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Discovery of molecular therapeutics for glaucoma: Challenges, successes, and promising directions

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Abstract

Glaucoma, a heterogeneous ocular disorder affecting ~60 million people worldwide, is characterized by painless neurodegeneration of retinal ganglion cells (RGCs), resulting in irreversible vision loss. Available therapies, which decrease the common causal risk factor of elevated intraocular pressure, delay, but cannot prevent, RGC death and blindness. Notably, it is changes in the anterior segment of the eye, particularly in the drainage of aqueous humor fluid, which are believed to bring about changes in pressure. Thus, it is primarily this region whose properties are manipulated in current and emerging therapies for glaucoma. Here, we focus on the challenges associated with developing treatments, review the available experimental methods to evaluate the therapeutic potential of new drugs, describe the development and evaluation of emerging Rho-kinase inhibitors and adenosine receptor ligands that offer the potential to improve aqueous humor outflow and protect RGCs simultaneously, and present new targets and approaches on the horizon.

Graphical Abstract

INTRODUCTION

Glaucoma: a brief background

Glaucoma, a group of neurodegenerative diseases of the optic nerve in the posterior eye, with varying etiologies, is a leading cause of irreversible blindness^{1, 2} and affects more than 60 million people worldwide.³ Glaucoma can be divided into two major subtypes, based on whether the iridocorneal angle (Figure 1) is closed (closed-angle glaucoma (CAG)), namely, in which the iris physically comes in contact with the trabecular meshwork (TM), or open

(open-angle glaucoma (OAG)). Primary open-angle glaucoma (POAG), the most common form overall, refers to presentation of glaucoma symptoms without attribution to other disease, injury, or closed iridocorneal angle.⁴ Secondary forms of glaucoma are those attributable to other causes; for example, secondary open angle glaucoma can be a result of pseudoexfoliation (PEX) syndrome⁵ or prolonged use of steroids.⁶ Glaucoma can be further divided based on age of onset into congenital, juvenile (JOAG, for those with onset at less than 35 years of age), and the more common, adult onset.

Patients with glaucoma are typically asymptomatic until vision loss, which is caused by damage to the nerve fiber layer surrounding retinal ganglion cells (RGCs) followed by RGC death.⁷ A clinically described feature referred to as ‘cupping’ differentiates glaucoma from other optic neuropathies involving RGC death.⁸ Namely, a change is apparent in the bundled optic nerve fibers at the center of the optic disc (i.e. the ‘cup’); the extent of cupping is used to monitor disease progression in high-risk patients before any changes in visual field are obvious.¹ A more readily monitored causal risk factor is elevated intraocular pressure (IOP), but it is not a necessary precondition for RGC death and vision loss in glaucoma, as in the case of a third glaucoma subtype, normal tension glaucoma (NTG).^{2, 9} As discussed in this Perspective, both for patients exhibiting elevated pressure as well as those with NTG, the standard of care is to slow loss of visual field by reduction of IOP.^{9–11} This approach has proven useful in reducing the loss of visual field over time, but IOP reduction alone does not halt disease progression.¹² As described below, IOP-reducing methods currently used in the clinic, as well as emerging therapies, are not developed *de novo* to target specific molecular underpinnings of glaucoma but rather are derived from other similar disorders, such as those of the cardiovascular system.

Efforts to improve our understanding of glaucoma pathogenesis, which remains unclear due to inherent disease heterogeneity and late age onset, involve identifying genetic susceptibility. The first, best-studied, and to date most strongly glaucoma-associated gene encodes for myocilin.¹³ Initially named the trabecular meshwork inducible glucocorticoid response (TIGR) gene due to its link with steroid-induced secondary glaucoma,¹⁴ numerous mutations in myocilin with autosomal dominant Mendelian inheritance patterns have since been linked with POAG and JOAG, in nearly every population and country throughout the world.^{15, 16} Other associated genes include those for (OPTN) and WDR36, which are seen in both inherited and sporadic forms of adult onset OAG.^{15–17} The Glu50Lys variant of OPTN is also associated with NTG.^{18, 19} Together, the known genetic variants of MYOC, OPTN, and WDR36 account for about 10% of glaucoma cases,^{15, 16} but relatives of all POAG patients are 20% more likely to develop glaucoma in their lifetime than controls, pointing to a wider genetic link.²⁰ Genome-wide association studies (GWAS), particularly the worldwide consortium with the acronym NEIGHBOR,²¹ continue to report susceptibility loci for glaucoma.^{22, 23} For example, a gene product that has received some attention among the over 20 genes identified by GWAS is CYP11B1, associated with primary congenital glaucoma as well as POAG.¹⁷ With the exception of myocilin discussed below, however, how these proteins contribute to pathogenesis remains yet unknown.

Relevant anatomical changes with age/glaucoma

Aqueous humor (AH), the fluid found in the anterior segment, supplies nutrients to nonvascular eye tissues such as the lens and cornea. The balance between AH production and outflow is intimately tied to IOP maintenance.²⁴ AH is produced in the non-pigmented epithelial (NPE) cells of the ciliary body, then flows to the anterior segment, and eventually drains through the (1) so-called *conventional pathway* via the trabecular meshwork (TM) to Schlemm's canal (SC) and finally into the episcleral veins (Figure 1), or via (2) the *uveoscleral pathway* through the ciliary body and iris root, which bypasses the TM and SC.²⁴ Active transport of chloride and sodium ions from the stroma to the AH, across both the pigmented epithelium and NPE of the ciliary body, is responsible for the majority of AH formation.²⁵ Control of ion movement across the ciliary epithelium is regulated at least in part by cyclic AMP (cAMP) modulation. Current and emerging therapeutic targets include those in signaling cascades that ultimately affect cAMP levels (see below), but studies to date do not yet converge on whether increased levels of cAMP increase²⁶ or decrease²⁷ AH formation.

AH outflow facility is altered in both normal aging and glaucoma.²⁸ In the healthy aging eye, IOP is maintained as both inflow and uveoscleral outflow tend to decrease; conventional outflow facility is unchanged.²⁹ By comparison, glaucoma-associated IOP increases are believed to be a result of reduced outflow predominantly through the conventional pathway, not increased AH formation or decreased outflow through the uveoscleral pathway.²⁸ Documented anatomical alterations include increased extracellular matrix (ECM) stiffness in the TM,³⁰ increased cell stiffness of the SC, decreased SC epithelial cell pore formation, a loss of TM cells,³¹ and increased sheath-derived plaque formation in the TM and SC.³² Nevertheless, the extent to which these are primary culprits for pathogenesis is complicated by the fact that changes to the TM and SC due to glaucoma versus the normal aging process are difficult to distinguish.³³

Model systems used to study glaucoma

A major impediment to developing new glaucoma therapeutics is the intrinsic heterogeneity of the disease. There is no established model system e.g. tissue or organ culture, animal model, or human postmortem tissue, to study the disease, develop, or systematically evaluate potential new treatments. Cell culture and organ models offer a controlled environment and are useful for initial screening of new drugs, but are derived from human postmortem tissues that vary widely³⁴. Often the medical history of a donor eye is not well documented and the eyes may be affected by undiagnosed disease, as well as long-term drug treatments for glaucoma and/or other disorders.

The use of cultures lacks a robust clinical translation such as that seen with use of animal models.³⁴ Yet, animal eyes, including those of birds, rodents, rabbits, dogs, cats and primates, vary greatly in their anatomical similarity to those of humans. Glaucoma in animal models may be induced by various methods including genetic mutation, treatment with steroids or photocoagulation, or in some species, occurs spontaneously.³⁵ Induced glaucoma offers quick, but non-natural, disease progression, while spontaneous glaucoma may take years for symptoms to appear.³⁵ Rat and mouse glaucoma models are the most commonly

used animals due to cost and access, but their eyes are not as similar to humans as other animal models and their small size can make testing difficult.^{34, 35} Primate eyes are the most similar to those of humans, but such animals are limited in availability and expensive to study.^{34, 36}

Standard experiments used to evaluate molecules of therapeutic potential for glaucoma

Typical drug discovery methods, namely high throughput screening of chemical libraries using biochemical or cell-based assays, cannot be used to detect reduction in IOP or changes in outflow resistance. Rather, IOP changes are typically assessed directly in animal models using rebound tonometry, a non-invasive method. Rebound tonometry uses a small probe that is rebounded off of the cornea, where the inverse of the deceleration speed of the probe correlates well with IOP.^{37, 38} Several measurements can be taken back-to-back or on different points of the cornea without harm. However, high variance is inherent in this measurement, and can be greater than the reductions in IOP afforded by treatment, thus complicating data interpretation when limited numbers of specimens or animals can be investigated.³⁹ To assess outflow facility, perfusion of mounted post-mortem eye tissues is commonly employed. In this experiment, solutions containing small molecules of interest are perfused through the sample, and changes in eye tissue with increased perfusion pressures are monitored.⁴⁰ Perfusion experiments are limited by availability of specimens, and variations in handling of these *ex vivo* samples will affect measurements.

TREATMENTS CURRENTLY USED IN THE CLINIC

Early detection is critical for preventing vision loss, as any loss of visual field due to retina degeneration is not reversible, but diagnosis is complicated by the fact that the age of onset and rate of glaucoma pathogenesis varies for different forms of the disease and varies widely among patients.⁴¹ Early signs of the disease can be missed if only IOP or variations in visual field are monitored, so ophthalmologists typically also look for changes or damage to the optic disk.¹ Once diagnosed, options to treat glaucoma fall into several classes all of which target lowering IOP as a method to slow disease progression.¹⁰ IOP is modulated by decreasing AH production or by increasing outflow. Even though drugs are generally applied topically to the eye, the five classes of current therapies (Table 1A) are not selective to eye tissues and were originally developed for other diseases. In addition, there are surgical options (Table 1B). Below we briefly summarize the five classes of currently marketed drugs and surgery used; for comprehensive descriptions and other clinical considerations, we refer the reader to a number of excellent review articles.^{42–45}

Cholinomimetics

The first class of drugs used to treat glaucoma, cholinergic agonists (also known as cholinomimetics, miotics, parasympathomimetics, acetylcholine receptor agonists), have been in use as glaucoma therapeutics since the 1870s.⁴⁶ Cholinergic agonists increase AH outflow through the conventional pathway by causing contraction of the ciliary body. Cholinergic agonists are divided into two classes, those that act directly by binding to muscarinic acetylcholine receptors in the ciliary body, or indirectly by inhibiting acetylcholinesterase.^{1, 47} Physostigmine, the first cholinergic agonist, was used during eye

surgery to constrict the pupil for over a decade before its IOP reducing effects were realized.⁴⁶ Pilocarpine, the most common⁴⁴ and oldest⁴⁶ cholinergic agonist glaucoma drug, is a nonselective muscarinic acetylcholine receptor inhibitor.⁴⁴ A third molecule, carbachol, works by both direct and indirect inhibition.⁴⁸

Adrenergic receptor agonists (sympathomimetics) and antagonists (β -blockers)

Adrenergic receptors are G-protein coupled receptors (GPCRs) that regulate the sympathetic nervous system. Epinephrine, the natural agonist of adrenergic receptors, was used as a glaucoma therapy in the 1950s.⁴⁶ Currently, the most commonly prescribed adrenergic receptor agonists are selectively targeted to the α_2 subclass (Table 1A),⁴⁹ and are sometimes coupled with a β -adrenergic receptor antagonist (next paragraph), which together are particularly effective for patients who do not tolerate prostaglandin analogs (see below). The α_2 -adrenergic receptors are involved in a cascade that maintains vascular constriction by activating G_i proteins, which inhibit adenylyl cyclase⁵⁰ and prevents protein kinase A (PKA) from phosphorylating myosin light chain (MLC) kinase, which remains active and prevents vascular relaxation.^{51, 52} Agonists of α_2 -adrenergic receptors are believed to decrease AH production by decreasing blood flow to the ciliary body,^{1, 52–54} and increase uveoscleral outflow.⁵⁵

The topical administration of β -adrenergic receptor antagonists, or β -blockers, which have been used to treat cardiovascular hypertension since the 1960s,^{56, 57} was a breakthrough in glaucoma treatment, since they significantly lowered IOP with fewer side effects than older glaucoma drugs. Before the introduction of prostaglandin analogs (see below), β -blockers were the most commonly prescribed treatment for glaucoma.^{58, 59} β -adrenergic receptors are found within the ciliary body⁶⁰ and early studies showed β -blocker propranolol decreased IOP in glaucoma patients,⁶¹ but its usefulness was limited due to side effects.⁴⁶ In the late 1970s, a topical timolol treatment became the first FDA approved β -blocker for the treatment of glaucoma.⁴⁶ Timolol decreases the formation of AH,^{62, 63} perhaps via the aforementioned mechanisms involving chloride transport in ciliary epithelium and cAMP,^{25, 64} or by cAMP-independent mechanisms,^{65, 66} but does not affect outflow facility.^{62, 63}

Carbonic Anhydrase Inhibitors

Carbonic anhydrase inhibitors (CAIs) are the best understood glaucoma therapeutic class on a molecular level. The production of bicarbonate by the Zn-dependent carbonic anhydrase enzyme is necessary for chloride transport, and thus AH formation (see above).²⁴ Inhibition of carbonic anhydrase decreases AH formation and in turn decreases IOP. The original CAIs were sulfonamides developed as diuretics and antimicrobials.⁴⁶ Current CAIs include the systemic acetazolamide and the topical dorzolamide.⁴⁸ While CAIs can offer decreases in IOP similar to β -blockers (see above), this effect is seen mostly with systemic treatment, which, with prolonged use, can cause side effects such as paresthesia of the hands and feet. Topical CAIs have fewer adverse effects, but are also less potent.⁵⁹

Prostaglandin Analogs

Prostaglandin analogs, the newest class of glaucoma drugs, have become the first line of treatment.⁶⁷ Naturally occurring prostaglandins bind a variety of cell surface receptors and are involved in mediating smooth muscle contraction and inflammation.⁶⁸ Since their discovery,⁶⁹ prostaglandins and their analogs have been studied as potential therapeutics in a range of diseases. For glaucoma, the finding that prostaglandin F2 α lowered IOP in monkeys bolstered the interest in further evaluating prostaglandins for therapeutic intervention.⁴⁶ Prostaglandin analogs have been shown to lower IOP mainly via increasing uveoscleral outflow,^{70, 71} along with conventional AH outflow.⁷² While the specific mechanism of action of increasing outflow remains unknown, studies indicate that prostaglandin analogs affect ECM remodeling^{73–75} and may relax the ciliary body.⁷⁶ Prostaglandin analogs have short half-lives and can be administered locally to the eye at low doses, which reduces the likelihood of occurrence of side effects.⁶⁷

Surgery and Implants

When drugs fail to lower IOP, more invasive measures such as laser treatment, surgery or shunt implant may be employed (Table 1B). Improvements in surgical techniques have lowered the risk of such procedures and are thus becoming more prevalent, including alongside drug treatments, for both CAG and POAG. Argon laser trabeculoplasty (ALT), or the newer, lower energy alternative, selective laser trabeculoplasty (SLT) mechanically increase outflow by damaging TM tissue, termed photocoagulation.⁷⁷ How photocoagulation of the TM leads to increased AH outflow is not understood, but the effect is thought to be due to either scarring of the TM or an increase in macrophages that clear debris via phagocytosis.^{78, 79} SLT is generally considered a safe treatment for IOP reduction as most complications are only present for a short time after the procedure; however, additional medication may need to be used and over time repeated treatment may be required to lower IOP to clinically acceptable levels.^{77, 78, 80} Trabeculectomy, also called glaucoma filtration surgery, is a third alternative. Due to a high 5-year failure rate⁸¹, and complications such as cataract, this surgery is reserved until other treatments have been unsuccessful.⁸² The surgery creates a fistula for AH to bypass the damaged TM, enabling increased outflow.⁷⁹ A shunt may be added after trabeculectomy to maintain the opening created by surgery.^{83, 84} Thus far, shunts appear to decrease the complications associated with trabeculectomy, but these devices are relatively new and lack long-term studies.⁸⁴ Finally, specific to CAG, a hole in the iris can be created either by a laser iridotomy⁸⁵ or surgical iridectomy,⁸⁶ to enable AH outflow despite the closed iridocorneal angle.

