



Glaucoma prevention and treatment: Novel therapeutic strategy to reduce RGC damage and disease progression

EXECUTIVE SUMMARY

- Glaucoma is a neurodegenerative disease often associated with increased Intraocular Pressure (IOP) and the number one cause of irreversible vision loss. Glaucoma causes Retinal Ganglion Cell (RGC) degeneration and excavation of the optic nerve head, leading to vision loss. Current management is limited to controlling the IOP, which only slows the progression, but does not cure the disease. Our strategy encompasses a novel, first-in-class protein therapeutic that can be delivered intravitreally as a naked protein or using various delivery vehicles (including AAV).
- This novel and **disease-modifying approach** is based on a validated Mechanism of Action. The team has previously shown that oxidative inactivation of neuroserpin is associated with increased plasmin activity, RGC degeneration and excavation of the optic nerve head in glaucoma. This approach delivers a mutant neuroserpin molecule that is resistant to oxidative inactivation. *In vivo* testing in glaucoma models has shown significant protection against glaucoma damage, including RGC degeneration. Its potential uses include both prevention and treatment of glaucoma as well as other conditions associated with plasmin activity or plasmin activator activity.
- **IP portfolio** includes a filed provisional patent application (Dec '20) and significant know-how.
- A multidisciplinary team with established knowledge and skills in the fields of optic neuropathy research, proteomics, knock-out animals and gene therapy.

BACKGROUND AND UNMET NEED

Glaucoma is the number one cause of irreversible vision loss and the second leading cause of blindness worldwide. Globally, around 76 million people live with glaucoma. This number is expected to increase to 111.8 million in 2040 due to demographic expansion and aging population. Over 300,000 Australians live with glaucoma and almost 10% of the population over the age of 80 are affected. The total community cost is expected to increase to \$4.3 billion pa by 2025 with the aging population. Increased Intraocular Pressure (IOP) is considered a prominent manifestation of glaucoma. Current primary management of glaucoma, which aims to control raised IOP, includes topical medications, laser therapy, non-penetrating glaucoma surgery (NPGS), invasive glaucoma surgeries, and the newer microinvasive glaucoma surgery (MIGS) procedures. Various medications include prostaglandin analogues, beta blockers, alpha-adrenergic agonists and combinations thereof. Current management is therefore limited to lowering the IOP, which only slows the progression of glaucoma, but does not cure the disease. It is extremely important to better understand mechanisms underlying Retinal Ganglion Cell (RGC) loss and structural damage in glaucoma. Loss of RGCs can occur in glaucoma cases even when IOP is reduced, and some patient still get glaucoma with normal IOP. Alternative treatment approaches are critically needed which target features of the disease other than or in addition to IOP.

NOVEL THERAPEUTIC APPROACH

Neuroserpin is a serine protease inhibitor which plays a role in inhibiting plasmin activity in the retina. Neuroserpin is well expressed in the retina and undergoes oxidative inactivation in glaucoma conditions. The research team has shown that oxidative inactivation of neuroserpin results in increased plasmin activity, and that decreased neuroserpin activity is associated with RGC degeneration and optic nerve damage, both of which are associated with glaucoma. The methionine residue in the neuroserpin active site makes the molecule labile to oxidative inactivation. The team has created a novel neuroserpin mutant (single amino acid) protein which retains the ability to inhibit serine proteases (such as plasmin, tissue plasminogen activator, and urokinase plasminogen activator) and is resistant to oxidative inactivation. Key supporting experimental *in vitro* and *in vivo* data are listed below.



