**AOC 1044 Mediated Exon 44 Skipping and Restoration of Dystrophin Protein in Cynomolgus Monkeys and DMD Patient Derived Myotubes**

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**Background**

DMD is a muscular disease caused by predominantly out-of-frame mutations in the dystrophin gene. Dystrophin protein expression can often be restored through oligonucleotide-mediated skipping of individual DMD exons to restore the reading frame. Although multiple oligonucleotides targeting different exons have been approved, their activity is limited due to poor uptake by the muscle.

Using Avidity’s antibody-oligonucleotide conjugate (AOC™) technology, we have previously demonstrated robust exon 23 skipping of the dystrophin gene and improved muscle function in mdx mice. AOC 1044 is an investigational drug comprised of a TR1 antibody conjugated to phosphorodiamidate morpholino oligomers (PMOs) that allows productive delivery of PMOs to muscle for treatment of DMD patients amenable to exon 44 skipping, for which there are no approved drugs.

**Avidity’s Approach to Treat DMD**

**1. PMO44 induces substantially greater skipping in DMD patient derived cells compared to healthy cells**

Myotubes derived from an immortalized healthy or DMD patient-derived myoblast cell line were treated with 10 nM of PMO44. The exon 44 skipping activity was determined by droplet digital PCR (ddPCR) 48 h post-treatment. Data shown represented as mean ± SEM of technical triplicates for a single donor for each cell type.

**2. PMO44 treatment results in robust dystrophin protein restoration and partial DAPC recovery in patient derived cells**

Human primary myoblasts from healthy and DMD patient were differentiated (MyoScreen™) for 8 days. At day 2 of differentiation, DMD myoblasts were treated with PMO44 for 6 days at 3, 10 and 30 nM in triplicates, with Endo-Porter (GeneTools) at 1 µl per delivery reagent. For each experiment, a mock condition corresponding to vehicle +/- Endo-Porter was included to be used as negative control. Representative images are shown dystrophin in green. Quantitative microscopy was performed using the Operetta HCS imaging system and images were analyzed using scripts developed in Acapella software (PerkinElmer). The mean intensity of dystrophin protein was quantified. Data represented as mean ± SEM of technical triplicates for each donor; n=3 DMD myoblasts, n=4 normal myoblasts.

**Results**

1. **PMO44 induces substantially greater skipping in DMD patient derived cells compared to healthy cells**

2. **PMO44 treatment results in robust dystrophin protein restoration and partial DAPC recovery in patient derived cells**

**Conclusion**

Data presented herein demonstrate that AOC 1044 and its PMO component produce robust exon 44 skipping that leads to restoration of dystrophin protein and DAPC formation in vitro. Furthermore, the clinical candidate AOC 1044 demonstrated activity in cynomolgus monkey and durable exon 44 skipping. These data, together with GLP toxicology data, support the development of AOC 1044 for the treatment of Duchenne muscular dystrophy patients amenable to exon 44 skipping. Avidity anticipates AOC 1044 will enter the clinic by the end of 2022.

**Abbreviations and References**

AOC, antibody-oligonucleotide conjugate; DAPC, dystrophin-associated glycoprotein complex; DAR, drug-antibody ratio; ddPCR, droplet digital PCR; DMD, Duchenne muscular dystrophy; ELISA, enzyme-linked immunosassay; IV, intravenous; mAb, monoclonal antibody; PCR, polymerase chain reaction; PMO, phosphorodiamidate morpholino oligomer; SEM, standard error of the mean.


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