

# Protecting retinal ganglion cells

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## Abstract

**Retinal ganglion cell degeneration underlies several conditions which give rise to significant visual compromise, including glaucoma, hereditary optic neuropathies, ischaemic optic neuropathies, and demyelinating disease. In this review, we discuss the emerging strategies for neuroprotection specifically in the context of glaucoma, including pharmacological neuroprotection, mesenchymal stem cells, and gene therapy approaches. We highlight potential pitfalls that need to be considered when developing these strategies and outline future directions, including the prospects for clinical trials.**

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## The clinical need for neuroprotection

Raised IOP is the strongest risk factor for glaucoma<sup>2,3</sup> and all current therapeutic strategies work by lowering the IOP either medically or surgically. IOP lowering is a powerful, well-tolerated approach for many patients but the clinical need for additional strategies remains as a proportion of patients continue to progress despite effective IOP reduction. Even in populations with access to the best treatments currently available, an estimated one in eight patients will still eventually become blind in at least one eye due to glaucoma progression.<sup>4,5</sup>

The precise pathogenesis underlying glaucoma progression is not fully understood but glaucoma is now considered to be a heterogeneous group of conditions giving rise to RGC damage. Differing underlying aetiologies contribute to the glaucoma phenotype with vascular, mitochondrial or connective tissue pathology likely to be involved to varying extents in different individuals. While effective neuroprotection strategies may not always require full knowledge of the primary degeneration process, the potential does exist for the development of individualised treatments according to the relative contributions of the various disease processes in individual patients. For example, myopic patients or those with connective tissue disorders who are theoretically more susceptible to RGC damage from IOP fluctuations due to a thinner lamina cribrosa and altered scleral elasticity may be less likely to respond to interventions that improve vascular dysregulation or mitochondrial function than those identified to have predominantly other pathologies on genetic testing. Individuals may also have a number of different genetic predispositions to develop glaucoma but with advances in gene editing technology, for example with the CRISPR/Cas9 system recently having been used to partially restore visual function in blind rodents,<sup>6</sup> perhaps it is only a matter of time before glaucoma patients will be

## Introduction

Retinal ganglion cells (RGCs) provide the final common pathway to transmit all visual information processed by the retina to the brain. RGC degeneration therefore has a significant visual impact and is the leading cause of irreversible blindness worldwide.<sup>1</sup> There are unmet clinical needs for many conditions affected by RGC degeneration including glaucoma, hereditary optic neuropathies, ischaemic optic neuropathies and demyelinating disease.

Lowering intraocular pressure (IOP) reduces the risk of progressive RGC loss in glaucoma but no currently available treatments directly prevent RGC damage. However, recent successes in the treatment of other progressive retinal pathologies have paved the way for the advancement of several strategies to target RGC degeneration. In this review, we outline the ongoing clinical need to develop approaches to protect RGCs specifically in the context of glaucoma and discuss emerging strategies (Figure 1) that show promise, potential pitfalls and future directions including the prospects for clinical trials of neuroprotective treatments.

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able to receive targeted treatments to overcome their innate susceptibility to the disease.

The ability to confer additional neuroprotection early to those deemed to be at highest lifetime risk may also prove to be beneficial and treatments that ultimately restore visual function to those at an advanced stage of the disease process remains an ambitious but important goal.

### Neuroprotective strategies

Neuroprotection aims to augment the benefit provided by IOP lowering in order to slow the rate of RGC cell death and preserving the remaining level of vision. Whether any potential neuroprotective strategies can also improve the function of injured retinal ganglion cells remains to be seen. Neuroregenerative approaches are a key target of the National Eye Institute Audacious Goals Initiative. Regeneration of the optic nerve has recently been shown to restore some visual function in animal models<sup>7</sup> and there is a compelling argument for a combined neuroprotective/neuroregenerative approach when considering future treatment strategies for glaucoma. Neuroregeneration of RGCs has been extensively reviewed elsewhere<sup>8–10</sup> and is discussed in detail in several other papers from the Cambridge Ophthalmological Symposium 2016, published in this edition of *Eye*.

### Pharmacological neuroprotection

A number of compounds targeting a variety of mechanisms including modulation of glutamate-induced excitotoxicity,<sup>11–14</sup> vascular regulation via inhibition of nitric oxide synthase<sup>15,16</sup> or the endothelin pathway,<sup>17,18</sup> oxidative stress,<sup>19–23</sup> and inhibition of glial activity<sup>24,25</sup> have been shown to be neuroprotective in animal models of glaucoma though few have been tested in formal clinical trials.

