AOC 1044: An Antibody Oligonucleotide Conjugate as a Novel Therapeutic Approach for DMD Patients Amenable to Exon 44 Skipping

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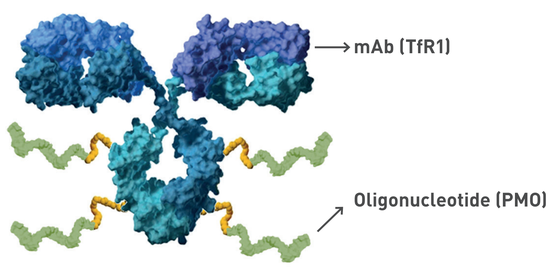
Background

Duchenne muscular dystrophy (DMD) is an X-linked muscular disease caused by mutations in the *DMD* gene that prevent the expression of a functional dystrophin protein.[1] Dystrophin protein expression can often be restored through oligonucleotide-mediated skipping of individual *DMD* exons to restore the reading frame.[2] Although several oligonucleotides targeting different exons have been approved, their clinical efficacy is limited due to poor muscle delivery.[3] Avidity has developed a transferrin receptor 1 (TfR1) antibody-based technology that can overcome this delivery issue. We have previously demonstrated that antibody-oligonucleotide conjugates (AOCs) can achieve substantial exon 23 skipping, dystrophin protein restoration, and muscle function improvement in mdx mice, a model of DMD.[4,5]

Here, we investigated the *in vivo* efficacy of AOC 1044, Avidity's clinical candidate for DMD patients amenable to exon 44 skipping.[6] First, we tested AOC 1044 in non-dystrophic non-human primates (NHP). In addition, using a novel humanized mouse model of DMD, which expresses the human *DMD* gene with exon 45 deletion that is amenable to exon 44 skipping (h*DMD*del45/*mdx*)[7], we investigated the therapeutic potential of a phosphorodiamidate morpholino oligomer (PMO) component conjugated to a mouse TfR1 antibody. It is important to note that exon 45 deletion accounts for roughly 50% of DMD patients with mutations amenable to exon 44 skipping therapy.[8,9]

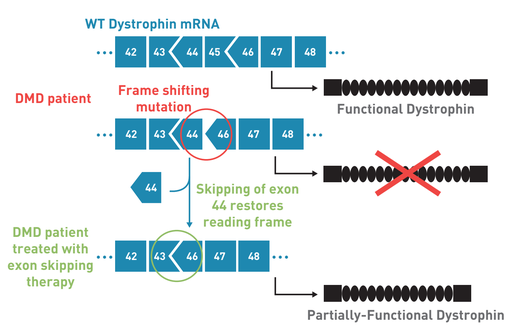
Avidity's Approach to Treating DMD

Avidity's AOC-PMOs technology



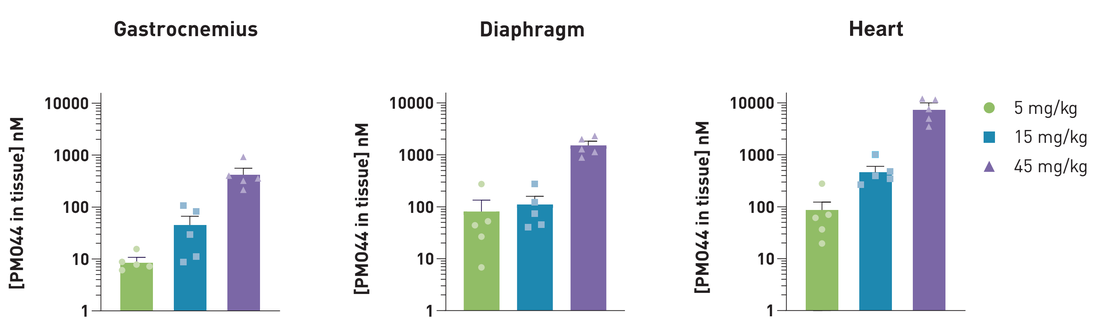
The PMO component can be changed to target different exons

Example of a DMD patient with exon 45 deletion, amenable to exon 44 skipping therapy[10]



