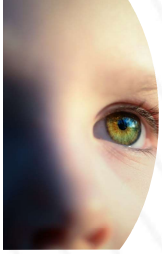

Cell penetrating peptides for the delivery of ASOs to the neural retina across rodents and NHPs

Adam D. Martin[1], Emily A. Woodward[1], Sue Fletcher[1,2], Sing Yee Yeung[1], Clinton Hall[1], Kristin Priebatsch, Hoang Vo[1], Anna Mills[1], Maria Kerfoot[1], Meenu Chopra[1], Paula T. Cunningham[1]

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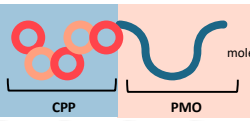
Antisense Therapies for Retinal Disease

- 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 PS-MOE delivery to the retinal layers.

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

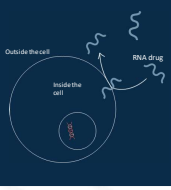


Precise, specific targeting of RNA molecules via complementary Watson-Crick base pairing

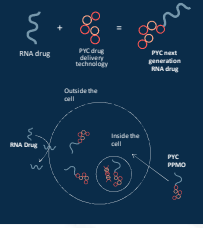
The Delivery Challenge of Antisense Therapies

PYC's technology overcomes the primary challenge for genetic medicines – delivering enough drug to the target

RNA therapies are an approved class of drug but their efficacy is limited by an inability to reach their target inside the cell



PYC's proprietary drug delivery technology is used to assist the RNA drug to reach its target inside the cell



Antisense Therapies for Retinal Disease

- Antisense oligomers (ASOs) are synthetic nucleic acid analogues that can be designed to modify pre-mRNA splicing or protein expression for treatment of diseases
- Suboptimal ASO delivery presents an ongoing challenge and limits the realisation of potential therapeutics[3]
- Antisense therapies for retinal disease are particularly limited in their ability to achieve adequate cellular uptake in the retina[4]
- PYC's cell-penetrating peptide platform facilitates phosphorodiamidate morpholino oligomer delivery to the cell layer in eye affected by ADOA

PYC's CPP-PMOs are translatable from *in vitro* assays to pharmacologically relevant in vivo models

Cell-based assays

- **Superior potency** versus alternative chemistries when applied gymnotically (i.e. not lipofected) – typical potencies low to sub-micromolar
- **High throughput screening** to rule out non-viable candidates – either cytotoxicity or lack of potency.

Mouse eye

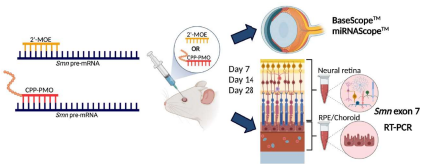
- **Rapid assessment** of distribution– ability to visualise localisation in retinal sub-layers and reference to target cell layer within the eye
- Moderate throughput screening for candidates; can rank on potency and tolerability

Larger eye

- **Quantitation of tissue concentration** in a relevant large eye, in target ocular layer
- Due **to well defined PK-PD relationship**, tissue concentration referenced to concentrations required to yield outcomes *in vitro*

Methods

Comparison of PPMO versus PS-MOE chemistry upon IVT administration in the mouse eye



- ASOs targeting *Smn* exon 7 exclusion included a PMO conjugated to a PYC CPP (CPP-PMO), and the same ASO 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).

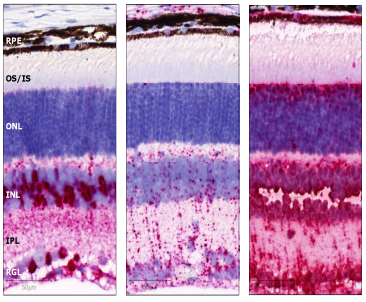
IVT administration of CPP-PMO into the rabbit eye

- ASOs targeting rabbit *Smn* exon 8 exclusion were designed and conjugated to a PYC-proprietary CPP, before being administered intravitreally to New Zealand White rabbits.
- Eyes were harvested at either Day 7, 14, 28 or 56 according to the designated endpoint of the study, with target engagement quantified through RT-PCR assessing the levels of *SmnΔ8* transcript and tissue concentration evaluated through an ASO-specific DELFIA.