NEW THERAPIES IN LATE-STAGE DEVELOPMENT

New drugs under development attempt to leverage our increasingly better understanding of the role eye tissues, particularly the TM⁸⁷ and optic nerve head (ONH),⁸⁸ play in glaucoma pathogenesis. As described below, Rho-associated protein kinase (ROCK) inhibitors and adenosine receptor (AR) ligands modulate the pathways involved in both ECM and cytoskeletal restructuring. In addition to lowering of IOP, these molecules appear to increase ONH blood flow or protect against RGC loss.⁸⁹ The neuroprotective benefits of these molecules are exciting because they are orthogonal to IOP reduction, which, from a clinical

perspective, already has inexpensive and successful options (see prior section). The lack of clinical tools with sufficient sensitivity to unequivocally demonstrate neuroprotection is an impediment, however. Currently available retina/optic nerve imaging tools and visual field tests cannot detect the subtle changes that occur at a cellular level early in the disease process, when neuroprotection would be most impactful.⁹⁰ Delivery of neuroprotective agents to the posterior eye is also an important area of consideration (see also Outlook).

ROCK inhibitors

ROCKs are serine/threonine kinases that are downstream effectors of Rho GTPases. ROCKs phosphorylate substrates involved in regulation of F-actin stress fiber formation, focal adhesion,^{91, 92} as well as cell-cell adhesion and migration properties.^{93, 94} Downstream effects on actin cytoskeleton organization and smooth muscle contractility are relevant to many cellular processes, and dysregulation of the ROCK-Rho signaling system is associated with diseases of tissues throughout the body.^{95, 96} Known ROCK substrates are numerous and include MLC kinase, Lin11, Isl1 and Mec3 Kinase (LIM Kinase), and adducin, among others.⁹³ ROCKs are composed of an N-terminal kinase domain (Figure 2a), a coiled-coil domain with a Rho-binding site, and a pleckstrin homology (PH) domain.^{96, 97} Upon binding of GTP-activated Rho GTPase, the ROCK autoinhibitory complex between the N-terminal kinase domain and the C-terminal Rho-binding and PH domains is disrupted to enhance ROCK phosphorylation activity.⁹³ There are two ~160 kDa ROCK isozymes, ROCK1 and ROCK2, which share 92% sequence similarity in their kinase domain.^{97, 98} ROCK1, predominantly expressed in non-neuronal tissues such as the skeletal muscles, heart and lung, and ROCK2, primarily expressed in the brain, phosphorylate the same substrates, suggesting they have at least partially overlapping functions.⁹⁹ ROCK1 is required for stress fiber and focal adhesion formation, whereas ROCK2 is involved in phagocytosis and formation of the fibronectin fibrillar matrix.^{100, 101}

The ROCK-Rho signaling system is pertinent to AH outflow and glaucoma.^{102, 103} Both ROCK isoforms are expressed in tissues throughout the eye including in the TM, ciliary body, corneal epithelium and the retina.¹⁰⁴ The selective role of ROCK1 and ROCK2 in glaucoma remains unknown, however. While ROCK1 is highly expressed in the ciliary epithelium, ROCK2 is expressed in the retina,¹⁰⁴ and both ROCK1 and ROCK2 knockout mice have lower IOP than wild type mice.¹⁰⁵

Development of ROCK inhibitors to treat glaucoma—Modulation of ROCK is an active therapeutic direction to treat cancers,¹⁰⁶ cardiovascular disorders,⁹⁵ metabolic disease,¹⁰⁷ neurodegenerative disorders,⁹⁶ as well as glaucoma (Table 2). As such, considerable effort has been placed in developing selective ROCK inhibitors, and then testing them in potential therapeutic contexts; review articles detailing the extended history of the discovery, improvement, and validation of the ROCK kinases have been written.^{96, 108–111} Briefly, ROCK inhibitors predate the discovery of the ROCK enzyme and were derived from naphthalene-based calmodulin (CaM) antagonists, namely, isoquinoline, which serendipitously inhibits protein kinases at high concentrations.¹¹² The isoquinoline derivative **R-1** (HA-1077/fasudil), which has been used clinically in Japan as a vasodilator to prevent cerebral vasospasm,¹¹³ and the related **R-2** (K-115), have been tested in clinical

trials for glaucoma (see below). Like other kinase inhibitor targets, these molecules competitively bind in the ROCK adenosine triphosphate (ATP) binding pocket and thus selectivity of ROCK over other kinases is difficult. For example, **R-1** has been shown to inhibit protein kinase C-related protein kinase 2 (PRK2),¹¹⁴ cyclic GMP-dependent protein kinase G (PKG) and protein kinase C (PKC).¹¹¹ Structures of ROCK1 and ROCK2 in complex with **R-1** (Figure 2b) and other inhibitors have revealed nuances in pocket shape and physicochemical properties compared to other kinases,⁹⁷ leading to compounds with more potent inhibition and specificity than **R-1**,^{111, 115} including some that have been tested in glaucoma models¹¹⁶ and are in clinical trials (Table 2).¹¹⁷ Many other newer-generation isoquinoline derivatives with nanomolar IC₅₀ values, better selectivity for ROCK, and promising pharmacokinetics properties^{118–123} have yet to be tested for treating glaucoma, however. Pyridine is a second well-studied ROCK inhibitor scaffold and has likewise been subjected to extensive optimization for potency and selectivity.^{124–126} **R-3** (Y-27632), the first selective inhibitor for ROCKs¹²⁷ and derivatives such as **R-4** (Y-39983),¹²⁸ **R-5**,¹²⁶ **R-6**, and **R-7**¹²⁹ have been examined for effects on IOP. Newer scaffolds identified via high throughput screening, such as pyrazole, indazole, aminofurazans, 2,4-diaminopyrimidines, benzodioxanes, urea derivatives and benzothiophenes, have expanded the chemical space for ROCK inhibition.^{108–110, 130, 131} Inhibitors based on these new scaffolds appear promising in other contexts, including overcoming issues of bioavailability,^{124, 132–139} which motivates their consideration for glaucoma. Thus far, treatment with **R-8** (SR-3677), a derivative of the pyrazole scaffold,^{109, 140, 141} improves outflow facility in perfused porcine eyes,¹⁴¹ **R-9**, a potent and selective ROCK2 inhibitor derived from additional structure-activity-relationship (SAR) studies of the urea scaffold¹³⁰, lowers IOP in a rat model; **R-10**, an SAR-based derivative of the benzothiophene scaffold, yielded a comparable reduction in IOP to **R-4**.¹³¹

Proposed IOP-lowering mechanisms (Figure 2c)—ROCK inhibitors have been tested in perfusion studies using porcine, bovine, and rabbit eyes, and *in vivo* in both ocular normotensive and hypertensive rabbit eyes.⁹⁴ These studies have repeatedly demonstrated that ROCK inhibitors decrease IOP by increasing AH drainage through the TM via cytoskeletal restructuring.^{94, 142, 143} For example, treatment of perfused bovine eyes with **R-3** leads to changes in cellular morphology of TM and SC cells, decreases the number of focal adhesions, increases permeability of SC cells,¹⁴⁴ and widens extracellular spaces in the TM of perfused eyes.¹⁴² Such changes have been attributed to a decrease in MLC phosphorylation upon ROCK inhibition.^{142, 145} Consistent with this model, MLC kinase inhibitors have also been shown to affect the actin cytoskeleton of cultured human TM cells and reduce IOP of rabbit eyes,¹⁴⁶ similar to the effects seen with ROCK inhibitors. In addition to inhibitors of MLC kinase, those targeted to LIM kinase have also been shown to reduce IOP.^{143, 147} ROCK activation of LIM kinase leads to inhibition of actin depolymerization by cofilin.¹⁴² Conversely, LIM kinase inhibition, either directly or through upstream inhibition of ROCK, appears to induce TM relaxation via depolymerization of actin filaments.¹⁴³ Thus, ROCK inhibitors have not only shown promise as glaucoma therapeutics, but studies of their downstream effects have opened up new potential IOP lowering targets, namely, MLC kinase and LIM kinase.

Other potential therapeutic benefits—Besides lowering IOP, ROCK inhibitors are active in the posterior eye. **R-1** increases ONH blood flow in rabbits by restoring induced blood flow impairment and related optic nerve damage.¹⁴⁸ Similar results were obtained for the **R-4** in restoring ONH blood flow, also in rabbits.¹⁴⁹ **R-1**, **R-3** and **R-4** have been further shown to protect RGCs from apoptosis. **R-1** protects the retina of rats from both ischemia/reperfusion induced damage¹⁵⁰ and N-methyl-D-aspartate (NMDA) induced excitotoxicity.¹⁵¹ In rats treated with **R-4**, the number of RGCs with regenerating axons was increased.¹⁴⁹ **R-3** protected RGCs from ischemia induced damage in rat eyes *ex vivo*¹⁵² and increased RGC regeneration after optic nerve crush both in rats and RGC culture.¹⁵³ Finally, **R-3** prevented postoperative scarring in a glaucoma surgery rabbit model by inhibiting collagen deposition and fibroproliferation, increasing positive surgery outcomes over controls.¹⁵⁴

Clinical Trials—With the consistent success of lowering IOP upon treatment in animal models and their neuroprotective effects, several ROCK inhibitors have entered clinical trials (clinicaltrials.gov).^{94, 109, 155} **R-2** (from Kowa Pharmaceuticals), is currently in Phase II trials as is the pyridine derivative **R-11** (AMA-0076, Amakem).¹⁵⁶ **R-4** (RKI-983 or SNJ-1656 from Senju and Novartis, respectively) was discontinued during Phase II. Aerie Pharmaceuticals has put forth several isoquinoline derivative ROCK inhibitors **R-12** (AR-12286), **R-13** (AR-13324), and **R-13** combined with the commonly prescribed IOP-reducing prostaglandin analog latanaprost (PG-324). At the time of writing this Perspective, Aerie Pharmaceuticals is recruiting subjects for Phase III trials for **R-13**.¹⁵⁷

Adenosine Receptor Ligands

Adenosine receptors (ARs), GPCRs that regulate adenylyl cyclase and thus affect the production of cAMP, are expressed in tissues throughout the eye including the ciliary body,¹⁵⁸ the TM,¹⁵⁹ SC,¹⁶⁰ and the retina¹⁵⁸ and have been implicated in maintenance of IOP.¹⁶¹ At the cellular level, AR classes A₁ and A₃ deactivate adenylyl cyclase via G_i proteins, and A_{2A} and A_{2B} activate adenylyl cyclase through G_s proteins.¹⁶² A₁AR and A₃AR activate phospholipase C (PLC), which is part of the mitogen-activated protein kinase (MAPK) and the extracellular signal related kinase (ERK1/2) pathway. A₁AR activation of ERK1/2 has been shown to lead to secretion of matrix metalloproteinases (MMPs) and accelerate ECM turnover.¹⁶³ A_{2A}AR and A_{2B}AR-mediated stimulation of cAMP production also modulates ERK1/2; however, this activates connective tissue growth factor (CTGF), increasing ECM deposition.¹⁶⁴ Thus, depending on the AR class, agonists or antagonists should elicit the desired effect of lowered IOP (Figure 3a).¹⁶⁵ For example, to promote IOP maintenance, enhancement of ECM turnover via an A₁AR agonist or a decrease in ECM deposition with an A_{2A}AR antagonist should lower IOP.

Development of AR ligands to treat glaucoma (Table 3)—**AR-1** (adenosine) was linked to heart rate modulation and arterial blood pressure more than 80 years ago¹⁶⁶ and soon after was used clinically to treat arrhythmia.¹⁶⁷ Ligands for particular AR classes have been developed for decades,¹⁶⁸ for a variety of diseases beyond cardiovascular, such as rheumatoid arthritis and Parkinson's disease,^{162, 169, 170} by taking advantage of their varying natural affinities for adenosine.¹⁶⁹ Because ARs are expressed throughout the body,

including in the brain, heart, lung and blood vessels,¹⁷¹ drug development for any particular disorder is fraught with significant side effects. Extensive SAR studies have been completed, guided by the available A_{2A}AR structures (Figure 3b), including those in complex with **AR-1** (PDB ID 2YDO),¹⁷² as well as synthetic agonists such as 5'-N-ethylcarboxamidoadenosine (**AR-2**, NECA, Figure 3b) (PDB ID 2YDV)¹⁷² and antagonists¹⁷³.

Most AR agonists shown to decrease IOP are derivatives of **AR-1** (Table 3, Figure 3c). In general, compounds with N⁶-cycloalkyl substitutions favor A₁AR (Figure 3c),^{155, 161, 168} and several including N⁶-cyclohexyladenosine (**AR-3**, CHA), (*R*)-N⁶-(2-phenyl-1-methylethyl) adenosine (**AR-4**, R-PIA), **AR-5** (INO8875), and N⁶-cyclopentyladenosine (**AR-6**, CPA) have been tested for their effect on IOP. A₃ARs are better targeted by N⁶-benzene or N⁶-substituted benzene ligands (Figure 3c), but to date, only **AR-7** (CF-101/IB-MECA) has shown promise for lowering IOP.^{155, 161, 169, 174, 175} Agonists selective for A_{2A}AR and A_{2B}AR are those typically modified at the C² position, and agonists selective for A_{2A}ARs are further substituted at the 5' C within the ribose portion of the scaffold (Figure 3c).^{169, 174, 176} A_{2A}AR agonists demonstrated to lower IOP include **AR-8** (CGS-21680), **AR-9** (ATL-313), **AR-10** (2-O-Ado) and **AR-11** (2-CN-Ado). The fewest number of described agonists are those selective for A_{2B}AR, which is less tolerant of substitutions at positions that confer selectivity to other ARs; to the best of our knowledge, available A_{2B}AR agonists have not been studied in the context of glaucoma.¹⁶⁵

AR antagonists shown to reduce IOP include one targeted to A_{2A}AR and several to A₃AR (Table 3). To date, A₁AR antagonists have shown little promise as IOP lowering drugs as they increase IOP in both rabbits and mice, and effects of A_{2B}AR antagonists in the context of IOP and glaucoma have not yet been reported.¹⁶⁵ A₁, A_{2A} and A_{2B}AR antagonists are largely derivatives of xanthine (Figure 3c), including the natural antagonist caffeine. A₃ARs have low affinity for caffeine and other xanthine derivatives, leading to a range of examined scaffolds, which vary in their core structure and include purine and pyridine derivatives, quinazoline derivatives, tricyclic xanthines, and nucleoside derivatives.^{177–180} A_{2A}AR antagonists include xanthine derivatives modified at C⁸ with styryl groups and nitrogen polyheterocyclic compounds,¹⁷⁹ such as **AR-13** (ZM241385), which reduces IOP in mice.¹⁶⁵ A₃AR antagonists tested for their effect on IOP include pyridine derivatives **AR-14** – **AR-16**, nucleoside derivatives **AR-17**–**AR-22**, and the tricyclic xanthine derivative **AR-23**.

