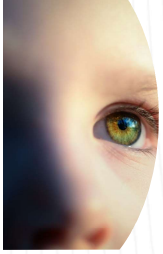

PYC-001, a peptide-conjugated phosphorodiamidate morpholino oligomer for the treatment of autosomal dominant optic atrophy

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ADOA is a progressive and blinding eye disease of childhood for which there are no available treatment options

Autosomal Dominant Optic Atrophy (ADOA) & PYC-001

- A progressive and **irreversible blinding eye disease with no available treatment**
- It is the most common inherited optic neuropathy with **9,000 – 16,000** addressable patients in the western world[1,2]
- Caused by **insufficient expression of one gene** (*OPA1*) in the cells that form the optic nerve in the eye
- *OPA1* gene variants account for 57-89% of ADOA cases, with over 200 pathogenic mutations having been reported[1,2]
- PYC's PPMO facilitates **delivery to target tissues** in the eye
- **PYC-001 increases *OPA1* protein levels** to enhance **mitochondrial structure** and improve **cellular bioenergetics** in models derived from patients with ADOA in a mutation independent manner

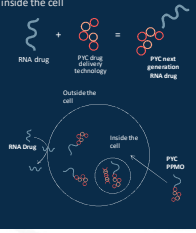
Deteriorating vision of an ADOA patient

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 reach its target inside the cell



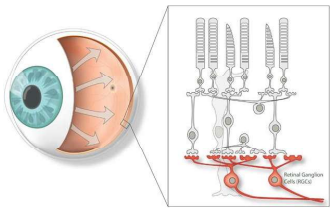
Antisense Therapies for Retinal Disease

- 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 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 (CPP) platform facilitates phosphorodiamidate morpholino oligomer (PMO) delivery to the cell layer in the eye affected by ADOA

ADOA is caused by insufficient expression of one gene *(OPA1)* in the cells that form the optic nerve in the eye

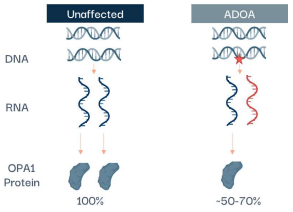
Affected cell type

In ADOA, the retinal ganglion cells (RGCs), that make up the optic nerve are affected



Mechanism of disease causation

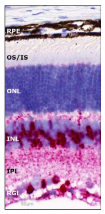
Patients with ADOA have a mutation in one copy of the *OPA1* gene causing an insufficient level of OPA1 protein in the RGCs



Pre-clinical data support the potential of PPMO for ADOA treatment

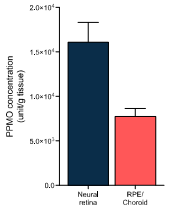
PYC's PPMO modality has an attractive distribution and favourable target engagement in the neural retinal *in vivo*

Attractive PPMO delivery to inner neural layers



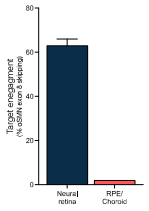
PPMO (red signal) in mouse retinal layers following intravitreal injection assessed by *in situ* hybridisation at 7 days post-treatment using probes specific for the PMO sequence

PPMO is concentrated in neural retina tissues



PPMO biodistribution in NZW rabbits at day 7 following a single intravitreal injection of Gen 2 CPP-oSMN_O8A(+7+31). n=2 eyes at 10 µg/eye, mean+SEM

PPMO mediates target engagement in the retina

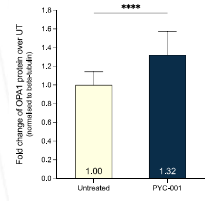


PPMO induced exon skipping in NZW rabbits at 7 days following a single intravitreal injection of Gen 2 CPP-oSMN_O8A(+7+31). n=2 eyes at 10 µg/eye, mean+SEM

PYC-001 rescues *OPA1* protein expression and improves functionality in ADOA patient-derived models

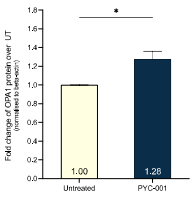
PYC has demonstrated the potential to address the root cause of ADOA in a mutation independent manner (validated in material derived from multiple patients), resulting in improved mitochondrial function critical for RGC health

Fibroblasts >1.3 fold upregulation



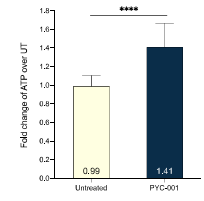
Bar graph represents mean+SD @ day 7 following PPMO incubation in fibroblasts. Patient 1, *OPA1* c.985-1G>A. n=3 biological replicates, 3 technical replicates. Student's *t*-test. ****p<0.0001

iPSC-RGC (target cell type) ~1.3 fold upregulation



Bar graph represents mean+SD @ day 5 following PPMO incubation in iPSC-RGCs. Patient 1, *OPA1* c.985-1G>A. n=3 biological replicates, 3 technical replicates. Student's *t* test *p=0.04

