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AAN Annual Meeting 2021

Optimization of AOC 1001, an antibody-oligonucleotide conjugate targeting the underlying cause of myotonic dystrophy type 1

Presenter: Dr. Barbora Malecova

Conflict of Interest and Disclosures

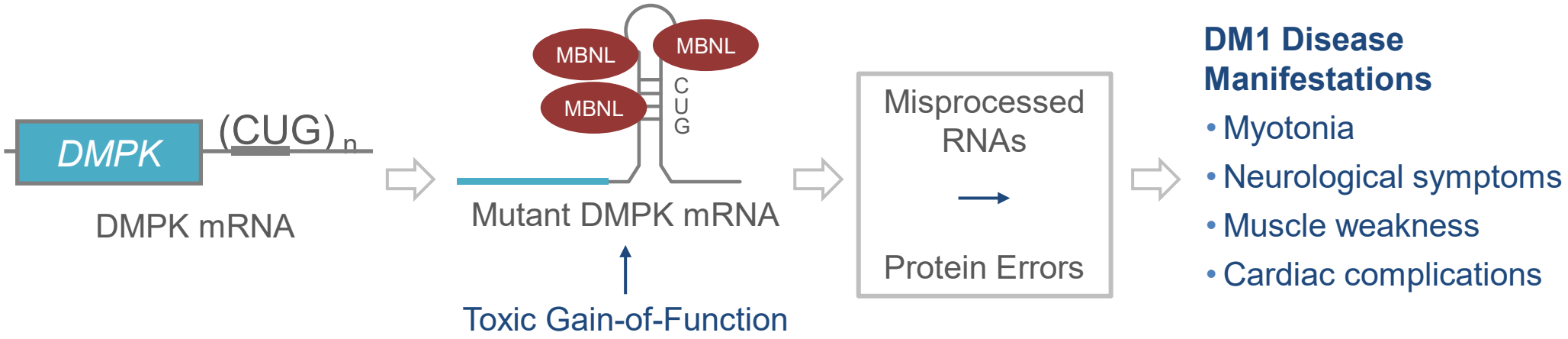
The following relationships exist related to this presentation:

- **Rob S. Burke, Barbora Malecova, Philip Kovach, Michael D. Hood, Samuel W. Beppler, Ramana Doppalapudi, Michael Cochran, Gulin Erdogan, J. Danny Arias, Christopher D. Miller, David Sala, Beatrice Darimont, Rachel Johns, Anneke K. Raney, Elizabeth J. Ackermann, Arthur A. Levin** are full-time employees of Avidity Biosciences who, in the course of this employment, have received stock and/or options exercisable for shares of Avidity Biosciences
- **Adam Pavlicek** is an employee of Monoceros Biosystems
- **Sole Gatto** is an employee of Monoceros Biosystems, the employer of an immediate family member has received research support from NIH
- **Vivienne Bunker is an** employee of Altasciences
- **Eve Duchemin-Pelletier, Oana Lorintiu, Joanne Young** are employees of CYTOO
- **Markus Hossbach, Martin Koegler, Lukas Perkams, Philipp Hadwiger** are employees of Axolabs
- **Andrew J. Geall** is a former employee of Avidity and is a shareholder in Avidity Biosciences
- This research is sponsored by Avidity Biosciences

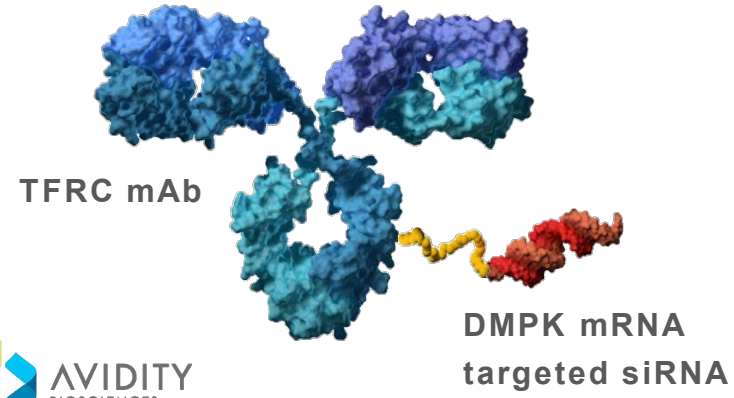
Avidity's AOC siRNA Targets Mutant DMPK mRNA - The Cause of Myotonic Dystrophy Type 1 (DM1)