Proposed IOP-lowering mechanisms (Figure 3a)—The molecular role(s) of the AR classes in IOP regulation is not well established, as there appears to be both issues of species specificity, as in the case of the A₂AR agonist **AR-8**,^{161, 181} as well as selectivity issues, as in the case of A₁AR agonists **AR-3** and **AR-4** respectively,¹⁶⁸ and is thus an important area for further investigation. Available evidence from studies conducted in bovine and monkey eyes indicates that A₁AR agonists lower IOP by increasing outflow^{182, 183} through the conventional pathway.¹⁸² At the molecular level, decreased outflow resistance from activation of A₁AR with agonists has been shown to result from enhanced signaling leading to elevated levels of relevant matrix metalloproteases 2 and 9 (MMP-2, MMP-9),^{163182, 184}

and thus ECM remodeling.^{185, 186} Consistent with this finding, outflow increase observed with A₁AR activation can be reversed by MMP inhibition.¹⁸² A₁AR,¹⁸⁷ A_{2A}AR, and A₃AR agonists have all also been shown to reduce TM cell volume,¹⁵⁹ which in turn, independently increases outflow facility.¹⁸⁸ A₃AR antagonists **AR-14**, **AR-15**, and **AR-16** prevent chloride release and reduce AH production in NPE cells in the ciliary body.^{189, 190} Decreased IOP has been documented in studies of mice after treatment with A₃AR antagonists as well as after A₃AR knockout,^{190, 191} but not all studies of A₃AR converge.¹⁶⁵ For example, in human clinical trials as a treatment for dry eye syndrome, the A₃AR agonist **AR-7**, i.e. not an antagonist, was found to lower IOP.¹⁹² It is suspected that chronic administration of **AR-7** in this study led to lower IOP via the documented “effect inversion” pharmacological mechanism seen for other AR ligands in other contexts,¹⁹³ or due to inadvertent cross-activation of A₁ARs.¹⁶⁹

Other potential therapeutic benefits—RGC death in glaucoma is associated with ATP-binding P2X7 purinergic receptors at the cell surface due to an influx of Ca²⁺^{194–197} and, more controversially, due to prolonged activation of glutamate-binding NMDA and non-NMDA receptors.^{198–200} Activation of A₁ARs and A₃ARs by adenosine can inhibit the effects of both glutamate- and ATP- induced Ca²⁺ influx to prevent RGC death.^{201–203} Treatment with adenosine has further been shown to increase ONH blood flow in healthy human eyes,²⁰⁴ and RGC loss has also been attributed to reduced ONH blood flow, as progression of the disease correlates with reduced ONH blood flow in humans^{205, 206} and in a rhesus monkey model of glaucoma.²⁰⁷ A₁ and A_{2A} subtypes were both specifically shown to be involved in ONH blood flow increase in rabbit eyes²⁰⁸ but involvement of A₃AR may also be relevant, as in one study, treatment of gerbils with A₃AR agonist **AR-7** improved post-ischemic neuronal blood flow.²⁰⁹ Finally, there may be a role for A_{2A}AR in neuroprotection by controlling inflammation, analogous to reports related to Alzheimer’s and Parkinson’s disease.²¹⁰

Clinical trials—As of the writing of this Perspective, selected compounds are in preclinical (**AR-17**)²¹¹, Phase I (**AR-9**)^{169, 174}, and Phase II (**AR-5**)¹⁵⁵, **AR-12**, and **AR-7**) trials (Table 3).^{155, 165}

SELECTED PROMISING NEW DIRECTIONS AND EMERGING TARGETS

Numerous targets are actively being pursued for IOP reduction with potential neuroprotective benefits.²¹² These include other targets to (a) increase ONH blood flow²¹³, (b) increase TM outflow via ECM remodeling or altering TM cell contractility, (c) increase uveoscleral outflow, and/or (d) decrease AH production. Here we highlight just a few examples of such targets, others are listed in Table 4.

Nerve Growth Factor (NGF)

NGF is an emerging treatment for glaucoma²¹⁴ based on its ability to prevent neural apoptosis, and its clinical evaluation in a number of non-ocular diseases including Alzheimer’s, Parkinson’s, diabetes, and HIV-related neuropathies.²¹⁵ NGF binds to the cell surface receptor tropomyosin kinase receptor A (TrKA) (Figure 4a), activating a signaling

cascade which includes ERK1/2 and, through subsequent upregulation of B-cell lymphoma 2 (Bcl-2), prevents apoptosis.^{216, 217} Treatment with exogenous NGF has been shown to protect RGCs from death in rats after optic nerve sectioning²¹⁸ and ischemic injury,²¹⁹ to reduce retinal damage due to increased IOP in rabbits,²²⁰ and to protect RGCs in a rat glaucoma model.²²¹ Interestingly, in a small human trial, three patients with advanced glaucoma treated with NGF-containing eye drops showed improvements in visual field and optic nerve function that lasted for several weeks after treatment, with minimal side effects.²²¹ Results from Phase I trials indicate NGF is safe with limited side effects with ocular administration,²²² and, as of the writing of this Perspective, Phase II trials are ongoing for NGF as a dry eye treatment.

Matrix Metalloproteases

Zinc-dependent matrix metalloproteases are involved in a wide range of functions including bone remodeling, blastocyst implantation, nerve growth, and their namesake, ECM remodeling.²²³ ECM remodeling, in particular, plays a role in maintaining IOP as MMPs 1, 2, 3, 9 and 14 are all upregulated under high IOP conditions,²²⁴ inhibition of MMPs increase outflow resistance,¹⁸⁶ and, as discussed earlier, increased activity of both MMP-2 (Figure 4b) and MMP-9 are involved in decreased outflow resistance in the TM.^{182, 183, 185, 186} Substrates of MMP-1, MMP-2 and MMP-9 include collagens, fibronectin and elastin; whereas MMP-1 is upregulated, MMP-2 and MMP-9 have been found to be reduced in POAG eyes.¹⁸⁵ In principle, increasing MMP activity in the TM could be useful as a glaucoma therapy to restore normal outflow through the TM by increasing turnover of the ECM, particularly in steroid induced glaucoma where MMPs are downregulated with prolonged steroid treatment.²²⁵ In a gene therapy-based study, induced MMP-1 expression successfully compensated for MMP-1 downregulation caused by steroid administration, and both prevented and reversed steroid-induced ocular hypertension in sheep.²²⁶ MMP-1 was secreted to the ECM, degraded collagen type 1 in the TM, and thereby increased outflow.²²⁵ There is also some evidence supporting a role for MMPs in neurogenesis,²²⁷ neurodegeneration,^{227, 228} death after neural injury,²²⁹ and repair,²²⁷ germane to reversing retina degeneration, but molecular details are still forthcoming. Thus, further studies are needed to determine the potential of modulating MMPs for glaucoma in both capacities of lowering IOP and neuroprotection.

Myocilin

Myocilin was the first gene linked with inherited forms of glaucoma¹³ and is also upregulated in steroid induced glaucoma.¹⁴ Missense mutations in myocilin account for ~5% of all POAG and ~10% of all JOAG cases^{4, 230}, and are clustered within its olfactomedin (myoc-OLF) domain.^{230, 231} Myocilin is expressed in anterior segment tissues including the TM,^{232, 233}. Recent studies have implicated myocilin in cell proliferation and survival through the ERK1/2 pathway, which involves the aforementioned MMPs,²³⁴ as well as in programmed RGC cell death.²³⁵ While interacting partner(s) of myocilin and precise biological function remains elusive, it is an attractive *de novo* therapeutic target for glaucoma patients with inherited mutations.

The prevailing mechanism proposed for myocilin pathogenesis caused by mutations within myoc-OLF is that of a gain of function, namely, instead of being secreted to the TM, mutant myocilins aggregate within the ER of TM cells, causing TM cell death. TM cells are highly phagocytotic and clear debris in surrounding extracellular tissue for proper AH filtration.²³⁶ Thus, TM cell death is thought to bring about dysregulation of outflow through the TM, leading to elevated IOP, and then glaucoma.⁴ Disease-causing myoc-OLF variants are less stable than wild type,²³⁷ and grow degradation-resistant amyloid-like fibrils *in vitro*, which have also been detected in cells.^{238, 239} Notably, the absence of myocilin, either due to the presence of a premature stop codon in humans, or due to its knock-out in mice, does not result in glaucoma,^{240, 241} suggesting that viable therapeutic strategies would prevent or alleviate aggregation and its toxic effects.

One avenue is to inhibit myocilin misfolding as a means to prevent aggregation and escape detection by ER-associated degradation (ERAD). The result would be the secretion of mutant myocilin, which could restore partial function or take advantage of TM cell phagocytosis for facile degradation. Early studies demonstrated that mutant myocilin secretion could be rescued by culturing TM cells at a lower temperature,^{242–244} presumably because aggregation rates are slowed at temperatures below the unfolding temperature of mutant myocilin. Thus far, attempts to rescue secretion using small molecules have included both chemical and pharmacological chaperone approaches. For the former, chemical chaperones trimethylamine-N-oxide (TMAO)²⁴⁵ and 4-phenylbutyrate (4PBA)²⁴⁶, which is approved to treat urea cycle disorders and is under consideration for other ER-stress inducing misfolding diseases like cystic fibrosis²⁴⁷, improved secretion of mutant myocilin in cell culture. Related, a series of osmolytes were shown to increase thermal stability of mutants to better than wild-type levels *in vitro*,^{237, 248} with simple compounds like sucrose exhibiting the most potent stability enhancement. The high concentrations of osmolytes required to enhance the stability and secretion of mutant myocilin limits their usefulness as therapeutics, however. With no known function for myocilin, there is no biochemical assay for the discovery of tailored molecules, so-called pharmacological chaperones, and secondary assays such as cell secretion and inhibition of aggregation are low-throughput, making their use as a primary screening method laborious. One high-throughput, target-independent stability assay was developed recently for myoc-OLF in which the protein is first slightly destabilized with denaturant, and potential ligand scaffolds are then identified by monitoring subsequent re-stabilization using a straightforward fluorescence read-out. From this assay, two compounds were found that restored wild type myoc-OLF thermal stability, inhibited amyloid formation *in vitro* and increased secretion in a cell model.²⁴⁹ The recent crystal structure of myoc-OLF (Figure 4c) should accelerate the discovery of new selective ligands with similar properties through *in silico* and rational drug design methods. To this end, several small molecules were found serendipitously to bind to the myoc-OLF surface in the crystal, suggesting the surface is druggable (Figure 4c).²³¹

An alternative method to prevent the intracellular build up of mutant myocilin is to manipulate ERAD-specific molecular chaperones, an approach also under consideration for a number of other diseases.²⁵⁰ For glaucoma, treatment of a Y437H-mutant myocilin mouse model with the aforementioned chemical chaperone 4PBA reduces IOP.²⁵¹ The targets of 4PBA are most likely chaperones, such as Hsc70,²⁵² Hsp70,²⁵³ and glucose regulated

protein 78 (Grp78),²⁵⁴ but not myocilin²⁴⁸. The lack of a clear molecular target for 4PBA, however, is a disadvantage for SAR studies to generate more potent and selective molecules. A more directed approach may be to target Grp94 (Figure 4d), which has been shown to recognize mutant myocilin in a tight interaction that abrogates retrotranslocation for degradation by the proteasome.²⁵⁵ Although the reasons for the unproductive interaction are not yet clear, Grp94 increases the rate of myoc-OLF aggregation and co-precipitates with myoc-OLF *in vitro*.²⁵⁶ In cell culture, if levels of Grp94 are reduced by treatment with RNAi, or activity is inhibited using molecules directed to the N-terminal ATP binding site (Figure 4d), mutant myocilin clearance is promoted by autophagy.²⁵⁵ Incubation of Grp94 with a more selective inhibitor than radamide (see Figure 4d) reestablishes baseline aggregation rates and prevents co-aggregation *in vitro*.²⁵⁶ From a medicinal chemistry perspective, this proteostasis approach is attractive for several reasons: (1) degradation of myocilin should not cause glaucoma (2) co-crystal structures of Grp94 with inhibitors are tractable, enabling a pathway for development of new molecules and SAR, (3) straightforward secondary assays to assess aggregation *in vitro* and in cells are accessible, and (4) myocilin-glaucoma mouse models are available for testing optimized compounds. Nevertheless, given that Grp94 levels are investigated in cancer^{257, 258} and are correlated with apoptosis,^{259, 260} this approach will require considerable work to prevent such activity and ensure selectivity over other similar chaperones.

OUTLOOK

Progress critical to the development of new glaucoma drugs will continue to require highly interdisciplinary teams composed of individuals spanning fields of expertise from physics to medicine, to biology, biochemistry, and engineering. New targets will emerge as our understanding of normal and pathological aspects of TM biomechanics and molecular details of the mechanisms regulating AH improves. Susceptibility genes found through GWAS and family studies worldwide will also continue to identify new potential targets and research directions. One area of growth for the field is to lower the barrier to testing potential glaucoma drugs with a high likelihood of translation to humans, for example, a three dimensional model of the TM in which changes correlate with IOP reduction;⁸⁷ until a broadly accepted and robust system is developed, testing molecules for IOP reduction will remain extremely low throughput, relying on perfusion studies or animal models. Besides small molecules, gene therapy with viral vectors and small interfering RNAs (siRNAs),^{261, 262} analogous to other diseases such as Alzheimer's and Parkinson's,²⁶³ are also in clinical trials. Facile and accurate methods to evaluate neuroprotection efficacy need to be developed. Finally, patient compliance is critical for therapeutic outcomes in the clinic. This issue is being addressed through advanced delivery methods such as implants, contact lenses, microneedles, and gels,^{264–267} which are being tested with current therapies. In the long term, such systems will offer localized, sustained, delivery of drugs, and better bioavailability in particular tissues, to improve the effectiveness of all classes of glaucoma therapies.

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ABBREVIATIONS

4PBA	4-phenylbutyrate
AH	aqueous humor
ALT	alternative laser trabeculoplasty
AR	adenosine receptor
Bcl-2	B-cell lymphoma 2
CAG	closed-angle glaucoma
CAI	carbonic anhydrase inhibitors
CaM	calmodulin
CTGF	connective tissue growth factor
ECM	extracellular matrix
ERAD	ER-associated degradation
ERK1/2	extracellular signal related kinase
GWAS	genome-wide association studies
IOP	intraocular pressure
JOAG	juvenile open-angle glaucoma
LIM Kinase	Lin11, Isl1 and Mec3 kinase
MLC	myosin light chain
myoc-OLF	myocilin olfactomedin domain
NGF	nerve growth factor
NPE	non-pigmented epithelial
NTG	normal tension glaucoma
OAG	open-angle glaucoma
ONH	optic nerve head
OPTN	optineurin

PEX	pseudoexfoliation
PH	pleckstrin homology
PKG	cyclic GMP-dependent protein kinase G
POAG	primary open-angle glaucoma
PRK2	protein kinase 2
RGC	retinal ganglion cell
ROCK	Rho-associated protein kinase
SAR	structure-activity-relationship
SC	Schlemm's canal
siRNAs	small interfering RNAs
SLT	selective laser trabeculoplasty
TGFβ	Transforming growth factor- β
TM	trabecular meshwork
TMAO	trimethylamine-N-oxide
TrKA	tropomyosin kinase receptor A

References

1. Alward WL. Medical management of glaucoma. *New Engl J Med*. 1998; 339:1298–1307. [PubMed: 9791148]
2. Casson RJ, Chidlow G, Wood JPM, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts. *Clin Experiment Ophthalmol*. 2012; 40:341–349. [PubMed: 22356435]
3. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006; 90:262–267. [PubMed: 16488940]
4. Kwon YH, Fingert JH, Kuehn MH, Alward WL. Primary open-angle glaucoma. *New Engl J Med*. 2009; 360:1113–1124. [PubMed: 19279343]
5. Schlötzer-Schrehardt U. Genetics and genomics of pseudoexfoliation syndrome/glaucoma. *Middle East Afr J Ophthalmol*. 2011; 18:30–36. [PubMed: 21572731]
6. Kersey JP, Broadway DC. Corticosteroid-induced glaucoma: a review of the literature. *Eye*. 2005; 20:407–416.
7. Vrabec JP, Levin LA. The neurobiology of cell death in glaucoma. *Eye*. 2007; 21:S11–S14. [PubMed: 18157171]
8. Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. *Br J Ophthalmol*. 2002; 86:238–242. [PubMed: 11815354]
9. Collaborative Normal-Tension Glaucoma Study Group. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. *Am J Ophthalmol*. 1998; 126:498–505. [PubMed: 9780094]
10. Schwartz K, Budenz D. Current management of glaucoma. *Curr Opin Ophthalmol*. 2004; 15:119–126. [PubMed: 15021223]