Translating to improved mitochondrial function

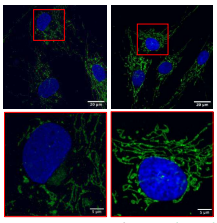


Bar graph represents mean+SD @ day 5 following PPMO incubation, Patient 2 derived fibroblast harbouring *OPA1* c.2708delTTAG mutation, n=3 biological replicates, 3 technical replicates. Student's *t*-test. **p<0.01, ****p<0.0001

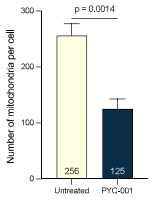
PYC-001 improves core characteristics of mitochondrial impairment in ADOA patient-derived fibroblast

PYC-001 treatment improves OPA1 function which results in enhanced mitochondrial morphology and network in ADOA patient-derived cells

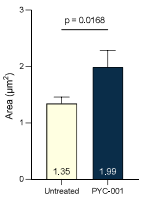
Untreated | **PYC-001 treated patient fibroblasts**



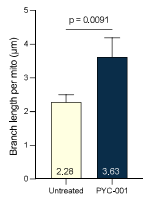
Less mitochondrial fragmentation



Enhanced mitochondrial morphometry



Improved mitochondrial network connectivity



Bar graph represents mean+SEM @ day 7 PPMO incubation, Patient 2 derived fibroblast harbouring *OPA1* c.2708delTTAG mutation, n=1 biological replicate, 1 technical replicate. Student's *t*-test.

Methods

Patient derived models

- Fibroblasts were obtained from skin biopsies from ADOA patients with an *OPA1* gene mutation
  - Patient cells were provided to PYC by Professor Alex Hewitt with consent via the Menzies Institute for Medical Research, and the Lions Eye Institute with consent via the University of Western Australia and Sir Charles Gairdner Hospital
  - To prepare retinal ganglion cell (RGC) cultures, patient fibroblasts were reprogrammed to iPSCs and then differentiated into RGCs.
  - RGC culture quality was confirmed by assessment of RGC-specific markers (γ-synuclein, Brn-3 and Thy-1)
- For OPA1 protein assays, fibroblasts or RGC cultures were treated with PPMO for 7 or 5 days, respectively, then protein expression assessed by western blot.
- To directly measure mitochondrial function in response to PYC-001 treatment, cells were treated for 7 days, and subsequently incubated with a glucose analogue (5 mM 2-Deoxy-D-glucose) plus a substrate for electron transport chain reaction (5 mM sodium pyruvate) for 18 h, then mitochondrial ATP quantified using a CellTiter-Glo Luminescent Cell Viability Assay.
- Mitochondrial network analysis was performed via immunocytochemical staining with the mitochondrial marker TOMM20.

Mouse model

- Assessment of PPMO distribution and function following intravitreal injection was performed using a reporter PMO targeting ubiquitously expressed *Smn*
- Distribution to the ganglion cell layer was assessed in 6–8-week-old C57BL/6 mice following a single dose of 3.2 µg/eye. PMO was detected by *in situ* hybridization at 7 days post-treatment using probes specific for the PMO sequence

Rabbit model

- Assessment of PPMO distribution following intravitreal injection was performed using a reporter PPMO (Gen 2 CPP-oSMN_O8A(+7+31)
- Tissue concentration in rabbit retina was measured by DELFIA utilizing probes specific to the PMO sequence
- Target engagement was assessed using a reporter PPMO targeting ubiquitously expressed *SMN* in New Zealand White rabbits 7 day following treatment with PPMO, 10 µg/eye.
  - Reporter PPMO induced *SMN* exon skipping was measured by RT-PCR

Conclusion: Preclinical findings support the development of PYC-001

- PYC's PPMO modality has an attractive delivery to the retinal ganglion cell layer in rabbits with favourable safety profile in non-human primates
- PYC-001 modulates OPA1 protein upregulation to a near healthy level (>1.3 fold) in patient-derived fibroblasts and iPSC-RGCs, sufficient for improvement of mitochondrial function
- PYC's approach leverages the healthy copy of the gene and can be used in a mutation-independent manner
- PYC-001 for ADOA is differentiated in it's ability to address the full restoration of ADOA phenotypes

References

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2. Yu-Wai-Man, P., *et al., Pattern of retinal ganglion cell loss in dominant optic atrophy due to OPA1 mutations.* Eye, 2011. **25**(5): p. 596-602.
3. Hoffmann, K., *et al., A platform for discovery of functional cell-penetrating peptides for efficient multi-cargo intracellular delivery.* Sci Rep, 2018. **8**, p12538.
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