Efficient Delivery of Antisense Oligonucleotides using Cell Penetrating Peptides Enables Potent, Durable Exon Skipping in Mouse and Human Disease Models

Anja Stirnweiss[1], Paula Cunningham[1], Shane Stone[1], Tatjana Heinrich[1], Clinton Hall[1], Tracy Chai[1], Robert Dewhurst[1], Ben Wylie[1], Ferrer Ong[1], Maria Kerfoot[1], Laura Florez[1], Suzy Juraja[1], Dan Zhang[2], Samuel McLenachan[2], Fred K Chen[2], Janya Grainok[3,4], Sue Fletcher[3], Kim Rice[1], Katrin Hoffmann[1]

[1]PYC Therapeutics, Australia; [2]Lions Eye Institute, University of Western Australia, Australia; [3]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Australia; [4]Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

BACKGROUND

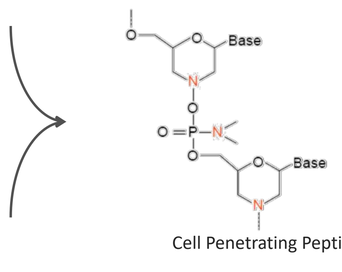
PYC Tx combines potent Cell Penetrating Peptides (CPPs) and precision RNA therapeutics such as phosphorodiamidate morpholino oligos (PMO) to create novel therapies

CPP

- Clinically validated for drug delivery[1]
- Enhanced delivery = lower dosing

PMO

- Safe and effective in vivo[2] and in the clinic[3]
- Highly stable = sustained splice modification



METHODS

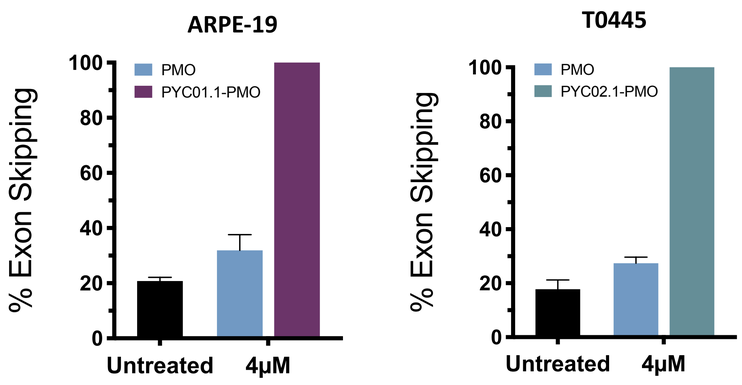
- Validation studies of PYC Tx CPP-PMO used Survival Of Motor Neuron 1 (*SMN1*) as a surrogate target. The *SMN1* gene product is ubiquitously expressed and plays important roles in the assembly of the spliceosome and biogenesis of ribonucleoproteins
- *In vitro* assays using cell lines and primary cells were assessed 48h post treatment at the indicated concentrations. The percentage of effect (exon skipping) was determined via RT-PCR and cell viability was measured using CellTiter-Glo® Luminescent Cell Viability Assays
- To assess the systemic delivery efficacy and safety, mice were injected via the tail vein and visually scored up to 48h before organs of interest were harvested for RNA extraction and analysis. For ocular delivery in mice, bilateral intravitreal (IVT) injections of 1.6µg drug or vehicle control were administered. Eyes were dissected into anterior segment, neural retina and RPE/choroid for assessment via RT-PCR

RESULTS

PYC Tx CPP Platform Creates Effective Therapeutics (Figure 1)

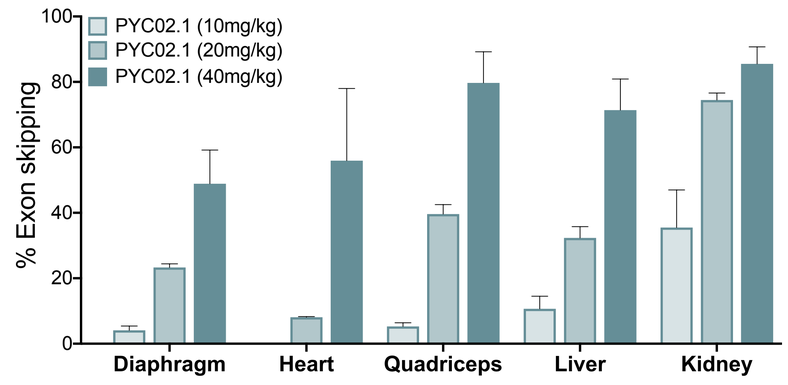
- ASOs penetrate cells through various mechanisms but high doses are required to achieve the desired effect. PYC Tx CPPs significantly enhance the delivery of ASOs, expanding their therapeutic reach to treat a range of genetic disorders.