Laboratory studies have demonstrated that systemic administration of brimonidine provides RGC neuroprotection in animal glaucoma models independent of its effect on IOP.<sup>26,27</sup> This neuroprotective effect is thought to be mediated via a variety of mechanisms including brain derived neurotrophic factor (BDNF) and basic fibroblast growth factor upregulation,<sup>28,29</sup> the activation of cell survival signalling pathways and prevention of apoptosis<sup>30</sup> and  $\alpha 2$  modulation of N-methyl-D-aspartate (NMDA) receptor function.<sup>31</sup>

In human clinical trials, brimonidine monotherapy lowered the incidence of visual field progression compared to timolol treated patients (9 vs 30%) in the Low Pressure Glaucoma Study Group<sup>32</sup> over a period of 30 months in patients who were able to tolerate the

treatment. In addition, brimonidine administered topically twice a day for 2 weeks prior to vitrectomy achieved 2 nM in the vitreous<sup>33</sup> which was at a sufficient concentration for neuroprotection in previous animal studies.<sup>34</sup> However, the repeated administration of topical agents is associated with side effects and non-compliance and therefore other approaches may have additional value.

Memantine is a non-competitive NMDA receptor antagonist which is used in the treatment of moderate to severe Alzheimer's disease and showed promising results in a monkey model of glaucoma.<sup>35</sup> However, in large scale multicentre, randomised double-masked placebo-controlled Phase III clinical trials<sup>36,37</sup> conducted to test the efficacy of oral memantine for glaucoma, there was no evidence of any statistical benefit compared to placebo in reducing visual field progression. The failure to demonstrate statistical significance relative to placebo in trials that took nearly 5 years at an estimated cost of over \$100 million highlighted a need for better glaucoma clinical trial design and more effective use of relevant endpoints as required by regulatory agencies.

Demonstrating that agents which have achieved neuroprotection in animal models have translational potential in human clinical trials presents several major challenges. First, accurately modelling a variable and complex multifactorial disease process such as glaucoma, where the underlying pathology is not fully understood, is difficult. Available animal models replicate some aspects of the disease but all have significant limitations. As an example, many glaucoma models involve induced elevation of IOP but do not model the increased susceptibility to glaucomatous damage that may be a contributing factor in many glaucoma patients.

Second, the way glaucoma is conventionally phenotyped is relatively unsophisticated and it is likely that different mechanisms are relevant to varying extents in different patients in ways that are not modelled effectively in animals at present.

Third, differences in outcome measures used when assessing laboratory studies and human patients are another potential issue. In addition, neuroprotective agents are often administered prior to the onset of damage in animal studies which limits their relevance when comparing any effect to therapeutic intervention in patients who already have the disease.

Slow progression of glaucoma in patients recruited to clinical trials of neuroprotection would suggest lengthy trials would be required and the individual variability that occurs when performing functional tests would be expected to necessitate large group sizes to determine whether there is evidence of any therapeutic effect.

It is therefore of considerable interest that recently, the UK Glaucoma Treatment Study (UKGTS),<sup>38</sup> which

evaluated vision preservation in patients taking the prostaglandin analogue Latanoprost for open-angle glaucoma in a randomised, multicentre, placebo-controlled trial demonstrated statistically significant differences between treatment groups in only one year with approximately 250 patients per group. It is conceivable that recruitment of patients progressing at a pre-defined rate prior to trial entry and enhancement of functional endpoints by clustering of measurements at the beginning and the end of studies could help to shorten neuroprotection trials and reduce the group sizes required still further.

### Mesenchymal stem cells

Mesenchymal stem cells (MSCs) have been shown to be strongly neuroprotective in models of Parkinson's disease, multiple sclerosis and spinal cord injury as well as many other disease models. Over 200 clinical trials using MSCs have been registered in the NIH database with 8 trials in multiple sclerosis alone. Advantages of MSCs compared to other types of stem cells include the fact that they are easy to obtain from a variety of sources including adult bone marrow, avoid ethical concerns and can be used without immune suppression.

