Drugs of the Future 2011, 36(4): 287-300

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.4.1587772

THOMSON REUTERS

REVIEW ARTICLE

NOVEL THERAPEUTIC APPROACHES FOR GLAUCOMA

C.A. Rasmussen and P.L. Kaufman

Department of Ophthalmology & Visual Sciences, University of Wisconsin, Madison, USA

CONTENTS

SUMMARY

Glaucoma refers to a collection of progressive optic neuropathies that can lead to irreversible damage to retinal ganglion cells and their axons, and eventual loss of vision if left untreated or treated unsuccessfully. The main goal in glaucoma therapeutics is to prevent functionally significant visual impairment. Glaucoma is the second leading cause of blindness worldwide, accounting for approximately 12.5% of all global blindness. The cost of intraocular pressure (IOP)-lowering treatment is estimated to reach $5 billion annually in the U.S. alone by 2011. With expected increases in disease prevalence and the number of patients seeking treatment for the disease, there remains a great need for novel products that offer significant improvements in efficacy, duration of action and side effect profile. Most glaucoma patients will be prescribed multiple drops of varying classes of compounds to control their disease. There is much interest in developing compounds with novel and complementary mechanisms of action, which are discussed herein.

INTRODUCTION

Glaucoma refers to a collection of progressive optic neuropathies that can lead to irreversible damage to retinal ganglion cells (RGC) and their axons and eventual loss of vision if left untreated or treated unsuccessfully. Because the disease is largely asymptomatic, many people are unaware they have glaucoma until loss of vision occurs. The main goal in glaucoma therapeutics is to prevent functionally significant visual impairment, which is generally assessed by standard automated perimetry. Unfortunately, by the time visual field deficits appear in this test, 20-40% (1) of the RGCs may already be damaged (2). A significant unmet need in glaucoma therapeutics is the validation of techniques to measure structural and functional changes in the eye; techniques that allow sensitive, specific and reproducible detection of small changes that can be reliably predictive of glaucoma and indicate progression for use clinically and in clinical trials.

Correspondence: Paul L. Kaufman, MD, Peter A. Duehr Professor and Chair, Department of Ophthalmology & Visual Sciences, University of Wisconsin School of Medicine and Public Health, 3310 University Ave., Ste 206, Madison, WI 53705-2135, USA. E-mail: kaufmanp@mhub.ophth.wisc.edu.

Glaucoma is the second leading cause of blindness worldwide, accounting for approximately 12.5% of all global blindness (3). It is estimated that about 8.4 million people will be bilaterally blind from glaucoma in 2010 (4). Primary open-angle glaucoma (POAG) is the most prevalent form of the disease, although in some regions of Asia angle closure glaucoma is more prevalent. An epidemiological study of POAG indicated that nearly 70 million people worldwide, 1-4% of the population over 45 years of age, might be affected (5). An estimated 2.2 million Americans have glaucoma (6), and an equal number may have the disease without knowing it. As the population ages and life expectancy increases, estimates target greater than 3 million cases of POAG in the U.S. by 2020. This is a large market that will experience change over the next few years due to a string of patent expirations, notably Pfizer's prostaglandin agonist Xalatan (latanoprost) in 2011 (7). Competing therapeutics for glaucoma include topical drops, penetrating and nonpenetrating surgical techniques (8), and implants or shunt devices (9). Newer treatment methods include canaloplasty (10), the iStent (11), Ex-PRESS mini glaucoma shunt (12) and Trabectome (13). The cost of IOP-lowering treatment is estimated to reach $5 billion annually in the U.S. alone by 2011 (14). With an expected increase in disease prevalence and treatment-seeking rates, there remains a need for novel products that offer significant improvements in efficacy, duration of effect and side effect profile.

CURRENT THERAPIES

Topical drops, generally small molecules acting at receptors or acting as mediators in signaling pathways, are the most common initial treatment prescribed for glaucoma. The two main mechanisms of action of common glaucoma therapeutics are to: 1) reduce IOP by decreasing aqueous humor production (β-blockers, α_2-adrenocep-

Table I. *Current medical therapies for glaucoma.*

Target	Examples	Mechanism of action	Side effects
Aqueous humor formation			
β-Adrenoceptor blockers	Timolol maleate, betaxolol HCl, carteolol HCl, levobunolol HCl, metipranolol	Block sympathetic nerve endings in the ciliary epithelium, causing a decrease in aqueous humor formation by the ciliary processes	Transient stinging or burning, low blood pressure, low heart rate, bronchospasm
α-Adrenoceptor blockers	Clonidine, apraclonidine, brimonidine tartrate	Inhibit cAMP formation, as well as activate presynaptic α_2-adrenoceptors to regulate noradrenaline release, reducing aqueous humor production and increasing uveoscleral outflow	Dry mouth, conjunctival redness, dry eye, fatigue, allergic conjunctivitis
Carbonic anhydrase inhibitors	Dorzolamide, brinzolamide	Inhibit carbonic anhydrase and bicarbonate production in ciliary process, decreasing aqueous humor formation	Transient burning or stinging, bitter taste, conjunctival redness
Aqueous humor outflow (conventional)			
Cholinergics	Pilocarpine HCl, carbachol	Increase in outflow through contraction of ciliary muscle	Unwanted accommodation, blurred vision, cataract, poor vision in dim light, headache
Aqueous humor outflow (uveoscleral)			
Prostaglandins	Latanoprost, travoprost, bimatoprost, unoprostone	Stimulate prostaglandin FP receptors, leading to an increase in MMP activation and ECM remodeling of ciliary body	Transient burning or stinging, irritation, eyelash growth, iris pigmentation changes (increase in brown pigment)

MMP, matrix metalloproteinases; ECM, extracellular matrix.

tor agonists, carbonic anhydrase inhibitors); or 2) increase aqueous humor outflow through the uveoscleral pathway (prostaglandin agonists, α_2-adrenoceptor agonists) (Table I). The most commonly prescribed drugs in these classes act on different parts of the ciliary body: β-blockers act on β-adrenoceptors in the ciliary processes (reducing aqueous humor production) and prostaglandin analogues effectively bind and activate prostaglandin F (FP) receptors in the ciliary smooth muscle (increasing uvescleral outflow) (15, 16) (Fig. 1).

Prostaglandin analogues are the most frequently used first-line topical drop agents. Prostaglandin analogues are hormones that are synthesized, released and act locally, and are thought to play a role in the normal regulation of aqueous outflow. They are produced by human trabecular endothelial and ciliary muscle cells in culture and are released by various ocular tissues during many types of ocular inflammation (17). Expression of cyclooxygenase-2 (COX-2), a key enzyme involved in prostaglandin synthesis, is lower in the ciliary epithelium in POAG patients (18). Stimulating endogenous FP receptors in the tissues of the monkey outflow pathway increases the production of matrix metalloproteinases MMP-1, -2 and -3 (19), and decreases levels of collagen I, III and IV (20) in the interbundle spaces of the ciliary muscle (CM) and the sclera, resulting in an increase in uveoscleral outflow. Prostaglandin analogues are well tolerated and their side effect profile compares favorably with other drops. The prostaglandin analogue with the largest market share (Pfizer's Xalatan, latanoprost) is reported to have annual sales of $1.6 billion. The patent expires in 2011, sparking a number of generic market entries, most of which have minor formulation changes aimed at boosting efficacy.

Formulation modifications

Conventional topical eyedrops have poor bioavailability. It is estimated that only 1-7% of the active ingredient is absorbed into the eye (15). Corneal permeability is hindered by solution drainage, lacrimation and tear turnover, tear evaporation and conjunctival absorption. Nasolacrimal drainage and conjunctival absorption can lead to systemic side effects from topical drops. Newer formulations aim to increase ocular contact time through manipulation of solution viscosity with polymers, collagen shields, gels, nanoparticles, microemulsions and liposomes. Nanoparticles for ophthalmic use are often small polymeric colloidal particles in which the therapeutic agent is either encapsulated in a polymer (nanocapsule) or dispersed in the polymer matrix (nanosphere) (21). An advantage is that they can be engineered to be relatively cell-specific (22). Liposomes can be produced from natural phospholipids and cholesterol. They are used to encapsulate drugs inside the cavity or between the bilayers, depending on the hydrophilicity or the hydrophobicity of the drug. Microemulsions use dispersions of water and oil with surfactant and cosurfactant in order to stabilize the areas of interface between the components.