A. Delivery of PMO with PYC Tx unique CPPs markedly increases efficacy in human RPE (ARPE-19) and cardiomyocytes (T0445)



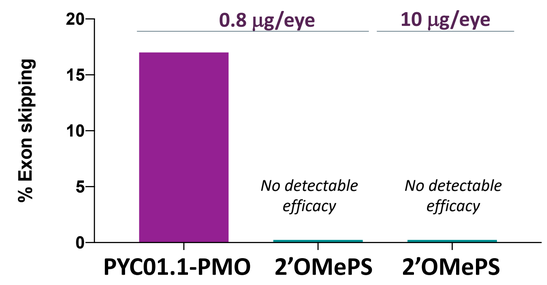
- ARPE-19 and T0445 cells were treated with PMO and CPP-PMO for 48 h and *SMN1* exon skipping was assessed by RT-PCR. Values are shown as mean ± SE (n=2 experiments, performed in duplicate)

B. A single injection (i.v) of PYC Tx CPP-PMOs increases exon skipping in multiple tissues linked to genetic disease



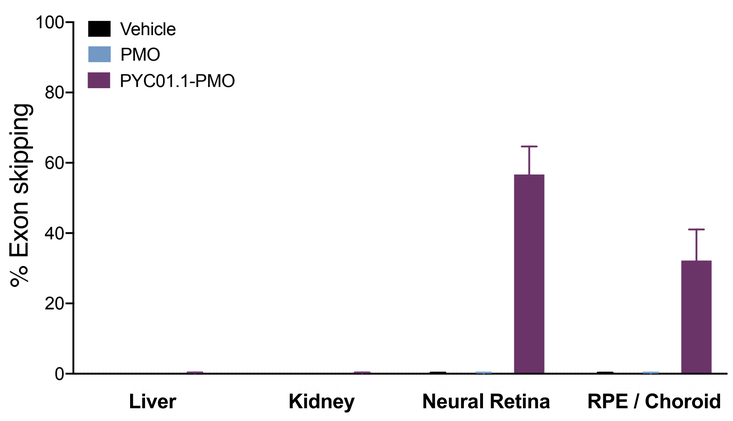
Mice (C57BL/6J) were treated with PMO and CPP-PMO for 48 h and *SMN1* exon skipping was assessed by RT-PCR. Values are shown as mean ± range (n=2)

C. PYC Tx CPP-PMOs are a potent alternative to 2'OMePS



Mice (C57BL/6J) were treated with a single IVT injection of CPP-PMO and naked 2'OMePS for 5d and *SMN1* exon skipping was assessed in RPE/choroid layer of the eye by RT-PCR.

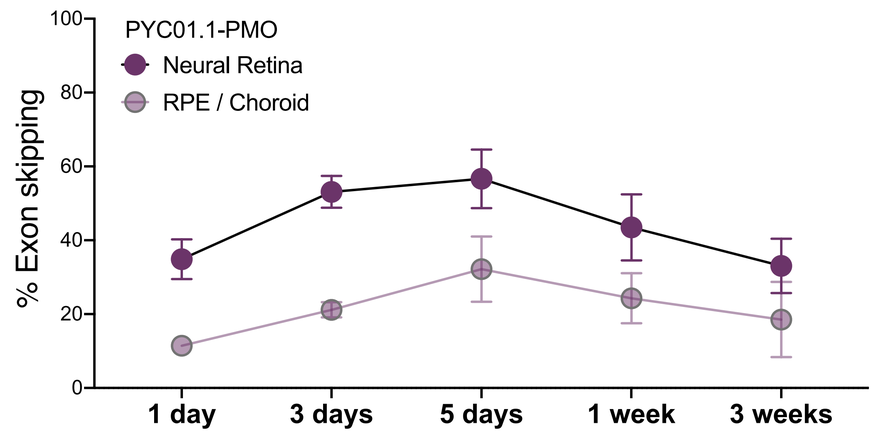
D. A single injection (IVT) of PYC Tx CPP-PMO into mouse eyes penetrates retinal layers to reach target cells for treatment of multiple ocular indications