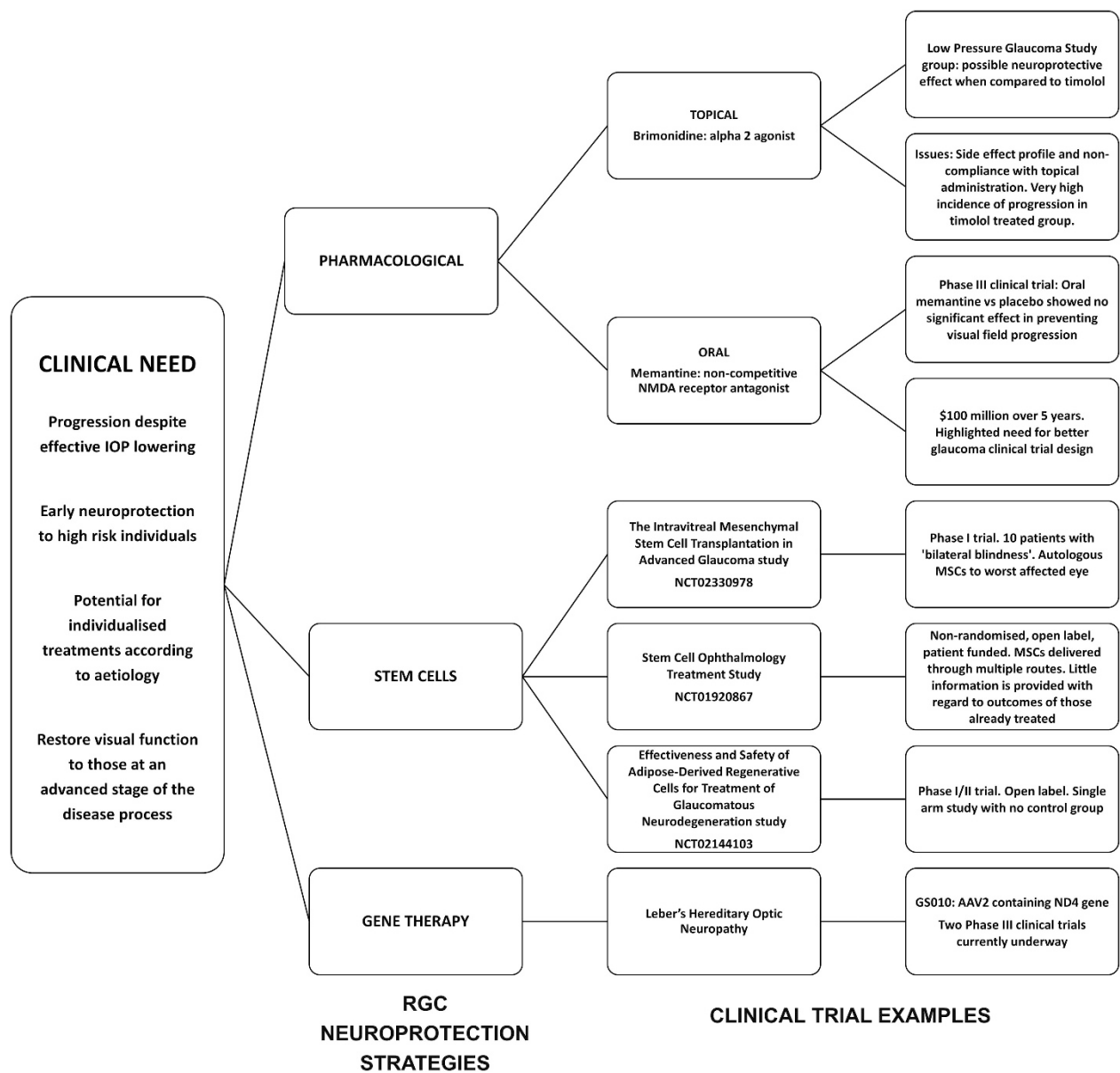


Figure 1 Summary of retinal ganglion cell (RGC) neuroprotection strategies.

MSCs have also been shown to be neuroprotective in a rat glaucoma model.<sup>39–42</sup> An analysis of the protective factors produced by MSCs strongly implicated platelet-derived growth factor (PDGF) and subsequent blockage of PDGF signalling prevents MSC-mediated neuroprotection in animal models.<sup>43</sup> We have also recently demonstrated a dose dependent protection against apoptosis with PDGF treatment with greater protection from MSCs in human post mortem retinal tissue (unpublished data). However, despite these encouraging results, it should be noted that PDGF and MSCs can induce reactive gliosis<sup>44</sup> in retinal Muller cells and astrocytes with upregulation of intermediate filament proteins and retinal folding.<sup>44</sup> Analysis of gene expression data obtained through microarray experiments suggest that both the STAT3 pathway and Lipocalin-2 (Lcn2) expression are upregulated in the presence of MSCs. This is consistent with increasing evidence that Lcn2 is involved in astrogliosis, neuroinflammation and reactive glia-mediated neurotoxicity in animal models of neurodegeneration,<sup>45</sup> perhaps through chemokine upregulation<sup>46</sup> and the promotion of glial migration.

