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

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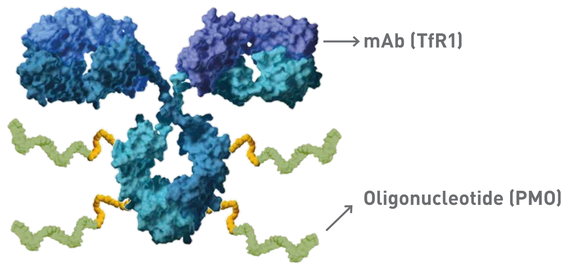
## Background

DMD is a muscular disease caused by predominantly out-of-frame mutations in the dystrophin gene[1]. Dystrophin protein expression can often be restored through oligonucleotide-mediated skipping of individual DMD exons to restore the reading frame[2]. Although multiple oligonucleotides targeting different exons have been approved, their activity is limited due to poor uptake by the muscle[3]. 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[4,5]. AOC 1044 is an investigational drug comprised of a TfR1 antibody conjugated to phosphorodiamidate morpholino oligomers (PMOs) that affords productive delivery of PMOs to muscle for treatment of DMD patients amenable to exon 44 skipping, for which there are no approved drugs.

Exon 44 skipping with PMO44, the PMO component of AOC 1044, was evaluated in healthy and DMD-derived cells. AOC 1044 was evaluated for activity in cynomolgus monkeys to evaluate tissue delivery and exon 44 skipping using an ELISA based assays and digital PCR as well as in DMD patient-derived cells to assess exon 44 skipping. Fluorescence microscopy was used to image restoration of the dystrophin-associated glycoprotein complex (DAPC), which is a marker of structural and functional myocyte recovery.

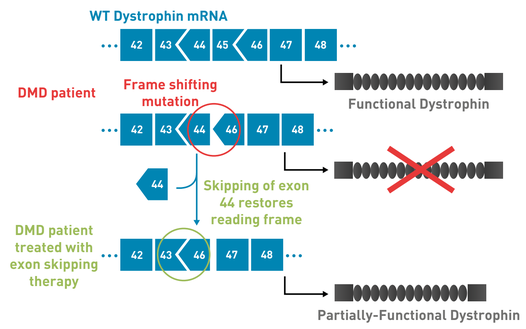
## Avidity's Approach to Treat DMD

### Avidity's AOC–PMOs Technology



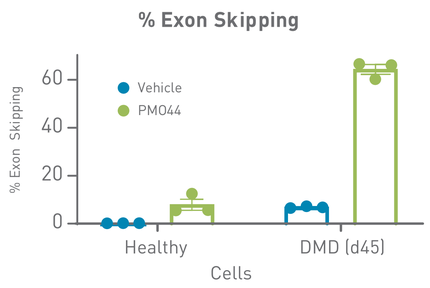

The PMO component can be changed to target different exons

### Example of a DMD Patient with Exon 45 Deletion, Amenable to Exon 44 Skipping Therapy



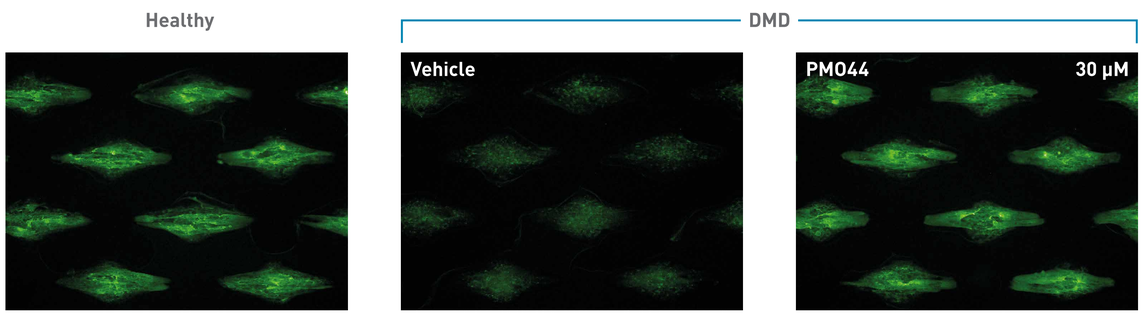

## Results

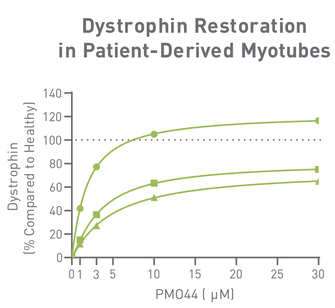
### 1. PMO44 induces substantially greater skipping in DMD patient derived cells compared to healthy cells

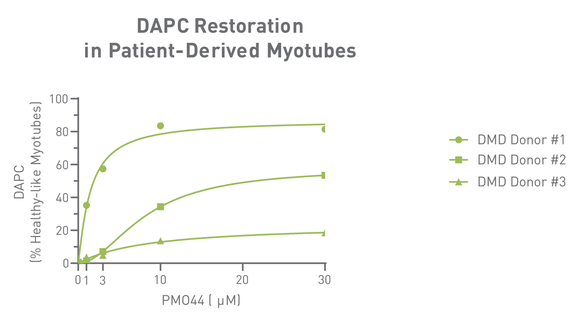



Myotubes derived from an immortalized healthy or DMD patient-derived myoblast cell line were treated with 10 µM of PMO44. The exon 44 skipping activity was determined by droplet digital PCR (ddPCR) 48 h post-treatment. Data shown represented as mean (+/-SEM) of technical triplicates for a single donor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