11. Collaborative Normal-Tension Glaucoma Study Group. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. *Am J Ophthalmol.* 1998; 126:487–497. [PubMed: 9780093]
12. Agis Investigators. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol.* 2000; 130:429–440. [PubMed: 11024415]
13. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC. Identification of a gene that causes primary open angle glaucoma. *Science.* 1997; 275:668–670. [PubMed: 9005853]
14. Polansky J, Fauss D, Chen P, Chen H, Lütjen-Drecoll E, Johnson D, Kurtz R, Ma ZD, Bloom E, Nguyen T. Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. *Ophthalmologica.* 1997; 211:126–139. [PubMed: 9176893]
15. Fan BJ, Wang DY, Lam DSC, Pang CP. Gene mapping for primary open angle glaucoma. *Clin Biochem.* 2006; 39:249–258. [PubMed: 16332362]
16. Allingham R, Liu Y, Rhee DJ. The genetics of primary open-angle glaucoma: a review. *Exp Eye Res.* 2009; 88:837–844. [PubMed: 19061886]
17. Vasilou V, Gonzalez FJ. Role of CYP1B1 in glaucoma. *Annu Rev Pharmacol Toxicol.* 2008; 48:333–358. [PubMed: 17914928]
18. Aung T, Rezaie T, Okada K, Viswanathan AC, Child AH, Brice G, Bhattacharya SS, Lehmann OJ, Sarfarazi M, Hitchings RA. Clinical features and course of patients with glaucoma with the E50K mutation in the optineurin gene. *Invest Ophthalmol Vis Sci.* 2005; 46:2816–2822. [PubMed: 16043855]
19. Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Héon E, Krupin T, Ritch R, Kreutzer D. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science.* 2002; 295:1077–1079. [PubMed: 11834836]
20. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma: population-based familial aggregation study. *Arch Ophthalmol.* 1998; 116:1640–1645. [PubMed: 9869795]
21. Wiggs JL, Hauser MA, Abdrabou W, Allingham RR, Budenz DL, DelBono E, Friedman DS, Kang JH, Gaasterland D, Gaasterland T. The NEIGHBOR consortium primary open-angle glaucoma genome-wide association study: rationale, study design, and clinical variables. *J Glaucoma.* 2013; 22:517–525. [PubMed: 22828004]
22. Thorleifsson G, Walters GB, Hewitt AW, Masson G, Helgason A, DeWan A, Sigurdsson A, Jonasdottir A, Gudjonsson SA, Magnusson KP. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet.* 2010; 42:906–909. [PubMed: 20835238]
23. Wiggs JL, Kang JH, Yaspan BL, Mirel DB, Laurie C, Crenshaw A, Brodeur W, Gogarten S, Olson LM, Abdrabou W. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma in caucasians from the United States. *Hum Mol Genet.* 2011; 20:4707–4713. [PubMed: 21873608]
24. Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. *Open Ophthalmol J.* 2010; 4:52–59. [PubMed: 21293732]
25. Do C, Civan M. Basis of chloride transport in ciliary epithelium. *J Membr Biol.* 2004; 200:1–13. [PubMed: 15386155]
26. Chen S, Inoue R, Inomata H, Ito Y. Role of cyclic AMP-induced Cl conductance in aqueous humor formation by the dog ciliary epithelium. *Br J Pharmacol.* 1994; 112:1137–1145. [PubMed: 7952875]
27. Do CW, Kong CW, To CH. cAMP inhibits transepithelial chloride secretion across bovine ciliary body/epithelium. *Invest Ophthalmol Vis Sci.* 2004; 45:3638–3643. [PubMed: 15452071]
28. Gabelt BAT, Kaufman PL. Changes in aqueous humor dynamics with age and glaucoma. *Prog Retin Eye Res.* 2005; 24:612–637. [PubMed: 15919228]
29. Toris CB, Yablonski ME, Wang YL, Camras CB. Aqueous humor dynamics in the aging human eye. *Am J Ophthalmol.* 1999; 127:407–412. [PubMed: 10218693]

30. Last JA, Pan T, Ding Y, Reilly CM, Keller K, Acott TS, Fautsch MP, Murphy CJ, Russell P. Elastic modulus determination of normal and glaucomatous human trabecular meshwork. *Invest Ophthalmol Vis Sci*. 2011; 52:2147–2152. [PubMed: 21220561]
31. Stamer WD, Braakman ST, Zhou EH, Ethier CR, Fredberg JJ, Overby DR, Johnson M. Biomechanics of Schlemm's canal endothelium and intraocular pressure reduction. *Prog Retin Eye Res*. 2015; 44:86–98. [PubMed: 25223880]
32. Tektas OY, Lutjen-Drecoll E. Structural changes of the trabecular meshwork in different kinds of glaucoma. *Exp Eye Res*. 2009; 88:769–775. [PubMed: 19114037]
33. Grossniklaus HE, Nickerson JM, Edelhauser HF, Bergman LA, Berglin L. Anatomic alterations in aging and age-related diseases of the eye. *Invest Ophthalmol Vis Sci*. 2013; 54:ORSF23–ORSF27. [PubMed: 24335063]
34. Weinreb RN, Lindsey JD. The importance of models in glaucoma research. *J Glaucoma*. 2005; 14:302–304. [PubMed: 15990612]
35. McNally S, O'Brien CJ. Drug discovery in glaucoma and the role of animal models. *Drug Discov Today Dis Models*. 2014; 10:e207–e214.
36. Bouhenni AR, Dunmire J, Sewell A, Edward DP. Animal models of glaucoma. *Biomed Res Int*. 2012; 2012 Article ID 692609.
37. Kontiola A. A new electromechanical method for measuring intraocular pressure. *Doc Ophthalmol*. 1997; 93:265–276.
38. Martinez-de-la-Casa JM, Garcia-Feijoo J, Castillo A, Garcia-Sanchez J. Reproducibility and clinical evaluation of rebound tonometry. *Invest Ophthalmol Vis Sci*. 2005; 46:4578–4580. [PubMed: 16303951]
39. Cervino A. Rebound tonometry: new opportunities and limitations of non-invasive determination of intraocular pressure. *Br J Ophthalmol*. 2006; 90:1444–1446. [PubMed: 17114589]
40. Hann CR, Vercnocke AJ, Bentley MD, Jorgensen SM, Fautsch MP. Anatomic changes in Schlemm's canal and collector channels in normal and primary open-angle glaucoma eyes using low and high perfusion pressures. *Invest Ophthalmol Vis Sci*. 2014; 55:5834–5841. [PubMed: 25139736]
41. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet*. 2004; 363:1711–1720. [PubMed: 15158634]
42. Bucolo C, Salomone S, Drago F, Reibaldi M, Longo A, Uva MG. Pharmacological management of ocular hypertension: current approaches and future prospective. *Curr Opin Pharm*. 2013; 13:50–55.
43. Beidoe G, Mousa SA. Current primary open-angle glaucoma treatments and future directions. *Clin Ophthalmol*. 2012; 6:1699–1707. [PubMed: 23118520]
44. Bagnis A, Papadia M, Scotto R, Traverso CE. Current and emerging medical therapies in the treatment of glaucoma. *Expert Opin Emerg Drugs*. 2011; 16:293–307. [PubMed: 21406029]
45. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014; 311:1901–1911. [PubMed: 24825645]
46. Realini T. A history of glaucoma pharmacology. *Optom Vis Sci*. 2011; 88:36–38. [PubMed: 21131876]
47. Wiederholt M, Schäfer R, Wagner U, Lepple-Wienhues A. Contractile response of the isolated trabecular meshwork and ciliary muscle to cholinergic and adrenergic agents. *Ger J Ophthalmol*. 1996; 5:146–153. [PubMed: 8803576]
48. Hoyng PJ, van Beek L. Pharmacological therapy for glaucoma. *Drugs*. 2000; 59:411–434. [PubMed: 10776828]
49. Arthur S, Cantor LB. Update on the role of alpha-agonists in glaucoma management. *Exp Eye Res*. 2011; 93:271–283. [PubMed: 21524649]
50. Remaury A, Larrouy D, Daviaud D, Rouot B, Paris H. Coupling of the alpha 2-adrenergic receptor to the inhibitory G-protein Gi and adenylate cyclase in HT29 cells. *Biochem J*. 1993; 292:283–288. [PubMed: 8099279]
51. Lim JJ, Liu YH, Khin ESW, Bian JS. Vasoconstrictive effect of hydrogen sulfide involves downregulation of cAMP in vascular smooth muscle cells. *Am J Physiol Cell Physiol*. 2008; 295:C1261–C1270. [PubMed: 18787076]

52. Bausher LP, Horio B. Regulation of cyclic AMP production in adult human ciliary processes. *Exp Eye Res.* 1995; 60:43–48. [PubMed: 7720804]
53. Sears ML, Neufeld AH. Editorial: Adrenergic modulation of the outflow of aqueous humor. *Invest Ophthalmol Vis Sci.* 1975; 14:83–86.
54. Reitsamer HA, Posey M, Kiel JW. Effects of a topical α_2 adrenergic agonist on ciliary blood flow and aqueous production in rabbits. *Exp Eye Res.* 2006; 82:405–415. [PubMed: 16198336]
55. Toris CB, Camras CB, Yablonski ME. Acute versus chronic effects of brimonidine on aqueous humor dynamics in ocular hypertensive patients. *Am J Ophthalmol.* 1999; 128:8–14. [PubMed: 10482088]
56. Baker JG, Hill SJ, Summers RJ. Evolution of β -blockers: from anti-anginal drugs to ligand-directed signalling. *Trends Pharmacol Sci.* 2011; 32:227–234. [PubMed: 21429598]
57. Prichard B, Gillam P. Use of propranolol (Inderal) in treatment of hypertension. *Br Med J.* 1964; 2:725–727. [PubMed: 14172036]
58. Katz I, Hubbard W, Getson A, Gould A. Intraocular pressure decrease in normal volunteers following timolol ophthalmic solution. *Invest Ophthalmol Vis Sci.* 1976; 15:489–492.
59. Marquis RE, Whitson JT. Management of glaucoma: focus on pharmacological therapy. *Drugs Aging.* 2005; 22:1–21.
60. Bromberg BB, Gregory DS, Sears ML. Beta-adrenergic receptors in ciliary processes of the rabbit. *Invest Ophthalmol Vis Sci.* 1980; 19:203–207. [PubMed: 6243269]
61. Phillips C, Howitt G, Rowlands D. Propranolol as ocular hypotensive agent. *Br J Ophthalmol.* 1967; 51:222–226. [PubMed: 6021164]
62. Coakes RL, Brubaker RF. The mechanism of timolol in lowering intraocular pressure. In the normal eye. *Arch Ophthalmol.* 1978; 96:2045–2048. [PubMed: 363105]
63. Zimmerman TJ, Harbin R, Pett M, Kaufman HE. Timolol and facility of outflow. *Invest Ophthalmol Vis Sci.* 1977; 16:623–624. [PubMed: 873723]
64. Gilbert SBWEM. Beta-adrenergic receptors, from their discovery and characterization through their manipulation to beneficial clinical application. *Cardiology.* 2012; 122:104–112. [PubMed: 22759389]
65. McLaughlin CW, Peart D, Purves RD, Carré DA, Peterson-Yantorno K, Mitchell CH, Macknight AD, Civan MM. Timolol may inhibit aqueous humor secretion by cAMP-independent action on ciliary epithelial cells. *Am J Physiol Cell Physiol.* 2001; 281:C865–C875. [PubMed: 11502564]
66. Neufeld AH. Experimental studies on the mechanism of action of timolol. *Surv Ophthalmol.* 1979; 23:363–370. [PubMed: 223248]
67. Cracknell KPB, Grierson I. Prostaglandin analogues in the anterior eye: Their pressure lowering action and side effects. *Exp Eye Res.* 2009; 88:786–791. [PubMed: 18930047]
68. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arterio Thromb Vasc Biol.* 2011; 31:986–1000.
69. Von Euler U. On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). *J Physiol.* 1936; 88:213–234. [PubMed: 16994817]
70. Nilsson SF, Drecoll E, Lütjen-Drecoll E, Toris CB, Krauss AHP, Kharlamb A, Nieves A, Guerra T, Woodward DF. The prostanoid EP2 receptor agonist butaprost increases uveoscleral outflow in the cynomolgus monkey. *Invest Ophthalmol Vis Sci.* 2006; 47:4042–4049. [PubMed: 16936121]
71. Weinreb RN, Toris CB, Gabelt BAT, Lindsey JD, Kaufman PL. Effects of prostaglandins on the aqueous humor outflow pathways. *Surv Ophthalmol.* 2002; 47(Supplement 1):S53–S64. [PubMed: 12204701]
72. Toris CB, Yablonski ME, Wang YL, Hayashi M. Prostaglandin A2 increases uveoscleral outflow and trabecular outflow facility in the cat. *Exp Eye Res.* 1995; 61:649–657. [PubMed: 8846836]
73. Hinz B, Rösch S, Ramer R, Tamm ER, Brune K. Latanoprost induces matrix metalloproteinase-1 expression in human nonpigmented ciliary epithelial cells through a cyclooxygenase-2-dependent mechanism. *FASEB J.* 2005; 19:1929–1931. [PubMed: 16076963]

74. Lindsey JD, Kashiwagi K, Kashiwagi F, Weinreb RN. Prostaglandins alter extracellular matrix adjacent to human ciliary muscle cells in vitro. *Invest Ophthalmol Vis Sci.* 1997; 38:2214–2223. [PubMed: 9344344]
75. Ocklind A. Effect of latanoprost on the extracellular matrix of the ciliary muscle. A study on cultured cells and tissue sections. *Exp Eye Res.* 1998; 67:179–191. [PubMed: 9733584]
76. Zhao X, Pearson KE, Stephan DA, Russell P. Effects of prostaglandin analogues on human ciliary muscle and trabecular meshwork cells. *Invest Ophthalmol Vis Sci.* 2003; 44:1945–1952. [PubMed: 12714628]
77. Melamed S, Simon GJB, Levkovitch-Verbin H. Selective laser trabeculoplasty as primary treatment for open-angle glaucoma: a prospective, nonrandomized pilot study. *Arch Ophthalmol.* 2003; 121:957–960. [PubMed: 12860797]
78. Damji KF, Shah KC, Rock WJ, Bains HS, Hodge WG. Selective laser trabeculoplasty vargon laser trabeculoplasty: a prospective randomised clinical trial. *Br J Ophthalmol.* 1999; 83:718–722. [PubMed: 10340983]
79. Johnson DH, Johnson M. How does nonpenetrating glaucoma surgery work? Aqueous outflow resistance and glaucoma surgery. *J Glaucoma.* 2001; 10:55–67. [PubMed: 11219641]
80. Ayala M, Chen E. Comparison of selective laser trabeculoplasty (SLT) in primary open angle glaucoma and pseudoexfoliation glaucoma. *Clin Ophthalmol.* 2011; 5:1469–1673. [PubMed: 22069348]
81. Gedde SJ, Schiffman JC, Feuer WJ, Herndon LW, Brandt JD, Budenz DL. Treatment outcomes in the tube versus trabeculectomy (TVT) study after five years of follow-up. *Am J Ophthalmol.* 2012; 153:789–803. e782. [PubMed: 22245458]
82. Edmunds B, Thompson J, Salmon J, Wormald R. The national survey of trabeculectomy. III. Early and late complications. *Eye.* 2002; 16:297–303. [PubMed: 12032721]
83. Spiegel D, Kobuch K. Trabecular meshwork bypass tube shunt: initial case series. *Br J Ophthalmol.* 2002; 86:1228–1231. [PubMed: 12386074]
84. Francis BA, Singh K, Lin SC, Hodapp E, Jampel HD, Samples JR, Smith SD. Novel glaucoma procedures: a report by the American Academy of Ophthalmology. *Ophthalmology.* 2011; 118:1466–1480. [PubMed: 21724045]
85. Nolan WP, Foster PJ, Devereux JG, Uranchimeg D, Johnson GJ, Baasanhu J. YAG laser iridotomy treatment for primary angle closure in east Asian eyes. *Br J Ophthalmol.* 2000; 84:1255–1259. [PubMed: 11049950]
86. Playfair TJ, Watson PG. Management of acute primary angle-closure glaucoma: a long-term follow-up of the results of peripheral iridectomy used as an initial procedure. *Br J Ophthalmol.* 1979; 63:17–22. [PubMed: 760771]
87. Dautriche CN, Xie Y, Sharfstein ST. Walking through trabecular meshwork biology: Toward engineering design of outflow physiology. *Biotechnol Adv.* 2014; 32:971–983. [PubMed: 24806891]
88. Yamamoto T, Kitazawa Y. Vascular pathogenesis of normal-tension glaucoma: a possible pathogenetic factor, other than intraocular pressure, of glaucomatous optic neuropathy. *Prog Retin Eye Res.* 1998; 17:127–143. [PubMed: 9537793]
89. Sena DF, Lindsley K. Neuroprotection for treatment of glaucoma in adults. *Cochrane Database Syst Rev.* 2013; 2:CD006539.
90. Chua B, Goldberg I. Neuroprotective agents in glaucoma therapy: recent developments and future directions. *Expert Rev Ophthalmol.* 2010; 5:627–636.
91. Leung T, Chen XQ, Manser E, Lim L. The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. *Mol Cell Biol.* 1996; 16:5313–5327. [PubMed: 8816443]
92. Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y, Kaibuchi K. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J Biol Chem.* 1996; 271:20246–20249. [PubMed: 8702756]
93. Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. *Nat Rev Mol Cell Biol.* 2003; 4:446–456. [PubMed: 12778124]

