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Review

# Neuroprotective Strategies in Glaucoma - Translation to Clinical Trials

Clarissa E. H. Fang [1], Li Guo [1], Daniel Hill [1], Timothy E. Yap [2], M. Francesca Cordeiro [1, 2, *]

1. Glaucoma & Retinal Neurodegeneration Research Group, Institute of Ophthalmology, University College London, London, EC1V 9EL, United Kingdom; E-mails: fangclarissa@gmail.com; l.guo@ucl.ac.uk; d.hill.13@ucl.ac.uk; m.cordeiro@ucl.ac.uk
2. Western Eye Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom; E-mail: timothyedward.yap@nhs.net

* **Correspondence:** M. Francesca Cordeiro; E-Mail: m.cordeiro@ucl.ac.uk

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**Abstract**

Neuroprotection in glaucoma is any medical treatment by which decline in visual function can be slowed or prevented by supporting the health and survival of neural cells, independent of lowering of intraocular pressure (IOP). This is achieved by targeting mechanisms to inhibit or delay retinal ganglion cell death and promote cell survival pathways. Despite demonstrating promising results in preclinical trials, many neuroprotective strategies have failed to show success in subsequent clinical trials. Of the clinical trials performed, many have been hampered by slow disease progression and questions surrounding biomarker sensitivity. Adaptive clinical trial design, enriched populations and the use of state-of-the-art clinical endpoints are required to improve assessment of therapeutic efficacy. We review the neuroprotective strategies in glaucoma that have been investigated in clinical trials, and appraise experimental designs, the strength of the original hypotheses and preceding work to examine why so few candidates have successfully translated into clinical research.

**Keywords**
Glaucoma; neuroprotection; translation

## 1. Introduction

Currently, the only clinically-approved treatments for glaucoma involve the reduction of intraocular pressure (IOP) despite much research into the development of neuroprotective strategies. Neuroprotection in glaucoma is understood as methods aimed at reducing or preventing loss of visual function, via an IOP-independent mechanism [1]. Despite numerous demonstrations of successful neuroprotective agents in experimental glaucoma, very few of these treatments have reached clinical trials. Of the treatments that have reached clinical trials, the results have often not reflected the preclinical research. Whether this is simply another example of the 'reproducibility crisis' [2] or in fact a failure of longitudinal study design or clinical endpoints that are unfit for purpose, is still to be decided.

Recent advances have suggested a broad range of potential molecular mechanisms of RGC death in glaucoma [3-5]. These include glutamate excitotoxicity [6, 7], mitochondrial dysfunction and oxidative stress [6-9], and the dysregulation and obstruction of neurotrophins such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF)[6, 7, 10, 11]. As understanding of glaucomatous pathology remains incomplete, learning from unsuccessful clinical trials is challenging [12]. This review article details the latest neuroprotective strategies in glaucoma that have reached clinical trials (Table 1, Figure 1), as well as discussing the strengths and limitations of the trials investigating their efficacy.