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Key supporting data:

- 1) **Mutant form of neuroserpin is significantly more resistant to oxidative stress conditions compared to the WT form**, as evident by MetS reactivity and Plasmin Inhibitory Activity (PIA) measurements. Methionine is converted to MetS upon oxidation and MetS reactivity reflects the oxidation of this amino acid. In cell lines expressing neuroserpin (directly exposed to H₂O₂), PIA of the WT neuroserpin was significantly decreased following H₂O₂ treatment while the PIA of mutant neuroserpin was preserved even after H₂O₂ treatment. Furthermore, cells subjected to H₂O₂ treatment had significantly increased MetS reactivity compared to the control cells. The WT neuroserpin had significantly more MetS reactivity compared to the mutant neuroserpin following H₂O₂ treatment (**Fig. 1 A & B**).
- 2) **Mutant neuroserpin rescues glaucoma model disease phenotype in mice**, conferring significant protection against glaucoma damage, as indicated by the measurements of Positive Scotopic Threshold Response (pSTR) amplitudes (a measurement of inner retinal function), Ganglion Cell Layer (GCL) loss, MetS reactivity and PIA (**Fig. 2 A, B & C**).
- 3) **Mutant neuroserpin rescues neuroserpin knockout retinal phenotype**, conferring significant protection against glaucoma damage (indicated by pSTR amplitudes) and GCL loss than the WT neuroserpin (**Fig. 3 A, B & C**).
- 4) Intraocular mutant neuroserpin administration did not give rise to any detectable safety concerns in the eyes. Blood biochemistry and clotting time remained unaltered in the neuroserpin treated animals. AAV mediated gene therapy to overexpress neuroserpin in the RGCs imparted significant protection against experimental glaucoma induced degeneration. No ocular side-effects were observed in the AAV expressing animals.

Stage of development:

- Comprehensive validation of the discovery that neuroserpin plays a neuroprotective role in glaucoma and that its modulation in glaucoma conditions influences RGC degeneration and excavation of the optic nerve.
- *In vivo* PoC in two animal models using intravitreal delivery of the novel oxidation-resistant mutant neuroserpin protein into the retina.
- Next steps include investigating novel ways of neuroserpin delivery to the retina (eg. using nanoparticles using eye drop instillation) and deepening our understanding of the protective role of neuroserpin in photoreceptors and other retinal neurons by using AAV mediated overexpression of neuroserpin.

› Oxidative inactivation of neuroserpin is associated with increased plasmin activity, retinal ganglion cell degeneration and excavation of the optic nerve head in glaucoma
 › Oxidatively resistant form of neuroserpin molecule can protect the retina, even under the oxidative stress conditions in glaucoma.
 › We developed a first-in-class, novel neuroserpin mutant protein that rescues a glaucoma model disease phenotype and a neuroserpin knock-out phenotype in mice

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FIGURE 1. Mutant form of neuroserpin is significantly more resistant to oxidative stress conditions compared to the WT form

