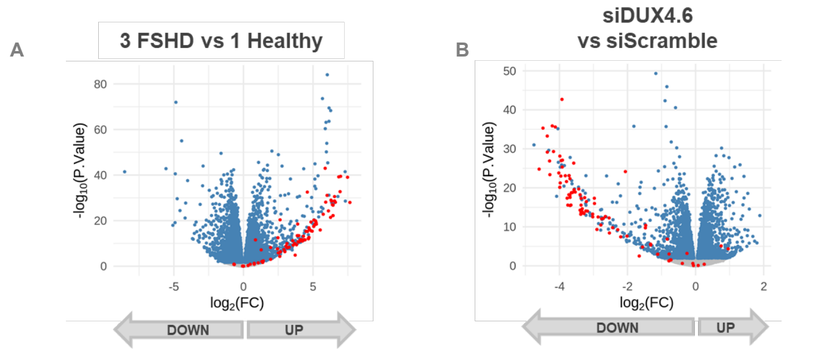
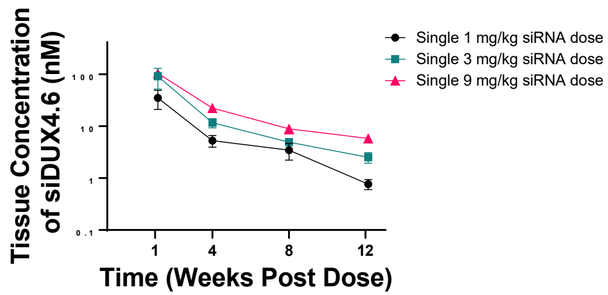
Results

4. siDUX4.6 Suppresses Many Known DUX4 Regulated Genes *In Vitro* in FSHD Primary Donor Myotubes



One healthy and three FSHD patient-derived primary myoblast cell lines were transfected with siDUX4.6 at 10 nM concentration. At 24 hours post transfection, myogenic differentiation was induced. Isolated RNA was analyzed by RNA-seq. Differential gene expression analysis was performed. In blue color are significant (false discovery rate [FDR]<0.05) differentially expressed genes. In red color are differentially expressed genes that were previously reported as DUX4 regulated genes.[3] In gray color are genes that are not significantly differentially expressed. A) Average differential gene expression in myotubes from three FSHD patients compared with one healthy donor is plotted for each gene. B) Average differential gene expression of three FSHD patient myotubes treated with siDUX4.6 versus non-targeting scramble siRNA for each gene is presented as volcano plots.

5. AOC 1020 Regimen Pharmacokinetic (PK) Results in Non-human Primate Muscle Tissue Support an Infrequent Dosing in Clinic



The PK profile of AOC 1020 following single dosing for up to 12 weeks was evaluated in skeletal muscles of cynomolgus monkeys. AOC 1020 was administered by IV infusion at 1, 3, and 9 mg/kg (dose reported as the siDUX4.6 component) on Day 1 as single dose. siRNA concentration in tissue was assessed by stem-loop RT-qPCR assay described previously (mean ± SEM, N=3).[4]

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Lukas Perkams[1], Vivienne Bunker[2]; and Satoru Oneda[2]

[1]LGC Axolabs GmbH, Germany; [2]Altasciences, United States

Summary

- 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*.
- siDUX4.6 demonstrates efficacy *in vitro* by downregulating a panel of known DUX4-regulated genes in FSHD patient-derived myotubes.
- siDUX4 demonstrates a dose-dependent activity and long duration of action (8 weeks) *in vivo* in FSHD mouse model expressing human DUX4.
- siDUX4 has minimal seed-mediated off-target profile in human muscle cells.
- AOC 1020 is currently in GLP toxicology studies
- Avidity is planning to enter the clinic with AOC 1020 for treatment of FSHD by end of 2022

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Abbreviations

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References

[1]Whiddon JL, et al. *Nat Genet.* 2017;49(6):935–40; [2]Jones T and Jones PL. *PLoS One.* 2018;13(2):20192657, [3]Yao Z, et al. *Hum Mol Genet.* 2014;23(20):5342–52; [4]Chen C, et al. *Nucleic Acids Res.* 2005;33(20):e179.