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



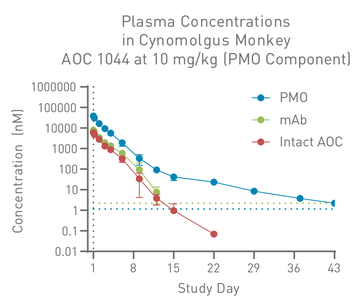



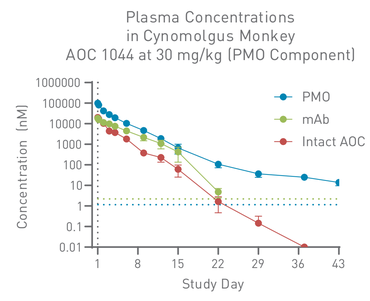



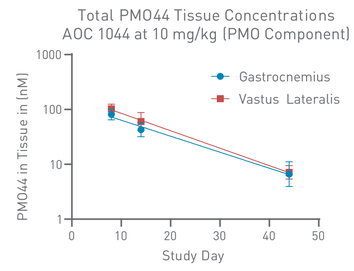

Human primary myoblasts from healthy and DMD patients were differentiated (MyoScreen™ Plates, Cytoo) for 8 days. At day 2 of differentiation, DMD myotubes were treated with PMO44 for 6 days at 3, 10 and 30 µM in triplicates, with Endo-Porter (GeneTools) at 1 µM as delivery reagent. For each experiment, 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 of technical triplicates for each donor, n=3 DMD myotubes, n=4 normal myotubes.

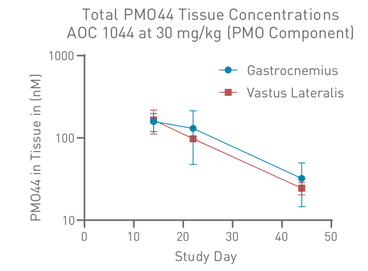
## Results

### 3. Plasma exposures of intact AOC 1044, total mAb, and total PMO44 as well as tissue exposure of PMO44, following the first dose of AOC 1044 in cynomolgus monkeys



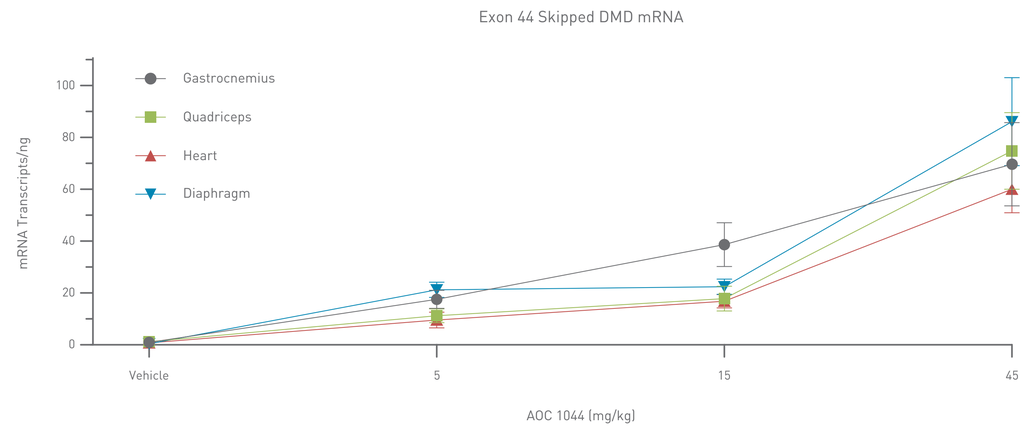




Animals received a single dose of AOC 1044 via IV infusion at dose levels of 10 or 30 mg/kg (PMO component dose). Plasma samples were obtained for PK analysis, and punch biopsies of the gastrocnemius and vastus lateralis muscles were obtained for tissue concentration analysis at several time points after dosing. Following AOC 1044 dosing, PMO concentrations were higher on a nM basis compared to intact AOC and mAb concentrations, whereas mAb concentrations were similar to intact AOC concentrations. This is consistent with the drug-to-antibody ratio (DAR) of AOC 1044 (DAR=3.8 on average). Mean total PMO concentrations in the gastrocnemius and vastus lateralis muscles were higher than in plasma at the tested timepoints. In general, mean muscle total PMO concentrations increased with increasing dose level. Data are represented as mean ± SD, n=4 animals per group.

### 4. AOC 1044 increases DMD exon 44 skipping levels in striated muscles of cynomolgus monkeys




AOC 1044 produced dose-dependent increases in the number of exon 44 skipped transcripts in a broad panel of muscle tissues, including the heart, following 4 doses, every 4 weeks of 5-45 mg/kg (PMO component dose) in male cynomolgus monkey. The number of skipped mRNA transcripts was evaluated using droplet digital PCR (ddPCR) and it was normalized to ng of total mRNA (shown on the Y axis). The number of total DMD transcripts (not shown) was similar in AOC 1044-treated animals and vehicle-treated animals, suggesting that AOC 1044 did not impact DMD expression up to the highest dose tested. Data are represented as mean ± SEM, n=4-5 animals per group.

## 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 immunoassay; IV, intravenous; mAb, monoclonal antibody; PCR, polymerase chain reaction; PMO, phosphorodiamidate morpholino oligomer; SEM, standard error of the mean

[1]Duan D, et al. *Nat Rev Dis Primers*. 2021;7(1):13;[2]Arechavala-Gomeza V, et al. *Curr Gene Ther*. 2012;12(3):152–60;
[3]Roberts TC, et al. *Nat Rev Drug Discov*. 2020; 19(10):673–94; [4]Etxaniz et al., Oral presentation, AAN 2022 Annual Meeting, Seattle, WA;
[5]Missinato et al., Poster presentation, New Directions in Biology and Disease of Skeletal Muscle Conference 2022, Ft. Lauderdale, FL.

## Acknowledgements

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