

Aptamer-enabled adsorber device for specific removal of anti-aquaporin-4 antibodies

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Objective

The aim of this study is to develop a first-in-class therapeutic medical device which will selectively remove pathogenic antibodies present in NMO-patients' blood. To tackle this challenge we used our modular SELEX platform termed PureApta™ to identify chemically modified DNA aptamers as ligands that specifically bind AQP4-IgG.

Introduction

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease associated with pathogenic autoantibodies directed against aquaporin-4 protein (AQP4) present in astrocytes' membranes. AQP4-IgG play an essential role in NMO pathogenesis and are regarded as highly specific biomarkers of NMO [1]. Current NMO therapies include generalized immunosuppression and plasma exchange, as well as of recently monoclonal antibodies, however their efficacy is limited and burdensome for patients [2], thus new targeted therapies are still desired.

SELEX process using PureApta™ platform

In vitro selection was performed using modular PureApta™ platform to identify chemically modified DNA aptamers that specifically bind AQP4-IgG. All deoxythymidines in the library were replaced with an unnatural hydrophobic uridine derivative (Fig. 1). After seven rounds of modified SELEX procedure with a recombinant anti-AQP4 antibody as target (AQP4-rAb), NGS and in-house bioinformatic analysis were applied to identify aptamer candidates. The most promising clone was tested against AQP4-rAb and later truncated down to 38 nucleotides (designated APS8.5). The chemical modification of the aptamer was proven to be required for binding.

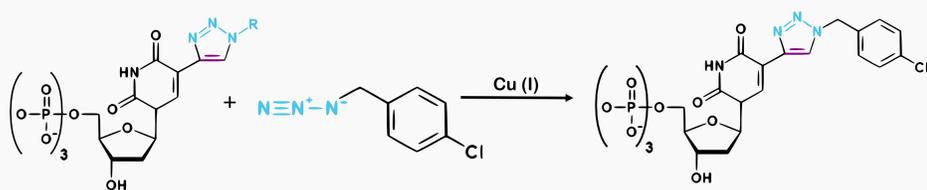


Figure 1. Click chemistry reaction between 5'-EdUTP and a hydrophobic azide that yielded an unnatural modified nucleotide which replaced all deoxythymidines during the SELEX.

Capturing of AQP4-IgG on aptamer-based adsorber

Aptamer APS8.5, immobilized on column-packed agarose resin via biotin-neutravidin interaction, was able to bind and deplete AQP4-rAb from healthy donor's human plasma spiked with AQP4-rAb, with at least 95% efficiency (densitometry in a flow-through fraction) and with minor depletion of other proteins. It was also shown that APS8.5 captures native AQP4-IgG from NMO-patient's plasma, which was confirmed using a clinically validated cell-based assay (Fig. 3 and 4).

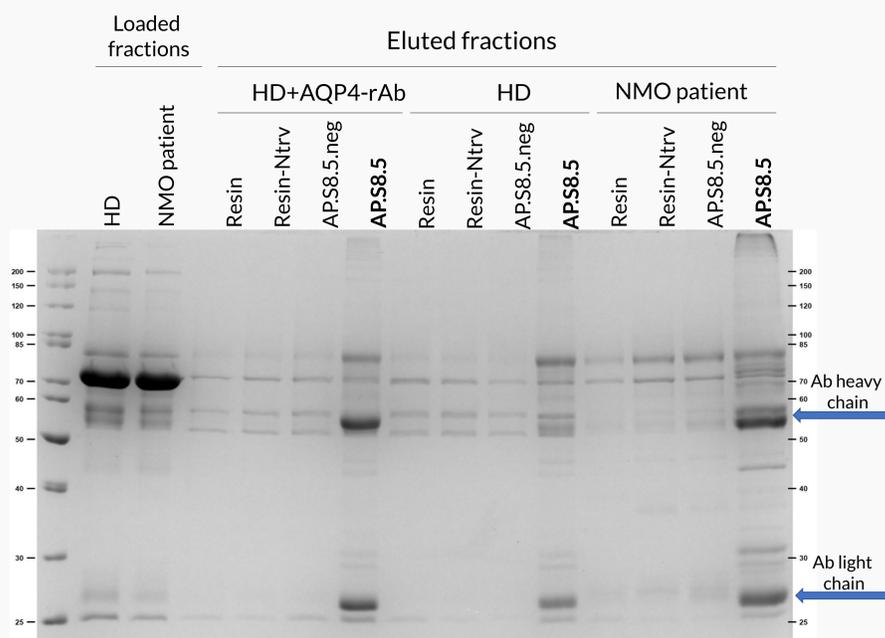


Figure 3. AQP4-IgG capture from human plasma using resin-immobilized aptamer. HD: healthy donor's plasma; HD+AQP4-rAb: healthy donor's plasma spiked with AQP4-rAb; NMO patient: AQP4-IgG positive plasma; Resin-Ntrv: resin-immobilized neutravidin (no aptamer); APS8.5.neg: unmodified aptamer (no chemical modification on thymidines).