Formulation changes can result in better corneal penetration, increased bioavailability and longer-lasting therapeutic effects,

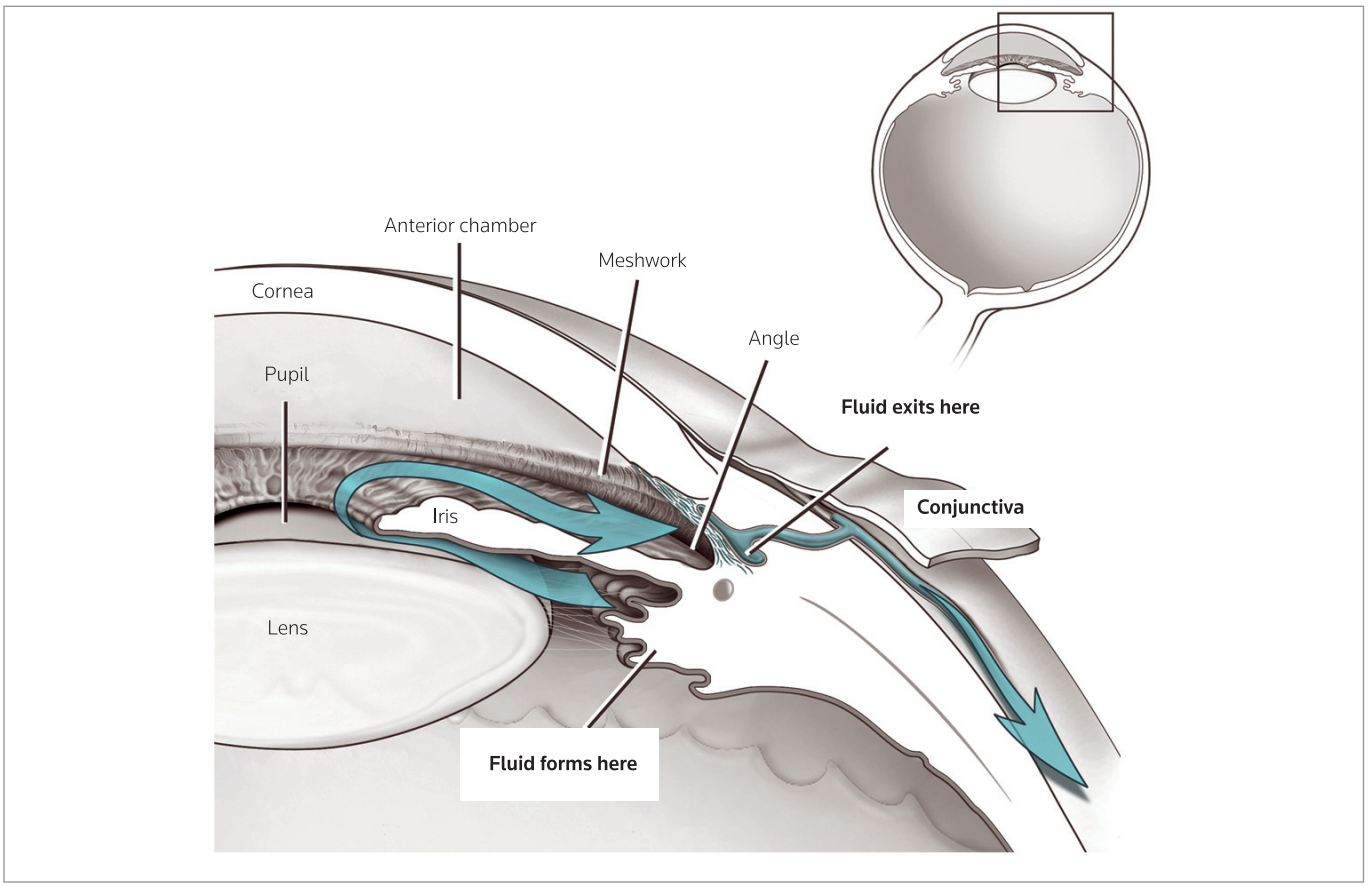

Figure 1. Aqueous humor formation and outflow relevant structures in the eye. Courtesy: National Eye Institute, National Institutes of Health (http://www.nei.nih.gov/photo/).

which can in turn result in less frequent dosing, better patient adherence and increased efficacy in controlling IOP. Several large studies have reported that even moderate decreases in IOP can halt or slow progression of the disease. The Early Manifest Glaucoma Trial Group (EMGT) reported that the progression hazard ratio increases by 11% for each 1 mmHg of higher IOP (23). The Ocular Hypertension Treatment Study (OHTS) found a 9.5% risk over 5 years to convert from ocular hypertension to POAG if untreated (24). Reducing IOPs is also critical to slow disease progression for those with normal-tension glaucoma (25). Formulation changes that result in greater IOP-lowering efficacy or patient compliance/adherence are highly sought after in glaucoma therapeutics.

Preservatives

The most common preservative in topical drops is the detergent quaternary ammonium compound benzalkonium chloride. While familiar to industry and possessing good antimicrobial properties, it has been associated with tear film and corneal surface irregularities (26). Ocular surface disease (OSD) refers to a constellation of disorders that affect the ocular surface (eyelids, conjunctiva and the multilayered corneal surface). Symptoms include burning, redness, irritation, fatigue, fluctuating visual acuity, infection and possible loss of vision. As many as 59% of patients with POAG or ocular hypertension report symptoms of OSD in at least one eye (27). Symptom severity is positively correlated with the number of IOP-lowering medications used and is commonly attributed to benzalkonium chloride (28). For the preservative-sensitive subset of patients, fixed-dose combination drops that reduce exposure by combining two active ingredients in one bottle, alternative preservatives or preservative-free drugs have been shown to reduce symptoms (29). Patients switching from latanoprost to benzalkonium chloride-free travoprost showed no significant decrease in hyperemia after 1 month, but showed significant decreases at 3 and 12 months compared with baseline ($P < 0.05$) (30). That reductions in symptoms can take 12 or more weeks may be the reason that shorter-term studies have not shown a difference between preservative and preservative-free groups. Also, not all symptoms of OSD are due to sensitivity to preservatives.

In addition to preservative-free unit-dose packaging or bottles with inherent antimicrobial properties (surface coatings and one-way valves), a number of companies are developing alternative classes of preservatives with better side effect profiles: sodium perborate (GenAqua), ionic-buffered preservative system (SofZia; e.g., used in Travatan-Z; Alcon), stabilized oxychloro complex (SOC/Purite) and polyquaternium-1 (Polyquad) (31). Global regulatory differences (between the FDA, EMEA, Japanese and Australian agencies) and

patient population preferences for the types of preservatives used in multiple-dose vials can make formulation decisions difficult.

Interestingly, benzalkonium chloride can have a beneficial effect beyond its strength as a preservative. It can enhance corneal permeability by disrupting cell–cell junctions, increasing the amount of drug that enters the anterior chamber (32). It is unclear to what extent these disruptions contribute to OSD in susceptible patients, but the effects may depend somewhat on the active ingredient benzalkonium chloride is paired with. In several in vitro and ex vivo studies, benzalkonium chloride alone in commercial formulation concentrations produced more cell death and inflammation than benzalkonium chloride with prostaglandin analogues (33-35). The authors suggest that the prostaglandin active ingredient may have a cytoprotective effect that can mitigate some of the deleterious corneal effects of benzalkonium chloride. More data correlating human and animal in vivo and in vitro data are needed to assess long-term effects.

While not all patients need to use preservative-free or benzalkonium chloride-free formulations, these changes do appear to benefit a subset of glaucoma patients. Treatment decisions will depend on individual patient reactions to preservative-containing drops. Some patients will benefit from switching to alternative preservatives or preservative-free medications, but the beneficial effects may not be seen for 3 months.

TOPICAL DROPS IN THE PIPELINE

Most glaucoma patients will be prescribed multiple drops of varying classes of compounds to control their IOP. There is much interest in developing compounds with a longer duration and novel mechanism of action that would be stand-alone products or additive with the prostaglandins.

Cytoskeletal agents

The newest class of compounds in the glaucoma armamentarium aims to increase conventional or trabecular outflow by directly targeting the extracellular matrix (ECM) and the actin cytoskeleton of the trabecular meshwork (TM) and CM. This pathway may account for 50-75% of aqueous humor outflow (36). Muscarinic agonists (e.g., pilocarpine) were the first class of compounds to target the TM, even if indirectly. These compounds contract the CM, creating traction on the scleral spur that in turn expands and in essence relaxes the TM, reducing resistance to outflow (37). In contrast, cytoskeletal agents affect the structural and functional biology of the TM itself by altering the dynamics of cell–cell and cell–matrix adhesions and contractility, thereby also relaxing and expanding the TM and reducing resistance. Together, CM- and TM-centered control of TM contraction/relaxation may function in synchrony to provide fine control of outflow resistance (36).

ECM synthesis and degradation is a dynamic process that can be manipulated to advantage to increase outflow, regardless of what the percentages of outflow through pathways in the juxtacanalicular meshwork and the inner wall of Schlemm's canal may be. Altering the actin microfilament system (by cytochalasins, latrunculins, etc.) or acto-myosin contractility (by myosin light chain kinase or rho-associated protein kinase inhibitors, or by overexpression of caldesmon) reduces outflow resistance in live monkeys and in human/monkey organ cultured perfused anterior segments. Morphological studies show that the common effect of these agents is the relaxation of TM, juxtacanicular tissue and inner wall of Schlemm's canal cells, as well as the TM overall. Cellular relaxation leading to a "relaxed" tissue configuration may be a geometrically/biomechanically critical event and may be a fundamental endogenous control mechanism for outflow resistance, providing validation of this as a therapeutic target for resistance reduction in glaucomatous eyes (38). Several compounds in this class are currently in phase I and II clinical trials.

