Enhancement of Antisense Oligomer Cell Penetration in Retinal Layers Using a Modular Cell Penetrating Peptide Platform

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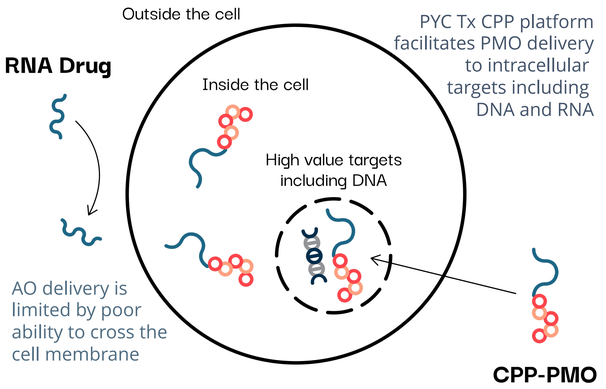
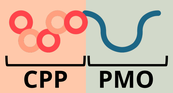
The Delivery Challenge



Antisense Therapies for Retinal Disease

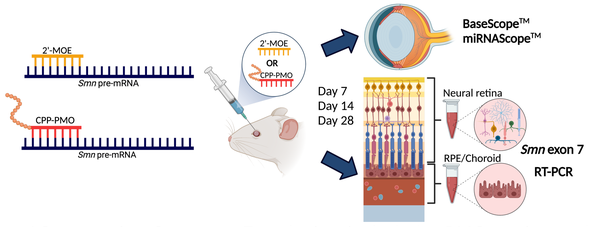
- Antisense oligomers (AOs) are synthetic nucleic acid analogues that can be designed to modify pre-mRNA splicing or protein expression for treatment of diseases.
- Suboptimal AO delivery presents an ongoing challenge and limits the realization of potential therapeutics[1].
- Antisense therapies for retinal disease are particularly limited in their ability to achieve adequate cellular uptake in the retina[2].
- PYC's cell-penetrating peptide (CPP) platform facilitates phosphorodiamidate morpholino oligomer (PMO) delivery[3] and is compared here with AO delivery to the retinal layers.

Peptides derived from bacteria, archaea, and viruses facilitate delivery across the cell membrane[3]



Precise, specific targeting of RNA molecules via complementary Watson-Crick base pairing

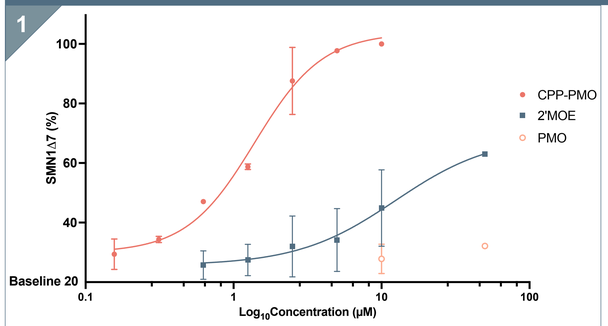
Methods



- AOs targeting *Smn* exon 7 exclusion included a PMO conjugated to a PYC CPP (CPP-PMO), and the same AO sequence with phosphorothioate 2′-*O*-methoxyethyl (PS 2′-MOE) modifications. Smn is ubiquitously expressed in the mouse retina and functions in spliceosome assembly.
- Mouse eyes were harvested 7-, 14-, and 28-days following intravitreal injection of CPP-PMO (1x) or 2′-MOE (1x, 10x or 30x molar concentration).

PYC CPP-PMOs Show Differential Uptake in Retinal Layers

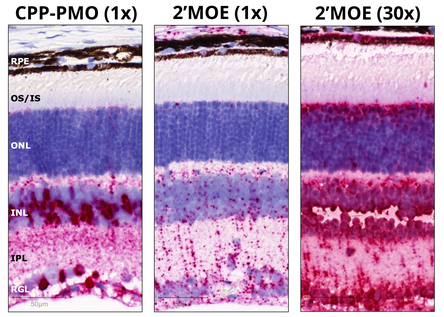
1



SMN1 exon 7 skipping induced by AOs in human primary fibroblasts. Each data point represents the mean ± SD from N=3 individual experiments.

PYC CPP-PMO has significantly improved AO delivery *in vitro*. 30-fold higher dose of 2′-MOE is required for similar exon skipping.

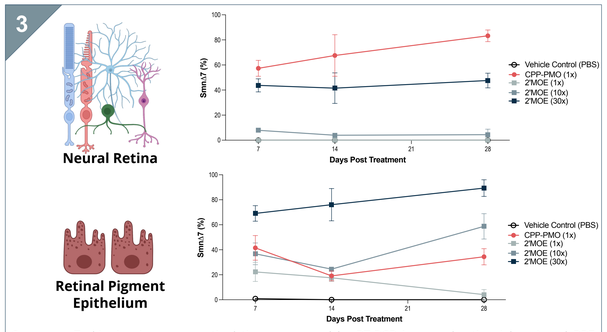
2



AO distribution (red signal) in mouse retinal layers following intravitreal injection assessed by *in situ* hybridization at 7 days post-treatment using probes specific for the AO sequence.