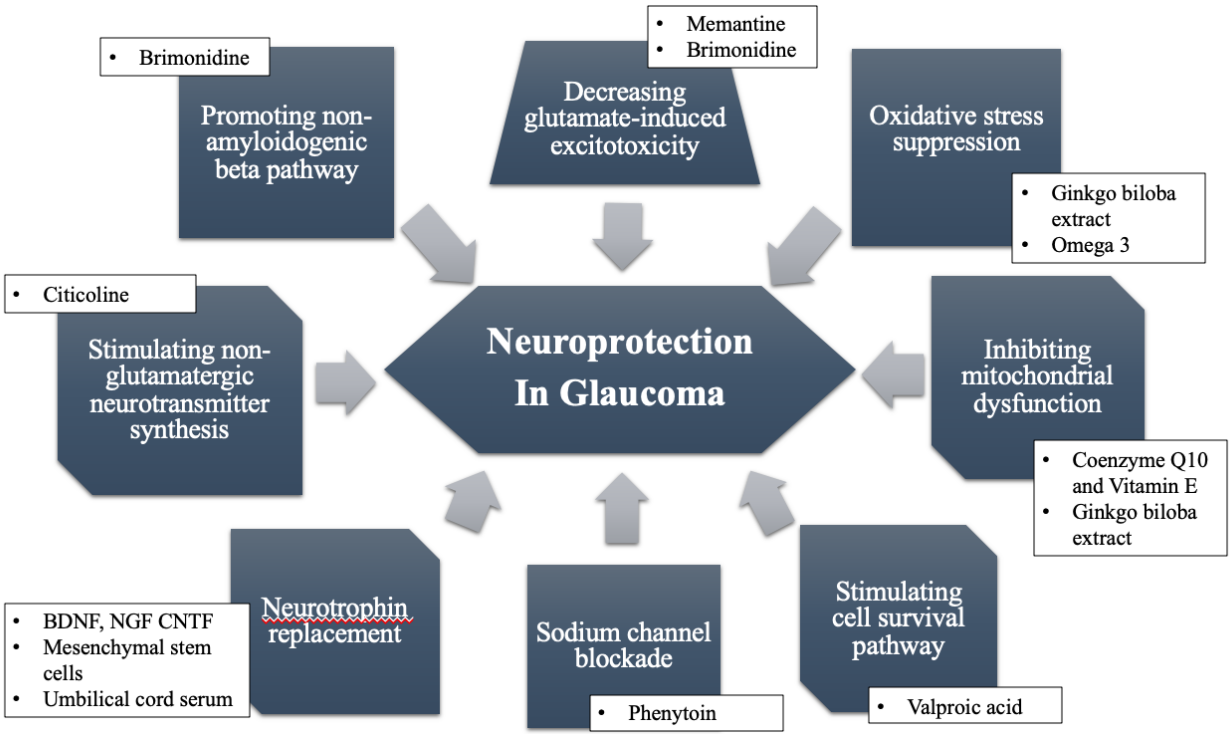


**Figure 1** Neuroprotective strategies in glaucoma translated to clinical trials.

**Table 1** Neuroprotective agents for glaucoma treatment into clinical trials.

| Agent | Class | Mechanism | Administration | Level of Evidence* | References |
|---|---|---|---|---|---|
| BDNF, NGF and CNTF | Neurotropic factors | Neurotrophin replacement | Topical | IIIB | [4, 6, 11, 13-20] |
| Ginkgo biloba extract | Antioxidant | Oxidative stress suppression Inhibiting mitochondrial dysfunction | Oral | IB | [21-28] |
| Coenzyme Q10 and Vitamin E | Antioxidant | Inhibiting mitochondrial dysfunction | Topical | IB | [29-31] |
| Omega 3 | Antioxidant | Oxidative stress suppression | Oral | IB | [32-37] |
| Memantine | NMDA receptor antagonist | Decreasing glutamate-induced excitotoxicity | Oral | IB | [38-44] |
| Brimonidine | Alpha 2 agonists | Decreasing glutamate-induced excitotoxicity Promoting non-amyloidogenic beta pathway | Topical | IA | [44-55] |
| Citicoline | | Stimulating non-glutamatergic neurotransmitter synthesis | Topical | IB | [56-66] |
| Valproic acid | Antiepileptic drug | Stimulating cell survival pathway | Oral | IIB | [67-71] |
| Phenytoin | Antiepileptic drug | Sodium channel blockade | Oral | IIB | [72-75] |
| Umbilical cord serum | | Neurotrophin replacement | Topical, implant | IV | [76-79] |
| Mesenchymal stem cells | | Neurotrophin replacement | Retrobulbar, subtenon, intravenous, intravitreal and intraocular injections | IV | [80-83] |

Abbreviations: N-methyl-D-aspartate (NMDA), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF)

*OCEBM Levels of Evidence Working Group. "The Oxford Levels of Evidence 2".

Oxford Centre for Evidence-Based Medicine. https://www.cebm.net/index.aspx?o=5653

## 2. Neurotropic Factors – BDNF, NGF, and CNTF

Neurotrophic factors have been shown to play a vital role in experimental models of glaucoma by supporting the growth [6], survival and repair of neurons, and inhibiting RGC apoptosis [13]. In comparison to NGF, more evidence supports the efficacy of BDNF for RGC survival [13]. In preclinical studies, upregulation of brain-derived neurotrophic factor (BDNF) using gene therapy was shown to be neuroprotective of RGCs in rat ocular hypertension models [11, 14, 15]. A similar effect was also seen using topical nerve growth factor (NGF) [16-18]. Over-expression of CNTF in an experimental rat model showed a significant protective effect on RGCs [19].