Fixed-dose combination

Fixed-dose combination drops containing two or more active molecules are a fast-growing segment in the IOP-lowering topical drop medication market. The first fixed-dose combinations all contained a β-blocker (timolol), but newer iterations contain prostaglandins or have been specifically developed to be used in conjunction with prostaglandins. Fixed-dose combinations can reduce the number of instillations per day, reduce the washout effect from subsequent drops, reduce exposure to preservatives and decrease patient inconvenience with multiple bottles and waiting times between drop instillations. Combination drugs may be advantageous in patients who need a large decrease in IOP. For many patients, fixed-dose combinations improve adherence and offer a better IOP-lowering effect than the individual compounds, but this is not always the case (39-42). Combinations currently approved by the FDA in the U.S. are Cosopt (Merck), which combines timolol and dorzolamide (a topical carbonic anhydrase inhibitor) and Combigan, which combines brinzolamide and timolol maleate. Fixed-dose combinations with prostaglandin analogues –travoprost/timolol (DuoTrav), latanoprost/timolol (Xalacom), bimatoprost/timolol maleate (Ganfort)– have been approved in Europe and elsewhere but are still in clinical trials in the U.S. Clinical trials for triple combination drops, bimatoprost/brimonidine tartrate/timolol ophthalmic solution (Triple Combination Therapy), are not yet recruiting.

Novel prostanoid FP and EP receptor agonists

Commercially available prostaglandins act on the prostanoid FP receptor, which is a receptor for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). A novel $PGF_{2\alpha}$ compound in phase II clinical trials for ocular hypertension is PF-3187207, which is thought to contain a nitric oxide (NO)-donating element that increases its efficacy in reducing IOP (43, 44). Other prostaglandin analogues target the prostanoid EP_2 and EP_4 receptors. While the prostanoid EP_2 receptor agonist butaprost appears to lower IOP by increasing uveoscleral outflow (45), other EP_2 receptor agonists (e.g., taprenepag isopropyl [formerly known as PF-04217329]) appear to be additive to latanoprost, suggesting that there may be a different mechanism of action with this class of compounds. Similarly, the selective prostanoid EP_4 receptor agonist 3,7-dithia-PGE_1 lowered IOP in monkeys. Total outflow facility was increased, but there was no effect on uveoscleral outflow or aqueous flow. Increased trabecular outflow facility was thought to account for a substantial proportion of the ocular hypotensive activity (46). The prostanoid EP_4 receptor agonist PF-04475270, a prodrug of CP-734432, lowered IOP in dogs after single and multiple days of dosing by up to 45% at 24 hours post-dose relative to vehicle (47).

Adenosine agonists

Adenosine A_1 receptor agonists, as well as A_3 receptor antagonists, show potential for development as IOP-lowering therapies. Aqueous

adenosine levels are positively correlated with IOP in ocular hypertensive individuals and could possibly serve as an endogenous modulator of IOP (48). IOP is decreased and outflow facility increased in monkeys following topical application of adenosine A_1 receptor agonists (49). In bovine organ cultured anterior segments, the outflow facility increase produced by the adenosine A_1 receptor agonist cyclohexyladenosine is also associated with MMP activation (50). A selective adenosine A_1 receptor agonist, INO-8875, which is now in clinical trials, reportedly has a favorable pharmacokinetic profile and increases outflow facility in pig organ cultured anterior segments (51). Knockout of adenosine A_3 receptors reduces IOP in the living mouse, likely through a reduction in inflow (52). Several observations obtained with isolated cells have suggested that adenosine A_3 receptors physiologically increase inflow of aqueous humor by activating Cl^- channels of the nonpigmented ciliary epithelial cells at the aqueous surface of the ciliary epithelium. Nucleoside-based derivatives are promising adenosine A_3 receptor antagonists for study in multiple animal models (53). The adenosine A_{2A} receptor agonist OPA-6566 is also under development for the treatment of glaucoma. The compound is thought to lower IOP by stimulating aqueous humor outflow via the trabecular meshwork through activation of adenosine A_{2A} receptors.

Endothelin antagonists

Endothelin, produced by endothelial cells, is a potent vasoconstrictor. Two receptors for endothelin-1 (ET-1), ET_A and ET_B, mediate vasoconstriction through the regulation (increase) of intracellular calcium levels (54). ET_A and ET_B receptors are found on both the ciliary body and trabecular meshwork and may contribute to contractility and outflow resistance, in turn affecting IOP (55). Endothelin is also associated with vascular dysregulation and altered ocular blood flow, which may lead to ischemic damage to the optic nerve head and retinal ganglion cells. ET-1 may mediate ECM remodeling, possibly contributing to increased collagen deposition, reduced aqueous humor outflow facility and progressive damage to the optic nerve head (56). Several ET-1 receptor antagonists have been tested in animals and humans. In glaucomatous monkeys, avosentan (SPP-301), an ET_A receptor antagonist, significantly reduced IOP (57). In a clinical trial, a dual ET_A/ET_B receptor blocker, bosentan, significantly increased blood flow to the retina, choroid and optic nerve head, but had no effect on IOP (58).

The ET-1 pathway appears to show promise as a target for a variety of glaucoma therapies. Current endothelin-based clinical trials are studying the effects of endothelin antagonists on IOP in pulmonary hypertension patients (59), ocular blood flow in glaucoma patients (60) and optic nerve head blood flow autoregulation (61).

Anecortave acetate

Patients with noninfectious posterior uveitis are usually treated with topical glucocorticoids administered several times daily or the synthetic corticosteroid fluocinolone acetonide in an implant (Retisert; Bausch & Lomb). Patients with a retinal vein occlusion that is causing macular edema may be treated with an implant containing the corticosteroid dexamethasone (Ozurdex; Allergan). Intraocular steroid injections are often used to treat diabetic and age-related retinal diseases. These treatments cause a significant increase in IOP in about one-third of the general population, with a substantial increase (> 15 mmHg) in about 5% (62, 63). An anterior juxtascleral depot of anecortave acetate (Retaane; Alcon), an angiostatic cortisene, has been reported to reduce IOP in patients with steroid-induced glaucoma (64, 65). A clinical study to evaluate if prophylactic anterior juxtascleral depot administration of anecortave acetate could prevent Retisert-induced elevated IOP did not meet its clinical target, but the search for steroid-induced glaucoma treatments is continuing. Recently, researchers developed both ovine and bovine animal models for glucocorticoid-induced ocular hypertension (66) and are working on a novel treatment strategy using a glucocorticoid-inducible adenovirus-based vector that overproduces MMP-1 only in the presence of dexamethasone. In primary human trabecular meshwork cells, during sequential off and on cycles, MMP-1 expression was only detected in the presence of dexamethasone (the on cycle). The goal is a single-administration therapy that is only active when a patient is on steroid treatment (67).

Protein Wnt signaling pathway

The Wnt signaling pathway consists of a network of proteins involved in multiple physiological processes in adult animals, including the regulation of hippocampal neural stem cell behavior, cell growth and apoptosis (68). Wnt signaling pathway components are found in the cornea, trabecular meshwork, ciliary body, lens epithelium and retina of the adult eye (69). Secreted frizzled-related protein 1 (sFRP-1, *SFRP1*) is a modulator of Wnt. When Wnt binds to sFRP-1, it inhibits the Wnt pathway. sFRP-1 expression is elevated in glaucomatous trabecular meshwork cells compared to normal trabecular meshwork cells (70). Overexpression of sFRP-1 in mouse eyes by intravitreal injection of an adenoviral vector encoding sFRP-1 produced a viral titer/sFRP-1 expression-dependent increase in IOP. This increase was inhibited when eyes were cotreated with a selective inhibitor of glycogen synthase kinase-3 (GSK-3), a downstream suppressor of Wnt signaling. Similarly, IOP was elevated in mice after overexpression of the canonical Wnt pathway inhibitor Dickkopf-related protein 1 (Dkk-1), and treatment with sFRP-1 protein resulted in downregulated β-catenin levels and decreased outflow facility in perfusion cultured human anterior segment (69). These data indicate that Wnt signaling plays a role in regulating IOP, that increased expression of sFRP-1 in the TM appears to be associated with elevated IOP, and that restoring Wnt signaling in the TM may be a novel disease intervention strategy for treating glaucoma.