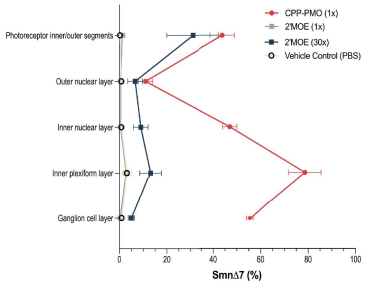
IVT administration of CPP-PMO into the NHP eye

- ASOs targeting human *CNOT3* exon 17 exclusion were designed and conjugated to a PYC-proprietary CPP, yielding the compound designated VP-001, before being administered intravitreally to cynomolgus monkeys.
- Eyes were harvested at pre-defined intervals between Day 1 and Day 112, according to the study design, with tissue concentration evaluated through a VP-001-specific hybridisation ELISA.

PYC's CPP-PMOs show enhanced productive uptake in mouse retina compared to other ASO chemistries



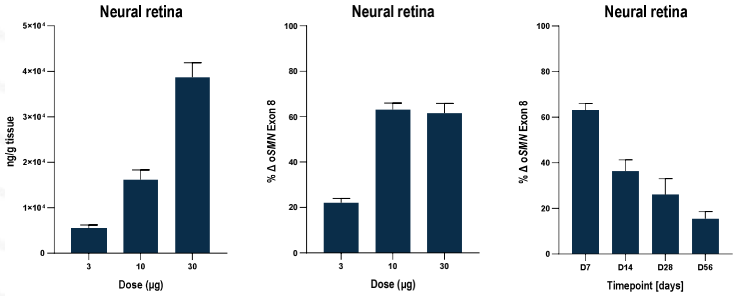
AO **distribution** (red signal) in mouse retinal layers following intravitreal injection assessed by *in situ* hybridization at 7 days post-treatment using probes specific for the AO sequence.



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

CPP-PMOs exhibit dose dependent uptake and target engagement in the rabbit neural retina

Dose dependent tissue concentration → **High target engagement at low administered dose** → **Extended duration of effect in retina**

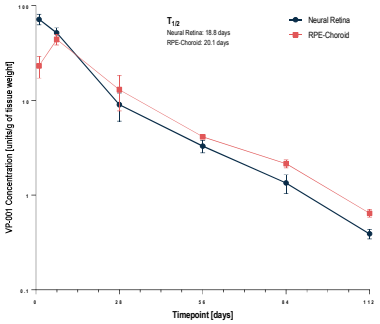


PYC's CPP-PMOs show dose dependent tissue concentration across all rabbit ocular tissues, including the neural retina

This tissue concentration translates to target engagement in a dose dependent manner, up until threshold is reached

The duration of target engagement is maintained across multiple months, supporting CPP-PMO as a Tx modality

CPP-PMOs exhibit a pharmacokinetic profile in the NHP retina that is supportive of extended dosing intervals in patients



~20 days
Half-life in target cell layers that are affected in RP11

~100 days
Anticipated dosing interval

Less frequent IVT dosing regimen
Preferred by patients

PYC's CPP-PMO, VP-001, has a half-life of 19 and 20 days in the retina (blue) and RPE-choroid (red), respectively in non-human primates after a single intravitreal injection, thus supporting a ~3-4 monthly dosing regimen.

Conclusions

- **Suboptimal delivery** presents an ongoing challenge and limits the realization of potential oligonucleotide drug therapeutics[1], particularly in retinal disease where non-productive uptake has hindered efforts.
- PYC's CPP-PMO technology, consisting of its proprietary cell-penetrating peptides conjugated to the clinically approved phosphorodiamidate morpholino oligomer chemistry, can effectively address the delivery challenge in the retina.
- In the mouse eye, PYC's CPP-PMOs achieve greater localization to the neural retina and higher target engagement versus an alternative PS-MOE ASO chemistry, across all cell layers in the eye.
- In the rabbit eye, PYC's CPP-PMOs show dose-dependent tissue concentration in all ocular tissues and excellent target engagement at low administered doses.
- In the NHP eye, pharmacokinetic data supports a multi-monthly dosing profile, with a half-life in the neural retina of approximately 20 days.
- PYC is currently leveraging its CPP-PMO platform technology to address inherited retinal disease, including retinitis pigmentosa and autosomal dominant optic atrophy.

References

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2. Kumar G Janoria, Sriram Gunda, Sai HS Boddu & Ashim K Mitra. Novel approaches to retinal drug delivery. *Expert Opinion on Drug Delivery*, **4:4**, 371-388 (2007). DOI: 10.1517/17425247.4.4.371
3. Hoffmann, K., Milech, N., Juraja, S.M. et al. A platform for discovery of functional cell-penetrating peptides for efficient multi-cargo intracellular delivery. *Sci Rep* **8**, 12538 (2018). DOI: 10.1038/s41598-018-30790-2

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