In human studies, Oddone et al. reported a case-control study showing reduced levels of BDNF and NGF in early and moderate, but not advanced, glaucoma patients [20], with potential for BDNF and NGF to be early biomarkers of glaucoma [20]. In a trial of 3 patients with advanced glaucoma, topical NGF has been shown to improve visual function on pattern electroretinography (PERG) and visual evoked potentials [18]. In addition, Neurotech Pharmaceuticals (Cumberland, Rhode Island, USA) have developed an implant (NT-501) which consists of encapsulated human cells genetically modified to secrete ciliary neurotrophic factor (CNTF). Phase I clinical trials for NT-501 in glaucoma patients demonstrated no serious adverse events (NCT01408472) and phase II clinical trials are currently in progress (NCT02862938)[4].

## 3. Antioxidants- Gingko Biloba Extract, Coenzyme Q10 and Vitamin E, and Omega 3

### *3.1 Gingko Biloba Extract*

Gingko biloba extract (GBE) is known to have antioxidant properties via stabilisation of mitochondria that have entered the apoptotic pathway [21], with neuroprotective actions shown in rat models of glaucoma [22]. A double-masked, randomized, placebo-controlled clinical trial (RCT) of 30 participants with normal tension glaucoma demonstrated that GBE can increase ocular blood flow as measured by Heidelberg retina flowmeter [23]. Lee et al. published a retrospective study reporting the reduction of visual field loss with 80 mg of GBE administered orally twice daily over 4 years [24]. In support, another retrospective study by Shim et al. showed improvement in visual fields in those taking 80mg GBE twice daily for at least 12 months [25]. A randomized controlled trial by Quaranta et al. also showed improvement of visual field parameters in those with visual field defects after 1 month of GBE treatment [26]. Whether these are truly regenerative effects, increases in retinal sensitivity, or a learning effect is unclear. However, GBE showed no effect on visual field or contrast sensitivity in patients with newly diagnosed normal tension glaucoma in another randomized clinical trial [27]. The effect of antioxidants on glaucoma progression has been studied in a phase III randomised trial (NCT01544192) comparing GBE, α−tocopherol and placebo, with results still to be reported [28].

### *3.2 Coenzyme Q10 With Vitamin E*

Topical coenzyme Q10 with Vitamin E has mitochondrial-targeted antioxidant properties. Topical coenzyme Q10 has been shown to reduce RGC loss and apoptosis compared to controls in a rat model of ocular hypertension [29]. A randomised clinical trial of 43 patients with primary open angle glaucoma demonstrated improvements in electrophysiological measurements of inner retinal

function after 12 months of coenzyme Q10 with Vitamin E treatment (as is commercially available) [30]. A randomised double masked placebo-controlled clinical trial with topical coenzyme Q10 and vitamin E administered to participants with primary open angle glaucoma is currently in progress (NCT03611530)[31].

### *3.3 Omega 3*

Dietary omega 3 is an antioxidant supplement, with pre-clinical studies having demonstrated neuroprotective properties [32] and increases in aqueous outflow leading to a lowering of IOP [33]. The majority of studies regarding dietary omega 3 are observational and suggest that a higher glaucoma risk is associated with a lack of dietary omega 3 [34, 35]. A pooled analysis from two double-masked, placebo-controlled randomized trials has shown a reduction of IOP in normotensive adults taking oral omega 3 for three months [36]. In a randomised placebo controlled study in patients with controlled IOP (less than 21mmHg) by Garcia-Medina et al., oral omega 3 was not shown to be beneficial in glaucoma progression [37], with no significant difference in visual field or structural measurements after a follow-up period of two years [37].

## 4. Glutamate-Induced Excitotoxicity

### *4.1 Memantine*

Memantine is a non-competitive glutamatergic NMDA (N-methyl-D-aspartate) receptor antagonist thought to improve cognitive function in moderate to severe Alzheimer's disease in combination with either acetylcholinesterase inhibitors or vitamin D [38]. When applied in animal models of glaucoma, memantine produced promising results in the reduction of RGC apoptosis rates [39-43]. In order to provide a topically applied sustained-release preparation that is able to deliver therapeutic concentrations to the retina, a nanoparticle delivery system has been investigated in vitro and in vivo, thus avoiding systemic side effects [41]. The results of this demonstrated RGC protection in a rat ocular hypertension model.

Orally administered memantine was tested in two large-scale, phase III, multicentre, randomised, placebo-controlled trials over 4 years, conducted 1 year apart [44]. Glaucoma progression was measured with standard automated perimetry (SAP), frequency doubling technology (FDT), and stereoscopic optic disc photographs. There was no significant benefit of memantine over placebo in the progression of open angle glaucoma. Barriers to reliably detecting significant results in this trial have been proposed including: IOP lowering therapies were left to the discretion of investigators rather than by a strict protocol, the inclusion of a wide range of disease severity including significantly advanced disease, variable criteria for the definition of low tension glaucoma patients, exclusion of subjects as soon as they showed visual field progression.