GENE TRANSFER STRATEGIES

Gene transfer is another proposed strategy for producing long-term IOP-lowering effects. The aim in this case is to up- or downregulate a biochemical or physiological process, i.e., to reprogram target cells to express or repress gene products that will have long-term effects on trabecular outflow, uveoscleral outflow, aqueous humor formation or retinal ganglion cell survival.

Nonviral gene transfer methods

Both viral and nonviral gene transfer methods have proponents. Nonviral gene delivery methods can be categorized as mechanical, physical and chemical. They have some important advantages over virus-based vectors, including low immunogenicity, a large capacity for DNA size, ease of manipulation and low-cost production (71). In

general, nonviral vectors are less efficient in gene delivery –it can take larger amounts of vector to get the same response as with a viral vector. Most also have a relatively short therapeutic duration (e.g., naked DNA), whereas viral vectors offer the possibility of effects/efficacy lasting several years (72-74). There are some limitations as to which cell types can be transfected easily by nonviral methods (including human cultured TM cells), but advances are rapidly being made in this field.

Viral gene transfer methods

Viral delivery systems have high transfection efficiencies but a limited loading capacity and can be difficult to produce in large scale. Newer engineered vectors have dramatically reduced the risk of insertional mutagenesis, but some risk of inflammatory and immunogenic effects remains. The eye is a good target for virus-mediated gene delivery, with its immune privilege, easily accessible and translucent media, which allows visual localization of the transfer process. Several different viral vectors have been investigated for the ocular delivery of genes, including adenovirus (Ad), herpes simplex virus (HSV), adeno-associated virus (AAV) and lentivirus (FIV, EIAV). Long-term green fluorescent protein (GFP) expression was monitored serially and noninvasively in the outflow pathways for over 1 year using a GFP.FIV construct (75, 76) in cats and monkeys (72). Until recently, AAV vectors had not been reported to transduce the TM. It was discovered that the vector was in fact able to enter the cell, but host downregulation of DNA replication did not occur. Self-replicating AAV vectors, referred to as scAAV, appear to be the answer to this and have demonstrated the ability to transduce the TM (77). Expression was documented for over 2 years using an scAAV-based vector with a GFP transgene marker attached (73). Eyes remained quiet and IOP was unaffected. These results demonstrate that transgenes can be safely delivered to specific sites in the eye relevant to glaucoma.

FIV is capable of producing long-term transgene expression in a large animal model and of transducing multiple ocular cell types, including terminally differentiated cells. FIV vectors are also subject to restriction factors (e.g., TRIM5 alpha) that are specific to primates (human and nonhuman). This could limit their usefulness in human gene therapy applications, as it might take a high dose to saturate the receptors and allow expression (78). The strategy of introducing elements of the prostaglandin biosynthesis and response pathways is being pursued using FIV. Vectors were injected, singly and in combination, into the anterior chambers of cats. Individual elements were a codon-optimized COX-2, the FP receptor and PGF synthase. Animals were monitored for 5 months for adverse effects and changes in IOP. The vectors were well tolerated and IOP remained decreased for the duration of the study. The greatest IOP lowering was seen with the COX-2/FP receptor combinations (79).

While AAV vectors account for only ~4% of human clinical trials (80), they are the most common vector used in ocular trials (81). AAV does not elicit the immune responses that Ad or HSV vectors do, is not associated with any human disease and provides long-term expression. AAV is also relatively easy to manipulate and able to carry a reasonably sized payload. AAV vectors are currently in phase II clinical trials for the inherited ocular condition Leber's congenital amaurosis (LCA), an autosomal recessive blinding disease. LCA patients show a loss of retinal and visual function early in life, with a progressive degeneration of retinal cellular structures (82). Versions of an AAV-RPE65 vector have been used by three separate groups in trials with LCA patients that have lasted up to 1.5 years after injection. No serious adverse events, systemic transmission of vector or humoral responses to vector or transgene were reported. Some efficacy was noted in some of the patients (82-84). The positive safety profile in these studies could help facilitate regulatory approval for the use of AAV vectors to treat other ocular conditions. The National Eye Institute (NEI) has an ocular gene therapy unit devoted to studying and developing the therapeutic potential of AAV vectors (85).

Different serotypes exist for AAV with different tropisms and there are now hybrid "pseudotyped" rAAV vectors. In the latter, an rAAV plasmid is packaged in a capsid from a different serotype of AAV. These hybrids have different times of onset, as well as tropism, making it easier to choose a vector for a particular application (86). The majority of 71 clinical trials using rAAV for gene delivery have used serotype 2 (80). This prompted the EMEA to issue a reflection paper on issues surrounding the use of AAV vectors, pointing out the difficulty of selecting a suitable animal model and encouraging extensive studies of biodistribution, which can depend on the route of administration (87).

Gene regulation

Regulation of transgene expression will be critical for gene therapy applications for glaucoma. A number of systems have been developed to effectively turn a transgene off or on in the presence or absence of a transactivator protein. Transactivator proteins most commonly used are mifepristone, ecdysone, rapamycin and tetracycline (88). Using the tetracycline system nomenclature, if transgene expression is allowed only in the absence of tetracycline, the system is called TetOff, whereas if it is allowed in the presence of tetracycline, the system is called TetOn. The first gene regulation system used in ocular studies of large animals was the rapamycin-dependent system (89). This approach expresses the two critical domains of a transcription factor as separate polypeptides. These interact only in the presence of a dimerizer, such as rapamycin. This system was well tolerated in a 2-year study in primates, where no neutralizing antibodies against the viral capsid proteins were seen in serum and histopathological study of the retina did not show anything other than minor scar tissue at the injection site. One drawback is the need to inject the two vectors so that they both transduce the same cells in the retina. Oral administration results in low bioavailability (10%), so high doses are needed for it to function well. Also, rapamycin has shown some immunosuppressive activity, although newer analogues are being developed (90).

The tetracycline (or its derivative doxycycline) regulatable system is attractive because the drugs used to induce transcription are well-known antibiotics with few side effects. Doxycycline and tetracycline have been widely used in patients and their pharmacological and toxicological characteristics are well documented. The serum half-life of doxycycline has been calculated to be 14-22 hours. Prolonged administration of doxycycline can result in side effects that include renal toxicity, dose-dependent photosensitivity and the possibility of raising resistance to the antibiotic itself. However, doxycycline at doses of 40 mg/day or less has not been shown to induce antibiot-

ic-resistant organisms. Also, it may be possible to use 4-epidoxycycline, a hepatic metabolite of doxycycline that has less antibiotic activity and yet was as effective as doxycycline in switching transgene expression on or off (90). Repeated induction cycles of transgene expression in the monkey retina using a tetracycline-inducible system via rAAV vector administration were sustained over 5 years, during which time no antibodies directed against the transactivator (rtTA) could be detected in the eye or in the serum (88). One issue with the tetracycline "on" system is that there is some "leak" and background levels of transgene expression can be problematic if too high. The second generation of tetracycline-responsive promoter elements shows tighter control of background levels (91, 92).

A significant issue with inducible promoters is that an immune response can develop against the proteins involved in transgene regulation, especially in large animal models. In a study in monkeys, humoral and cellular immune responses against the transactivator involved in the tetracycline "on" system (rtTA) were seen, which resulted in a loss of transgene regulation and expression. A later study by the same group showed that the immune response was largely due to the route of administration. Comparing anti-rtTA antibodies in the serum of monkeys that received an i.m. or regional i.v. dose, the regional i.v. group showed good transgene regulation with no detectable antibodies against the rtTA transgene product in naive immunocompetent animals, while the i.m. group mounted an immune response and lost expression (93).

RNA interference (RNAi)

RNAi is a way of silencing the activity of a specific gene of interest. It is a useful strategy in situations where suppressing the expression of a single protein will address the symptoms or pathology of the disease and is being used in 10 clinical trials (80). Briefly, RNAi post-transcriptional gene silencing is triggered by double-stranded (ds) RNA molecules called short interfering RNAs (siRNAs). siRNAs bind to a nuclease-containing multiprotein complex called RISC (RNA-induced silencing complex) and then pair with the complementary target mRNA. The RISC complex cleaves the target mRNA strand, which results in degradation of the mRNA molecule, effectively preventing its translation into protein (94). RNAi therapies can be effective at lower concentrations than small molecules, which potentially means lower doses and fewer adverse effects. While dsRNAs longer than 30 nucleotides can trigger immune responses, siRNAs are smaller (19 and 25 nucleotides are thought to be the optimal size) (95) and do not seem to trigger cellular toxicity (96). Appropriate target identification is critical in RNAi therapeutics. Some companies develop proprietary targets, as well as therapeutics. Quark Pharmaceuticals designed the synthetic siRNA molecule PF-4523655 (RTP-801i-14) to inhibit its proprietary target RTP801, to treat wet age-related macular degeneration (AMD). Sylentis also has a product in development (SYL-040012) to treat ocular hypertension/glaucoma, targeting a β_2-adrenoceptor gene involved in aqueous humor production. A phase I trial has been completed and recruitment is under way for a phase I/II clinical trial.

