

Novel Pharmacologic Candidates for Treatment of Primary Open-Angle Glaucoma

Louise J. Lu^a, James C. Tsai^b, Ji Liu^{a,*}

^aDepartment of Ophthalmology and Visual Science, Yale School of Medicine, New Haven, Connecticut; ^bDepartment of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York Eye and Ear Infirmary of Mount Sinai, New York, New York

Primary open-angle glaucoma (OAG⁺) affects approximately 45 million people worldwide and more than 2.5 million people aged 40 years or older in the United States. Pharmacologic treatment for glaucoma is directed towards lowering intraocular pressure (IOP) to slow disease progression and delay visual field loss. Current medical treatment options for the lowering of IOP include the following classes of topical medications: beta-adrenergic antagonists, alpha-adrenergic agonists, cholinergic agonists, carbonic anhydrase inhibitors, and prostaglandin analogs. Issues with existing drugs include failure to achieve target IOP with monotherapy, drug-related side effects, and low patient compliance with multiple daily administration of eye drops. In recent years, the scientific and medical community has seen encouraging development of novel classes of drugs for primary OAG, the majority of which lower IOP by targeting the trabecular meshwork outflow pathway to increase aqueous humor outflow. Among the most promising new pharmacologic candidates are rho kinase inhibitors including ripasudil (K-115), netarsudil (AR-13324), and AMA0076; adenosine receptor agonists including trabodenson (INO-8875); and modified prostaglandin analogs including latanoprostene bunod (LBN, BOL-303259-X) and ONO-9054. This study aims to systematically review and summarize the most recent developments in clinical trials for new pharmacologic options for the treatment of primary open-angle glaucoma.

BACKGROUND

Primary open-angle glaucoma (OAG) affects approximately 45 million people worldwide and more than 2.5 million people aged 40 years or older in the United States [1-3]. Patients with OAG suffer from progressive visual field loss due to deterioration of the optic nerve [4]. Although OAG is a multifactorial disease, intraocular pressure (IOP) remains the major and only treatable risk factor. Reduction of IOP is the main therapeutic mechanism for slowing disease progression in patients with existing OAG, as well as reducing the risk of progression to glaucoma development in non-glaucomatous eyes with persistently higher than normal IOP (> 21mmHg), or ocular hypertension (OHT) [4-8]. IOP

is a product of aqueous humor circulation in the anterior eye, as aqueous humor is produced by the ciliary body epithelium and flows out through two pathways, the trabecular and uveoscleral pathways. Evidence suggests that homeostatic regulation of IOP predominantly occurs in the trabecular meshwork outflow pathway, where there is the highest degree of normal aqueous humor outflow resistance [9].

First-line intervention for IOP reduction is through the use of pharmacologic drugs, administered as topically-applied eye drops. When medical therapy becomes insufficient, laser or surgical interventions including trabeculectomy, drainage implants, or newer microinvasive glaucoma surgeries (MIGS) are performed [10]. The cur-

*To whom all correspondence should be addressed: Ji Liu, MD, 40 Temple St., Department of Ophthalmology and Visual Science, Yale School of Medicine, New Haven, CT 06510; Tele: 203-785-2020; Fax: 203-785-7090; Email: liu.ji@yale.edu.

†Abbreviations: OAG, open-angle glaucoma; IOP, intraocular pressure; OHT, ocular hypertension.

Keywords: glaucoma, drug development, ocular hypertension, intraocular pressure, rho kinase inhibitor, adenosine receptor agonist, modified prostaglandin analogs

rent line-up of pharmacologic treatment options for the lowering of IOP include the following classes of topical medications: beta-adrenergic antagonists (e.g. timolol), alpha-2-adrenergic agonists (e.g. brimonidine), cholinergic agonists (e.g. pilocarpine), carbonic anhydrase inhibitors (e.g. brinzolamide), and prostaglandin analogs (e.g. latanoprost) [11]. Mechanisms of current drugs for glaucoma include increasing aqueous humor outflow and/or decreasing aqueous humor production [12]. Beta-blockers and prostaglandin analogs are commonly used as the initial drugs for patients with OAG, with evidence indicating that these drugs may reduce IOP by up to 25 percent and 33 percent, respectively [13,14]. Fixed-combination drugs are now available, such as brinzolamide-brimonidine, dorzolamide-timolol and brimonidine-timolol, that have been found to significantly reduce IOP by up to 15 percent more than any of the single drug components [15-17]; these drugs have the additional advantage of reducing the total number of drops that patients must administer per day. Despite evidence of overall efficacy, monotherapy with current drugs is unable to achieve target IOP for many patients [18]. Additionally, notable side effects have been reported for most drug classes, including stinging and dry eyes with beta-blocker therapy and hyperemia with prostaglandin analogs [14,19-20]. Furthermore, pharmacologic therapy is often compromised by patient compliance, as current treatment options (including fixed-combination drugs) require multiple daily administration; a once-daily formulation may improve compliance and therefore, efficacy of treatment [21].

