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

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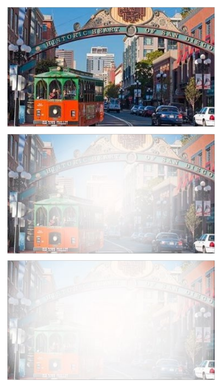
ADOA is a progressive and blinding eye disease of childhood for which there are no available treatment options

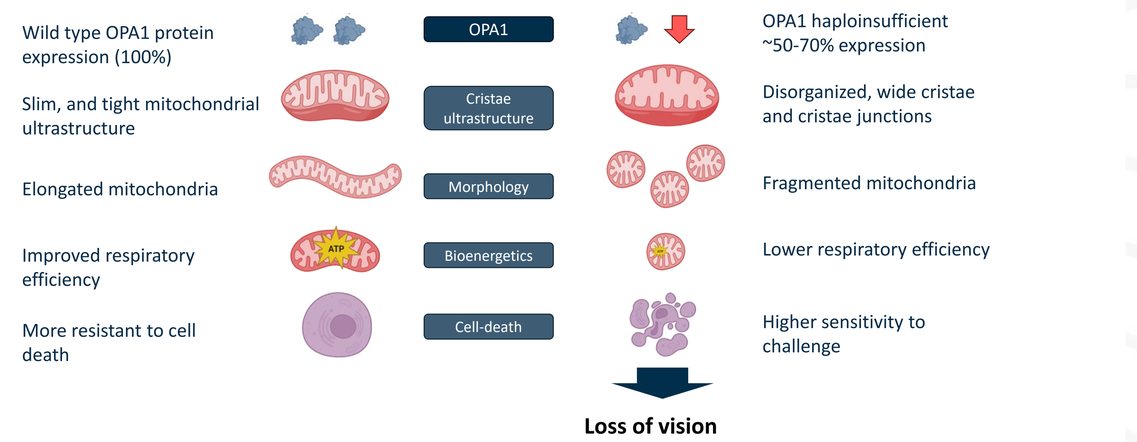
Autosomal Dominant Optic Atrophy (ADOA) & PYC-001

- A progressive and irreversible blinding eye disease
- It is the most common inherited optic neuropathy with **9,000 – 16,000** addressable patients in the western world[1,2]
- Median age of onset at 7 years of age, with 80% of patients symptomatic before age 10[1]
- **There are no treatments available** for patients with ADOA
- Caused by **haploinsufficiency of the *OPA1* gene** in RGCs that form the optic nerve of 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



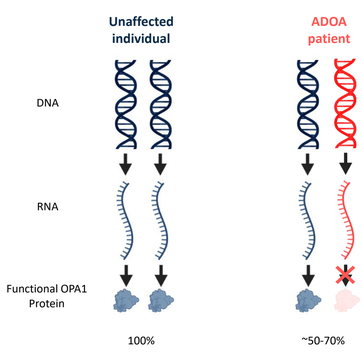
The deficiency of OPA1 protein in ADOA patients triggers a cascade of bioenergetic deficits that culminate in cell death and loss of vision



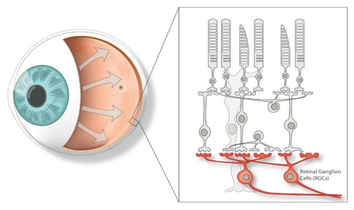
PYC-001 addresses the root cause of ADOA – insufficient expression of OPA1 protein in retinal ganglion cells

Mechanism of disease causation

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



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

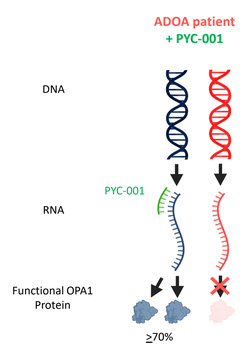


Decreased OPA1 protein leads to:

- Compromised mitochondrial function
- Reduced cellular bioenergetics
- Increased ROS and apoptosis
- RGC atrophy and vision decline