### *4.2 Brimonidine*

Brimonidine is an alpha-2 adrenergic agonist, used topically in patients with glaucoma to lower IOP by reducing aqueous production and increasing uveoscleral outflow [45]. Brimonidine is thought to have a dual mechanism of action in glaucoma, as suggested by IOP-independent neuroprotective effects in experimental models of glaucoma [46]. It is proposed that this is accomplished via modulation of glutamate transporters and NMDA receptors [47, 48], and

promoting the non-amyloidogenic beta pathway [46]. In a small clinical study involving patients with POAG, those that were treated with brimonidine over the course of 1 year demonstrated reduced loss of retinal nerve fibre layer thickness (independent of IOP reduction) compared to patients treated with timolol [49]. In another study comparing brimonidine with timolol, brimonidine appeared to improve contrast sensitivity after 3 months of treatment, a finding which was also independent of IOP reduction [50]. In a RCT comparing topical brimonidine to argon laser trabeculoplasty (ALT) in patients with POAG, brimonidine significantly reduced visual field deterioration over 18 months despite the IOP reduction having been less than that of ALT [51].

The Low-Pressure Glaucoma Treatment Study (LoGTS), showed that topical brimonidine twice daily preserved visual field to a greater extent than topical timolol in low tension glaucoma patients, despite similar IOP-lowering effects after a follow-up period of 4 years [52, 53]. Although this would support the neuroprotective actions of brimonidine, the high dropout in the brimonidine group (28.3%) compared to the timolol group (11.4%) must be considered. An attrition bias causing progressing patients in the brimonidine group to leave the study may otherwise explain the results. Alternatively, the neuroprotective effects of brimonidine may in some way have been related to those who did not have ocular allergy. Furthermore, the IOP target in this study was a reduction of ≥20% which was achieved by only 44% of brimonidine group and 39% of timolol group. This could suggest that the subjects were undertreated or had poor compliance with drops. Other factors in the interpretation of the results of this study include the possible adverse systemic hypotensive effects of timolol on optic nerve perfusion, and the differences in diurnal variation [54]. Clinical evidence still exists to refute these findings, albeit with similar issues surrounding the withdrawal rates and also low prevalence of visual field progression [55].

## 5. Citicoline

Citicoline is an endogenous compound that is thought to be protective of damaged neurons by altering non-glutamatergic neurotransmitter synthesis. It is thought to accomplish this via influencing the synthesis of phosphatidylcholine, a cell membrane phospholipid [56]. Intraperitoneal administration of citicoline was shown to increase retinal dopamine concentrations in rabbits [57]. Citicoline was also demonstrated to decrease neuron damage in a rat optic nerve crush model [58] and model of kainic acid-induced retinal damage [56]. In the same model, a reduction in concentration of apoptotic proteins such as mitogen-activated protein kinase was found [59]. Citicoline was shown to support regeneration of RGCs in a tissue culture of mouse retina [60]. In patients, intramuscular citicoline appeared to be beneficial for glaucoma, and visual field progression was shown to slow in 9 out of 11 patients following 6-monthly intramuscular citicoline administration over 10 years, compared to the control group. [61, 62] Oral administration of citicoline was also reported to improve visual evoked potentials in 13 out of 21 patients with glaucoma [63]. In a study of 41 patients with POAG, 2 years of oral citicoline was reported to decrease the rate of visual field loss [64]. Using pattern electroretinography (PERG), 16 patients with progressive open angle glaucoma showed RGC function improvement after treatment with topical citicoline three times daily for two months, but regressed after one month of stopping treatment [65]. Similarly, a randomized clinical trial showed topical citicoline could improve PERG and visual evoked potentials after being administered three times daily for 4 months but the effect stopped

two months later [66], suggesting a lack of permanent regenerative effects, or the need for continued citicoline treatment to prevent retinal functional deterioration.