94. Wang SK, Chang RT. An emerging treatment option for glaucoma: Rho kinase inhibitors. *Clin Ophthalmol*. 2014; 8:883–890. [PubMed: 24872673]
95. Satoh K, Fukumoto Y, Shimokawa H. Rho-kinase: important new therapeutic target in cardiovascular diseases. *Am J Physiol Heart Circ Physiol*. 2011; 301:H287–H296. [PubMed: 21622831]
96. Mueller BK, Mack H, Teusch N. Rho kinase, a promising drug target for neurological disorders. *Nat Rev Drug Discov*. 2005; 4:387–398. [PubMed: 15864268]
97. Jacobs M, Hayakawa K, Swenson L, Bellon S, Fleming M, Taslimi P, Doran J. The structure of dimeric ROCK I reveals the mechanism for ligand selectivity. *J Biol Chem*. 2006; 281:260–268. [PubMed: 16249185]
98. Nakagawa O, Fujisawa K, Ishizaki T, Saito Y, Nakao K, Narumiya S. ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. *FEBS Lett*. 1996; 392:189–193. [PubMed: 8772201]
99. Hahmann C, Schroeter T. Rho-kinase inhibitors as therapeutics: from pan inhibition to isoform selectivity. *Cell Mol Life Sci*. 2010; 67:171–177. [PubMed: 19907920]
100. Yoneda A, Multhaupt HAB, Couchman JR. The Rho kinases I and II regulate different aspects of myosin II activity. *J Cell Biol*. 2005; 170:443–453. [PubMed: 16043513]
101. Yoneda A, Ushakov D, Multhaupt HA, Couchman JR. Fibronectin matrix assembly requires distinct contributions from Rho kinases I and-II. *Mol Biol Cell*. 2007; 18:66–75. [PubMed: 17065553]
102. Kjller L, Hall A. Signaling to Rho GTPases. *Exp Cell Res*. 1999; 253:166–179. [PubMed: 10579921]
103. Fukata Y, Kaibuchi K, Amano M. Rho–Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol Sci*. 2001; 22:32–39. [PubMed: 11165670]
104. Fukiage C, Mizutani K, Kawamoto Y, Azuma M, Shearer TR. Involvement of phosphorylation of myosin phosphatase by ROCK in trabecular meshwork and ciliary muscle contraction. *Biochem Biophys Res Commun*. 2001; 288:296–300. [PubMed: 11606042]
105. Whitlock NA, Harrison B, Mixon T, Yu XQ, Wilson A, Gerhardt B, Eberhart DE, Abuin A, Rice DS. Decreased intraocular pressure in mice following either pharmacological or genetic inhibition of ROCK. *J Ocul Pharmacol Ther*. 2009; 25:187–194. [PubMed: 19456252]
106. Aznar S, Fernndez-Valern P, Espina C, Lacal JC. Rho GTPases: potential candidates for anticancer therapy. *Cancer Lett*. 2004; 206:181–191. [PubMed: 15013523]
107. Noda K, Nakajima S, Godo S, Saito H, Ikeda S, Shimizu T, Enkhjargal B, Fukumoto Y, Tsukita S, Yamada T, Katagiri H, Shimokawa H. Rho-kinase inhibition ameliorates metabolic disorders through activation of AMPK pathway in mice. *PLoS ONE*. 2014; 9:e110446. [PubMed: 25365359]
108. Guan R, Xu X, Chen M, Hu H, Ge H, Wen S, Zhou S, Pi R. Advances in the studies of roles of Rho/Rho-kinase in diseases and the development of its inhibitors. *Eur J Med Chem*. 2013; 70:613–622. [PubMed: 24211637]
109. Pan P, Shen M, Yu H, Li Y, Li D, Hou T. Advances in the development of Rho-associated protein kinase (ROCK) inhibitors. *Drug Discov Today*. 2013; 18:1323–1333. [PubMed: 24076262]
110. LoGrasso PV, Feng Y. Rho kinase (ROCK) inhibitors and their application to inflammatory disorders. *Curr Top Med Chem*. 2009; 9:704–723. [PubMed: 19689376]
111. Tamura M, Nakao H, Yoshizaki H, Shiratsuchi M, Shigyo H, Yamada H, Ozawa T, Totsuka J, Hidaka H. Development of specific Rho-kinase inhibitors and their clinical application. *Biochim Biophys Acta*. 2005; 1754:245–252. [PubMed: 16213195]
112. Hidaka H, Inagaki M, Kawamoto S, Sasaki Y. Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide-dependent protein kinase and protein kinase C. *Biochemistry*. 1984; 23:5036–5041. [PubMed: 6238627]
113. Ono-Saito N, Niki I, Hidaka H. H-Series protein kinase inhibitors and potential clinical applications. *Pharmacol Ther*. 1999; 82:123–131. [PubMed: 10454191]
114. Davies S, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J*. 2000; 351:95–105. [PubMed: 10998351]

115. Shibuya M, Hirai S, Seto M, Satoh S-i, Ohtomo E. Group FISS. Effects of fasudil in acute ischemic stroke: results of a prospective placebo-controlled double-blind trial. *J Neurol Sci*. 2005; 238:31–39. [PubMed: 16005902]
116. Sumi K, Inoue Y, Nishio M, Naito Y, Hosoya T, Suzuki M, Hidaka H. IOP-lowering effect of isoquinoline-5-sulfonamide compounds in ocular normotensive monkeys. *Bioorg Med Chem Lett*. 2014; 24:831–834. [PubMed: 24412071]
117. Yamamoto K, Maruyama K, Himori N, Omodaka K, Yokoyama Y, Shiga Y, Morin R, Nakazawa T. The novel Rho kinase (ROCK) inhibitor K-115: A new candidate drug for neuroprotective treatment in glaucoma. *Invest Ophthalmol Vis Sci*. 2014; 55:7126–7136. [PubMed: 25277230]
118. Lavogina D, Kalind K, Bredihina J, Hurt M, Vaasa A, Kasari M, Enkvist E, Raidaru G, Uri A. Conjugates of 5-isoquinolinesulfonylamides and oligo-D-arginine possess high affinity and selectivity towards Rho kinase (ROCK). *Bioorg Med Chem Lett*. 2012; 22:3425–3430. [PubMed: 22521647]
119. Ray P, Wright J, Adam J, Bennett J, Boucharens S, Black D, Cook A, Brown AR, Epemolu O, Fletcher D, Haunso A, Huggett M, Jones P, Laats S, Lyons A, Mestres J, de Man J, Morphy R, Rankovic Z, Sherborne B, Sherry L, van Straten N, Westwood P, Zaman GZR. Fragment-based discovery of 6-substituted isoquinolin-1-amine based ROCK-I inhibitors. *Bioorg Med Chem Lett*. 2011; 21:97–101. [PubMed: 21145740]
120. Iwakubo M, Takami A, Okada Y, Kawata T, Tagami Y, Sato M, Sugiyama T, Fukushima K, Taya S, Amano M, Kaibuchi K, Iijima H. Design and synthesis of Rho kinase inhibitors (III). *Bioorg Med Chem*. 2007; 15:1022–1033. [PubMed: 17084087]
121. Wu F, Büttner FH, Chen R, Hickey E, Jakes S, Kaplita P, Kashem MA, Kerr S, Kugler S, Paw Z. Substituted 2H-isoquinolin-1-one as potent Rho-Kinase inhibitors. Part 1: Hit-to-lead account. *Bioorg Med Chem Lett*. 2010; 20:3235–3239. [PubMed: 20462760]
122. Ginn JD, Bosanac T, Chen R, Cywin C, Hickey E, Kashem M, Kerr S, Kugler S, Li X, Prokopowicz A. Substituted 2H-isoquinolin-1-ones as potent Rho-kinase inhibitors: part 2, optimization for blood pressure reduction in spontaneously hypertensive rats. *Bioorg Med Chem Lett*. 2010; 20:5153–5156. [PubMed: 20678931]
123. Bosanac T, Hickey ER, Ginn J, Kashem M, Kerr S, Kugler S, Li X, Olague A, Schlyer S, Young ER. Substituted 2H-isoquinolin-1-ones as potent Rho-kinase inhibitors: part 3, aryl substituted pyrrolidines. *Bioorg Med Chem Lett*. 2010; 20:3746–3749. [PubMed: 20471253]
124. Li R, Martin MP, Liu Y, Wang B, Patel RA, Zhu JY, Sun N, Pireddu R, Lawrence NJ, Li J, Haura EB, Sung SS, Guida WC, Schonbrunn E, Sehti SM. Fragment-based and structure-guided discovery and optimization of Rho kinase inhibitors. *J Med Chem*. 2012; 55:2474–2478. [PubMed: 22272748]
125. Ho KK, Beasley JR, Belanger L, Black D, Chan JH, Dunn D, Hu B, Klon A, Kultgen SG, Ohlmeyer M. Triazine and pyrimidine based ROCK inhibitors with efficacy in spontaneous hypertensive rat model. *Bioorg Med Chem Lett*. 2009; 19:6027–6031. [PubMed: 19800787]
126. Henderson AJ, Hadden M, Guo C, Douglas N, Decornez H, Hellberg MR, Rusinko A, McLaughlin M, Sharif N, Drace C. 2, 3-Diaminopyrazines as Rho kinase inhibitors. *Bioorg Med Chem Lett*. 2010; 20:1137–1140. [PubMed: 20022494]
127. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature*. 1997; 389:990–994. [PubMed: 9353125]
128. Tokushige H, Inatani M, Nemoto S, Sakaki H, Katayama K, Uehata M, Tanihara H. Effects of topical administration of Y-39983, a selective Rho-associated protein kinase inhibitor, on ocular tissues in rabbits and monkeys. *Invest Ophthalmol Vis Sci*. 2007; 48:3216–3222. [PubMed: 17591891]
129. Chen HH, Namil A, Severns B, Ward J, Kelly C, Drace C, McLaughlin MA, Yacoub S, Li B, Patil R. In vivo optimization of 2, 3-diaminopyrazine Rho kinase inhibitors for the treatment of glaucoma. *Bioorg Med Chem Lett*. 2014; 24:1875–1879. [PubMed: 24684843]
130. Yin Y, Cameron MD, Lin L, Khan S, Schröter T, Grant W, Pocas J, Chen YT, Schürer S, Pachori A. Discovery of potent and selective urea-based ROCK inhibitors and their effects on intraocular pressure in rats. *ACS Med Chem Lett*. 2010; 1:175–179. [PubMed: 24900192]

131. Davis RL, Kahraman M, Prins TJ, Beaver Y, Cook TG, Cramp J, Cayanan CS, Gardiner EMM, McLaughlin MA, Clark AF, Hellberg MR, Shiau AK, Noble SA, Borchardt AJ. Benzothiophene containing Rho kinase inhibitors: efficacy in an animal model of glaucoma. *Bioorg Med Chem Lett.* 2010; 20:3361–3366. [PubMed: 20434334]
132. Feng Y, Cameron MD, Frackowiak B, Griffin E, Lin L, Ruiz C, Schröter T, LoGrasso P. Structure–activity relationships, and drug metabolism and pharmacokinetic properties for indazole piperazine and indazole piperidine inhibitors of ROCK-II. *Bioorg Med Chem Lett.* 2007; 17:2355–2360. [PubMed: 17368019]
133. Goodman KB, Cui H, Dowdell SE, Gaitanopoulos DE, Ivy RL, Sehon CA, Stavenger RA, Wang GZ, Viet AQ, Xu W. Development of dihydropyridone indazole amides as selective Rho-kinase inhibitors. *J Med Chem.* 2007; 50:6–9. [PubMed: 17201405]
134. Sehon CA, Wang GZ, Viet AQ, Goodman KB, Dowdell SE, Elkins PA, Semus SF, Evans C, Jolivet LJ, Kirkpatrick RB. Potent, selective and orally bioavailable dihydropyrimidine inhibitors of Rho kinase (ROCK1) as potential therapeutic agents for cardiovascular diseases. *J Med Chem.* 2008; 51:6631–6634. [PubMed: 18842034]
135. Shaw D, Hollingworth G, Soldermann N, Sprague E, Schuler W, Vangrevelinghe E, Duggan N, Thomas M, Kosaka T, Waters N, van Eis MJ. Novel ROCK inhibitors for the treatment of pulmonary arterial hypertension. *Bioorg Med Chem Lett.* 2014; 24:4812–4817. [PubMed: 25248678]
136. Stavenger RA, Cui H, Dowdell SE, Franz RG, Gaitanopoulos DE, Goodman KB, Hilfiker MA, Ivy RL, Leber JD, Marino JP. Discovery of aminofurazan-azabenzimidazoles as inhibitors of Rho-kinase with high kinase selectivity and antihypertensive activity. *J Med Chem.* 2007; 50:2–5. [PubMed: 17201404]
137. Pireddu R, Forinash K, Sun NN, Martin MP, Sung SS, Alexander B, Zhu JY, Guida WC, Schönbrunn E, Sebt SM. Pyridylthiazole-based ureas as inhibitors of Rho-associated protein kinases. *Cancer Res.* 2012; 72:3904–3904.
138. Patel RA, Forinash KD, Pireddu R, Sun Y, Sun N, Martin MP, Schönbrunn E, Lawrence NJ, Sebt SM. RKI-1447 is a potent inhibitor of the Rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer. *Cancer Res.* 2012; 72:5025–5034. [PubMed: 22846914]
139. Schirok H, Kast R, Figueroa-Pérez S, Bennabi S, Gnoth MJ, Feurer A, Heckroth H, Thutewohl M, Paulsen H, Knorr A. Design and synthesis of potent and selective azaindole-based Rho kinase (ROCK) inhibitors. *ChemMedChem.* 2008; 3:1893–1904. [PubMed: 18973168]
140. Chen YT, Bannister TD, Weiser A, Griffin E, Lin L, Ruiz C, Cameron MD, Schürer S, Duckett D, Schröter T, LoGrasso P, Feng Y. Chroman-3-amides as potent Rho kinase inhibitors. *Bioorg Med Chem Lett.* 2008; 18:6406–6409. [PubMed: 18990570]
141. Feng Y, Yin Y, Weiser A, Griffin E, Cameron MD, Lin L, Ruiz C, Schürer SC, Inoue T, Rao PV. Discovery of substituted 4-(pyrazol-4-yl)-phenylbenzodioxane-2-carboxamides as potent and highly selective Rho kinase (ROCK-II) inhibitors. *J Med Chem.* 2008; 51:6642–6645. [PubMed: 18834107]
142. Rao PV, Deng PF, Kumar J, Epstein DL. Modulation of aqueous humor outflow facility by the Rho kinase–specific inhibitor Y-27632. *Invest Ophthalmol Vis Sci.* 2001; 42:1029–1037. [PubMed: 11274082]
143. Harrison BA, Almstead ZY, Burgoon H, Gardyan M, Goodwin NC, Healy J, Liu Y, Mabon R, Marinelli B, Samala L. Discovery and development of LX7101, a dual LIM-kinase and ROCK inhibitor for the treatment of glaucoma. *ACS Med Chem Lett.* 2014; 6:84–88. [PubMed: 25589936]
144. Lu Z, Overby DR, Scott PA, Freddo TF, Gong H. The mechanism of increasing outflow facility by Rho-kinase inhibition with Y-27632 in bovine eyes. *Exp Eye Res.* 2008; 86:271–281. [PubMed: 18155193]
145. Rao PV, Deng P, Sasaki Y, Epstein DL. Regulation of myosin light chain phosphorylation in the trabecular meshwork: role in aqueous humour outflow facility. *Exp Eye Res.* 2005; 80:197–206. [PubMed: 15670798]
146. Honjo M, Inatani M, Kido N, Sawamura T, Yue BYJT, Honda Y, Tanihara H. A myosin light chain kinase inhibitor, ML-9, lowers the intraocular pressure in rabbit eyes. *Exp Eye Res.* 2002; 75:135–142. [PubMed: 12137759]