Binding affinity of APS8.5 to AQP4-rAb

Using biolayer interferometry technology (BLI) for affinity measurements, it was determined that APS8.5, immobilized on a sensor via biotin-streptavidin interaction, specifically binds AQP4-rAb with an average K_D of $5.86 \times 10^{-11} \pm 4.67 \times 10^{-12}$ M (Fig. 2).

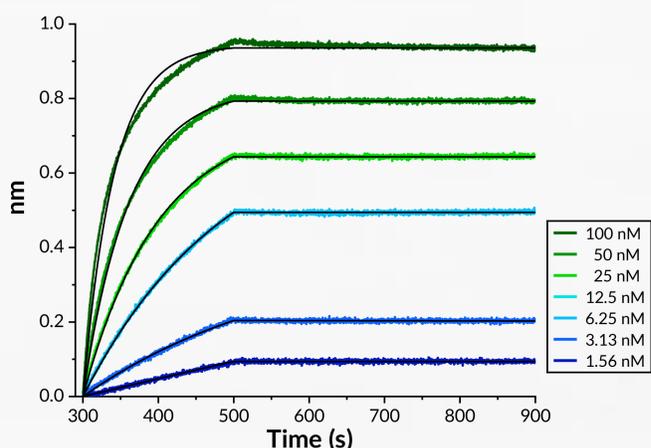


Figure 2. Affinity measurements of APS8.5 aptamer towards AQP4-rAb using BLI technology. 1:1 kinetic binding model was applied in a series of dose-response experiments.

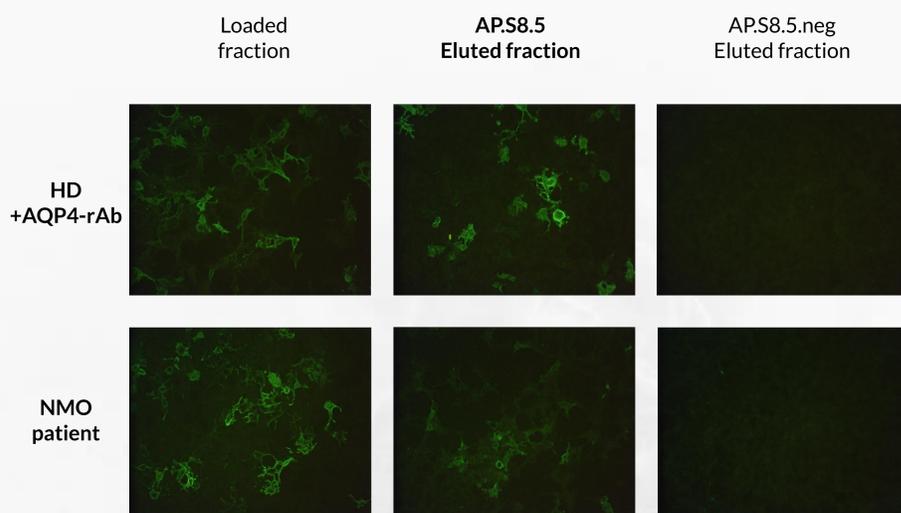


Figure 4. Column-eluted proteins were tested in a clinically validated cell-based assay for the presence of AQP4-IgG binding to AQP4-overexpressing cells. Green signal in microscopic images shows samples positive for AQP4-IgG. HD+AQP4-rAb: sample captured from healthy donor's plasma spiked with AQP4-rAb, NMO patient: sample captured from AQP4-IgG positive plasma; APS8.5.neg: unmodified aptamer (no chemical modification on thymidines).

Conclusions

Our results provide a proof-of-concept for the novel targeted therapy of NMO with the use of aptamer-enabled adsorber, capturing AQP4-IgG, and thus clearing pathogenic autoantibodies from anti-AQP4-positive plasma, without marked interference with other plasma proteins. The project is planned to enter *in vivo* studies in 2021.

Acknowledgements

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References

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- [2] Araki M., Yamamura T. (2017). Neuromyelitis optica spectrum disorders: emerging therapies. *Clinical and Experimental Neuroimmunology* 8, 107-116.



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