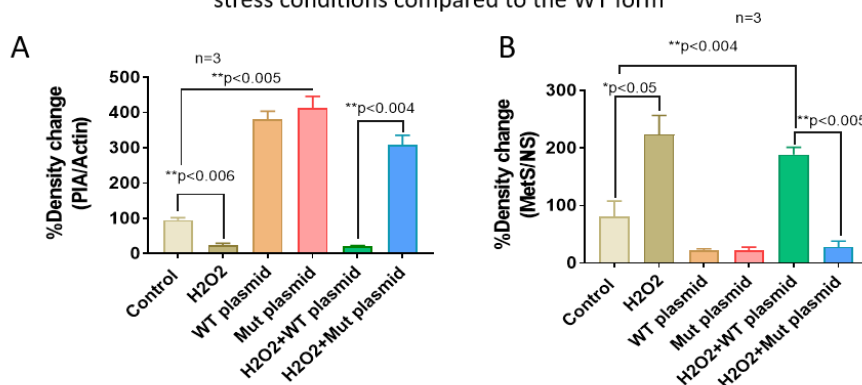


FIGURE 2. Mutant neuroserpin rescues glaucoma model disease phenotype in mice

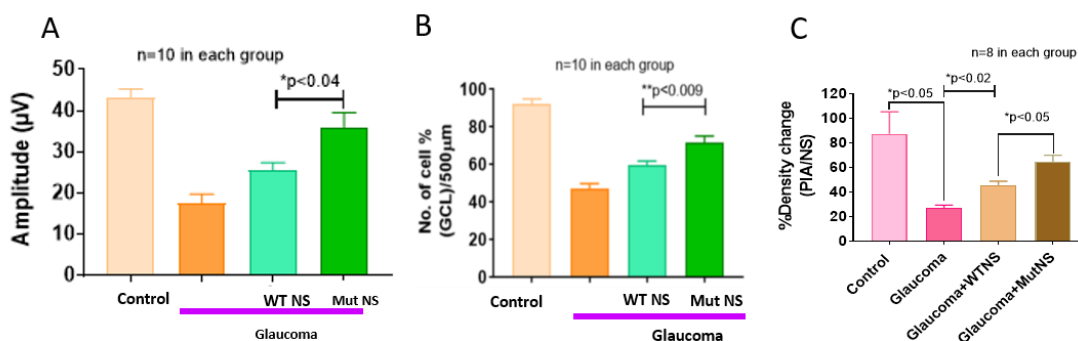
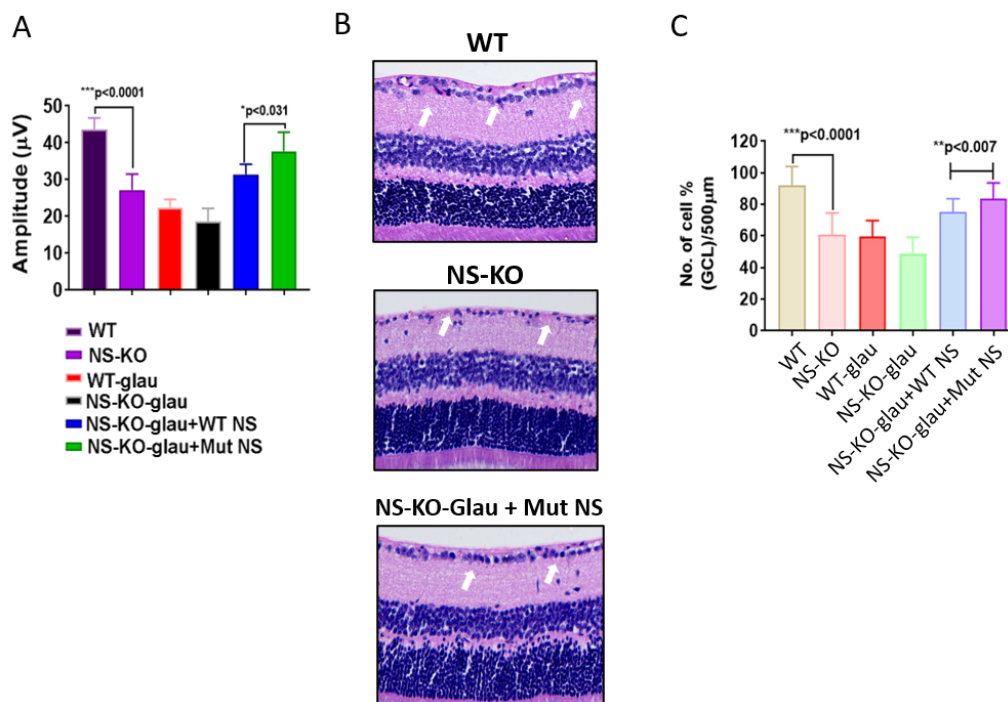


FIGURE 3. Mutant neuroserpin rescues neuroserpin knock-out retinal phenotype in mice



Glaucoma prevention and treatment: Novel therapeutic strategy to reduce RGC damage and disease progression