147. Harrison BA, Whitlock NA, Voronkov MV, Almstead ZY, Gu K-j, Mabon R, Gardyan M, Hamman BD, Allen J, Gopinathan S. Novel class of LIM-kinase 2 inhibitors for the treatment of ocular hypertension and associated glaucoma. *J Med Chem.* 2009; 52:6515–6518. [PubMed: 19831390]
148. Sugiyama T, Shibata M, Kajiura S, Okuno T, Tonari M, Oku H, Ikeda T. Effects of fasudil, a Rho-associated protein kinase inhibitor, on optic nerve head blood flow in rabbits. *Invest Ophthalmol Vis Sci.* 2011; 52:64–69. [PubMed: 20720232]
149. Tokushige H, Waki M, Takayama Y, Tanihara H. Effects of Y-39983, a selective Rho-associated protein kinase inhibitor, on blood flow in optic nerve head in rabbits and axonal regeneration of retinal ganglion cells in rats. *Curr Eye Res.* 2011; 36:964–970. [PubMed: 21950703]
150. Song H, Gao D. Fasudil, a Rho-associated protein kinase inhibitor, attenuates retinal ischemia and reperfusion injury in rats. *Int J Mol Med.* 2011; 28:193–198. [PubMed: 21455561]
151. Kitaoka Y, Kitaoka Y, Kumai T, Lam TT, Kuribayashi K, Isenoumi K, Munemasa Y, Motoki M, Kobayashi S, Ueno S. Involvement of RhoA and possible neuroprotective effect of fasudil, a Rho kinase inhibitor, in NMDA-induced neurotoxicity in the rat retina. *Brain Res.* 2004; 1018:111–118. [PubMed: 15262212]
152. Hirata A, Inatani M, Inomata Y, Yonemura N, Kawaji T, Honjo M, Tanihara H. Y-27632, a Rho-associated protein kinase inhibitor, attenuates neuronal cell death after transient retinal ischemia. *Graefes Arch Clin Exp Ophthalmol.* 2008; 246:51–59. [PubMed: 17763865]
153. Lingor P, Tönges L, Pieper N, Bermel C, Barski E, Planchamp V, Bähr M. ROCK inhibition and CNTF interact on intrinsic signalling pathways and differentially regulate survival and regeneration in retinal ganglion cells. *Brain.* 2008; 131:250–263. [PubMed: 18063589]
154. Honjo M, Tanihara H, Kameda T, Kawaji T, Yoshimura N, Araie M. Potential role of Rho-associated protein kinase inhibitor Y-27632 in glaucoma filtration surgery. *Invest Ophthalmol Vis Sci.* 2007; 48:5549–5557. [PubMed: 18055804]
155. Chen J, Runyan SA, Robinson MR. Novel ocular antihypertensive compounds in clinical trials. *Clin Ophthalmol.* 2011; 5:667–677. [PubMed: 21629573]
156. Boland S, Defert O, Alen J, Bourin A, Castermans K, Kindt N, Boumans N, Panitti L, Van de Velde S, Stalmans I. 3-[2-(Aminomethyl)-5-[(pyridin-4-yl) carbamoyl] phenyl] benzoates as soft ROCK inhibitors. *Bioorg Med Chem Lett.* 2013; 23:6442–6446. [PubMed: 24119557]
157. deLong MA, Yingling J, Lin CW, Sherman B, Sturdivant J, Heintzelman G, Lathem C, van Haarlem T, Kopczynski C. Discovery and SAR of a class of ocularly-active compounds displaying a dual mechanism of activity for the treatment of glaucoma. ARVO Meeting Abstracts. *Invest Ophthalmol Vis Sci.* 2012; 53:3867.
158. Kvanta A, Seregard S, Sejersen S, Kull B, Fredholm BB. Localization of Adenosine Receptor Messenger RNAs in the Rat Eye. *Exp Eye Res.* 1997; 65:595–602. [PubMed: 9367639]
159. Fleischhauer J, Mitchell C, Stamer W, Karl M, Peterson-Yantorno K, Civan M. Common actions of adenosine receptor agonists in modulating human trabecular meshwork cell transport. *J Membr Biol.* 2003; 193:121–136. [PubMed: 12879160]
160. Karl MO, Fleischhauer JC, Stamer WD, Peterson-Yantorno K, Mitchell CH, Stone RA, Civan MM. Differential P1-purinergic modulation of human Schlemm's canal inner-wall cells. *Am J Physiol Cell Physiol.* 2005; 288:C784–C794. [PubMed: 15590899]
161. Avila MY, Stone RA, Civan MM. A1-, A2A- and A3-subtype adenosine receptors modulate intraocular pressure in the mouse. *Br J Pharmacol.* 2001; 134:241–245. [PubMed: 11564641]
162. Fredholm BB. Adenosine receptors as drug targets. *Exp Cell Res.* 2010; 316:1284–1288. [PubMed: 20153317]
163. Husain S, Shearer TW, Crosson CE. Mechanisms linking adenosine A1 receptors and extracellular signal-regulated kinase 1/2 activation in human trabecular meshwork cells. *J Pharmacol Exp Ther.* 2007; 320:258–265. [PubMed: 17015637]
164. Agarwal R, Agarwal P. Newer targets for modulation of intraocular pressure: focus on adenosine receptor signaling pathways. *Expert Opin Ther Targets.* 2014; 18:527–539. [PubMed: 24579961]
165. Zhong Y, Yang Z, Huang WC, Luo X. Adenosine, adenosine receptors and glaucoma: An updated overview. *Biochim Biophys Acta.* 2013; 1830:2882–2890. [PubMed: 23328492]

166. Drury A, Szent-Györgyi Av. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J Physiol.* 1929; 68:213–237. [PubMed: 16994064]
167. Chen JF, Eltzhig HK, Fredholm BB. Adenosine receptors as drug targets - what are the challenges? *Nat Rev Drug Discov.* 2013; 12:265–286. [PubMed: 23535933]
168. Jacobson KA, Van Galen PJ, Williams M. Adenosine receptors: pharmacology, structure-activity relationships, and therapeutic potential. *J Med Chem.* 1992; 35:407–422. [PubMed: 1738138]
169. Müller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochim Biophys Acta.* 2011; 1808:1290–1308. [PubMed: 21185259]
170. Haskó G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov.* 2008; 7:759–770. [PubMed: 18758473]
171. Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. Structure and function of adenosine receptors and their genes. *Naunyn-Schmiedeberg's Arch Pharmacol.* 2000; 362:364–374. [PubMed: 11111830]
172. Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AGW, Tate CG. Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. *Nature.* 2011; 474:521–525. [PubMed: 21593763]
173. Doré, Andrew S., Robertson, N., Errey, James C., Ng, I., Hollenstein, K., Tehan, B., Hurrell, E., Bennett, K., Congreve, M., Magnani, F., Tate, Christopher G., Weir, M., Marshall, Fiona H. Structure of the adenosine A2A receptor in complex with ZM241385 and the xanthines XAC and caffeine. *Structure.* 2011; 19:1283–1293. [PubMed: 21885291]
174. Yan L, Burbiel JC, Maaß A, Müller CE. Adenosine receptor agonists: from basic medicinal chemistry to clinical development. *Expert Opin Emerg Drugs.* 2003; 8:537–576. [PubMed: 14662005]
175. Zhang K, Zhang L, Weinreb RN. Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. *Nat Rev Drug Discov.* 2012; 11:541–559. [PubMed: 22699774]
176. Konno T, Murakami A, Uchibori T, Nagai A, Kogi K, Nakahata N. Involvement of adenosine A2a receptor in intraocular pressure decrease induced by 2-(1-octyn-1-yl) adenosine or 2-(6-cyano-1-hexyn-1-yl) adenosine. *J Pharmacol Sci.* 2005; 97:501–509. [PubMed: 15821340]
177. Daly J. Caffeine analogs: biomedical impact. *Cell Mol Life Sci.* 2007; 64:2153–2169. [PubMed: 17514358]
178. Okamura T, Kurogi Y, Hashimoto K, Sato S, Nishikawa H, Kiryu K, Nagao Y. Structure–activity relationships of adenosine A3 receptor ligands: new potential therapy for the treatment of glaucoma. *Bioorg Med Chem Lett.* 2004; 14:3775–3779. [PubMed: 15203160]
179. Li AH, Moro S, Melman N, Ji X-d, Jacobson KA. Structure-activity relationships and molecular modeling of 3, 5-diacyl-2, 4-dialkylpyridine derivatives as selective A3 adenosine receptor antagonists. *J Med Chem.* 1998; 41:3186–3201. [PubMed: 9703464]
180. Wang Z, Do CW, Avila MY, Peterson-Yantorno K, Stone RA, Gao ZG, Joshi B, Besada P, Jeong LS, Jacobson KA, Civan MM. Nucleoside-derived antagonists to A3 adenosine receptors lower mouse intraocular pressure and act across species. *Exp Eye Res.* 2010; 90:146–154. [PubMed: 19878673]
181. Crosson CE. Adenosine receptor activation modulates intraocular pressure in rabbits. *J Pharmacol Exp Ther.* 1995; 273:320–326. [PubMed: 7714784]
182. Crosson CE, Sloan CF, Yates PW. Modulation of conventional outflow facility by the adenosine A1 agonist N6-cyclohexyladenosine. *Invest Ophthalmol Vis Sci.* 2005; 46:3795–3799. [PubMed: 16186365]
183. Tian B, Gabelt BAT, Crosson CE, Kaufman PL. Effects of adenosine agonists on intraocular pressure and aqueous humor dynamics in cynomolgus monkeys. *Exp Eye Res.* 1997; 64:979–989. [PubMed: 9301479]
184. Shearer TW, Crosson CE. Adenosine A1 receptor modulation of MMP-2 secretion by trabecular meshwork cells. *Invest Ophthalmol Vis Sci.* 2002; 43:3016–3020. [PubMed: 12202524]
185. Keller KE, Aga M, Bradley JM, Kelley MJ, Acott TS. Extracellular matrix turnover and outflow resistance. *Exp Eye Res.* 2009; 88:676–682. [PubMed: 19087875]

186. Bradley J, Vranka J, Colvis CM, Conger DM, Alexander JP, Fisk AS, Samples JR, Acott TS. Effect of matrix metalloproteinases activity on outflow in perfused human organ culture. *Invest Ophthalmol Vis Sci*. 1998; 39:2649–2658. [PubMed: 9856774]
187. Karl MO, Peterson-Yantorno K, Civan MM. Cell-specific differential modulation of human trabecular meshwork cells by selective adenosine receptor agonists. *Exp Eye Res*. 2007; 84:126–134. [PubMed: 17070802]
188. Al-Aswad LA, Gong H, Lee D, O'Donnell ME, Brandt JD, Ryan WJ, Schroeder A, Erickson KA. Effects of Na-K-2Cl cotransport regulators on outflow facility in calf and human eyes in vitro. *Invest Ophthalmol Vis Sci*. 1999; 40:1695–1701. [PubMed: 10393038]
189. Mitchell CH, Peterson-Yantorno K, Carré DA, McGlinn AM, Coca-Prados M, Stone RA, Civan MM. A3 adenosine receptors regulate Cl channels of nonpigmented ciliary epithelial cells. *Am J Physiol Cell Physiol*. 1999; 276:C659–C666.
190. Yang H, Avila MY, Peterson-Yantorno K, Coca-Prados M, Stone RA, Jacobson KA, Civan MM. The cross-species A3 adenosine-receptor antagonist MRS 1292 inhibits adenosine-triggered human nonpigmented ciliary epithelial cell fluid release and reduces mouse intraocular pressure. *Curr Eye Res*. 2005; 30:747–754. [PubMed: 16146920]
191. Avila MY, Stone RA, Civan MM. Knockout of A3 adenosine receptors reduces mouse intraocular pressure. *Invest Ophthalmol Vis Sci*. 2002; 43:3021–3026. [PubMed: 12202525]
192. Avni I, Garzozzi HJ, Barequet IS, Segev F, Varssano D, Sartani G, Chetrit N, Bakshi E, Zadok D, Tomkins O. Treatment of dry eye syndrome with orally administered CF101: data from a phase 2 clinical trial. *Ophthalmology*. 2010; 117:1287–1293. [PubMed: 20304499]
193. Jacobson KA, von Lubitz DKJE, Daly JW, Fredholm BB. Adenosine receptor ligands: differences with acute versus chronic treatment. *Trends Pharmacol Sci*. 1996; 17:108–113. [PubMed: 8936347]
194. Mitchell CH, Lu W, Hu H, Zhang X, Reigada D, Zhang M. The P2X7 receptor in retinal ganglion cells: A neuronal model of pressure-induced damage and protection by a shifting purinergic balance. *Purinergic Signal*. 2008; 4:313–321. [PubMed: 18923921]
195. Zhang X, Zhang M, Laties AM, Mitchell CH. Stimulation of P2X7 receptors elevates Ca²⁺ and kills retinal ganglion cells. *Invest Ophthalmol Vis Sci*. 2005; 46:2183–2191. [PubMed: 15914640]
196. Kakurai K, Sugiyama T, Kurimoto T, Oku H, Ikeda T. Involvement of P2X7 receptors in retinal ganglion cell death after optic nerve crush injury in rats. *Neurosci Lett*. 2013; 534:237–241. [PubMed: 23262079]
197. Niyadurupola N, Sidaway P, Ma N, Rhodes JD, Broadway DC, Sanderson J. P2X7 receptor activation mediates retinal ganglion cell death in a human retina model of ischemic neurodegeneration. *Invest Ophthalmol Vis Sci*. 2013; 54:2163–2170. [PubMed: 23449724]
198. Ullian E, Barkis W, Chen S, Diamond J, Barres B. Invulnerability of retinal ganglion cells to NMDA excitotoxicity. *Mol Cell Neurosci*. 2004; 26:544–557. [PubMed: 15276156]
199. Sucher NJ, Lipton SA, Dreyer EB. Molecular basis of glutamate toxicity in retinal ganglion cells. *Vision Res*. 1997; 37:3483–3493. [PubMed: 9425525]
200. Vorwerk CK, Gorla MS, Dreyer EB. An experimental basis for implicating excitotoxicity in glaucomatous optic neuropathy. *Surv Ophthalmol*. 1999; 43:S142–S150. [PubMed: 10416757]
201. Hartwick AT, Lalonde MR, Barnes S, Baldrige WH. Adenosine A1-receptor modulation of glutamate-induced calcium influx in rat retinal ganglion cells. *Invest Ophthalmol Vis Sci*. 2004; 45:3740–3748. [PubMed: 15452085]
202. Zhang X, Zhang M, Laties AM, Mitchell CH. Balance of purines may determine life or death of retinal ganglion cells as A3 adenosine receptors prevent loss following P2X7 receptor stimulation. *J Neurochem*. 2006; 98:566–575. [PubMed: 16805847]
203. Zhang M, Hu H, Zhang X, Lu W, Lim J, Eysteinnsson T, Jacobson KA, Laties AM, Mitchell CH. The A3 adenosine receptor attenuates the calcium rise triggered by NMDA receptors in retinal ganglion cells. *Neurochem Int*. 2010; 56:35–41. [PubMed: 19723551]
204. Polska E, Ehrlich P, Luksch A, Fuchsjäger-Mayrl G, Schmetterer L. Effects of adenosine on intraocular pressure, optic nerve head blood flow, and choroidal blood flow in healthy humans. *Invest Ophthalmol Vis Sci*. 2003; 44:3110–3114. [PubMed: 12824258]