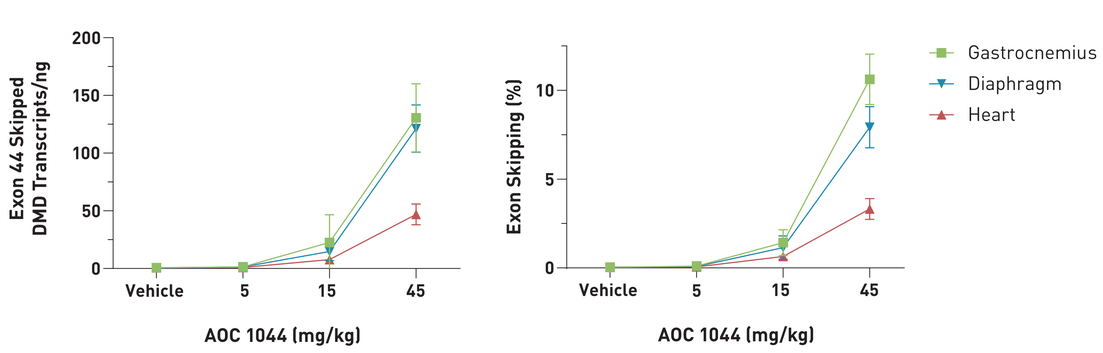
Results

Figure 1. Repeated dosing of AOC 1044 produces dose-proportional increase in PMO muscle tissue concentrations in cynomolgus monkeys



Animals were dosed with AOC 1044 via IV infusion at dose levels of 5, 15, or 45 mg/kg (PMO component dose) every 4 weeks. Tissues were obtained at necropsy for tissue concentration analysis following 9 months of repeat Q4W dosing. These data demonstrated effective delivery to skeletal muscle and cardiac tissue. Following AOC 1044 multiple dosing, mean muscle total PMO concentrations increased with increasing dose level. Data are represented as mean ± standard error of the mean (SEM), n=5 animals per group.

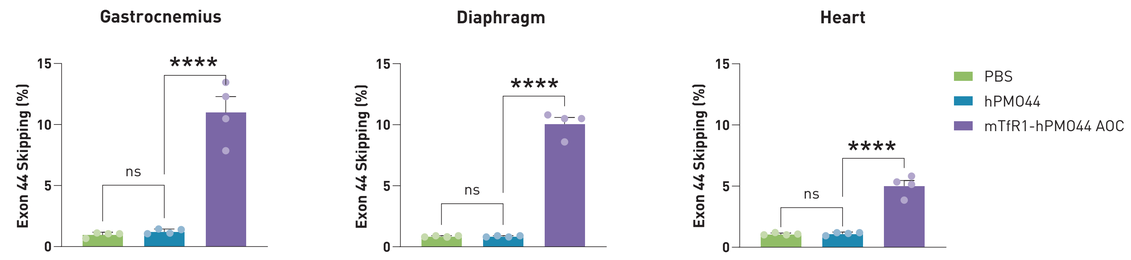
Figure 2. Repeated dosing of AOC 1044 increases DMD exon 44 skipping in skeletal muscles and heart in cynomolgus monkeys



Animals were dosed with AOC 1044 via IV infusion at dose levels of 5, 15, or 45 mg/kg (PMO component dose) every 4 weeks. Tissues were obtained at necropsy for exon skipping analysis following 9 months of repeat Q4W dosing. This study demonstrated that treatment with AOC 1044 was well tolerated in male cynomolgus monkeys and resulted in up to 11% exon 44 skipping in skeletal muscle at the highest dose. Indeed, AOC 1044 produced dose-dependent increases in the number of exon 44 skipped *DMD* transcripts in skeletal and cardiac muscles. 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. Exon skipping was analyzed by droplet digital PCR (ddPCR). Data are represented as mean ± SEM, n=5 animals per group.

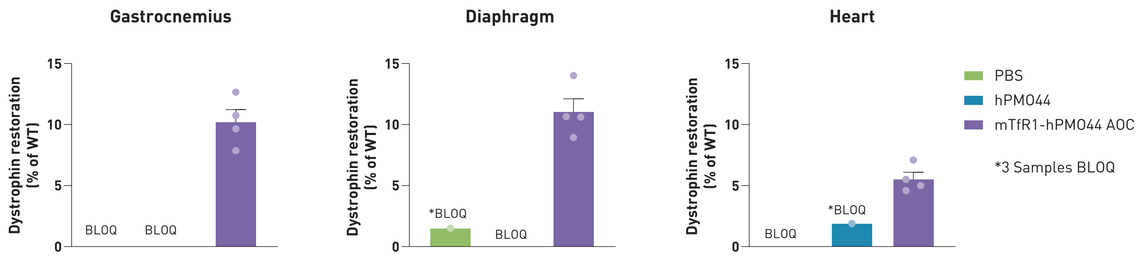
Results (Continued)