Challenges remain in advancing RNAi therapeutics. In vivo delivery is the most problematic aspect of RNAi therapeutic development. Effects can be transient, as unmodified, naked siRNAs are quickly degraded by endo- and exonucleases and RNases present in many tissue microenvironments. Delivery to the eye bypasses contact with serum, high in RNases, but it is not clear whether RNases are present in human aqueous humor, although even if enzyme degradation occurs, it may only take a small amount of siRNA to silence the gene (94). Off-target effects are difficult to predict with this emerging technology. While some off-target effects may be the result of formulation issues (e.g., lipid-based transfection reagent) rather than the siRNA itself (97), careful consideration of species differences during preclinical testing is essential. It remains to be seen if species-specific surrogate sequences will need to be designed for preclinical toxicity testing done in parallel with human-specific sequences. In other development aspects, siRNA-based drugs are attractive. Since an RNA synthesizer manufactures the strands of RNA, production scale-up is easier. siRNAs directed against proto-oncogene c-Jun, apoptotic protease-activating factor 1 (APAF-1) or BAX have been used to investigate gene function in apoptosis of RGCs (98). As with other novel strategies, RNAi may be as beneficial in modeling diseases, studying the effects of silencing-specific genes in vitro and in vivo, as it is in treating them.

Encapsulated cell therapy

A novel delivery strategy for ciliary neurotrophic factor (CNTF), a compound used to treat degenerative retinal diseases, is encapsulated cell therapy (ECT). ECT implants allow controlled, continuous delivery of biologics directly to the back of the eye. The implants contain cells that are genetically modified to produce the desired therapeutic factor. To avoid immune reaction, the cells are encapsulated within a semipermeable, hollow-fiber membrane that can be anchored to the sclera but within the vitreous cavity. The CNTF protein is released to ocular tissues, but the modified RPE cells are immunologically isolated from the patient (99). ECT is currently in phase II and III clinical trials for the treatment of geographic atrophy associated with atrophic (dry) AMD and retinitis pigmentosa (100). This same strategy might prove to be effective for glaucoma, although clinical trial endpoints that are difficult and time-consuming to meet can be deterrents to companies with potential neuroprotective compounds.

Constraints and challenges

Gene therapy constraints and challenges include developing tissue-specific promoters; determining the degree and extent of immune/inflammatory responses with subsequent injections should they be needed; developing vectors with multiple cloning sites so that multiple elements of a pathway can be combined in a single injection; identifying which preclinical screening tests could provide a quantitative predictor of immune responses (humoral or cell-mediated cytotoxic responses); development of analytical methods for assessing the quality and activity of the delivery vectors; defining a maximum tolerated dose; biodistribution (e.g., are detected vector sequences due to migration of immune cells that engulfed the vector or vector spread to other organs by moving through the vascular system?); determining how vectors are cleared from the eye in the absence of either an inflammatory response or significant immune response; and the need for dosing levels and timing of systemic corticosteroids.

Despite the many constraints and challenges, the nature of the eye as a target (immune privilege), the small surface area of the TM tis-

sue to be transduced and the small amount of vector needed to do so, the safety demonstrated by the LCA clinical trials using AAV vectors, the number of current ocular gene therapy clinical trials and the number of gene therapy trials worldwide will all positively impact the feasibility of developing this technology.

NEUROPROTECTION

Compounds that might have a neuroprotective effect are of intense interest as glaucoma therapeutics.

The incidence of disease progression in normal-tension glaucoma patients and in patients with seemingly well-controlled IOP implicates mechanisms independent of IOP in glaucomatous degeneration. This has led to expanded research into treatments targeting the cellular basis of apoptosis. Although, as yet, there has been no proof of clinical efficacy for any neuroprotective compound in glaucoma (101, 102), a number of targets have been identified.

β-Amyloid

Recent studies have implicated β-amyloid (Aβ) in the apoptotic cascade responsible for retinal ganglion cell death in glaucoma (103). Aβ levels are decreased in glaucomatous eyes, which is thought to be consistent with Aβ retinal deposition (104). Aβ, injected intravitreally in a rodent model, caused atrophy of large RGCs and a significant increase in the percentage of smaller RGCs. There is evidence that RGC morphological changes, specifically shrinkage and atrophy, are associated with cell death in glaucoma (105). This neuronal damage was thought to be due to a chronic glial response. In a rat ocular hypertension model, Aβ was increased and colocalized with apoptotic RGCs. Intravitreal injection of Aβ peptides resulted in a dose- and time-dependent increase in RGC apoptosis. Intervention by targeting different elements of the Aβ pathway resulted in a reduction in RGC apoptosis. A combination of treatment elements affecting multiple stages in the pathway was the most effective at blocking apoptosis (resulting in a reduction of ~80%) (106). This has potential as a new therapeutic strategy for protecting RGCs in glaucoma. Local delivery of these elements would make it easier to meet the desired profile of a neuroprotectant, which is to reach the optic nerve head and/or ganglion cells at therapeutically meaningful levels with minimal off-target effects.

Memantine

Memantine, approved by the FDA for the treatment of Alzheimer's disease and used for Parkinson's disease in Europe, is a low- to moderate-affinity, noncompetitive NMDA receptor antagonist that binds to the receptor molecule for glutamate on the cell surface, preventing glutamate from attaching to the cell (107). Without glutamate, calcium cannot enter the nerve cells, which are thus protected from toxic levels of calcium. This process does not interfere with the normal functioning of the cell, making memantine an attractive compound.

Phase III clinical trials investigating memantine as a neuroprotectant in glaucoma were conducted. The trials enrolled 2,200 patients worldwide and followed them for 4 years. It is estimated that the trials cost approximately $80 million, spanning a 7-year period (108). Patients were heterogeneous, having diverse types of glaucoma, at varying stages, with multiple IOP-lowering treatment strategies, including surgery. The trials failed to meet the primary functional endpoint of visual field progression, as assessed by full-threshold examinations performed every 6 months. That memantine did not meet the study endpoint criteria may say as much about the study design and endpoints themselves as it does for the efficacy of the compound. It has been suggested that future neuroprotection trials attempt to enroll a more homogeneous population (same glaucoma diagnosis, similar levels of optic nerve and visual field damage, IOP limits and glaucoma treatments) to better assess efficacy and benefit to subgroups, although this could prolong recruitment (109, 110). Biologically and clinically relevant endpoints need to be developed that can be used in both animal and human trials (109). A key issue is the current inability to translate laboratory evidence of neuroprotection into clinical evidence of neuroprotection (111). While the FDA currently requires visual function as the primary endpoint, new endpoint criteria based on advances in clinical imaging technologies may be forthcoming (see below) (112).

Another novel method of determining the efficacy in neuroprotection may be magnetic resonance imaging (MRI), which allows visualization of the lateral geniculate nucleus (LGN) and assessment of shrinkage of visual structures along the geniculo-cortical pathway. The LGN is atrophied in both human (113) and experimental models (114) of glaucoma, demonstrating that there is central neural degeneration in glaucoma away from the retina and optic nerve head, and that this has potential as a biomarker (113, 115).

Clinically, cost/benefit decisions for neuroprotective agents would need to consider that any neuroprotective therapy will likely be required for life, be adjunctive to current IOP-lowering treatments (in cost as well), and initially be reserved for patients still progressing after current therapeutic strategies have been exhausted. As with any treatment decision, a careful, individualized review of a patient's overall medical situation, taking into account ocular and systemic contraindications, would be necessary. Opinion varies widely among ophthalmologists about the advisability of life-long systemic therapy, especially for patients whose disease appears to be stable on existing therapeutics.

It has been suggested that, given the variability of ganglion cell receptor profiles, axonal length and position within the globe, apoptotic triggers will vary (116). It may be that a combination of antagonists/agonists acting directly on multiple receptors (not only NMDA receptors) will be most effective for treating glaucoma (108). A combination of administration methods (intravitreal and/or continuous infusion to the visual cortex) was used to deliver brain-derived neurotrophic factor (BDNF) in a cat optic nerve crush model. Animals that received BDNF via both routes showed increased ganglion cell survival and improved pattern electroretinographic (PERG) responses compared to animals that received only intravitreal BDNF (117, 118). In addition to BDNF, ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) are being investigated for their role in the pathogenesis of glaucoma and as potential neuroprotective treatments (119).