Figure legend: Fig. 1A. In cells expressing neuroserpin (SHSY5Y exposed to H₂O₂ (10μM, 6h)), PIA of the WT neuroserpin was significantly decreased following H₂O₂ treatment while the PIA of mutant neuroserpin was preserved; **Fig 1B.** Cells subjected to H₂O₂ treatment had significantly increased MetS reactivity compared to the control cells. The WT neuroserpin had significantly more MetS reactivity compared to the mutant neuroserpin following H₂O₂ treatment. WT: wild-type; Mut: mutant neuroserpin; **Fig 2.** A mouse model of chronic glaucoma was generated by repeated microbead injections of 10μm fluorospheres. Weekly injections into the anterior chamber of eye resulted in sustained increase in IOP for 2 months (control 10.5 ± 1.2; Glaucoma 24.6 ± 1.3 mmHg) following which the tissues were harvested for further biochemical analysis. There was a significant reduction of Positive Scotopic Threshold Response (pSTR) amplitudes in animals subjected to increased IOP. Control and glaucoma mice were analysed as well as the animals that were subjected to either WT or mutant neuroserpin treatment; **2A.** While in both WT and mutant neuroserpin treated animals the retinas were protected, the latter demonstrated significantly more protection compared to both glaucoma alone as well as glaucoma + WT neuroserpin treated mice. For electrophysiological recordings, the animals were dark-adapted overnight and anaesthetised with ketamine and medetomidine (75 and 0.5 mg/kg, respectively), and pupils were dilated using 2.5% phenylephrine, after which 1% tropicamide and topical anaesthetic (1% proparacaine) were applied to the cornea. pSTR recordings, which indicate inner retinal function, were recorded using flash intensities of - 4.3 log cd-s/m² delivered 30 times at a frequency of 0.5 Hz. The pSTR amplitudes were measured from baseline to the positive peak observed around 120 ms. **2B.** Cell density in the GCL was quantified for each eye by counting the number of cells in the GCL over a distance of 500 μm from the edge of the optic disc for both superior and inferior retina. While both WT and mutant neuroserpin administered groups showed protection of the GCL against glaucoma damage, the latter showed a significantly greater number of cells in the GCL compared to both glaucoma alone and glaucoma + WT neuroserpin treated mice eyes. **2C.** Mutant neuroserpin is able to better retain its PIA when compared to the WT neuroserpin under the glaucomatous stress conditions. While the PIA activity was much reduced in glaucoma conditions, it was significantly rescued in the WT and mutant neuroserpin groups. The later group demonstrated a much higher PIA compared to the WT neuroserpin treated mice. WT NS: wild-type neuroserpin; Mut NS: mutant neuroserpin; **Fig 3.** Mutant neuroserpin administration can rescue the neuroserpin knockout retinal phenotype and can impart much higher protection compared to the WT native neuroserpin protein. Global neuroserpin knockout mice were generated that are genetically deficient in neuroserpin from birth. Retinal electrophysiological recordings from these animals showed reduced pSTR amplitudes, which indicates that neuroserpin plays an important role in maintenance of inner retinal function. **3A.** Quantification of pSTR amplitudes revealed that both WT and neuroserpin administered neuroserpin knockout mice were significantly protected against the glaucoma damage, with mutant neuroserpin treated mice showing greater protection as compared to the WT neuroserpin; **3B & C.** Histological analysis revealed that both WT and mutant neuroserpin administered animals showed protection against GCL loss, where mutant neuroserpin administration conferred significantly greater protection when compared to the WT neuroserpin in mice. H&E staining and quantification of neuroserpin knockout mice retinas (thinning of the GCL was observed in the neuroserpin knockout animals compared to the WT mice). Arrows point to the Ganglion Cell Layer (GCL). WT: wild-type; NS-KO: neuroserpin knock-out; WT-glau: induced glaucoma on wild-type mice; NS-KO-glau: induced glaucoma on neuroserpin knockout mice; Mut NS: mutant neuroserpin; Neuroserpin protein (wt or mutant) was administered to the mice through weekly intravitreal injections under anaesthesia (1μg/μL, 2μL vol, for 2 months). Western blots of protein lysates were probed for using specific antibodies against neuroserpin, MetS and actin. Additionally, the proteins were subjected to native gelatin gel electrophoresis under non-denaturing conditions to assess PIA using gelatin gel zymography.