Twenty years have passed since the development of the last drug class in glaucoma: the FDA approved latanoprost (Xalatan), the first prostaglandin analog, in 1996. Given the persistently high incidence of primary OAG and issues with current medications, there is a significant incentive for the development of new drugs for OHT and primary OAG patients. Various lines of new drug classes for glaucoma are currently in development, lowering IOP via alternate mechanisms by targeting aqueous humor outflow in the trabecular meshwork outflow pathway [22].

This study aims to systematically review and summarize evidence regarding the most up-to-date developments in new pharmacologic options for the treatment of primary OAG, including rho kinase inhibitors, adenosine receptor agonists, and additives to prostaglandin therapy.

RHO KINASE INHIBITORS

Rho kinase inhibitors are a novel class of drugs in development for primary OAG. Rho kinase inhibitors are cytoskeletal-modulating drugs, that lower IOP by altering the arrangement of the cytoskeleton and focal adhesions in trabecular meshwork cells [23,24]. Evidence

indicates that the actin cytoskeleton plays a major role in the regulation of aqueous humor outflow in the trabecular meshwork outflow pathway [23,24]. By altering cellular components of the trabecular meshwork and Schlemm's canal, rho kinase inhibitors decrease resistance in the trabecular meshwork outflow pathway and promote reduction of IOP [25]. Rho kinase inhibitors that are currently being studied in clinical and preclinical trials include ripasudil (K-115), netarsudil (AR-13324), and AMA0076.

Ripasudil (K-115)

Ripasudil (K-115) is a rho kinase inhibitor that has shown promising results in clinical trials and has been approved for ocular use in Japan since 2014. The most recently published results of a clinical study assessing the IOP-lowering effects and safety of ripasudil found that a 52-week administration of 0.4 percent ripasudil successfully lowered IOP when used in both monotherapy and additive therapy [26]. Tanihara et al. conducted a multicenter, prospective study of 354 patients with primary OAG or OHT who were divided into groups of ripasudil monotherapy and additive therapy to prostaglandin analogs, beta-blockers, and fixed-combination drugs. Mean IOP reduction at trough and peak at the conclusion of the study were -2.5 and -3.7 mmHg for monotherapy, and -1.4 and -2.4, -2.2 and -3.0, and -1.7 and -1.7 mmHg for additive therapy to prostaglandin analogs, beta-blockers, and fixed combination drugs, respectively [26].

Adverse drug reactions were experienced in 301 of 354 (85.0 percent) of patients, with the most frequent drug-related adverse events being conjunctival hyperemia (74.6 percent), blepharitis (20.2 percent), and allergic conjunctivitis (15.0 percent). Despite the high incidence, the study authors reported that the majority of adverse effect presentations were mild and transient, and frequently resolved spontaneously, consistent with findings from previous clinical studies of ripasudil in patients [27-29].

Previous phase III clinical trials had already established the additive effects of IOP reduction to latanoprost and timolol after eight weeks of treatment [25]. Results from the most recent clinical study have now supported and expanded findings of ripasudil's additive effects of IOP reduction to other prostaglandin analogs, beta blockers, fixed combination drugs over a longer treatment period. Additionally, the study also confirmed ripasudil's effectiveness at IOP reduction even in monotherapy compared to current first-line anti-glaucoma drugs [26], adding to previous findings of ripasudil's IOP-lowering effects compared to second-line medications such as carbonic anhydrase inhibitors and brimonidine [30,31]. Positive findings from recent clinical studies are encouraging for ophthalmologists, as ripasudil appears to be a highly

Table 1. Summary of novel glaucoma drugs in clinical development

Drug	Drug class	Mechanism of IOP reduction	Phase of drug development	Mean IOP lowering effect in phase II/ III trials
Ripasudil (K-115)	Rho kinase inhibitor	Decrease resistance in the trabecular meshwork outflow pathway	Phase III	3.7 mmHg
Netarsudil (AR-13324)	Rho kinase inhibitor	Decrease resistance in the trabecular meshwork outflow pathway and aqueous humor production	Phase III	5.5 mmHg
AMA0076	Rho kinase inhibitor	Decrease resistance in the trabecular meshwork outflow pathway	Phase II	3.7 mmHg
Trabodenson (INO-8875)	Adenosine receptor agonist	Increase aqueous humor outflow through the trabecular meshwork	Phase III	4.1 mmHg
Latanoprostene bunod (LBN, BOL-303259-X)	Modified prostaglandin analog	Increase aqueous humor outflow through the trabecular meshwork and uveoscleral pathway	Phase III	8.4 mmHg
ONO-9054	Modified prostaglandin analog	Increase aqueous humor outflow through the trabecular meshwork and uveoscleral pathway	Phase II	7.2 mmHg

promising new drug for the reduction of intraocular pressure in patients with primary open-angle glaucoma and ocular hypertension (Table 1).