MECHANISM OF DISEASE:

DM1 is Caused by a Toxic Gain-of-Function of the Mutant DMPK mRNA



ANTIBODY OLIGONUCLEOTIDE CONJUGATE (AOC)



- AOCs represent a new class of therapeutics allowing delivery of oligonucleotides to target tissues
- Avidity's AOC technology combines monoclonal antibodies and oligonucleotides
  - ✓ Specificity of targeting with mAbs
  - ✓ Potency & precision of oligonucleotides
  - ✓ Targets tissues with potent and durable agents

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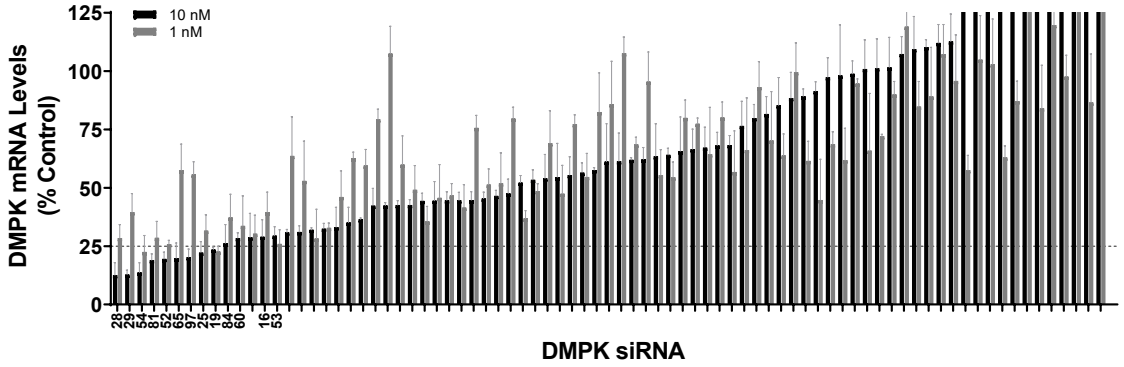
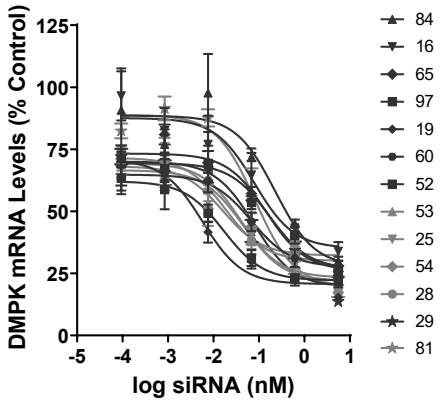
Selection of Active siRNAs Targeting Human *DMPK* by Assessment of *In Vitro* Potency