The absence of effect in kidney and liver indicates that the drug is not distributed systemically when delivered via intravitreal injections

Mice (C57BL/6J) were treated with a single IVT injection of PMO and CPP-PMO (1.6µg/eye) for 5 days and *SMN1* exon skipping was assessed by RT-PCR. Values are shown as mean ± SE (n=5)

E. Therapeutic effect of PYC Tx CPP-PMO effect is durable after a single IVT injection

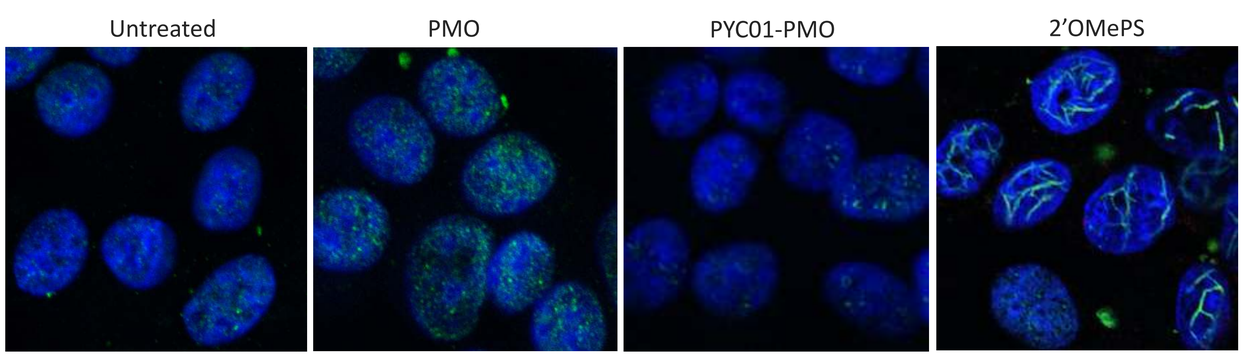


Mice (C57BL/6J) were treated with a single IVT injection of CPP-PMO (1.6µg/eye) and *SMN1* exon skipping was assessed by RT-PCR at indicated times. Values are shown as mean ± SE (n ≥ 3)

PYC Tx CPP-PMOs - A Safe Alternative to 2'-OMePS ASOs (Figure 2)

- PYC Tx CPP-PMOs are highly effective compared to alternative ASO chemistries and exhibit a favorable toxicity profile.

A. 2'-OMePS transfected ARPE-19 cells contain intranuclear inclusions linked to disruptions in apoptosis, signalling, chromatin silencing and other pathways[4,5]. PMO and CPP-PMO treated cells resemble untreated controls

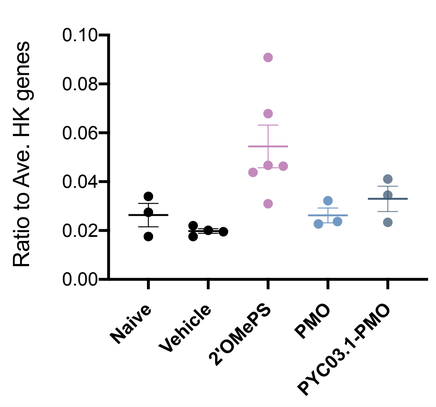


Images represent immunocytochemistry (ICC) of the paraspeckle protein, SFPQ (Green), following treatment of ARPE-19 cells with PMO (5µM), CPP-PMO (5µM), or 2'OMePS (100nM) for 48 h