Treatment with PYC-001

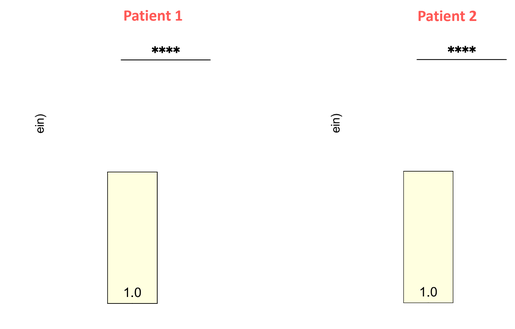
PYC-001 treatment **increases functional OPA1 protein expression** in a **mutation agnostic manner** while maintaining **OPA1 isoform balance**



PYC-001 increases OPA1 protein expression and improves functionality in ADOA patient-derived iPSC-RGCs

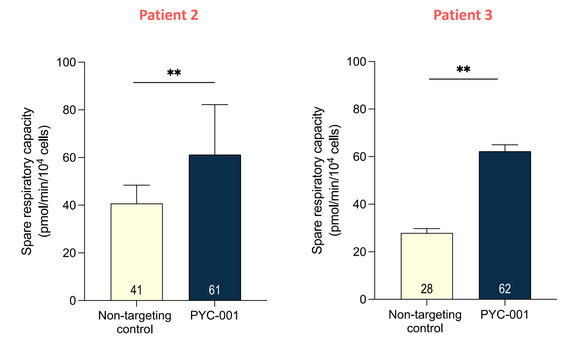
PYC-001 has demonstrated the potential to address the root cause of ADOA in a mutation independent manner (validated in material derived from multiple patients), resulting in improved mitochondrial function critical for RGC health

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>C.; Patient 2, *OPA1* c.2608delA. n=1 biological replicate, 3 technical replicates. Student's *t* test ****p<0.0001

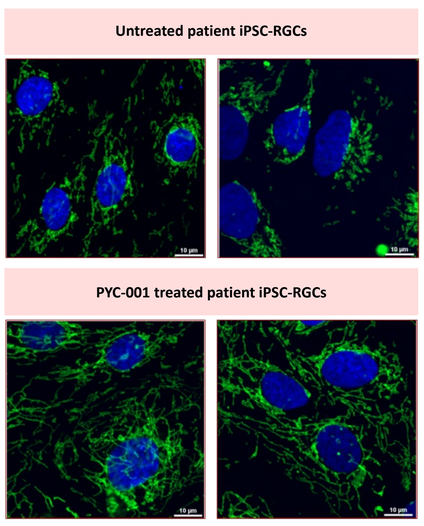
Translating to improved mitochondrial bioenergetics

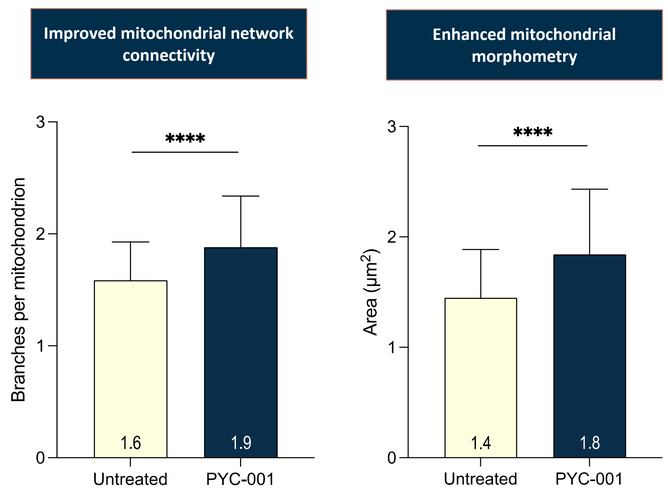


Bar graph represents mean+SEM @ day 7 following PPMO incubation in iPSC-RGCs., Patient 2, *OPA1* c.2608delA; Patient 3, *OPA1* c.2708-2711delTTAG. n=1 biological replicates, >4 technical replicates. Student's *t*-test. **p<0.01

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

PYC-001 treatment corrects mitochondrial structural defects in ADOA patient-derived iPSC-RGCs