DMPK siRNA Library Screening in DM1 Patient-Derived Myoblasts

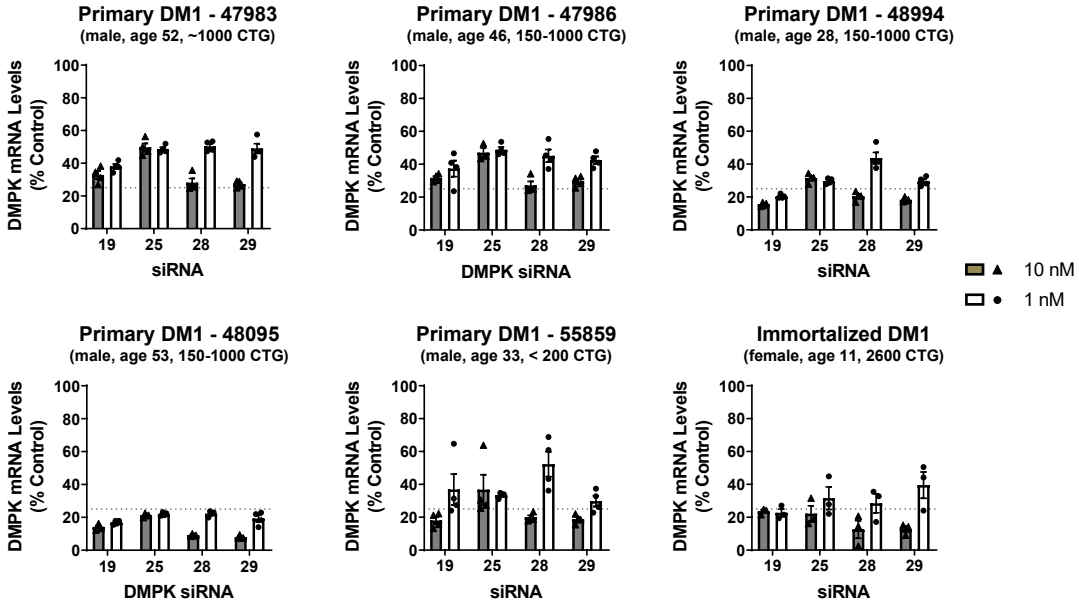


Potency of DMPK siRNAs



siRNA	IC_{50} (nM)	E_{max} (%)
19	0.006	79.3
53	0.016	67.8
52	0.017	77.9
25	0.018	70.0
28	0.042	78.1
54	0.043	76.7
60	0.068	72.4
29	0.078	80.5
16	0.080	64.9
81	0.084	80.6
65	0.146	74.0
97	0.171	73.3
84	0.228	72.3

Activity of DMPK siRNAs in Variety of DM1 Patient Donors

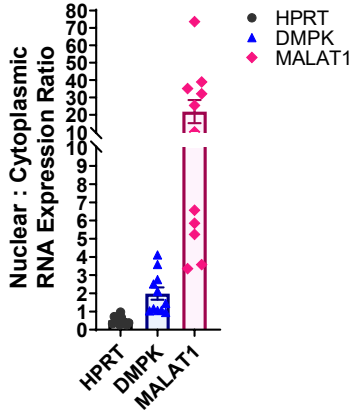


Highly potent DMPK-targeted siRNAs were selected in screening

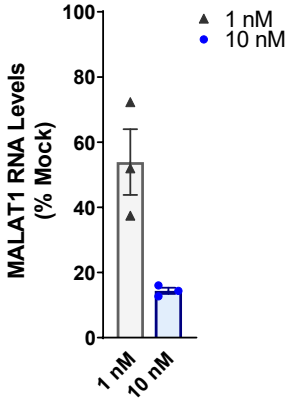
- Many siRNAs capable of markedly reducing *DMPK* expression were identified.
- Concentration-response curves in DM1 patient-derived myoblasts were generated for 13 of the siRNAs identified as hits in the initial screen, where IC_{50} values ranged from 6 to 228 pM and maximal *DMPK* mRNA reduction ranged from 65% to 81%.
- Top selected *DMPK*-targeted siRNA induced substantial reduction of *DMPK* mRNA in the five primary myoblast cultures at 1 and 10 nM concentration, independent of the CUG repeat length in the *DMPK* mRNA.