CPP-PMO is concentrated in the nuclei of the retinal ganglion and inner nuclear layers of the mouse retina.

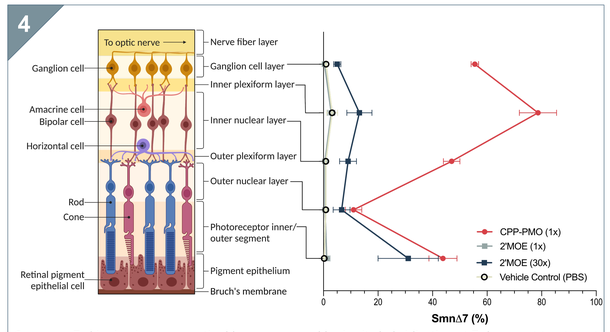
3



Smn exon 7 skipping in mouse retinal tissue assessed by RT-PCR in neural retina (above) and RPE (below). Each data point represents N=6 mice; error bars represent SEM.

The CPP-PMO outperformed the 2′-MOE *in vivo* at a 30-fold lower dose in neural retina and at an equimolar dose in the RPE.

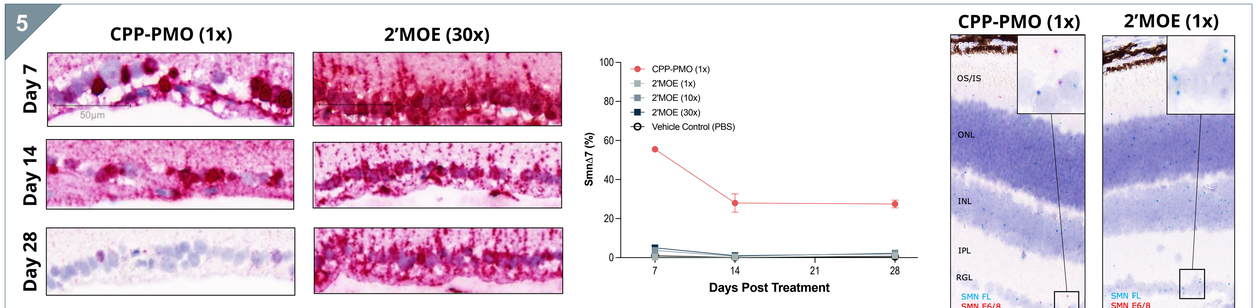
4



Smn exon 7 skipping in mouse retinal layers assessed by *in situ* hybridization at 7 days post-treatment using full-length *Smn* and *Smn*Δ7-specific probes in CPP-PMO or 2′MOE treated eyes. N=3 eyes ± SEM

PYC CPP-PMO confers a delivery advantage in the inner layers of the mouse retina, reaching multiple target cell types.

5

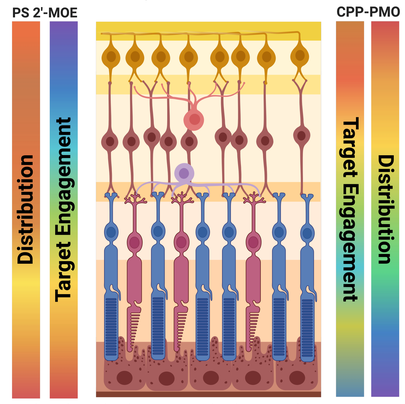


(Left) AO distribution (red signal) assessed by *in situ* hybridization in the mouse retinal ganglion layer using probes specific for the AO sequence. (Middle) *Smn* exon 7 skipping in the retinal ganglion layer over time (N=3 eyes ± SEM) detected by RNA *in situ* hybridization signals (right) using full-length *Smn* (blue signal) and *Smn*Δ7 (red signal)-specific probes in CPP-PMO or 2′MOE AO treated mouse eyes.

The CPP-PMO achieves durable exon skipping in the retinal ganglion layer, where minimal exon skipping was induced by a 2′-MOE AO. The localization of the 2′-MOE AO is not correlated with exon skipping, suggesting lower cellular uptake compared with the CPP-PMO.

Conclusion

- The CPP-PMO was detected in the retina up to 28 days with associated *Smn* exon 7 skipping, indicating intracellular penetration.
- Although the 2′-MOE was evenly distributed across the retina, this did not always correlate with *Smn* exon 7 skipping in different retinal layers, suggesting lower cellular penetration.
- This CPP-PMO confers a delivery advantage in the inner layers of the retina, including the retinal ganglion layer, where minimal exon skipping was induced by the 2′-MOE modified AO in this study.
- The improved outcome from CPP-PMO application in the inner retina may offer distinct advantages for therapeutics to treat diseases characterized by retinal ganglion cell degeneration, such as glaucoma.
- PYC's naturally derived peptide libraries have the potential to enhance oligomer delivery within the eye and other tissues.
- The modular CPP-PMO platform allows modification to fine-tune oligomer delivery and distribution, facilitating the development of oligomer-based therapeutics.



Heatmap depicting the relationship between AO distribution and target engagement (*Smn* exon 7 skipping) in the mouse retina for 2′-MOE (left) and CPP-PMO (right).

References & Acknowledgements

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