Netarsudil (AR-13324)

Netarsudil (AR-13324), another compound in development for open-angle glaucoma and ocular hypertension, is a small-molecular inhibitor of rho kinase and the norepinephrine transporter [32]. As a rho kinase inhibitor, netarsudil functions to increase aqueous humor outflow by dilating the trabecular meshwork juxtacanalicular connective tissue and episcleral veins, similar to the mechanism of ripasudil [33,34]. Netarsudil appears to also decrease the production of aqueous humor to lower intraocular pressure, a mechanism that may be attributed to the additional inhibition of the norepinephrine transporter [31]. The earliest preclinical studies of netarsudil conducted in rabbits and monkeys established that netarsudil was capable of lowering IOP by 20 to 25 percent with a long duration of action [35].

Bacharach et al. recently reported results from a clinical trial of 224 patients randomized to receive netarsudil 0.01 percent solution, AR-11324 0.02 percent solution, or latanoprost 0.005 percent (control). At the 28-day mark, an IOP reduction of 5.5 and 5.7 mmHg was achieved

with the netarsudil 0.01 percent and netarsudil 0.02 percent solutions, respectively. Netarsudil did not achieve a significantly greater IOP reduction, whereas latanoprost yielded a 6.8 mmHg reduction in IOP ($P < 0.001$) [34]. The most common adverse event associated with netarsudil was conjunctival hyperemia, present in 18 percent and 24 percent of patients randomized to netarsudil 0.01 percent and netarsudil 0.02 percent, respectively. This was more frequently reported for netarsudil than for latanoprost, in which 11 percent of patients reported conjunctival hyperemia at the conclusion of the study. Thus, although daily doses of netarsudil 0.01 percent and 0.02 percent produced significant reductions in IOP, with 0.02 percent yielding greater reductions than 0.01 percent solution, netarsudil was overall found to be less effective than latanoprost by IOP reductions of approximately 1 mmHg and had a higher incidence of ocular hyperemia [34].

The efficacy of netarsudil (AR-13324) in IOP reduction has also been studied as part of a fixed-dose combination with latanoprost. Lewis et al. conducted a clinical study in 298 patients with OAG or OHT to evaluate the efficacy of fixed-dose combinations of AR-13324 0.02 percent and latanoprost 0.005 percent relative to the individual active components at the same concentrations

[36]. Results after treatment for 28 days showed that the fixed-dose combination of AR-13324 and latanoprost provided significantly improved efficacy in IOP reduction relative to the individual active components, providing an additional IOP reduction of 1.9 and 2.6 mmHg compared to individually-administered AR-13324 and latanoprost, respectively [36].

AMA0076

AMA0076 is a novel, potent rho kinase inhibitor that has undergone both *in vitro* and *in vivo* experiments to establish its safety profile and efficacy in IOP reduction. A unique characteristic of AMA0076 is its locally-acting nature: outside the aqueous humor, it undergoes rapid conversion to an inactive form to be subsequently eliminated, thereby potentially minimizing off-target activity and adverse effects from topical and systemic absorption after ocular application [37].

Results from *in vitro* experiments, in which human trabecular meshwork cell cultures were exposed to AMA0076, found that AMA0076 induced significant alterations to the actin filament organization and focal adhesions of the human trabecular meshwork cells and successfully modified trabecular meshwork cell morphology [37].

An *in vivo* animal model study found that in normotensive rabbits, AMA0076 achieved an IOP reduction of 48 percent, 39 percent, and 23 percent at concentrations of 0.5 percent, 0.3 percent, and 0.1 percent, respectively [37]. Thus, AMA0076 significantly decreased IOP in a dose-dependent manner. When compared to latanoprost, it was found that AMA0076 and latanoprost had comparable performance when IOP measurements were conducted at night, with IOP reductions of 25.3 percent for AMA0076 and 22.2 percent for latanoprost. However, AMA0076 also reduced IOP during the day, while latanoprost did not [37]. A further experiment examining the efficacy of AMA0076 compared to prostaglandin analogs was conducted in a rabbit model of ocular hypertension. AMA0076 was significantly more effective in lowering IOP than the prostaglandin analogs latanoprost and bimatoprost ($P < 0.0001$) [37]. Finally, regarding adverse effects, AMA0076 was found to cause conjunctival hyperemia, but to a significantly less effect ($P = 0.002$) than Y-39983, a less potent rho kinase inhibitor [37].

A phase I clinical trial for AMA0076 was completed in 2013. The study was a multicenter, randomized, double-masked, placebo-controlled study conducted among 82 OAG/OHT patients. Results showed that AMA0076 achieved IOP reduction of 3.7 mmHg, and was observed to be safe and well-tolerated with no significant adverse effects reported [38].