Demonstration of Nuclear Activity of *Malat1* siRNA and AOC

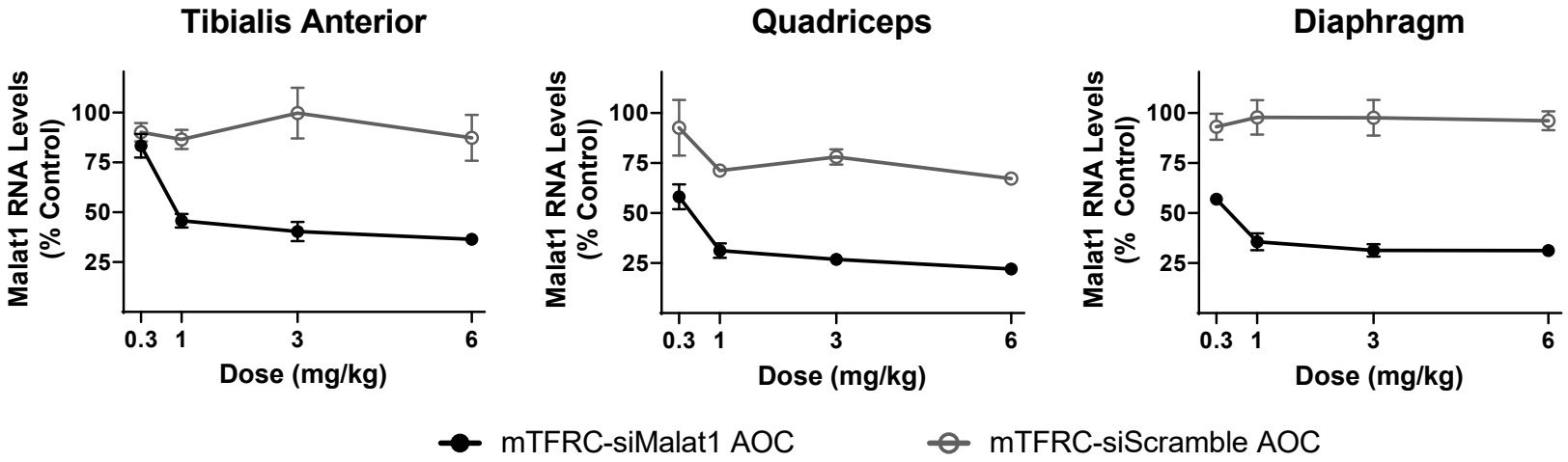
MALAT1 is Nuclear Located RNA



siRNA-Mediated Downregulation of *MALAT1 in vitro*



In vivo Activity of *Malat1* AOC in Murine Muscles 2 Weeks Post Single Dose



The RNAi enzymatic machinery is present and active in the cell nucleus

- The long non-coding RNA *MALAT1* is known to be primarily located in nucleus. We observed an approximately 20-fold enrichment of *MALAT1* mRNA in the nucleus compared to the cytoplasm in DM1 patient-derived muscle cells.
- siRNA targeting *MALAT1* produced a robust reduction of *MALAT1* expression.
- *Malat1* siRNA was conjugated to a murine TFRC (mTFRC) antibody and intravenously injected into wild type mice. A single administration of Malat1 AOC up to 6 mg/kg (siRNA dose) into mice reduced nuclear *Malat1* expression up to 80% in skeletal muscle 2 weeks post-dose.

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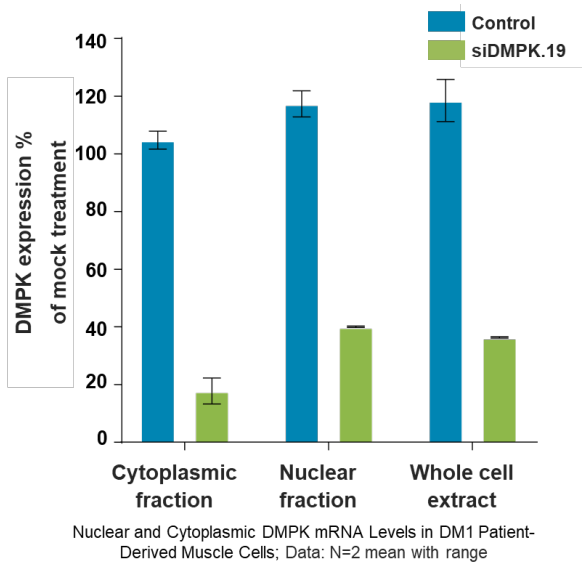
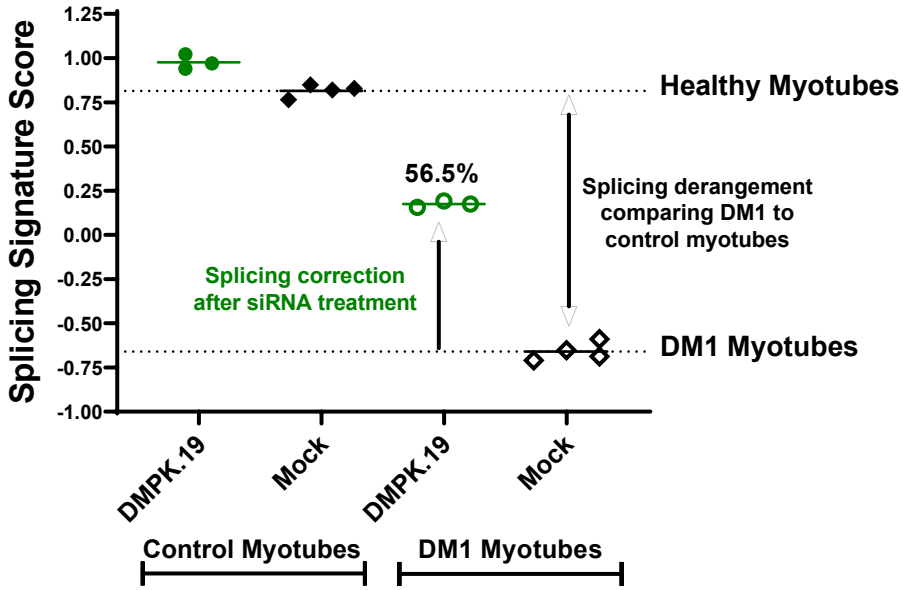
Avidity's Lead *DMPK*-Targeted siRNA Demonstrates Activity in the Nucleus and Efficacy in DM1 Patient-Derived Muscle Cells