Figure 3. AOC treatment produces robust and durable exon skipping for up to 4 weeks after a single dose in h*DMD*del45/*mdx* mice



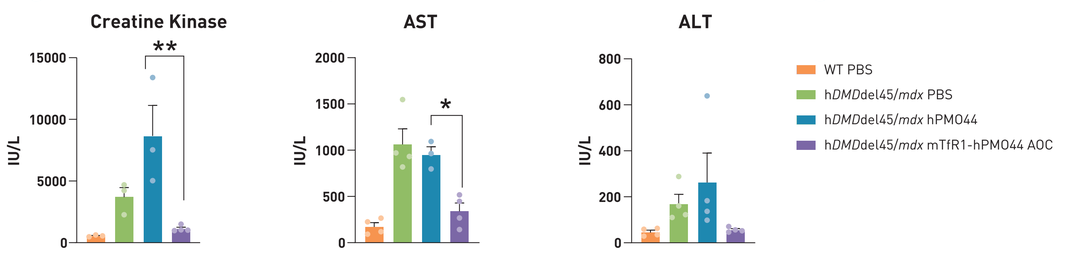
Exon skipping data in h*DMD*del45/*mdx* mouse model of DMD disease that expresses human *DMD* transgene with exon 45 deletion. Skeletal muscles and cardiac tissue were collected at 28 days post single IV bolus of vehicle PBS, 30 mg/kg of unconjugated hPMO44 or 30 mg/kg of AOCs (PMO dose), which was generated by conjugating the murine TfR1 antibody to the hPMO44. Exon skipping was analyzed by ddPCR. Data are represented as mean ±SEM, n=4 animals per group. ****p<0.0001.

Figure 4. AOC treatment effectively restores dystrophin protein in skeletal and cardiac muscle for up to 4 weeks after a single dose in h*DMD*del45/*mdx* mice



In the same study, dystrophin protein restoration in skeletal muscles and cardiac tissue of h*DMD*del45/*mdx* mice analyzed by Protein Simple Jess Capillary Western Blot. Tissues were collected at 28 days post single IV bolus of vehicle PBS, 30 mg/kg of unconjugated hPMO44, or 30 mg/kg of AOCs (PMO dose). Data were normalized relative to dystrophin concentrations in wild-type mice tissues. Data are represented as mean ±SEM, n=4 animals per group.

Figure 5. AOC treatment improves serum biomarkers of muscle damage to control levels



In the same study, serum biomarkers of muscle damage were analyzed at 28 days post dose. Marked reduction in serum biomarkers was observed in h*DMD*del45/*mdx* treated with AOC-hPMO44, compared to naked hPMO44-treated mice. Data are represented as mean ± SEM, n=3-4 animals per group. **p<0.01, *p<0.05.

Conclusions

- In non-human primates, treatment with the clinical candidate AOC 1044 was well tolerated and resulted in dose-proportional increase of drug in muscle tissue, and up to 11% exon 44 skipping in skeletal muscle at the highest dose as measured by ddPCR
- In a DMD mouse model expressing human *DMD* with exon 45 deletion, effective and durable exon skipping activity and dystrophin restoration in skeletal muscle and heart were observed for at least 4 weeks after a single IV dose of the surrogate AOC molecule with hPMO44
  - Dystrophin protein levels in gastrocnemius and heart increased by up to 10% and 5% of the wild-type animals' dystrophin, respectively
  - AOC treatment also improved serum biomarkers of muscle damage (CK, ALT, AST) to control levels, suggesting a functional improvement in muscle physiology
- Data presented here support the evaluation of AOC 1044 in the Phase 1/2 EXPLORE44™ trial for the treatment of Duchenne Muscular Dystrophy patients amenable to exon 44 skipping (NCT05747924)[11]

Abbreviations and References

ALT, alanine transaminase; AOC, antibody-oligonucleotide conjugate; AST, Aspartate aminotransferase; BLOQ, below limit of quantification; CK, creatine kinase; ddPCR, droplet digital PCR; DMD, Duchenne muscular dystrophy; IV, intravenous; mAb, monoclonal antibody; NHP, non-human primate; NS, not significant; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PMO, phosphorodiamidate morpholino oligomer; SEM, standard error of the mean; TfR1, transferrin receptor 1; WT, wild type.

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DISCLOSURES

- This poster is sponsored by Avidity Biosciences, Inc.
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