Results from phase I clinical trials demonstrated

AMA0076 to be a powerful rho kinase inhibitor with similar or potentially greater efficacy at lowering IOP than prostaglandin inhibitors. Due to its locally-acting nature, AMA0076 may also have an improved tolerability profile with less incidence of hyperemia compared to other rho kinase inhibitors. A phase II clinical trial evaluating the safety, tolerability, and efficacy of AMA0076 was recently completed, although results have not yet been published in the literature. Ongoing clinical studies are needed to assess the potential of AMA0076 to be a new drug candidate for the treatment of open-angle glaucoma.

ADENOSINE RECEPTOR AGONISTS

Adenosine receptor agonists are a novel class of drugs that reduce intraocular pressure by increasing the outflow of aqueous humor through the trabecular meshwork pathway. Adenosine functions in many physiological processes in the human body, including a role in modulating intraocular pressure, through interactions with four known adenosine receptor subtypes— A_1 , A_{2A} , A_{2B} , and A_3 [11,39-41]. Animal models have demonstrated the effect of IOP reduction via selective A_1 receptor agonist, as well as IOP elevation via non- A_1 receptor agonism [42,43]. The mechanism of IOP reduction for adenosine receptor agonists is that stimulation of the A_1 receptor enhances the secretion of matrix metalloproteinase-2 (MMP-2) which promotes digestion of type IV collagen components of the extracellular matrix in the trabecular meshwork [41,44]. As levels of MMP-2 rise, increased extracellular matrix turnover in the trabecular meshwork removes protein from the trabecular meshwork outflow pathway, lowering outflow resistance and reducing intraocular pressure [45].

Trabodенoson (INO-8875)

Trabodенoson (INO-8875) is a highly-selective A_1 adenosine receptor agonist that is being studied for its activity in lowering IOP in glaucoma and ocular hypertension. Preclinical studies found that normotensive cynomolgus monkeys and pigmented rabbits that were administered doses of trabodенoson experienced significant IOP decreases of up to 25 percent [41]. A previously-conducted phase I study among volunteers without primary OAG or ocular hypertension confirmed the safety and tolerability of trabodенoson in healthy subjects [39].

Myers et al. conducted a randomized, placebo-controlled dose-escalation phase II clinical trial among 144 subjects with primary OAG or OHT to assess the safety, tolerability, and efficacy of trabodенoson compared to placebo for 28 days [39]. The study found that trabodенoson produced a dose-dependent reduction in IOP, with the 500 mcg trabodенoson group achieving significantly greater IOP reductions than the placebo group. The

500 mcg trabodenoson group had a mean change of -4.1 mmHg compared to -1.6 mmHg for the placebo group. In addition, trabodenoson demonstrated both long duration of action, with persistent IOP reduction 24 hours after the final 500 mcg dose ($P = 0.048$) as well as evidence that trabodenoson has greater efficacy with longer treatment time, given that IOP reduction at day 28 was significantly greater than at day 14 ($P = 0.0163$) [39].

The most frequent drug-related adverse event was conjunctival and ocular hyperemia, with 18.8 percent of patients randomized to the trabodenoson group developing hyperemia during the course of the study [39]. The researchers graded the majority of hyperemia cases as mild cases, with only three subjects receiving a grade of moderate hyperemia. No other clinically significant drug-related adverse events were observed.

The results from the latest clinical study on trabodenoson indicate that this highly selective A_1 adenosine receptor agonist has met safety and tolerability goals and achieves statistically significant IOP reductions in patients with primary OAG or ocular hypotension. In addition, the dose-efficacy study of trabodenoson found that increasing doses of administered trabodenoson correlated with dose-related improvements in IOP reduction, with no plateau in efficacy in the highest dose tested in the study (500 mcg). Phase III clinical trials with higher doses of trabodenoson are currently ongoing.

MODIFIED PROSTAGLANDIN ANALOGS

Modified prostaglandin analogs are a novel class of drugs that combine the IOP-lowering effects of prostaglandin analogs with a novel mechanism of nitric-oxide-mediated relaxation of the trabecular meshwork and Schlemm's canal. This works to increase aqueous humor outflow through the trabecular meshwork and further reduce IOP. Latanoprostene bunod is a modified prostaglandin analog that has demonstrated promising results in recent phase III clinical trials. Another modified prostaglandin analog that is undergoing investigation for glaucoma therapy is ONO-9054, a compound that targets both the prostanoid F (FP) receptor and the prostanoid EP3 receptors found in the trabecular meshwork and ciliary muscle.

Latanoprostene Bunod

Latanoprostene bunod (LBN; BOL-303259-X) is a nitric oxide-donating prostanoid FP receptor agonist that becomes metabolized, upon topical administration in the eye, into latanoprost acid and butanediol mononitrate [46-48]. Latanoprost acid reduces intraocular pressure by increasing aqueous humor outflow via the uveoscleral pathway through remodeling of the extracellular matrices of the ciliary body [49,50]. Butanediol mononitrate adds

to the IOP-lowering effect by acting as a nitric oxide donor, which induces relaxation of the trabecular meshwork and Schlemm's canal, resulting in increased aqueous humor outflow through the trabecular meshwork outflow pathway [51-54].