205. Sugiyama, T., Shibata, M., Kojima, S., Ikeda, T. Optic nerve head blood flow in glaucoma. In: Kubena, T., editor. *The Mystery of Glaucoma*. InTech; 2011. p. 207-218.
206. Flammer J, Orgül S. Optic nerve blood-flow abnormalities in glaucoma. *Prog Retin Eye Res*. 1998; 17:267–289. [PubMed: 9695795]
207. Wang L, Cull GA, Piper C, Burgoyne CF, Fortune B. Anterior and posterior optic nerve head blood flow in nonhuman primate experimental glaucoma model measured by laser speckle imaging technique and microsphere method. *Invest Ophthalmol Vis Sci*. 2012; 53:8303–8309. [PubMed: 23169886]
208. Hirao M, Oku H, Goto W, Sugiyama T, Kobayashi T, Ikeda T. Effects of adenosine on optic nerve head circulation in rabbits. *Exp Eye Res*. 2004; 79:729–735. [PubMed: 15500831]
209. Von Lubitz DK, Lin RCS, Popik P, Carter MF, Jacobson KA. Adenosine A3 receptor stimulation and cerebral ischemia. *Eur J Pharmacol*. 1994; 263:59–67. [PubMed: 7821362]
210. Santiago AR, Baptista FI, Santos PF, Cristóvão G, Ambrósio AF, Cunha RA, Gomes CA. Role of microglia adenosine A 2A receptors in retinal and brain neurodegenerative diseases. *Mediators Inflamm*. 2014; 2014 Article ID 465694.
211. Pal S, Choi WJ, Choe SA, Heller CL, Gao ZG, Chinn M, Jacobson KA, Hou X, Lee SK, Kim HO. Structure–activity relationships of truncated adenosine derivatives as highly potent and selective human A 3 adenosine receptor antagonists. *Bioorg Med Chem*. 2009; 17:3733–3738. [PubMed: 19375920]
212. Rocha-Sousa A, Rodrigues-Araújo J, Gouveia P, Barbosa-Breda J, Azevedo-Pinto S, Pereira-Silva P, Leite-Moreira A. New therapeutic targets for intraocular pressure lowering. *Int Sch Res Notices*. 2013; 2013 Article ID 261386.
213. Wentz SM, Kim NJ, Wang J, Amireskandari A, Siesky B, Harris A. Novel therapies for open-angle glaucoma. *F1000Prime Rep*. 2014; 6:102. [PubMed: 25580256]
214. Wang H, Wang R, Thrimawithana T, Little PJ, Xu J, Feng Z-P, Zheng W. The nerve growth factor signaling and its potential as therapeutic target for glaucoma. *Biomed Res Int*. 2014; 2014 Article ID 759473.
215. Aloe L, Rocco ML, Bianchi P, Manni L. Nerve growth factor: from the early discoveries to the potential clinical use. *J Transl Med*. 2012; 10:239–239. [PubMed: 23190582]
216. Kaplan DR, Miller FD. Signal transduction by the neutrophin receptors. *Curr Opin Cell Biol*. 1997; 9:213–221. [PubMed: 9069267]
217. Roberti G, Mantelli F, Macchi I, Massaro-Giordano M, Centofanti M. Nerve growth factor modulation of retinal ganglion cell physiology. *J Cell Physiol*. 2014; 229:1130–1133. [PubMed: 24501088]
218. Carmignoto G, Maffei L, Candeo P, Canella R, Comelli C. Effect of NGF on the survival of rat retinal ganglion cells following optic nerve section. *J Neurosci*. 1989; 9:1263–1272. [PubMed: 2467970]
219. Sivilia S, Giuliani A, Fernández M, Turba ME, Forni M, Massella A, De Sordi N, Giardino L, Calzà L. Intravitreal NGF administration counteracts retina degeneration after permanent carotid artery occlusion in rat. *BMC Neurosci*. 2009; 10:52. [PubMed: 19473529]
220. Lambiase A, Centofanti M, Micera A, Manni GL, Mattei E, De Gregorio A, de Feo G, Bucci MG, Aloe L. Nerve growth factor (NGF) reduces and NGF antibody exacerbates retinal damage induced in rabbit by experimental ocular hypertension. *Graefes Arch Clin Exp Ophthalmol*. 1997; 235:780–785. [PubMed: 9439971]
221. Lambiase A, Aloe L, Centofanti M, Parisi V, Mantelli F, Colafrancesco V, Manni GL, Bucci MG, Bonini S, Levi-Montalcini R. Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: implications for glaucoma. *Proc Natl Acad Sci U S A*. 2009; 106:13469–13474. [PubMed: 19805021]
222. Ferrari MP, Mantelli F, Sacchetti M, Antonangeli MI, Cattani F, D'Anniballe G, Sinigaglia F, Ruffini PA, Lambiase A. Safety and pharmacokinetics of escalating doses of human recombinant nerve growth factor eye drops in a double-masked, randomized clinical trial. *BioDrugs*. 2014; 28:275–283. [PubMed: 24327173]
223. Nagase H, Woessner JF. Matrix metalloproteinases. *J Biol Chem*. 1999; 274:21491–21494. [PubMed: 10419448]

224. De Groef L, Van Hove I, Dekeyser E, Stalmans I, Moons L. MMPs in the trabecular meshwork: promising targets for future glaucoma therapies? *Invest Ophthalmol Vis Sci.* 2013; 54:7756–7763. [PubMed: 24265206]
225. Spiga MG, Borrás T. Development of a gene therapy virus with a glucocorticoid-inducible MMP1 for the treatment of steroid glaucoma. *Invest Ophthalmol Vis Sci.* 2010; 51:3029–3041. [PubMed: 20089870]
226. Gerometta R, Spiga MG, Borrás T, Candia OA. Treatment of sheep steroid-induced ocular hypertension with a glucocorticoid-inducible MMP1 gene therapy virus. *Invest Ophthalmol Vis Sci.* 2010; 51:3042–3048. [PubMed: 20089869]
227. Rosenberg GA. Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. *Lancet Neurol.* 2009; 8:205–216. [PubMed: 19161911]
228. Yong VW. Metalloproteinases: mediators of pathology and regeneration in the CNS. *Nat Rev Neurosci.* 2005; 6:931–944. [PubMed: 16288297]
229. Chintala SK, Zhang X, Austin JS, Fini ME. Deficiency in matrix metalloproteinase gelatinase B (MMP-9) protects against retinal ganglion cell death after optic nerve ligation. *J Biol Chem.* 2002; 277:47461–47468. [PubMed: 12354772]
230. Fingert JH, Heon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, Kawase K, Hoh ST, Buys YM, Dickinson J, Hockey RR, Williams-Lyn D, Trope G, Kitazawa Y, Ritch R, Mackey DA, Alward WL, Sheffield VC, Stone EM. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. *Hum Mol Genet.* 1999; 8:899–905. [PubMed: 10196380]
231. Donegan RK, Hill SE, Freeman DM, Nguyen E, Orwig SD, Turnage KC, Lieberman RL. Structural basis for misfolding in myocilin-associated glaucoma. *Hum Mol Genet.* 2015; 24:2111–2124. [PubMed: 25524706]
232. Lutjen-Drecoll E, May CA, Polansky JR, Johnson DH, Bloemendal H, Nguyen TD. Localization of the stress proteins alpha B-crystallin and trabecular meshwork inducible glucocorticoid response protein in normal and glaucomatous trabecular meshwork. *Invest Ophthalmol Vis Sci.* 1998; 39:517–525. [PubMed: 9501861]
233. Takahashi H, Noda S, Mashima Y, Kubota R, Ohtake Y, Tanino T, Kudoh J, Minoshima S, Oguchi Y, Shimizu N. The myocilin (MYOC) gene expression in the human trabecular meshwork. *Curr Eye Res.* 2000; 20:81–84. [PubMed: 10617907]
234. Joe MK, Kwon HS, Cojocaru R, Tomarev SI. Myocilin regulates cell proliferation and survival. *J Biol Chem.* 2014; 289:10155–10167. [PubMed: 24563482]
235. Koch MA, Rosenhammer B, Koschade SE, Braunger BM, Volz C, Jägle H, Tamm ER. Myocilin modulates programmed cell death during retinal development. *Exp Eye Res.* 2014; 125:41–52. [PubMed: 24837143]
236. Buller C, Johnson DH, Tschumper RC. Human trabecular meshwork phagocytosis. Observations in an organ culture system. *Invest Ophthalmol Vis Sci.* 1990; 31:2156–2163. [PubMed: 2211012]
237. Burns JN, Turnage KC, Walker CA, Lieberman RL. The stability of myocilin olfactomedin domain variants provides new insight into glaucoma as a protein misfolding disorder. *Biochemistry.* 2011; 50:5824–5833. [PubMed: 21612213]
238. Orwig SD, Perry CW, Kim LY, Turnage KC, Zhang R, Vollrath D, Schmidt-Krey I, Lieberman RL. Amyloid fibril formation by the glaucoma-associated olfactomedin domain of myocilin. *J Mol Biol.* 2012; 421:242–255. [PubMed: 22197377]
239. Hill SE, Donegan RK, Lieberman RL. The glaucoma-associated olfactomedin domain of myocilin forms polymorphic fibrils that are constrained by partial unfolding and peptide sequence. *J Mol Biol.* 2014; 426:921–935. [PubMed: 24333014]
240. Lam DS, Leung YF, Chua JK, Baum L, Fan DS, Choy KW, Pang CP. Truncations in the TIGR gene in individuals with and without primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 2000; 41:1386–1391. [PubMed: 10798654]
241. Kim BS, Savinova OV, Reedy MV, Martin J, Lun Y, Gan L, Smith RS, Tomarev SI, John SW, Johnson RL. Targeted disruption of the myocilin gene (myoc) suggests that human glaucoma-causing mutations are gain of function. *Mol Cell Biol.* 2001; 21:7707–7713. [PubMed: 11604506]

242. Vollrath D, Liu Y. Temperature sensitive secretion of mutant myocilins. *Exp Eye Res.* 2006; 82:1030–1036. [PubMed: 16297911]
243. Gobeil S, Letartre L, Raymond V. Functional analysis of the glaucoma-causing TIGR/myocilin protein: integrity of amino-terminal coiled-coil regions and olfactomedin homology domain is essential for extracellular adhesion and secretion. *Exp Eye Res.* 2006; 82:1017–1029. [PubMed: 16466712]
244. Liu Y, Vollrath D. Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. *Hum Mol Genet.* 2004; 13:1193–1204. [PubMed: 15069026]
245. Jia LY, Gong B, Pang CP, Huang Y, Lam DSC, Wang N, Yam GHF. Correction of the disease phenotype of myocilin-causing glaucoma by a natural osmolyte. *Invest Ophthalmol Vis Sci.* 2009; 50:3743–3749. [PubMed: 19234343]
246. Yam GHF, Gaplovska-Kysela K, Zuber C, Roth J. Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis. *Invest Ophthalmol Vis Sci.* 2007; 48:1683–1690. [PubMed: 17389500]
247. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *science.* 2008; 319:916–919. [PubMed: 18276881]
248. Burns JN, Orwig SD, Harris JL, Watkins JD, Vollrath D, Lieberman RL. Rescue of glaucoma-causing mutant myocilin thermal stability by chemical chaperones. *ACS Chem Biol.* 2010; 5:477–487. [PubMed: 20334347]
249. Orwig SD, Chi PV, Du Y, Hill SE, Cavitt MA, Suntharalingam A, Turnage KC, Dickey CA, France S, Fu H, Lieberman RL. Ligands for glaucoma-associated myocilin discovered by a generic binding assay. *ACS Chem Biol.* 2013; 9:517–525. [PubMed: 24279319]
250. Guerriero CJ, Brodsky JL. The delicate balance between secreted protein folding and endoplasmic reticulum-associated degradation in human physiology. *Physiol Rev.* 2012; 92:537–576. [PubMed: 22535891]
251. Zode GS, Bugge KE, Mohan K, Grozdanic SD, Peters JC, Koehn DR, Anderson MG, Kardon RH, Stone EM, Sheffield VC. Topical ocular sodium 4-phenylbutyrate rescues glaucoma in a myocilin mouse model of primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 2012; 53:1557–1565. [PubMed: 22328638]
252. Rubenstein RC, Zeitlin PL. Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of F508-CFTR. *Am J Physiol Cell Physiol.* 2000; 278:C259–C267. [PubMed: 10666020]
253. Gong B, Zhang LY, Lam DSC, Pang CP, Yam GHF. Sodium 4-phenylbutyrate ameliorates the effects of cataract-causing mutant gammaD-crystallin in cultured cells. *Mol Vis.* 2010; 16:997–1003. [PubMed: 20577655]
254. Basseri S, Lhoták Š, Sharma AM, Austin RC. The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response. *J Lipid Res.* 2009; 50:2486–2501. [PubMed: 19461119]
255. Suntharalingam A, Abisambra JF, O’Leary JC, Koren J, Zhang B, Joe MK, Blair LJ, Hill SE, Jinwal UK, Cockman M. Glucose-regulated protein 94 triage of mutant myocilin through endoplasmic reticulum-associated degradation subverts a more efficient autophagic clearance mechanism. *J Biol Chem.* 2012; 287:40661–40669. [PubMed: 23035116]
256. Stothert AR, Suntharalingam A, Huard DJ, Fontaine SN, Crowley VM, Mishra S, Blagg BS, Lieberman RL, Dickey CA. Exploiting the interaction between Grp94 and aggregated myocilin to treat glaucoma. *Hum Mol Genet.* 2014; 23:6470–6480. [PubMed: 25027323]
257. Zhang X, Zhang L, Wang S, Wu D, Yang W. Decreased functional expression of Grp78 and Grp94 inhibits proliferation and attenuates apoptosis in a human gastric cancer cell line in vitro. *Oncol Lett.* 2015; 9:1181–1186. [PubMed: 25663878]
258. Wang Q, He Z, Zhang J, Wang Y, Wang T, Tong S, Wang L, Wang S, Chen Y. Overexpression of endoplasmic reticulum molecular chaperone GRP94 and GRP78 in human lung cancer tissues and its significance. *Cancer Detect Prev.* 2005; 29:544–551. [PubMed: 16297569]
259. Reddy RK, Lu J, Lee AS. The endoplasmic reticulum chaperone glycoprotein GRP94 with Ca²⁺-binding and antiapoptotic properties is a novel proteolytic target of calpain during etoposide-induced apoptosis. *J Biol Chem.* 1999; 274:28476–28483. [PubMed: 10497210]