Reduction of Both Nuclear and Cytoplasmic DMPK mRNA



Nuclear and Cytoplasmic DMPK mRNA Levels in DM1 Patient-Derived Muscle Cells; Data: N=2 mean with range

Correction of Aberrant RNA Splicing

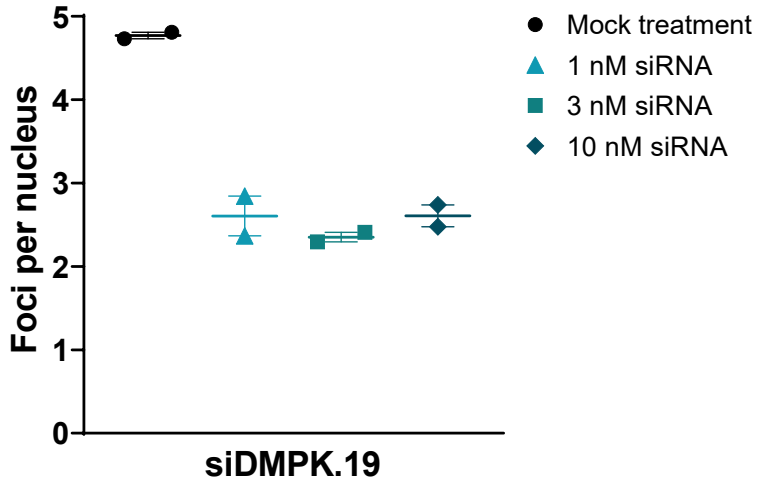


siDMPK.19 treated DM1 patient-derived cells show:

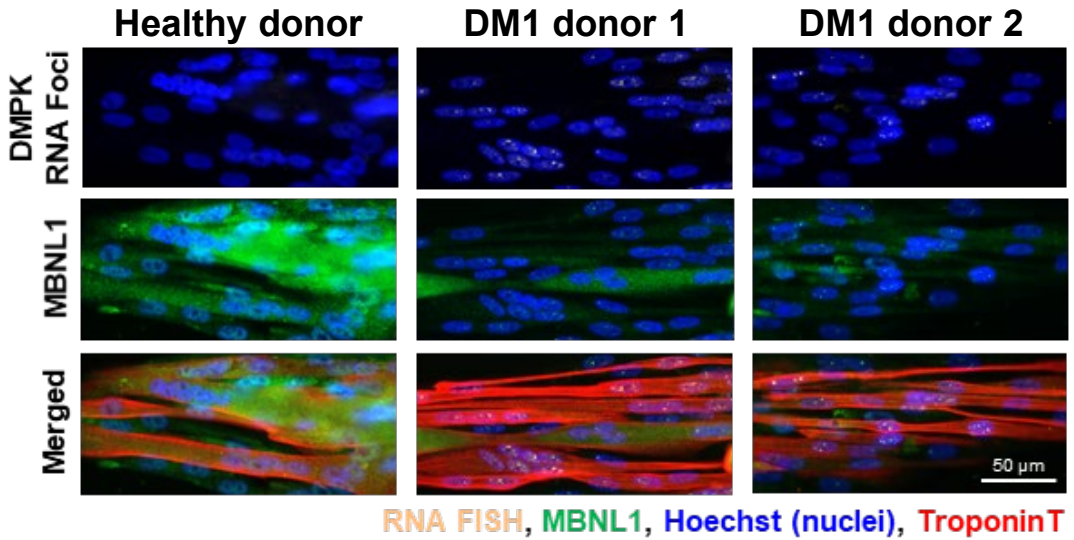
- Reduced *DMPK* mRNA levels in nucleus (60%) and cytoplasm (80%) after 7 days of siDMPK.19 treatment of DM1 myoblasts.
- Corrected the aberrant RNA splicing in DM1 differentiated myotubes, treated with siDMPK.19 for 6 days, towards healthy control cells.
- The immortalized human myoblasts were derived from infantile onset DM1 patient with 2,600 CTG repeats in the *DMPK* gene (Arandel et al. 2017).