Preclinical studies of latanoprostene bunod demonstrated significant lowering of IOP in rabbits, even those that were unaffected by latanoprost alone, indicating that the contribution of the mechanism of nitric oxide to the prostaglandin analog is additive for IOP reduction [48]. A phase II clinical trial conducted among 413 patients with primary OAG or OHT found that latanoprostene bunod 0.024 percent achieved a significantly greater reduction in IOP compared to latanoprost 0.005 percent at the conclusion of the 28-day study [46].

Weinreb et al. conducted a phase III, randomized, controlled clinical trial of 420 patients with OAG or OHT to compare the IOP-lowering efficacy of latanoprostene bunod 0.024 percent administered daily with timolol 0.5 percent administered twice daily (BID) [47]. IOP was measured at nine assessment time points throughout the three-month period of the study. At the conclusion of the study, patients randomized to the latanoprostene bunod 0.024 percent group achieved significantly greater IOP reduction (range, -7.7 to -9.1 mmHg) at all nine time points compared to the timolol 0.5 percent group (range, -6.6 to -8.0 mmHg) ($P \leq 0.002$). The percentage of patients with a mean IOP ≤ 18 mmHg was 22.9 percent and 11.3 percent in the LBN 0.024 percent and timolol 0.5 percent groups, respectively ($P = 0.005$). The percentage of patients with an IOP reduction ≥ 25 percent compared to baseline was also significantly higher among those who received LBN, with rates of 34.9 percent for the LBN 0.024 percent group compared to 19.5 percent for the timolol 0.5 percent group ($P = 0.001$) [47].

The frequency of adverse effects occurring during the three-month study was generally low, with eye irritation and conjunctival hyperemia being the most common reported adverse events. The percentage of patients experiencing a treatment-related adverse effect was comparable in the LBN 0.024 percent and timolol 0.5 percent groups [47]. However, although the overall percentage of subjects with ocular hyperemia was similar between the two groups, a greater percentage of subjects randomized to the LBN group experienced moderate to severe hyperemia compared with subjects randomized to the timolol group. The study authors noted that such findings are consistent with the reported rates of hyperemia in patients administered latanoprost [47,55].

Overall, the results of the phase III clinical study concluded that latanoprostene bunod 0.024 percent, a nitric oxide-donating prostaglandin administered as monotherapy, was achieved adequate safety and tolerability and was significantly more effective than timolol 0.5 per-

cent (administered BID) in lowering IOP in patients with primary OAG or OHT.

ONO-9054

ONO-9054 is a novel compound that aims to enhance the mechanism of prostaglandin analogs by targeting both the FP receptor as well as the prostanoid EP3 receptors. Targeting the EP3 receptors, which are located in the trabecular meshwork and ciliary muscle, allows ONO-9054 to increase outflow of aqueous humor through the trabecular meshwork outflow pathway in addition to the uveoscleral pathway for an additive effect to lower IOP [56,57].

Preclinical experiments of ONO-9054 demonstrated a significantly greater IOP-lowering effect compared to currently available prostaglandin analogs in monkeys [58]. Suto et al. conducted the first human study of ONO-9054 in a phase I clinical trial of 48 healthy volunteers [59]. The researchers found that ONO-9054 was safe and well-tolerated; in addition, ONO-9054 achieved a significant reduction in IOP at the conclusion of the seven-day study, with a maximum IOP change of -7.2 mmHg (-28.23 percent from baseline) with a 30.0 mcg/ml dose [59]. Similar rates of adverse events were reported in the ONO-9054 and placebo groups, with mild conjunctival hyperemia being the most frequent ocular adverse event [59].

A phase I clinical trial investigating the pharmacodynamic potential of ONO-9054 in nine subjects with early primary OAG or OHT found both single and multiple-day dosing of ONO-9054 led to significant IOP reductions across all treatment groups, with a maximum IOP change of -7.0 mmHg following single dosing and -8.2 mmHg following multiple-day dosing [60]. A phase II clinical trial was subsequently conducted to compare the safety, tolerability, and mean IOP reduction effects of ONO-9054 versus latanoprost. Results of the trial, which was conducted among 62 adults with an un-medicated IOP \geq 24 mmHg, indicate that ONO-9054 achieved a greater reduction in mean diurnal IOP compared to latanoprost (-7.2 mmHg versus -6.6 mmHg) [62]. Encouraging results from the phase II clinical study support additional, larger clinical trials to further evaluate the efficacy of ONO-9054 for the treatment of primary OAG and OHT.