Brimonidine

The commonly prescribed hypotensive agent brimonidine, an α_2-adrenoceptor agonist, is thought to have some neuroprotective properties/effects. It can induce the release of several neurotrophins

(BDNF, CNTF and basic fibroblast growth factor [bFGF]), which may prevent apoptosis (120-122). While there is currently insufficient evidence to say that α_2-adrenoceptor agonists are neuroprotective clinically, this is under investigation. An intravitreal implant containing brimonidine tartrate is now in a phase II clinical study for the evaluation of safety and effects on visual function in patients with glaucomatous optic neuropathy. Brimonidine is also in a clinical study to determine its effects on retinal blood flow autoregulation (123). Ocular blood flow disturbances may play a role in the pathogenesis of glaucoma and are therefore of interest for glaucoma therapeutics. Changes in posture can affect retinal blood flow (124). Normally, the vasculature automatically dilates and constricts as needed to maintain a steady rate of blood flow. Patients with normal-tension glaucoma do not always autoregulate blood flow –when lying down, blood flow increases (125). Some patients taking brimonidine were able to maintain a steady blood flow rate similar to control subjects (123, 126).

FUTURE DIRECTIONS

Biomarkers

Another brimonidine clinical trial is aimed at studying the relationship between the IOP-lowering efficacy of topically administered brimonidine and α_2-adrenoceptor polymorphisms. Responses to treatment with brimonidine show much interindividual variability. Some of this could be due to corneal differences influencing the pharmacokinetic properties of the drug, but polymorphisms of the α_2-adrenoceptor could also have an effect. This trial highlights a significant unmet need in glaucoma therapeutics –the identification of biomarkers. Although investigation into the association of Aβ and RGC degeneration in glaucoma is preliminary, it is possible that serum levels of Aβ oligomers could be a biomarker for the RGC dysfunction or for treatment efficacy (127). Serum antibodies have potential as biomarkers. Elevated serum antibodies to rhodopsin, retinal antigens and heat shock proteins have been detected in patients with normal-tension glaucoma, suggesting that immunoregulation and autoimmune mechanisms play a role in glaucoma, particularly in normal-pressure glaucoma (128, 129). MRI used to measure atrophy of the LGN associated with glaucoma has shown potential as a biomarker in animal and human studies (113, 114).

Patient adherence

A number of clinical trials currently recruiting patients are investigating ways of increasing adherence to topical drop therapies (130, 131). Over time, most patients will need more than a single class of topical drop to control their disease (132). Patient adherence with topical drop therapies decreases with increased complexity and frequency of dosing regimens. Estimates of nonadherence range from 24% to 59% (133-135). Patients diagnosed with glaucoma had better adherence to topical drop therapy than those diagnosed as glaucoma suspects (136). An area of interest in glaucoma therapeutics is the development of strategies to monitor patient adherence and prompt patients at the appropriate dosing times (137). In a report on barriers to adherence (138), four main problem areas and their contribution to poor adherence were identified: situational/environmental (49%), regimen factors (32%), individual patient factors and medical provider factors (19%). Surprisingly, older age was not a consistent risk factor for poor adherence to medication regimens. Strategies for increasing adherence include simplifying medication regimens, lowering costs and better patient education about the risks of skipping doses. Other things noted to improve adherence are good family/social support, regular physician visits and teaching patients to pair medication administration with specific activities (139).

IOP monitoring

More comprehensive monitoring of IOP may have some predictive value in disease progression (140). Studies of IOP range indicate that office visit IOP measurements do not reflect the true extent of a patient's IOP fluctuation and risk of disease progression (23). Measurement of IOP over a 24-hour span in glaucoma patients shows that peak IOP and a larger fluctuation in IOP values may be found outside of office hours (141, 142). Current home measurement of IOP with hand-held devices varies in accuracy across patients. Several measurement devices are in development that use contact lenses or sensors to provide continuous IOP measurement and remove some of the patient variability. Until reproducible, accurate measurements can be collected, it will not be known how much this information will add to the treatment decision-making process.

Models for neuroprotective drugs

Current in vitro and in vivo models of glaucomatous neurodegeneration parallel putative aspects of the disease process (e.g., ischemia, biomechanics of the optic nerve), but do not accurately model the complex neurodegenerative process. In the case of neuroprotection, although animal and cell models have shown benefits for neuroprotective strategies, effective translation to humans has not been made. There are a number of reasons for this: the models may not be similar enough to the human disease; the pathophysiology of the animal model may be fundamentally different from humans; the variability in humans is much greater than in animal models; and the timing of interventions is different (110). Additionally, earlier and more accurate methods for measuring disease progression are needed.

Clinical trial endpoints

The current gold standard clinical trial endpoints in glaucoma are changes in IOP and visual field progression for assessment of clinical IOP-lowering efficacy and functional changes in the optic nerve. Approximately 20-40% of RGCs may be lost before visual field defects are detected (1), and it may take 5 or more years to detect progression (143). This lengthens the time and increases the costs of clinical trials (143), hindering introduction of new therapeutics to the market. Advances in the sensitivity and specificity of optic nerve head and retinal nerve fiber layer imaging technologies may allow their use as outcome measures in clinical trials, provided they are shown to be predictive of function that is clinically relevant to a patient (112). The three main technologies used for detecting structural changes are scanning laser polarimetry, confocal scanning laser ophthalmoscopy and optical coherence tomography. In this rapidly evolving field, changes in technology, compatibility issues and global regulatory differences have limited longitudinal data collection. Nevertheless, examples of structural change preceding visual field change have been reported using all three of the commercially available ocular imaging instrument platforms (144-147). The same imaging instruments used in the clinic can be used in experi-

mental animal models. There is significant value in maintaining continuity in imaging techniques throughout basic research, preclinical testing, clinical trials and post-marketing surveillance.

CONCLUSIONS

Treatment strategies for glaucoma will continue to see an increase in specificity for the individual patient, i.e., their type of glaucoma, underlying mechanisms, comorbid conditions, genetic makeup and speed of progression. Novel targets will continue to be in demand, as glaucoma is a life-long condition that requires a multifaceted, additive approach to medical treatment.

DISCLOSURES

The authors received support from the NIH/NEI R01 EY002698, NEI P30 EY016665, Research to Prevent Blindness, Ocular Physiology Research and Education Foundation.