## 6. Antiepileptics Drugs – Valproic Acid and Phenytoin

### *6.1 Valproic Acid*

Valproic acid, a commonly used antiepileptic and mood stabiliser, has been shown to improve RGC survival in cell culture and in rats with optic nerve crush injury, by stimulating BDNF-trkB(tropomyosin-related kinase B) signalling pathway and inhibiting histone deacetylase [67-69]. Preliminary clinical results for use in retinitis pigmentosa demonstrate some short-term benefits in terms of visual acuity and visual field preservation, however follow-up in the study was only 6 months in duration.[70]. Oral valproate has been reported to significantly improve visual acuity when taken for 3 months, and the effect lasted for 9 months after cessation in advanced glaucoma patients. However, in this unmasked randomized clinical trial, there was no improvement in visual field or electroretinography (ERG) [71]. Given this trial was unmasked to the patients, an interesting confounder in mood improvement may have otherwise explained the beneficial effects of valproic acid [71].

### *6.2 Phenytoin*

The anti-epileptic drug, phenytoin, has been shown to exhibit neuroprotective effects on RGC density and decrease axon loss in the optic nerve in a rat model of glaucoma. This neuroprotection is thought to be the result of sodium channel blockade [72]. In a study conducted in the 1970s, phenytoin showed neuroprotective effects on retinal degeneration in patients with glaucoma [73]. In that cohort of 17 patients, 11 showed improvement of visual fields after two months of treatment. Phenytoin has now been used in the treatment of optic neuritis in a phase II RCT, in which it demonstrated a reduction in the loss of retinal nerve fibre layer thickness [74]. In glaucoma, however, there have been fewer advancements using phenytoin. Notably, a cohort study of orally administered phenytoin in glaucoma patients in Israel has yet to publish their results (NCT00739154) [75].

## 7. Umbilical Cord Serum

Umbilical cord serum is known for its high content of neurotrophic growth factors [76]. Topical application of cord serum has been used for the treatment of severe ocular surface disease [77, 78]. A case report by Campos et al. described improvement in visual fields in two patients with primary open angle glaucoma after topical administration of cord serum over two months, however the improvement of their co-existing ocular surface disease may well have improved their visual field test performance. The same research group are currently carrying out a non-randomised clinical study with 4 month follow-up using topical cord serum in glaucoma patients (NCT03609125) [79].

## 8. Mesenchymal Stem Cells

Mesenchymal stem cells can proliferate and differentiate into glial cells and neurons, as well as secrete BDNF [80]. Intravitreal administration of mesenchymal stem cells has demonstrated an

increase in RGC survival in a rat model of glaucoma [81]. The use of mesenchymal stem cells for optic nerve and retinal conditions has been assessed by the Stem Cell Ophthalmology Treatment Study (SCOTS), an open-label non-randomized efficacy study (NCT01920867) [82]. Routes of administration include retrobulbar, subtenon, intravenous, intravitreal and intraocular injection with vitrectomy. Early reports have shown positive results for Leber's hereditary optic neuropathy and optic neuritis. The SCOTS study is currently enrolling participants with glaucoma. Presently, there are two phase I clinical trials using mesenchymal stem cells in glaucoma patients; one is being conducted in Brazil, in patients with advanced glaucoma (NCT02330978), and the other in Russia (NCT02144103)[83]. The outcomes are awaited.

## 9. Future of Neuroprotection

There are many barriers to successful translation of glaucoma treatment into clinical practice (Figure 2). The main factors amongst these are inherent in the delivery of the drug to the target tissue, and the biomarkers available to monitor therapeutic response.