CONCLUSION

Glaucoma is the second leading cause of blindness worldwide, affecting tens of millions of patients [59]. Pharmacologic treatment for glaucoma is directed towards lowering intraocular pressure to slow disease progression and delay visual field loss [5,61]. In recent years, the scientific and medical community has seen encourag-

ing development of novel drug classes for IOP reduction in patients with primary open-angle glaucoma and ocular hypertension. Among the most promising new pharmacologic candidates are rho kinase inhibitors including ripasudil (K-115), netarsudil (AR-13324), and AMA0076; adenosine receptor agonists including trabodenson (INO-8875); and modified prostaglandin analogs including latanoprostene bunod (LBN, BOL-303259-X) and ONO-9054. Continued clinical investigation into these promising drug candidates may soon produce a novel class of IOP-reducing drugs to the current line-up of pharmacologic therapy for patients with primary open-angle glaucoma and ocular hypertension. While IOP reduction is the primary outcome in current drug development studies, additional clinical trials assessing visual outcomes may be required before novel drug compounds can be shown to be beneficial in the clinical setting.

Acknowledgments: This work was funded in part by an unrestricted departmental grant from Research to Prevent Blindness (RPB), Inc.

REFERENCES

1. Friedman DS, et al. Prevalence of open-angle glaucoma among adults in the United States. *Archives of ophthalmology*. 2004;122:532-538.
2. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311:1901-1911.
3. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet*. 2004;363:1711-1720.
4. Varma R, Lee PP, Goldberg I, Kotak S. An assessment of the health and economic burdens of glaucoma. *Am J Ophthalmol*. 2011;152:515-522.
5. Kass MA, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Archives of Ophthalmology*. 2002;120:701-713; discussion 829-730.
6. Heijl A, et al. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Archives of Ophthalmology*. 2002;120:1268-1279.
7. Leske MC, et al. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. *Archives of Ophthalmology*. 2003;121:48-56.
8. Garway-Heath DF, et al. Latanoprost for open-angle glaucoma (UKGTS): a randomised, multicentre, placebo-controlled trial. *Lancet*. 2015;385:1295-1304.
9. Tamm ER. The trabecular meshwork outflow pathways: structural and functional aspects. *Exp Eye Res*. 2009;88:648-655.
10. Burr J, Azuara-Blanco A, Avenell A, Tuulonen A. Medical versus surgical interventions for open angle glaucoma. *Cochrane Database Syst Rev*. 2012: Cd004399.
11. Fishman P, Cohen S, Bar-Yehuda S. Targeting the A3 adenosine receptor for glaucoma treatment (review). *Mol Med Rep*. 2013;7:1723-1725.
12. Damji KF, Allingham RR, Shields MB, Freedman SF. Principles of Medical Therapy and Management. In: Damji KF, Allingham RR, Shields MB, Freedman SF. *Shields Textbook of Glaucoma*. Philadelphia, PA: Walters Kluwer