REFERENCES

1. Kerrigan-Baumrind, L.A., Quigley, H.A., Pease, M.E., Kerrigan, D.F., Mitchell, R.S. *Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons*. Invest Ophthalmol Vis Sci 2000, 41(3): 741-8.
2. Guo, L., Cordeiro, F.M. *Assessment of neuroprotection in the retina with DARC*. Prog Brain Res 2008, 173: 437-50.
3. Resnikoff, S., Pascolini, D., Etya'ale, D. et al. *Global data on visual impairment in 2002*. Bull WHO 2004, 82(11): 84-51.
4. Quigley, H.A., Broman, A.T. *The number of people with glaucoma worldwide in 2010 and 2020.* Br J Ophthalmol 2006, 90(3): 262-7.
5. Clark, A.F., Yorio, T. *Ophthalmic drug discovery*. Nat Rev Drug Discov 2003, 2(6): 448-59.
6. Friedman, D.S., Wolfs, R.C., O'Colmain, B.J. et al.; Eye Diseases Prevalence Research Group. *Prevalence of open-angle glaucoma among adults in the United States*. Arch Ophthalmol 2004, 122(4) : 532-8.
7. http://www.businesswire.com, Accessed July 30, 2010.
8. Mosaed, S., Dustin, L., Minckler, D.S. *Comparative outcomes between newer and older surgeries for glaucoma*. Trans Am Ophthalmol Soc 2009, 107: 127-33.
9. Yalvac, I.S., Eksioglu, U., Satana, B., Duman, S. *Long-term results of Ahmed glaucoma valve and Molteno implant in neovascular glaucoma*. Eye 2007, 21(1): 65-70.
10. Godfrey, D.G., Fellman, R.L., Neelakantan, A. *Canal surgery in adult glaucomas*. Curr Opin Ophthalmol 2009, 20(2): 116-21.
11. Minckler, D.S., Hill, R.A. *Use of novel devices for control of intraocular pressure.* Exp Eye Res 2009, 88(4): 792-8.
12. Hendrick, A.M., Kahook, M.Y. *Ex-PRESS mini glaucoma shunt: Surgical technique and review of clinical experience.* Expert Rev Med Devices 2008, 5(6): 673-7.
13. Filippopoulos, T., Rhee, D.J. *Novel surgical procedures in glaucoma: Advances in penetrating glaucoma surgery.* Curr Opin Ophthalmol 2008, 19(2): 149-54.
14. Lee, P.P., Walt, J.G., Doyle, J.J. et al. *A multicenter, retrospective pilot study of resource use and costs associated with severity of disease in glaucoma.* Arch Ophthalmol 2006, 124(1): 12-9.
15. Ghate, D., Edelhauser, H.F. *Barriers to glaucoma drug delivery*. J Glaucoma 2008, 17(2): 147-56.
16. Medeiros, F.A., Weinreb, R.N. *Medical backgrounders: Glaucoma*. Drugs Today 2002, 38(8): 563.
17. Matsumoto, S., Yorio, T., Magnino, P.E., DeSantis, L., Pang, I.H. *Endothelin-induced changes of second messengers in cultured human ciliary muscle cells.* Invest Ophthalmol Vis Sci 1996, 37(6): 1058-66.
18. Maihöfner, C., Schlötzer-Schrebardt, U., Gühring, H. et al. *Expression of cyclooxygenase-1 and -2 in normal and glaucomatous human eyes.* Invest Ophthalmol Vis Sci 2001, 42(11): 2616-24.
19. Gaton, D.D., Sagara, T., Lindsey, J.D., Gabelt, B.T., Kaufman, P.L., Weinreb, R.N. *Increased matrix metalloproteinases 1, 2, and 3 in the monkey uveoscleral outflow pathway after topical prostaglandin F(2 alpha)-isopropyl ester treatment*. Arch Ophthalmol 2001, 119(8): 1165-70.
20. Sagara, T., Gaton, D.D., Lindsey, J.D., Gabelt, B.T., Kaufman, P.L., Weinreb, R.N. *Topical prostaglandin F2alpha treatment reduces collagen types I, III, and IV in the monkey uveoscleral outflow pathway.* Arch Ophthalmol 1999, 117(6): 794-801.
21. Barbu, E., Sarvaiya, I., Green, K.L., Nevell, T.G., Tsibouklis, J. *Vinylpyrrolidone-co-(meth)acrylic acid inserts for ocular drug delivery: synthesis and evaluation*. J Biomed Mater Res A 2005, 74(4): 598-606.
22. Cai, X., Conley, S.M., Nash, Z., Fliesler, S.J., Cooper, M.J., Naash, M.I. *Gene delivery to mitotic and postmitotic photoreceptors via compacted DNA nanoparticles results in improved phenotype in a mouse model of retinitis pigmentosa.* FASEB J 2010, 24(4): 1178-91.
23. Bengtsson, B., Leske, M.C., Hyman, L., Heijl, A.; Early Manifest Glaucoma Trial Group. *Fluctuation of intraocular pressure and glaucoma progression in the early manifest glaucoma trial*. Ophthalmology 2007, 114(2): 205-9.
24. Kass, M.A., Heuer, D.K., Higginbotham, E.J. et al. *The ocular hypertension treatment study*. Arch Ophthalmol 2002, 120(6): 701-13.
25. Collaborative Normal-Tension Glaucoma Study Group. *Comparison of glaucomatous progression between untreated patients with normal tension glaucoma and patients with therapeutically reduced intraocular pressure.* Am J Ophthalmol 1998, 126(4): 487-97.
26. Noecker, R.J., Herrygers, L.A., Anwaruddin, R. *Corneal and conjunctival changes caused by commonly used glaucoma medications.* Cornea 2004, 23(5): 490-6.
27. Leung, E.W., Medeiros, F.A., Weinreb, R.N. *Prevalence of ocular surface disease in glaucoma patients.* J Glaucoma 2008, 17(5): 350-5.
28. Fechtner, R.D., Godfrey, D.G., Budenz, D., Stewart, J.A., Stewart, W.C., Jasek, M.C. *Prevalence of ocular surface complaints in patients with glaucoma using topical intraocular pressure-lowering medications*. Cornea 2010, 29(6): 618-21.
29. Katz, G., Springs, C.L., Craven, E.R., Montecchi-Palmer, M. *Ocular surface disease in patients with glaucoma or ocular hypertension treated with either BAK-preserved latanoprost or BAK-free travoprost*. Clin Ophthalmol 2010, 4: 1253-61.
30. Aihara, M., Otani, S.I., Kozaki, J., Unoki, K., Takeuchi, M., Minami, K., Miyata, K. *Long-term effect of BAK-free travoprost on ocular surface and intraocular pressure in glaucoma patients after transition from latanoprost*. J Glaucoma 2011, Epub ahead of print.
31. Freeman, D.P., Kahook, M.Y. *Preservatives in topical ophthalmic medications: Historical and clinical perspectives*. Expert Rev Ophthalmol 2009, 4(1): 59-64.
32. Ramselaar, J.A., Boot, J.P., van Haeringen, N.J., van Best, J.A., Oosterhuis, J.A. *Corneal epithelial permeability after instillation of ophthalmic solutions containing local anaesthetics and preservatives.* Curr Eye Res 1988, 7(9): 947-50.

33. Guenoun, J.M., Baudouin, C., Rat, P., Pauly, A., Warnet, J.M., Brignole-Baudouin, F. *In vitro study of inflammatory potential and toxicity profile between latanoprost, travoprost, and bimatoprost on conjunctiva-derived epithelial cells.* Invest Ophthalmol Vis Sci 2005, 46(7): 2444-50.
34. Pisella, P.J., Debbasch, C., Hamard, P., Creuzot-Garcher, C., Rat, P., Brignole, F., Baudouin, C. *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(5): 1360-8.
35. Guenoun, J.M., Baudouin, C., Rat, P., Pauly, A., Warnet, J.M., Brignole-Baudouin, F. *In vitro comparison of cytoprotective and antioxidative effects of latanoprost, travoprost, and bimatoprost on conjunctiva-derived epithelial cells*. Invest Ophthalmol Vis Sci 2005, 46(12): 4594-45.
36. Kaufman, P.L. *Enhancing trabecular outflow by disrupting the actin cytoskeleton, increasing uveoscleral outflow with prostaglandins, and understanding the pathophysiology of presbyopia interrogating Mother Nature: Asking why, asking how, recognizing the signs, following the trail.* Exp Eye Res 2008, 86(1): 3-17.
37. Kaufman, P.L., Barany, E.H. *Loss of acute pilocarpine effect on outflow facility following surgical disinsertion and retrodisplacement of the ciliary muscle from the scleral spur in the cynomolgus monkey.* Invest Ophthalmol 1976, 15(10): 793-807.
38. Tian, B., Gabelt, B.T., Geiger, B., Kaufman, P.L. *The role of the actomyosin system in regulating trabecular fluid outflow.* Exp Eye Res 2009, 88(4): 713-7.
39. Hutzelmann, J., Owens, S., Shedden, A., Adamsons, I., Vargas, E. *Comparison of the safety and efficacy of the fixed combination of dorzolamide/timolol and the concomitant administration of dorzolamide and timolol: A clinical equivalence study.* Br J Ophthalmol 1998, 82(11): 1249-53.
40. Sherwood, M.B., Craven, E.R., Chou, C., DuBiner, H.B., Batoosingh, A.L., Schiffman, R.M., Whitcup, S.M. *Twice-daily 0.2% brimonidine-0.5% timolol fixed-combination therapy vs monotherapy with timolol or brimonidine in patients with glaucoma or ocular hypertension.* Arch Ophthalmol 2006, 124(9): 1230-8.
41. Brandt, J.D., Cantor, L.B., Katz, L.J., Batoosingh, A.L., Chou, C., Bossowska, I.; Ganfort Investigators Group II. *Bimatoprost/timolol fixed combination: A 3-month double-masked, randomized parallel comparison to its individual components in patients with glaucoma or ocular hypertension*. J Glaucoma 2008, 17(3): 211-6.
42. Tabet, R., Stewart, W.C., Feldman, R., Konstas, A.G.P. *A review of additivity to prostaglandin analogs: Fixed and unfixed combinations.* Surv Ophthalmol 2008, 53(Suppl. 1): S85-92.
43. Krauss, A.H., Toris, C.B., Kallberg, M.E. et al. *Ocular hypotensive activity of PF-3187207, a nitric oxide donating prostaglandin analog, in preclinical models*. Annu Meet Assoc Res Vision Ophthalmol (ARVO) (May 3-7, Fort Lauderdale) 2009, Abst 1471.
44. Borghi, V., Bastia, E., Guzzetta, M. et al. *A novel nitric oxide releasing prostaglandin analog, NCX 125, reduces intraocular pressure in rabbit, dog, and primate models of glaucoma*. J Ocul Pharmacol Ther 2010, 26(2): 125-32.
45. Nilsson, S.F., Lütjen-Drecoll, E., Toris, C.B. et al. *The prostanoid EP2 receptor agonist butaprost increases uveoscleral outflow in the cynomolgus monkey*. Invest Ophthalmol Vis Sci 2006, 47(9): 4042-9.
46. Woodward, D.F., Nilsson, S.F., Toris, C.B., Kharlamb, A.B., Nieves, A.L., Krauss, A.H. *Prostanoid EP4 receptor stimulation produces ocular hypotension by a mechanism that does not appear to involve uveoscleral outflow*. Invest Ophthalmol Vis Sci 2009, 50(7): 3320-8.
47. Prasanna, G., Fortner, J., Xiang, C. et al. *Ocular pharmacokinetics and hypotensive activity of PF-04475270, an EP4 prostaglandin agonist in preclinical models.* Exp Eye Res 2009, 89(5): 608-17.
48. Daines, B.S., Kent, A.R., McAleer, M.S., Crosson, C.E. *Intraocular adenosine levels in normal and ocular-hypertensive patients*. J Ocul Pharmacol Ther 2003, 19(2): 113-9.
49. Tian, B., Gabelt, B.T., Crosson, C.E., Kaufman, P.L. *Effects of adenosine agonists on intraocular pressure and aqueous humor dynamics in cynomolgus monkeys.* Exp Eye Res 1997, 64(6): 979-89.
50. Crosson, C.E., Sloan, C.F., Yates, P.W. *Modulation of conventional outflow facility by the adenosine A1 agonist N6-cyclohexyladenosine.* Invest Ophthalmol Vis Sci 2005, 46(10): 3795-9.
51. Kim, N., Crosson, C., Lam, T. et al. *INO-8875, an adenosine A1 agonist, lowers intraocular pressure through the conventional outflow pathway.* Annu Meet Assoc Res Vision Ophthalmol (ARVO) (May 2-6, Fort Lauderdale) 2010, Abst 3238-D780.
52. Avila, M.Y., Stone, R.A., Civan, M.M. *Knockout of A3 adenosine receptors reduces mouse intraocular pressure.* Invest Ophthalmol Vis Sci 2002, 43(9): 3021-6.
53. Wang, Z., Do, C.W., Avila, M.Y. et al. *Nucleoside-derived antagonists to A3 adenosine receptors lower mouse intraocular pressure and act across species*. Exp Eye Res 2010, 90(1): 146-54.
54. Arai, H., Hori, S., Aramori, I., Ohkubo, H., Nakanishi, S. *Cloning and expression of a cDNA encoding an endothelin receptor*. Nature 1990, 348(6303): 730-2.
55. Cellini, M., Versura, P., Trere, D., Campos, E.C. *Effects of endothelin-1 on human trabecular meshwork cell contraction*. Ophthalmic Res 2005, 37(1): 43-9.
56. Rao, V.R., Krishnamoorthy, R.R., Yorio, T. *Endothelin-1 mediated regulation of extracellular matrix collagens in cells of human lamina cribrosa.* Exp Eye Res 2008, 86(6): 886-94.
57. Podos, S.M., Wang, R.F., Serle, J.B., Baltatu, O.C. *Effect of SPP 301, an endothelin antagonist, on intraocular pressure in glaucomatous monkey eyes.* Annu Meet Assoc Res Vision Ophthalmol (ARVO) (May 3-7, Fort Lauderdale) 2009, Abst 1476.
58. Resch, H., Karl, K., Weigert, G., Wolzt, M., Hommer, A., Schmetterer, L., Garhöfer, G. *Effect of dual endothelin receptor blockade on ocular blood flow in patients with glaucoma and healthy subjects.* Invest Ophthalmol Vis Sci 2009, 50(1): 358-63.
59. *Changes in eye pressure and anterior chamber depth with oral endothelin antagonist therapy (NCT00452283*). ClinicalTrials.gov Web site, April 13, 2011.
60. *Effects of non-specific endothelin-A receptor blockade on ocular blood flow in patients with glaucoma (NCT00701597).* ClinicalTrials.gov Web site, April 13, 2011.
61. *Role of endothelin- and nitric oxide-system in the regulation of optic nerve head blood flow during changes in ocular perfusion pressure (NCT00406731).* ClinicalTrials.gov Web site, April 13, 2011.
62. Becker, B. *Intraocular pressure response to topical corticosteroids*. Invest Ophthalmol Vis Sci 1965, 4: 198-205.
63. Jonas, J.B., Degenrigh, R.F., Kreissig, I., Akkoyun, I., Kamppeter, B.A. *Intraocular pressure elevation after intravitreal triamcinolone acetonide injection.* Ophthalmology 2005, 112(4): 593-8.
64. Robin, A.L., Suan, E.P., Sjaarda, R.N., Callanan, D.G., Defaller, J; Alcon Anecortave Acetate for IOP Research Team. *Reduction of intraocular pressure with anecortave acetate in eyes with ocular steroid injection-related glaucoma*. Arch Ophthalmol 2009, 127(2): 173-8.