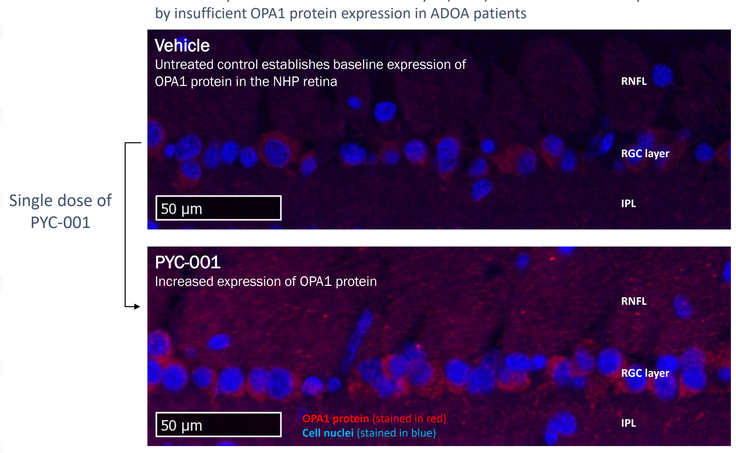


Bar graph represents mean+SD @ day 7 PPMO incubation, Patient derived iPSC-RGC harbouring *OPA1* c.2608delA mutation, n=1 biological replicate, 3 technical replicate. Minimum of 406 cells used for mitochondrial analysis. Student's *t* test ****p<0.0001.

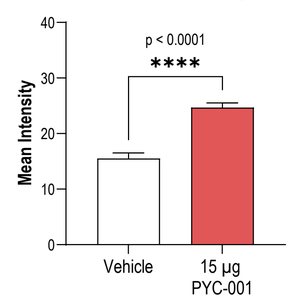
PYC-001 increases OPA1 protein in NHP retina with a single safe and well tolerated dose

PYC-001 increases OPA1 protein in target cell type (RGCs) in NHPs

The RGC layer and Retinal Nerve Fibre Layer (RNFL) are the two cellular layers affected by insufficient OPA1 protein expression in ADOA patients



RGC OPA1 staining



- A single 15 µg dose of PYC-001 increases OPA1 protein expression in NHPs in the two cellular layers affected by ADOA at 29 days following treatment
- This result was achieved with a dose of of PYC-001 that is safe and well tolerated in NHPs
- The 1.6-fold increase in OPA1 protein expression seen *in vivo* in NHPs is associated with rescue of the functional deficits seen in ADOA patient-derived models *in-vitro*

Methods

Patient derived models

- Cells from ADOA patient with an *OPA1* mutation 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 consequently differentiated into RGCs. RGC culture quality was confirmed by assessment of RGC-specific markers (γ-synuclein, Brn-3 , ISL-1 and Thy-1).
- For OPA1 protein assays, iPSC-RGCs were treated with PPMO for 5 days, then protein expression assessed by western blot.
- To measure mitochondrial bioenergetics, iPSC-RGCs were treated with PYC-001 for 7 days, and Oxygen Consumption Rate (OCR) were measured using Seahorse Cell Mito Stress assay. The rates of O_2 (OCR) were first measured under basal condition and then different parameters of mitochondrial functions were determined by sequentially adding oligomycin (1 .5 µM), carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) (1 µM), rotenone /antimycin A (0.5 µM).
- Mitochondrial network analysis was performed via immunocytochemical staining with the mitochondrial marker TOMM20 followed by mitochondrial network analysis using Mitochondrial Analyzer. Images were captured using a Confocal microscopy (60x magnification).

Non-human primate model

- Cynomolgus monkeys were dosed bilaterally with PYC-001 by intravitreal injections of 15 µg/eye. Quantification of OPA1 protein expression was performed by immunofluorescence in the RGC layer in NHP at 29 days post-treatment.

Conclusion: Preclinical findings support the development of PYC-001

- PYC-001 was found to be both safe and effective in Non-Human Primates following a single dose and resulted in OPA1 protein upregulation in target cells
- PYC-001 modulated OPA1 protein upregulation to a near healthy level (>1.3 fold) in patient-derived iPSC-RGCs, sufficient for improvement of mitochondrial function
- The current preclinical data supports PYC-001 potential as a functional cure for ADOA
- PYC is progressing PYC-001 through to human trials in 2024

References

1. 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.
2. Amati-Bonneau, P. et al. OPA1-associated disorders: phenotypes and pathophysiology. The international journal of biochemistry & cell biology, 2009;41(10), 1855–1865. doi: 10.1016/j.biocel.2009.04.012

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