- Lippincott Williams and Wilkins; 2011.
13. Strahlman E, Tipping R, Vogel R. A double-masked, randomized 1-year study comparing dorzolamide (Trusopt), timolol, and betaxolol. International Dorzolamide Study Group. *Archives of Ophthalmology*. 1995;113:1009-1016.
 14. Feldman RM. Conjunctival hyperemia and the use of topical prostaglandins in glaucoma and ocular hypertension. *J Ocul Pharmacol Ther*. 2003;19:23-35.
 15. Lee NY, Park HL, Park CK. Effects of a dorzolamide/timolol fixed combination on diurnal intraocular pressure, heart rate, blood pressure, and ocular perfusion pressure in normal-tension glaucoma. *Jpn J Ophthalmol*. 2016.
 16. Fechtner RD, Myers JS, Hubatsch DA, Budenz DL, Dubiner HB. Ocular hypotensive effect of fixed-combination brinzolamide/brimonidine adjunctive to a prostaglandin analog: a randomized clinical trial. *Eye*. 2016.
 17. Takagi Y, Osaki H, Yamashita T, Kai Y. Prospective Observational Post-marketing Study of Tafluprost 0.0015%/Timolol 0.5% Combination Ophthalmic Solution for Glaucoma and Ocular Hypertension: Short-Term Efficacy and Safety. *Ophthalmology and Therapy*. 2016.
 18. Kass MA, et al. Delaying treatment of ocular hypertension: the ocular hypertension treatment study. *Archives of Ophthalmology*. 2010;128:276-287.
 19. Baudouin C, et al. Conjunctival epithelial cell expression of interleukins and inflammatory markers in glaucoma patients treated over the long term. *Ophthalmology*. 2004;111:2186-2192.
 20. Pisella PJ, et al. Conjunctival proinflammatory and proapoptotic effects of latanoprost and preserved and unpreserved timolol: an ex vivo and in vitro study. *Invest Ophthalmol Vis Sci*. 2004;45:1360-1368.
 21. Reardon G, Kotak S, Schwartz GF. Objective assessment of compliance and persistence among patients treated for glaucoma and ocular hypertension: a systematic review. *Patient Preference and Adherence*. 2011;5:441-463.
 22. Chowdhury UR, Hann CR, Stamer WD & Fautsch MP. Aqueous humor outflow: dynamics and disease. *Invest Ophthalmol Vis Sci*. 2015;56:2993-3003.
 23. Honjo M, et al. Effects of rho-associated protein kinase inhibitor Y-27632 on intraocular pressure and outflow facility. *Invest Ophthalmol Vis Sci*. 2001;42:137-144.
 24. Koga T, et al. Rho-associated protein kinase inhibitor, Y-27632, induces alterations in adhesion, contraction and motility in cultured human trabecular meshwork cells. *Exp Eye Res*. 2006;82:362-370.
 25. Tanihara H, et al. Intra-ocular pressure-lowering effects of a Rho kinase inhibitor, ripasudil (K-115), over 24 hours in primary open-angle glaucoma and ocular hypertension: a randomized, open-label, crossover study. *Acta Ophthalmol*. 2015;93:e254-260.
 26. Tanihara H, et al. One-year clinical evaluation of 0.4% ripasudil (K-115) in patients with open-angle glaucoma and ocular hypertension. *Acta Ophthalmol*. 2016;94:e26-34.
 27. Tanihara H, et al. Phase 2 randomized clinical study of a Rho kinase inhibitor, K-115, in primary open-angle glaucoma and ocular hypertension. *Am J Ophthalmol*. 2013;156:731-736.
 28. Tanihara H, et al. Additive Intraocular Pressure-Lowering Effects of the Rho Kinase Inhibitor Ripasudil (K-115) Combined With Timolol or Latanoprost: A Report of 2 Randomized Clinical Trials. *JAMA Ophthalmol*. 2015;133:755-761.
 29. Williams RD, Novack GD, van Haarlem T, Kopczynski C. Ocular hypotensive effect of the Rho kinase inhibitor AR-12286 in patients with glaucoma and ocular hypertension. *Am J Ophthalmol*. 2011;152:834-841.e831.
 30. Tanna AP, Rademaker AW, Stewart WC, Feldman RM. Meta-analysis of the efficacy and safety of alpha2-adrenergic agonists, beta-adrenergic antagonists, and topical carbonic anhydrase inhibitors with prostaglandin analogs. *Archives of Ophthalmology*. 2010;128:825-833.
 31. Kitazawa Y, Araie M. [Double-masked, phase III comparative study of the combination ophthalmic solution of the 1% dorzolamide hydrochloride/0.5% timolol maleate (MK-0507A) in patients with glaucoma and ocular hypertension]. *Nippon Ganka Gakkai Zasshi*. 2011;115:495-507.
 32. Bacharach J, et al. Double-masked, randomized, dose-response study of AR-13324 versus latanoprost in patients with elevated intraocular pressure. *Ophthalmology*. 2015;122:302-307.
 33. Ren R, Li G, Le T et al. Netarsudil Increases Outflow Facility in Human Eyes Through Multiple Mechanisms. *Invest Ophthalmol Vis Sci*. 2016;6197-6209.
 34. Li G, Mukherjee D, Navarro I, et al. (2016) Visualization of conventional outflow tissue responses to netarsudil in living mouse eyes. *Eur J Pharmacol*. 2016;20-31.
 35. Wang RF, Williamson JE, Kopczynski C, Serle JB. Effect of 0.04% AR-13324, a ROCK, and norepinephrine transporter inhibitor, on aqueous humor dynamics in normotensive monkey eyes. *J Glaucoma*. 2015;24:51-54.
 36. Lewis RA, Levy B, Ramirez N, et al. Fixed-dose combination of AR-13324 and latanoprost: a double-masked, 28-day, randomised, controlled study in patients with open-angle glaucoma or ocular hypertension. *Br J Ophthalmol*. 2016;100:339-344.
 37. Van de Velde S, et al. AMA0076, a novel, locally acting Rho kinase inhibitor, potentially lowers intraocular pressure in New Zealand white rabbits with minimal hyperemia. *Invest Ophthalmol Vis Sci*. 2014;55:1006-1016.
 38. Hall J, et al. Initial Clinical Evaluation of Safety, Tolerability and Pharmacodynamics of the Locally-Acting ROCK Inhibitor AMA0076 [abstract]. *Investigative Ophthalmology & Visual Science*. 2014;55:565.
 39. Myers JS, et al. A Dose-Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Efficacy of 2 and 4 Weeks of Twice-Daily Ocular Trabodensin in Adults with Ocular Hypertension or Primary Open-Angle Glaucoma. *J Ocul Pharmacol Ther*. 2016.
 40. Daines BS, Kent AR, McAleer MS, Crosson CE. Intraocular adenosine levels in normal and ocular-hypertensive patients. *J Ocul Pharmacol Ther*. 2003;19:113-119.
 41. Zhong Y, Yang Z, Huang WC, Luo X. Adenosine, adenosine receptors and glaucoma: an updated overview. *Biochim Biophys Acta*. 2013;1830:2882-2890.
 42. Avila MY, Stone RA, Civan MM. A(1)-, A(2A)- and A(3)-subtype adenosine receptors modulate intraocular pressure in the mouse. *British journal of pharmacology*. 2001;134:241-245.
 43. Crosson CE, Gray T. Characterization of ocular hypertension induced by adenosine agonists. *Invest Ophthalmol Vis Sci*. 1996;37:1833-1839.
 44. Shearer TW, Crosson CE. Adenosine A1 receptor modulation of MMP-2 secretion by trabecular meshwork cells. *Invest Ophthalmol Vis Sci*. 2002;43:3016-3020.
 45. Bradley JM, et al. Effect of matrix metalloproteinases activity on outflow in perfused human organ culture. *Invest Ophthalmol Vis Sci*. 1998;39:2649-2658.
 46. Weinreb RN, et al. A randomised, controlled comparison of latanoprostene bunod and latanoprost 0.005% in the treatment of ocular hypertension and open angle glaucoma: the VOYAGER study. *Br J Ophthalmol*. 2015;99:738-745.
 47. Weinreb RN, Scassellati Sforzolini B, Vittitov J, Liebmann J. Latanoprostene Bunod 0.024% versus Timolol Maleate 0.5% in Subjects with Open-Angle Glaucoma or Ocular Hypertension: The APOLLO Study. *Ophthalmology*.