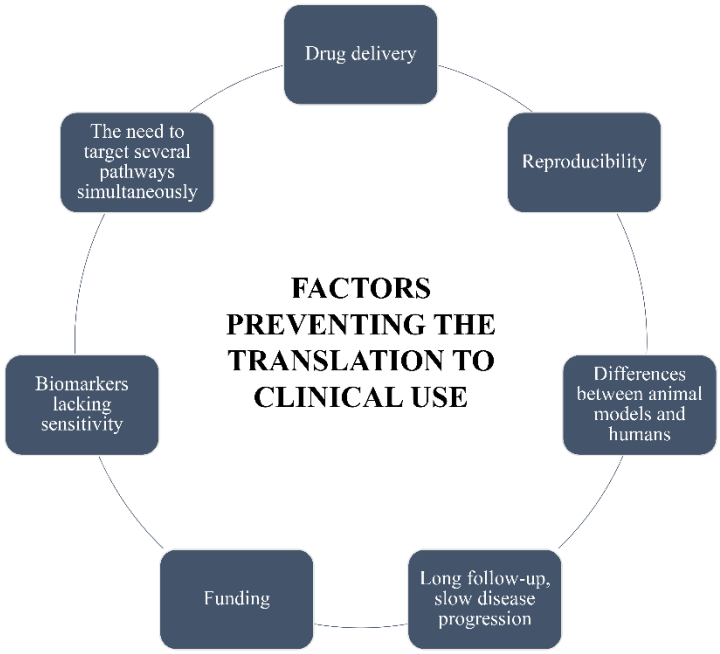


**Figure 2** Factors preventing the translation to clinical use.

Besides having a therapeutic effect, successful novel treatments require sufficient bioavailability with suitable dosing supporting compliance from the patient. This is being tackled by using novel formulations and routes of delivery, whilst also exploring new classes of treatment such as neurotrophic factors and stem cells. In terms of detecting therapeutic effect, other conditions such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and ageing itself also demonstrate retinal neurodegeneration and can confuse the clinical picture [84, 85]. Other confounders in glaucoma can also include lifestyle factors, ethnicity and systemic medications.

In line with ethical regulations, trials are carried out with patients already receiving IOP-lowering treatment, with the purpose of diminishing the remaining glaucoma progression below that achieved by IOP lowering treatment alone [86]. Of the clinical trials mentioned in the manuscript,

there is paucity of information on potential interactions of current or previous medications with the novel agents. It may be unlikely that novel agents will interact with traditional IOP lowering agents. It is thought that the traditional IOP lowering medications may also have non IOP lowering neuroprotective effects, such as in the case of the alpha agonists brimonidine which have neuroprotective features in addition to lowering IOP [52]. There are purported alternative neuroprotective mechanisms such as the anti-apoptotic effects of prostaglandin analogues and blood flow regulation of carbonic anhydrase inhibitors [87]. This may infer that novel agents targeting similar pathways would not bring additional benefit.

Since glaucoma is typically a slowly progressing disease, a long period of observation is often required to assess therapeutic efficacy in a clinical trial [88, 89]. Furthermore, damage to the optic nerve occurs before clinically measurable changes in visual fields, with up to 20-40% of RGCs are lost before visual field defects are detected [90]. However, strategies for assessing rates of progression of visual fields and OCT imaging are increasingly advocated to reduce the length of clinical trials [91-93]. Novel techniques such as DARC (Detection of Apoptosing Retinal Cells) have been developed as biomarkers [94] in the hope of more accurately quantifying drug efficacy by disease activity, especially in the early stages, and indeed has been utilised for this purpose experimentally, testing neuroprotective drugs including topical curcumin, topical Coenzyme Q10, glutamate modulation, and stem cell transplantation [27, 29, 43, 95-97]. With clinical trials showing promising results, [98, 99] it is hoped that DARC can be used as a surrogate endpoint in clinical trials for novel neuroprotective strategies.

## 10. Conclusions

Currently, there are a number of different neuroprotective strategies that have reached clinical trials but there are many and common limitations to evidence in this field of research. The difficulty in clinical translation is that many drugs, despite having positive results from preclinical data, failed to pass phase II clinical trials [6]. This may be attributed to the complex and as yet incompletely understood pathogenesis of disease, in addition to its slow progression requiring long term follow-up for clinically detectable changes to occur with current methodologies. Alternative trial designs for clinical trials may be beneficial to close the translation gap. An adaptive trial design increases flexibility and efficacy, providing the opportunity to modify the trial with prespecified criteria [99]. Another potential design is a futility trial design which tests the non-superiority of the intervention and removes unfavourable treatment with fewer subjects and in a shorter study period. It is important to adopt emerging highly sensitive biomarkers, have better visual field testing protocols, and have tight control for confounding factors. There may be an argument for treating at a much earlier disease stage when RGCs are believed to be less vulnerable and perhaps more amenable to neuroprotection [100]. Future clinical trials need to focus on the preservation of vision in patients. The detection of early glaucoma with novel biomarkers is the first stage in the future of diagnosis and treatment of glaucoma. Additionally, the development of tools enabling the objective measurement of neuroprotection outcomes is required. Hopefully, future researchers will take on board lessons learnt from previous trials' experiences to maximise the chances of providing new sight-saving therapies for our patients.

## Author Contributions

CF drafted and constructed the manuscript, carried out the literature review and summarization of studies. LG and DH validated the studies and provided the main revisions. TY contributed critical revision and editing of the manuscript. MFC conceptualized the paper, completed final revisions and oversaw the research.

## Competing Interests

Professor M. Francesca Cordeiro is a named inventor on a patent application covering the DARC technology disclosed in this manuscript. The remaining authors declare no conflict of interest.

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