RESEARCH EXPERTISE AND CAPABILITIES

A multidisciplinary team with established knowledge and skills in the fields of optic neuropathy research, proteomics, knock-out animals and gene therapy. Led by Prof. Stuart Graham, who is an internationally recognised clinician-scientist with major contributions in the fields of glaucoma, multiple sclerosis, electrophysiology and investigating neurodegenerative processes in the visual system (>160 publications, >4300 citations). He has established a strong research group at Macquarie University in 2009, pioneering work in visual electrophysiology, ocular imaging of humans and animal models including transgenic mice and investigating cellular and molecular changes in neurodegeneration. He is a former Chairman of the Ophthalmic Research Institute of Australia between 2012-2016. The team also includes post-doctoral protein chemists and molecular biologists. Vivek Gupta joined Macquarie from Dean McGee Eye Institute (US) and has strong expertise in vision research investigating cellular signalling. Nitin Chitranshi has critical skills such as the use of transgenic mice models in vision research and AAV-based gene delivery. The group has access to state-of-the-art electrophysiology equipment, optical coherence tomography, fundus imaging equipment for small animals, a dedicated animal facility and molecular biology and microscopy labs. Prof. Graham is the head of Ophthalmology and has been involved in several clinical trials, which will help in the clinical translation of the research findings.

INTELLECTUAL PROPERTY PORTFOLIO

- 1) Provisional patent application titled “*Treatment of glaucoma*” filed in December 2020; includes composition of matter and method claims as well as various therapeutic modalities amenable to glaucoma treatment, including AAV-based delivery
- 2) Significant know-how



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PARTNERSHIP OPPORTUNITY

We are seeking an industry partner for further development and commercialisation of this unique and promising therapeutic approach through flexible and optimal partnership models. Furthermore, we are seeking collaboration opportunities in the area of ophthalmological neurodegenerative disease.

SELECTED PUBLICATIONS

1. Gupta, V., et al. (2017) Glaucoma is associated with plasmin proteolytic activation mediated through oxidative inactivation of neuroserpin. *Scientific Reports*. 10.1038/s41598-017-08688-2.
2. Chitranshi, N., et al. (2018) Shp2 gene therapy protects against retinal ganglion cell degeneration in glaucoma. *Molecular Therapy* 27 (2), 424-441.
3. Abbassi, M., et al. (2018) Regulation of growth factor and neurotrophin signalling pathways by protein tyrosine phosphatase Shp2 in the retina: A brief review. *Frontiers in Cellular Neuroscience*. <https://doi.org/10.3389/fncel.2018.00085>
4. Gupta, V., et al (2020) Retinal changes in Alzheimer's disease—integrated prospects of imaging, functional and molecular advances. *Progress in Retinal and Eye Research*. doi.org/10.1016/j.preteyeres.2020.100899.
5. Abbasi, M., et al. (2021). Inner retinal injury in experimental glaucoma is prevented upon AAV mediated Shp2 silencing in a caveolin dependent manner. *Theranostics* (Minor revision).
6. Abbasi, M., et al. (2020) Caveolin-1 ablation imparts partial protection against inner retinal injury in experimental glaucoma and reduces apoptotic activation. *Molecular Neurobiology*, 57, 9, 3759-3784
7. Dheer, Y., et al. (2019) Retinoid x receptor modulation protects against ER stress response and rescues glaucoma phenotypes in adult mice. *Experimental Neurology*, 314, 111-125.

INTERESTED TO LEARN MORE?

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