- 2016;123:965-973.
48. Krauss AH, et al. Ocular hypotensive activity of BOL-303259-X, a nitric oxide donating prostaglandin F₂alpha agonist, in preclinical models. *Exp Eye Res.* 2011;93:250-255.
 49. 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.
 50. Richter M, Krauss AH, Woodward DF, Lutjen-Drecoll E. Morphological changes in the anterior eye segment after long-term treatment with different receptor selective prostaglandin agonists and a prostamide. *Invest Ophthalmol Vis Sci.* 2003;44:4419-4426.
 51. Heyne GW, Kiland JA, Kaufman PL, Gabelt BT. Effect of nitric oxide on anterior segment physiology in monkeys. *Invest Ophthalmol Vis Sci.* 2013;54:5103-5110.
 52. Cavet ME, Vittitow JL, Impagnatiello F, Ongini E, Bastia E. Nitric oxide (NO): an emerging target for the treatment of glaucoma. *Invest Ophthalmol Vis Sci.* 2014;55:5005-5015.
 53. Lei Y, Zhang X, Song M, Wu J, Sun X. Aqueous Humor Outflow Physiology in NOS3 Knockout Mice. *Invest Ophthalmol Vis Sci.* 2015;56:4891-4898.
 54. Chang DF, et al. Antibiotic prophylaxis of postoperative endophthalmitis after cataract surgery: Results of the 2014 ASCRS member survey. *J Cataract Refract Surg.* 2015;41:1300-1305.
 55. Zhang WY, Po AL, Dua HS, Azuara-Blanco A. Meta-analysis of randomised controlled trials comparing latanoprost with timolol in the treatment of patients with open angle glaucoma or ocular hypertension. *Br J Ophthalmol.* 2001;85:983-990.
 56. Gabelt BT, et al. Prostaglandin subtype-selective and non-selective IOP-lowering comparison in monkeys. *J Ocul Pharmacol Ther.* 2009;25:1-8.
 57. Schlotzer-Schrehardt U, Zenkel M, Nusing RM. Expression and localization of FP and EP prostanoid receptor subtypes in human ocular tissues. *Invest Ophthalmol Vis Sci.* 2002;43:1475-1487.
 58. Yamane S, et al. IOP-Lowering Effect of ONO-9054, A Novel Dual Agonist of Prostanoid EP3 and FP Receptors, in Monkeys. *Invest Ophthalmol Vis Sci.* 2015;56:2547-2552.
 59. Suto F, Rowe-Rendelman CL, Ouchi T, Jamil A, Wood A, Ward CL. A Novel Dual Agonist of EP3 and FP Receptors for OAG and OHT- Safety, Pharmacokinetics, and Pharmacodynamics of ONO-9054 in Healthy Volunteers. *Invest Ophthalmol Vis Sci.* 2015;56:7963-7970.
 60. Harris A, et al. Ocular Hypotensive Effect of ONO-9054, an EP3/FP Receptor Agonist: Results of a Randomized, Placebo-controlled, Dose Escalation Study. *J Glaucoma.* 2016.
 61. Resnikoff S, Pascolini D, Mariotti SP, Pokharel GP. Global magnitude of visual impairment caused by uncorrected refractive errors in 2004. *Bulletin of the World Health Organization.* 2008;86:63-70.
 62. Miller Ellis E, Berlin MS, Ward CL, Sharpe JA, Jamil A, Harris A. Ocular hypotensive effect of the novel EP3/FP agonist ONO-9054 versus Xalatan: results of a 28-day, double-masked, randomised study. *Br J Ophthalmol.* 2016.