There have been similar recent reports of proinflammatory vitreous clumping of MSCs injected intravitreally<sup>47</sup> as well as thick epiretinal membrane formation following MSC administration in humans.<sup>48</sup> These adverse effects may also be due in part to the inconsistency in MSC isolation and preparation. An attempt to standardise the identification of these cells led to the development of the International Society for Cellular Therapy criteria<sup>49</sup> but problems continue to be reported despite this and may limit the degree to which MSCs can successfully be used to confer neuroprotection to RGCs.

### *Stem cells in glaucoma clinical trials*

Although there are significant concerns with regard to the unregulated use of MSCs in various centres around the world, there are currently at least three registered clinical trials evaluating the use of stem cells in glaucoma:

1. The Intravitreal Mesenchymal Stem Cell Transplantation in Advanced Glaucoma study:<sup>50</sup> a Phase 1 safety study in 10 patients who meet the legal definition of bilateral blindness, with intravitreal injection of autologous MSCs administered to the worst-affected eye. Outcome measures include visual acuity, visual fields, optical coherence tomography and electroretinography.
2. Stem Cell Ophthalmology Treatment Study (SCOTS):<sup>51</sup> a non-randomised, open-label efficacy study evaluating the use of MSCs in multiple eye diseases including glaucoma delivered through either the sub-Tenon's, retrobulbar or intravenous route. The study is patient funded and claims to be associated with a risk of potential complications of 0.0008–5% depending on the physician-selected treatment protocol chosen for individual patients. The study website declares that it hopes for visual improvement in the vast majority of individuals enrolled, but very little information is provided with regard to outcomes of those already treated.
3. Effectiveness and Safety of Adipose-Derived Regenerative Cells for Treatment of Glaucomatous Neurodegeneration study:<sup>52</sup> an open-label safety and efficacy study in Russia. Stem cells are delivered via the sub-Tenon's route with structural and functional endpoints and is a single-arm study with no control group.

It remains conceivable that stem cells do have the potential to provide a useful treatment strategy for glaucoma, either by a neuroprotective or neuroregenerative mechanism. However, in order to assess such strategies we need randomised, masked, controlled clinical trials and further work on the optimal mode of delivery. Most 'stem cell' treatments for glaucoma administered worldwide are unregulated and currently most registered trials are patient funded, open-label studies. Care needs to be taken to prevent the exploitation of vulnerable patients and false promise of success with unproven treatments.

### *Gene therapy*

There has been significant progress in the use of retinal gene replacement strategies designed to augment loss of function mutations in conditions such as Leber's congenital amaurosis and choroideraemia, with encouraging safety and efficacy results and reports of some possible improvement in visual function in some patients.<sup>53,54</sup>

Advances are also being made in RGC disease and clinical trials are currently being conducted to evaluate the efficacy of GS010, an adeno-associated virus type 2 (AAV2) construct containing the human wild-type NADH dehydrogenase subunit 4 (*ND4*) gene to treat patients with Leber's Hereditary Optic Neuropathy due to the G11778A *ND4* mitochondrial mutation. Preliminary data from the Phase I/II trial<sup>55,56</sup> showed promising results and provided the foundation for two Phase III clinical trials which are currently underway.<sup>57,58</sup>

Additional gene-based strategies being investigated include the delivery of genes encoding therapeutic proteins such as neurotrophic factors. AAV2 mediated delivery of BDNF<sup>59</sup> and ciliary neurotrophic factor



(CNTF)<sup>60</sup> have been shown to confer RGC neuroprotection in experimental glaucoma.

Furthermore, optogenetic approaches to transform light-naïve retinal cells such as RGCs and introduce additional light sensitivity to the retina where the rod and cone photoreceptors have degenerated are also being developed. RetroSense Therapeutics are currently recruiting for a Phase I/II trial to test the safety profile of intravitreal RST-001, which delivers a gene encoding channelrhodopsin-2 to RGCs. Channelrhodopsin-2 depolarises when expressed and exposed to light, thereby generating a signal for transmission along RGC axons to the brain.

### Neuroprotection and the future

The delivery of intravitreal agents has revolutionised the treatment of retinal conditions such as age related macular degeneration, diabetic retinopathy and retinal vascular pathologies and their success and widespread therapeutic use demonstrates that patients are able to tolerate intravitreal injections repeatedly. There remains a strong clinical need for RGC neuroprotection with gene therapy and cell-based therapies showing considerable promise. As we continue to develop these strategies, the need to ensure that vulnerable patients are not taken advantage of with the false promise of success from unregulated trials that rely on patient funding is paramount. Refinement of clinical trial design to ensure that endpoints are feasible and clear also remains a priority.

### Conflict of interest

KRM is a consultant to Quethera Ltd. TZK declares no conflict of interest.

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