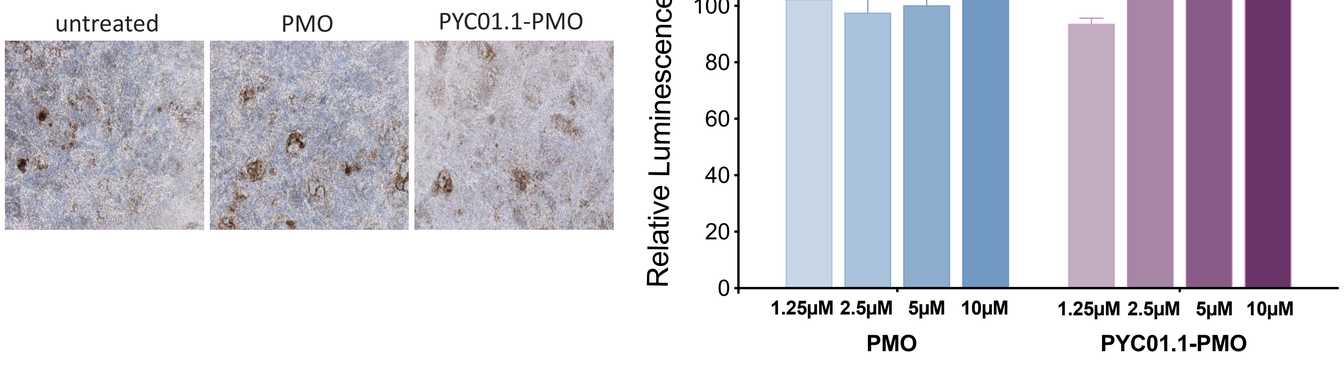
B. GFAP transcript levels are elevated in the neural retinas of 2'OMePS treated rodent eyes (single IVT injection, 1.6µg /eye) compared to UT, PMO alone and CPP-PMO treated eyes

Glial fibrillary acidic protein (GFAP) is a sensitive biomarker for inflammation induced activation of Mueller cells in the retina

GFAP mRNA levels in PYC Tx CPP-PMO treated eyes resemble untreated controls, 5 days post-treatment

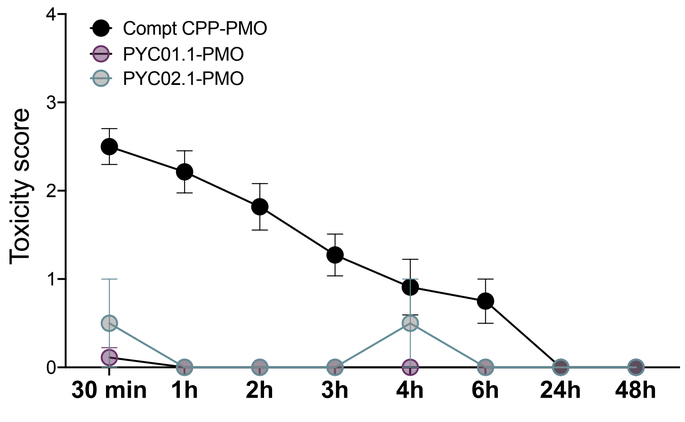


C. No morphological changes or cytotoxicity in human iPSC-derived RPE after treating with PYC Tx CPP-PMO at concentrations significantly higher than the expected therapeutic dose



Images represent brightfield images of control retinal pigment epithelium (RPE) treated with PMO (5µM) and CPP-PMO (5µM) for 48 h. Cell viability was assessed in RPE cells following treatment at indicated concentration for 48 h using CellTiter-Glo® Luminescent Cell Viability Assays. Values are shown as mean ± SE (n=3)

D. Mice treated systemically with PYCTx CPP-PMOs display an improved toxicity profile compared to a competitor CPP-linked PMO



In vivo toxicity was assessed following i.v injection of C57BL/6J mice with CPP-PMOs indicated (20 mg/kg)(n ≥ 3/group)

REFERENCES

1. Guidotti G, et al. *Trends Pharm Sci*. 2017; 38(4): 406-424.
2. Fletcher S, et al. *J Gene Med*. 2006; 8(2): 217-216.
3. Mendell J, et al. *Expert Opinion on Orphan Drugs*. 2017; 5(9):683-90.
4. Flynn L, et al. *BioRxiv*. 2018; **doi:** https://doi.org/10.1101/446773
5. Shen W, et al. *Nat Biotechnol*. 2019; 37(6):640-650.

Lions Eye Institute