Avidity's Lead *DMPK*-Targeted siRNA Reduces Nuclear Foci in DM1 Patient-Derived Muscle Cells

Reduction of Nuclear Foci Containing Mutant DMPK mRNA



Nuclear foci in DM1 muscle cells containing mutant *DMPK* mRNA and MBNL1 protein



siDMPK.19 treated DM1 patient-derived cells show:

- Reduced mutant DMPK m-RNA-associated nuclear foci by 50% in the myotubes cultured from DM1 patients treated with siDMPK.19.
- DM1 patient derived myotubes were cultured on MyoScreen CYTOOplates (CYTOO) and treated for 7-9 days with siDMPK.19. *DMPK* mRNA-containing nuclear foci were detected by Fluorescence *in situ* hybridization using a Cy3-labeled $(CAG)_5$ oligonucleotide probe.

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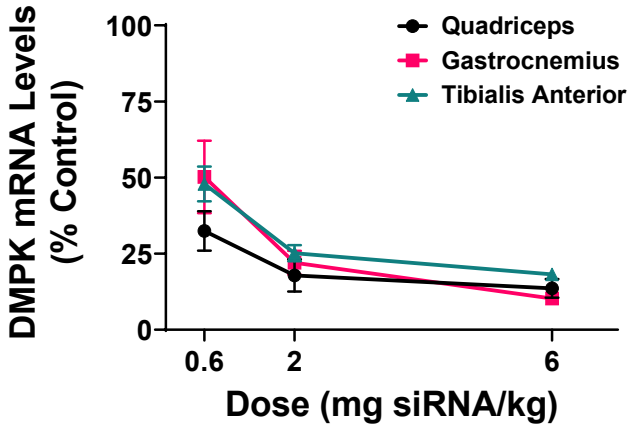
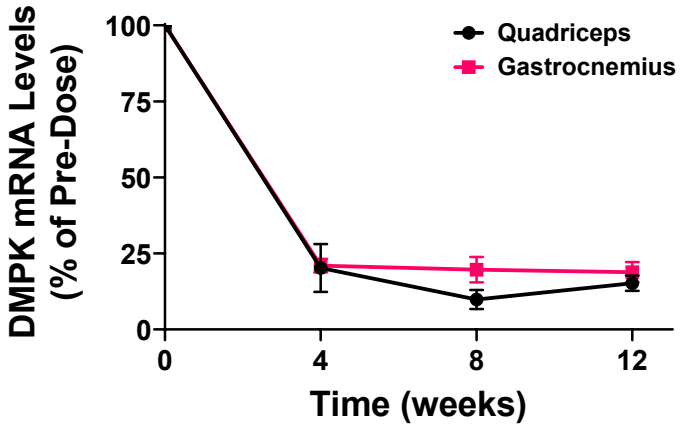
Single Intravenous Infusion of AOC 1001 Produced a Robust and Durable Reduction in *DMPK* Expression in Skeletal Muscles of Non-Human Primates

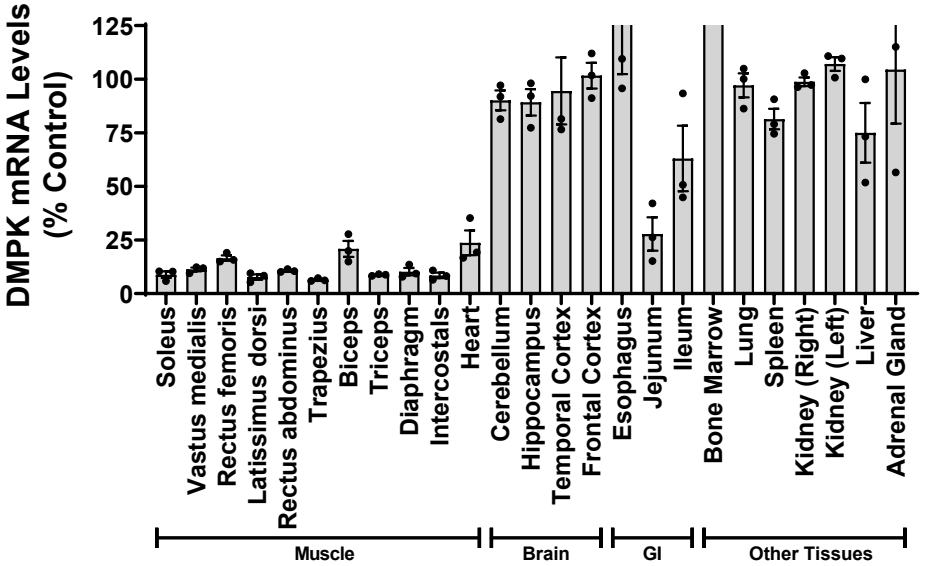
AOC 1001 dose response at 4 weeks



AOC 1001 durable activity at 2 mg/kg siRNA dose



AOC 1001 activity across tissues at 6 mg/kg siRNA at 4 weeks



Conclusions:

- AOC 1001 is in development for the treatment of DM1 and is composed of siDMPK.19 conjugated to TFRC mAb
- Robust activity and long duration of action in all examined skeletal and cardiac muscle of non-human primates after a single IV dose of AOC 1001
- Clinical investigation of AOC 1001 is planned to initiate in second half of 2021

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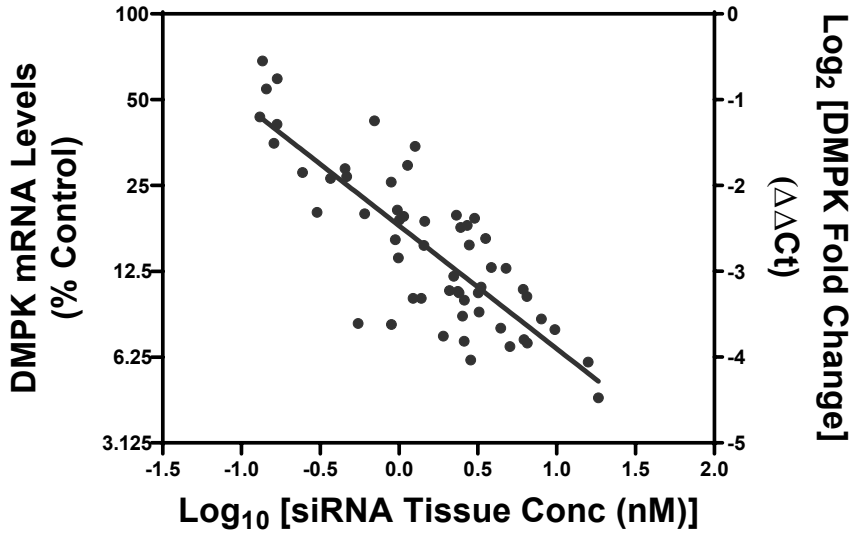
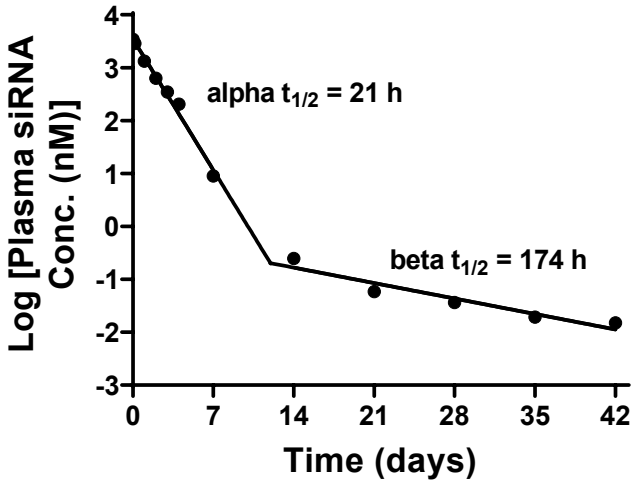
Plasma and Tissue Pharmacokinetic Evaluation of Avidity's Lead *DMPK* siRNA in Non-Human Primates

Tissue PK and Activity correlation for siDMPK.19



Biphasic Plasma PK Curve



Conclusions:

- Strong correlation was observed between the amount of *DMPK* mRNA downregulation and siDMPK.19 muscle tissue concentration 4 weeks after single IV AOC 1001 dose (R^2 = 0.6759; $p < 0.0001$).
- Plasma siRNA concentration-time curve was obtained after a single IV dose of AOC 1001 at 2.5 mg/kg (siRNA dose).
- The measurement of siDMPK.19 circulating in plasma for days is an evidence that the majority of AOC 1001 remains intact, with the large size of the conjugated antibody preventing renal filtration and elimination in the urine.