260. Bando Y, Katayama T, Aleshin A, Manabe T, Tohyama M. GRP94 reduces cell death in SH-SY5Y cells perturbed calcium homeostasis. *Apoptosis*. 2004; 9:501–508. [PubMed: 15192333]
261. Borrás T. Advances in glaucoma treatment and management: gene therapy. *Invest Ophthalmol Vis Sci*. 2012; 53:2506–2510. [PubMed: 22562852]
262. Martínez T, González MV, Roehl I, Wright N, Pañeda C, Jiménez AI. In vitro and in vivo efficacy of SYL040012, a novel siRNA compound for treatment of glaucoma. *Mol Ther*. 2014; 22:81–91. [PubMed: 24025749]
263. Simonato M, Bennett J, Boulis NM, Castro MG, Fink DJ, Goins WF, Gray SJ, Lowenstein PR, Vandenberghe LH, Wilson TJ. Progress in gene therapy for neurological disorders. *Nat Rev Neurol*. 2013; 9:277–291. [PubMed: 23609618]
264. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: An overview. *World J Pharmacol*. 2013; 2:47–64. [PubMed: 25590022]
265. Guzman-Aranguez A, Colligris B, Pintor J. Contact lenses: promising devices for ocular drug delivery. *J Ocul Pharmacol Ther*. 2013; 29:189–199. [PubMed: 23215541]
266. Molokhia SA, Thomas SC, Garff KJ, Mandell KJ, Wirostko BM. Anterior eye segment drug delivery systems: current treatments and future challenges. *J Ocul Pharmacol Ther*. 2013; 29:92–105. [PubMed: 23485091]
267. Achouri D, Alhanout K, Piccerelle P, Andrieu V. Recent advances in ocular drug delivery. *Drug Dev Ind Pharm*. 2013; 39:1599–1617. [PubMed: 23153114]
268. Wehrman T, He X, Raab B, Dukipatti A, Blau H, Garcia KC. Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. *Neuron*. 2007; 53:25–38. [PubMed: 17196528]
269. Morgunova E, Tuuttila A, Bergmann U, Isupov M, Lindqvist Y, Schneider G, Tryggvason K. Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed. *Science*. 1999; 284:1667–1670. [PubMed: 10356396]
270. Dollins DE, Warren JJ, Immormino RM, Gewirth DT. Structures of GRP94-nucleotide complexes reveal mechanistic differences between the hsp90 chaperones. *Mol Cell*. 28:41–56.
271. Immormino RM, Metzger LE IV, Reardon PN, Dollins DE, Blagg BJS, Gewirth DT. Different poses for ligand and chaperone in inhibitor-bound Hsp90 and GRP94: implications for paralog-specific drug design. *J Mol Biol*. 2009; 388:1033–1042. [PubMed: 19361515]
272. Garnock-Jones KP. Ripasudil: first global approval. *Drugs*. 2014; 74:2211–2215. [PubMed: 25414122]
273. Fredholm BB, Irenius E, Kull B, Schulte G. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells1. *Biochem Pharmacol*. 2001; 61:443–448. [PubMed: 11226378]
274. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov*. 2006; 5:247–264. [PubMed: 16518376]
275. Cunha RA, Constantino M, Sebastião A, Johansson B, Fredholm BB. Evidence for high-affinity binding sites for the adenosine A2A receptor agonist [3H] CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal A2A receptors. *Naunyn-Schmiedeberg Arch Pharmacol*. 1996; 353:261–271. [PubMed: 8692280]
276. Mathôt R, Wenden E, Soudijn W, IJzerman A, Danhof M. Deoxyribose analogues of N6-cyclopentyladenosine (CPA): partial agonists at the adenosine A1 receptor in vivo. *Br J Pharmacol*. 1995; 116:1957–1964. [PubMed: 8640332]
277. Day YJ, Li Y, Rieger JM, Ramos SI, Okusa MD, Linden J. A2A adenosine receptors on bone marrow-derived cells protect liver from ischemia-reperfusion injury. *J Immunol*. 2005; 174:5040–5046. [PubMed: 15814735]
278. Jacobson KA, Park KS, Jiang JL, Kim YC, Olah ME, Stiles GL, Ji XD. Pharmacological characterization of novel A(3) adenosine receptor-selective antagonists. *Neuropharmacology*. 1997; 36:1157–1165. [PubMed: 9364471]
279. Jeong LS, Choe SA, Gunaga P, Kim HO, Lee HW, Lee SK, Tosh DK, Patel A, Palaniappan KK, Gao ZG, Jacobson KA, Moon HR. Discovery of a new nucleoside template for human A3 adenosine receptor ligands: d-4'-thioadenosine derivatives without 4'-hydroxymethyl group as highly potent and selective antagonists. *J Med Chem*. 2007; 50:3159–3162. [PubMed: 17555308]

280. Comes N, Buie LK, Borrás T. Evidence for a role of angiopoietin-like 7 (ANGPTL7) in extracellular matrix formation of the human trabecular meshwork: implications for glaucoma. *Genes Cells*. 2011; 16:243–259. [PubMed: 21199193]
281. Goel M, Sienkiewicz AE, Picciani R, Wang J, Lee RK, Bhattacharya SK. Cochlin, intraocular pressure regulation and mechanosensing. *PLoS ONE*. 2012; 7:e34309. [PubMed: 22496787]
282. Iyer P, Maddala R, Pattabiraman PP, Rao PV. Connective tissue growth factor-mediated upregulation of neuromedin: expression in trabecular meshwork cells and its role in homeostasis of aqueous humor outflow. *Invest Ophthalmol Vis Sci*. 2012; 53:4952–4962. [PubMed: 22761259]
283. Junglas B, Kuespert S, Seleem AA, Struller T, Ullmann S, Bösl M, Bosserhoff A, Köstler J, Wagner R, Tamm ER, Fuchshofer R. Connective tissue growth factor causes glaucoma by modifying the actin cytoskeleton of the trabecular meshwork. *Am J Pathol*. 2012; 180:2386–2403. [PubMed: 22542845]
284. Rosenthal R, Fromm M. Endothelin antagonism as an active principle for glaucoma therapy. *Br J Pharmacol*. 2011; 162:806–816. [PubMed: 21054341]
285. Rocha-Sousa A, Pereira-Silva P, Tavares-Silva M, Azevedo-Pinto S, Rodrigues-Araújo J, Pinho S, Avelino A, Falcão-Reis F, Leite-Moreira A. Identification of the ghrelin-GHSR 1 system and its influence in the modulation of induced ocular hypertension in rabbit and rat eyes. *Peptides*. 2014; 57:59–66. [PubMed: 24780389]
286. Rasmussen CA, Kaufman PL, Ritch R, Haque R, Brazzell RK, Vittitow JL. Latrunculin B reduces intraocular pressure in human ocular hypertension and primary open-angle glaucoma. *Transl Vis Sci Technol*. 2014; 3:1.
287. Pintor J, Peláez T, Hoyle CH, Peral A. Ocular hypotensive effects of melatonin receptor agonists in the rabbit: further evidence for an MT3 receptor. *Br J Pharmacol*. 2003; 138:831–836. [PubMed: 12642384]
288. Wiederholt M, Sturm A, Lepple-Wienhues A. Relaxation of trabecular meshwork and ciliary muscle by release of nitric oxide. *Invest Ophthalmol Vis Sci*. 1994; 35:2515–2520. [PubMed: 7512945]
289. Kaufman PL, Rasmussen CA. Advances in glaucoma treatment and management: outflow drugs. *Invest Ophthalmol Vis Sci*. 2012; 53:2495–2500. [PubMed: 22562850]
290. Fuchshofer R, Tamm ER. The role of TGF- β in the pathogenesis of primary open-angle glaucoma. *Cell Tissue Res*. 2012; 347:279–290. [PubMed: 22101332]

Biographies

Rebecca K. Donegan earned her B.Sc. in chemistry from Middle Tennessee State University and defended her Ph.D. dissertation in April 2015. Her doctoral work under the supervision of Raquel Lieberman included solving the crystal structure and characterizing the calcium binding of the olfactomedin domain of myocilin.

Raquel L. Lieberman received her B. Sc. In Chemistry from Massachusetts Institute of Technology in 1998 and her Ph. D. in Chemistry from Northwestern University in 2005 where she worked with Prof. Amy C. Rosenzweig on biophysical and structural studies of an intramembrane metalloenzyme. After conducting postdoctoral work with Prof. Michael S. Wolfe (Center for Neurological Diseases, Harvard Medical School) and Prof. Gregory A. Petsko (Brandeis) where she expanded to molecular aspects of protein misfolding, she joined the faculty in the School of Chemistry and Biochemistry at Georgia Institute of Technology and is currently an Associate Professor. Research projects in Dr. Lieberman's lab focus on molecular aspects of proteins implicated in misfolding disorders.

Figure 1. Overview of the anterior segment of the eye

Key anatomical structures discussed in this Perspective are labeled. The conventional pathway for aqueous humor outflow is highlighted with blue arrows.

Figure 2. ROCK inhibition

(a) Cartoon representation of ROCK-1 bi-lobed kinase domain in complex with **R-1** (PDB code 2F2U). Canonical domain features highlighted: Small N-terminal lobe; larger C-terminal lobe; P-loop, phosphate binding loop containing glycine-rich motif; C-loop, catalytic loop; A-loop, activation loop. (b) Zoom into active site and residues within hydrogen bonding distance of fasudil presented in ball-and-stick. (c) Summary of proposed signaling cascade for ROCK inhibition leading to reduced IOP.

Figure 3. AR ligands

(a) Summary of proposed signaling cascade for AR agonist and antagonist leading to reduced IOP. Dashed arrows imply the possibility of additional intervening steps in the signaling cascade. (b) Left: Cartoon representation of A_{2A}AR with bound AR-2 colored in rainbow from N-terminus (blue) to C-terminus (red). Perpendicular helix 8 (red) may be involved in protein-protein interactions. Right: Zoom into ligand binding pocket and residues within hydrogen bonding distance of AR-2 presented in ball-and-stick. (c) Summary of SAR efforts using adenosine and xanthine scaffolds.

Figure 4. Emerging new potential targets

(a) NGF in complex with TrkA extracellular domain (PDB code 2IFG).²⁶⁸ (b) Full-length pro-MMP-2 (PDB code 1CK7), with domains highlighted.²⁶⁹ (c) myoc-OLF 5-bladed propeller. Top: cartoon representation. Bottom: surface representation with bound glycerol molecules on surface presented as magenta sticks.²³¹ (d) Grp94 overall domain structure (PDB code 2O1U)²⁷⁰ and zoom into N-terminal ATP binding pocket with radamide inhibitor bound (PDB code 2GFD).²⁷¹

Table 1

Current glaucoma therapies used in the clinic

A. IOP Lowering Drugs		
Class	Example	Method of IOP Reduction
Cholinomimetics (Cholinergic Agonists)	Pilocarpine	Increase AH outflow
Adrenergic receptor agonists (sympathomimetics)	Brimonidine	Reduce AH production, increase outflow
β-Adrenergic receptor antagonists (β-Blockers)	Timolol	Reduce AH production
Carbonic Anhydrase inhibitors	Dorzolamide	Reduce AH production
Prostaglandin analogues	Latanoprost	Increase uveoscleral outflow
B. Surgery		
CAG		
Type	Purpose	
Laser peripheral iridotomy (LPI)	Widen iridocorneal angle	
Iridoplasty	Widen iridocorneal angle	
Argon laser peripheral iridoplasty (ALPI)	Contraction burns to create space between the anterior iris surface and the TM	
Phacoemulsification cataract extraction (phaco/IOL)	Removal of lens alleviates pressure	
POAG		
Type	Purpose	
Glaucoma filter surgery/trabeculectomy	Partial removal of drainage angle allowing AH to bypass TM and alleviate pressure	
Trabeculectomy with Shunt/Stent	Trabeculectomy with addition of shunt or stent to alleviate pressure	
Trabeculoplasty	Lasers open drainage channels to alleviate pressure	

Table 2

ROCK inhibitors

Compound (Other Names or Identifiers)	Structure	Potency (nM) <i>a,b</i>	Clinical Trials for IOP reduction
		ROCK1 ROCK2	
R-1 (Fasudil, HA- 1077)		260 320	
R-2 (K-115, Ripasudil)		51 19	Phase II (Approved for treating glaucoma and ocular hypertension in Japan)
R-3 ^c (Y-27632)		200 140	
R-4 ^c (Y-39983, RKI-983, SNJ-1656)		N/A 3.6	Discontinued after Phase II
R-5		300 78	

Compound (Other Names or Identifiers)	Structure	Potency (nM) <i>a,b</i>	Clinical Trials for IOP reduction
		ROCK1 ROCK2	
R-6	<p>R = (CH₂)₃NHAc</p>	346 75	
R-7		105 24	
R-8 ^c SR-3677		56 3	
R-9		N/A <1	
R-10		20 N/A	

Compound (Other Names or Identifiers)	Structure	Potency (nM) ^{a,b}	Clinical Trials for IOP reduction
		ROCK1 ROCK2	
R-11 Scaffold for AMA-0076		N/A 2.5 (reported for compound where X=F R= CH ₂ -oxolan-2-yl)	Phase II - completed
R-12 (AR-12141 Precursor to AR-12286)		6 1.5	AR-12286 in Phase II
R-13 (AR-12432 Precursor to AR-13324)		1.2 1.1	AR-13324 in Phase III

^aIC₅₀ values unless otherwise noted.

^bReferences R-1¹¹⁵, R-2²⁷², R-3¹⁰⁹, R-4,¹¹⁹ R-5¹²⁶, R-6,¹²⁹ R-7,¹²⁹ R-8,^{109, 141} R-9,¹³⁰ R-10,¹³¹ R-11,¹⁵⁶ R-12, ¹⁵⁷ R-13¹⁵⁷.

^cTested in perfusion studies. N/A: not available.

Table 3

AR agonists, antagonists

Compound	Function	Structure	Affinity/Potency ^{a,b} (nM)	Clinical Trials
AR-1 (Adenosine)	Non-selective agonist		A ₁ 310 A _{2A} 730 A _{2B} 23500 A ₃ 290 (EC ₅₀ values)	
AR-2 (NECA)	Non-selective agonist		A ₁ 14 A _{2A} 20 A _{2B} 140 A ₃ 25	
AR-3 (CHA)	A ₁ agonist		1.3–2.3	
AR-4 (R-PIA)	A ₁ agonist		A ₁ 2 A ₃ 16	
AR-5 (INO-8875)	A ₁ agonist		0.97	Phase I/II
AR-6 (CPA)	A ₁ agonist		5.9	

Compound	Function	Structure	Affinity/Potency ^{a,b} (nM)	Clinical Trials
AR-7 (CF-101, IB-MECA)	A ₃ agonist		A ₃ 1.8 A ₁ 51	Phase II
AR-8 (CGS-21680)	A _{2A} agonist		A _{2A} 27 A ₃ 67	
AR-9 (ATL-313)	A _{2A} agonist		0.7 nM (K _d)	Phase I
AR-10 (2-O-Ado)	A _{2A} agonist		31.8	
AR-11 (2-CN-Ado)	A _{2A} agonist		49.3	
AR-12 (OPA-6566)	A _{2A} agonist	N/A	N/A	Phase II
AR-13 (ZM241385)	A _{2A} antagonist		1.6	
AR-14 (MRS-1097)	A ₃ antagonist		108	

Compound	Function	Structure	Affinity/Potency ^{a,b} (nM)	Clinical Trials
AR-15 (MRS-1191)	A ₃ antagonist		31.4	
AR-16 (MRS-1523)	A ₃ antagonist		19	
AR-17 (LJ-1251)	A ₃ antagonist		4.16	
AR-18 (MRS-1292)	A ₃ antagonist		42 (IC ₅₀)	
AR-19 (LJ-979)	A ₃ antagonist		15.5	
AR-20 (MRS-3826)	A ₃ antagonist		910	

Compound	Function	Structure	Affinity/Potency ^{a,b} (nM)	Clinical Trials
AR-21 (MRS-3827)	A ₃ antagonist	<p>R' = COCH₃ and R'' = H or R' = H and R'' = COCH₃</p>	N/A	
AR-22 (MRS-3771)	A ₃ antagonist		29	
AR-23 (OT-7999)	A ₃ antagonist		0.61	

^aK_i values unless otherwise noted.

^bK_i/IC₅₀/EC₅₀/K_d value references: AR-1,²⁷³ AR-2²⁷⁴, AR-3²⁷⁵, AR-4¹⁵⁵, AR-5¹⁵⁵, AR-6²⁷⁶, AR-7¹⁵⁵, AR-8^{274, 275} AR-9²⁷⁷, AR-10¹⁷⁶, AR-11¹⁷⁶, AR-13¹⁷³, AR-14²⁷⁸, AR-15²⁷⁸, AR-16¹⁸⁹, AR-17^{180, 211, 279}, AR-18¹⁸⁹, AR-19¹⁸⁰, AR-20¹⁸⁰, AR-22¹⁸⁰, AR-23²⁷⁴ N/A: not available.

Table 4

Other therapeutic strategies under consideration.

Drug/target/pathway	Proposed Therapeutic Mode of Action
Angiopoietin-like molecules	Increase TM outflow through reorganization of the ECM. ²⁸⁰
Cannabinoids	Decrease AH production and increases TM outflow, also may be neuroprotective. ²¹³
Cochlin	Regulation may modulate TM outflow. ²⁸¹
CTGF	Outflow increase by modification of TM actomyosin cytoskeleton. ^{282, 283}
Endothelin-1	Increase TM outflow by relaxation of TM, also shown to be neuroprotective of RGCs. ²⁸⁴
Ghrelin	Increase TM outflow by relaxation of the TM. ²⁸⁵
Latrunculins	Increase TM outflow by reducing cell contractility and cell-cell and cell-matrix adhesions. Drugs have reached human trials. ²⁸⁶
MMP-2, MMP-9	Increase TM turnover, possible neuron regeneration in retina (see text).
Melatonin	Increase TM outflow through adrenoceptor and cholinceptor modulation. ²⁸⁷
Myocilin	Decrease of TM cell stress and death (see text).
NGF	Protect RGCs from neurodegeneration (see text).
Nitric oxide (NO)	Increase TM outflow by relaxation of the TM and ciliary muscle. ²⁸⁸ A NO-donating prostaglandin drug has entered clinical trials. ²⁸⁹
Transforming Growth Factor- β (TGF- β)	Increase TM outflow through ECM remodeling or modification of the actin cytoskeleton. ²⁹⁰