65. Robin, A.L., Clark, A.F., Covert, D.W. et al. *Anterior juxtascleral delivery of anecortave acetate in eyes with primary open-angle glaucoma: A pilot investigation*. Am J Ophthalmol 2009, 147(1): 45-50.e2.
66. Candia, O.A., Gerometta, R., Millar, J.C., Podos, S.M. *Suppression of corticosteroid-induced ocular hypertension in sheep by anecortave*. Arch Ophthalmol 2010, 128(3): 338-43.
67. Spiga, M.G., 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(6): 3029-41.
68. Lie, D.C., Colamarino, S.A., Song, H.J. et al. *"Wnt signalling regulates adult hippocampal neurogenesis".* Nature 2005, 437(7063): 1370-5.
69. Mao, W., Wordinger, R.J., Clark, A.F. *Focus on molecules: SFRP1.* Exp Eye Res 2010, 91(5): 552-3.
70. Wang, W.-H., McNatt, L.G., Pang, I.-H. et al. *Increased expression of the WNT antagonist sFRP-1 in glaucoma elevates intraocular pressure*. J Clin Invest 2008, 118(3): 1056-64.
71. Andrieu-Solere, C., Bejjani, R.A., de Bizemont, T., Normand, N., BenEzra, D., Behar-Cohen, F. *Ocular gene therapy: A review of nonviral strategies*. Mol Vis 2006, 12: 1334-47.
72. Barraza, R.A., Rasmussen, C.A., Loewen, N. et al. *Prolonged transgene expression with lentiviral vectors in the aqueous humor outflow pathway of nonhuman primates*. Hum Gene Ther 2009, 20(3): 191-200.
73. Buie, L.K., Rasmussen, C.A., Porterfield, E.C. et al. *Self-complementary AAV virus (scAAV) safe and long-term gene transfer in the trabecular meshwork of living rats and monkeys*. Invest Ophthalmol Vis Sci 2010, 51(1): 236-48.
74. Lebherz, C., Auricchio, A., Maguire, A.M. et al. *Long-term inducible gene expression in the eye via adeno-associated virus gene transfer in nonhuman primates*. Hum Gene Ther 2005, 16(2): 178-86.
75. Loewen, N., Fautsch, M.P., Teo, W.L., Bahler, C.K., Johnson, D.H., Poeschla, E.M. *Long-term, targeted genetic modification of the aqueous humor outflow tract coupled with noninvasive imaging of gene expression in vivo*. Invest Ophthalmol Vis Sci 2004, 45(9): 3091-8.
76. Khare, P.D., Loewen, N., Teo, W. et al. *Durable, safe, multi-gene lentiviral vector expression in feline trabecular meshwork.* Mol Ther 2008, 16(1): 97-106.
77. Borrás, T., Xue, W., Choi, V.W., Bartlett, J.S., Li, G., Samulski, R.J., Chisolm, S.S. *Mechanisms of AAV transduction in glaucoma-associated human trabecular meshwork cells.* J Gene Med 2006, 8(5): 589-602.
78. Diaz-Griffero, F., Kar, A., Lee, M., Stremlau, M., Poeschla, E., Sodroski, J. *Comparative requirements for the restriction of retrovirus infection by TRIM5alpha and TRIMCyp*. Virology 2007, 369(2): 400-10.
79. Barraza, R.A., McLaren, J.W., Poeschla, E.M. *Prostaglandin pathway gene therapy for sustained reduction of intraocular pressure.* Mol Ther 2010, 18(3): 491-501.
80. *Gene Therapy Clinical Trials Worldwide*. J Gene Med 2010, March.
81. Clinical trials.gov, Accessed March 12, 2010.
82. Simonelli, F., Maguire, A.M., Testa, F. et al. *Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration*. Mol Ther 2010, 18(3): 643-50.
83. Maguire, A.M., High, K.A., Auricchio, A. et al. *Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: A phase 1 dose-escalation trial*. Lancet 2009, 374(9701): 1597-605.
84. Cideciyan, A.V., Hauswirth, W.W., Aleman, T.S. et al. *Human RPE65 gene therapy for Leber congenital amaurosis: Persistence of early visual improvements and safety at 1 year*. Hum Gene Ther 2009, 20(9): 999-1004.
85. NEI website - http://www.nei.nih.gov/intramural/ocular_gene.asp, Accessed May 2, 2010.
86. Bainbridge, J.W.B. *Prospects for gene therapy of inherited retinal disease*. Eye 2009, 23(10): 1898-903.
87. Quality, Non-Clinical And Clinical Issues Relating Specifically To Recombinant Adeno-Associated Viral Vectors, EMEA/CHMP/GTWP/587488/2007, London, March 19, 2009.
88. Stieger, K., Le Meur, G., Lasne, F. et al. *Long-term doxycycline-regulated transgene expression in the retina of nonhuman primates following subretinal injection of recombinant AAV vectors*. Mol Ther 2006, 13(5): 967-75.
89. Stieger, K., Lhériteau, E., Moullier, P., Rolling, F. *AAV-mediated gene therapy for retinal disorders in large animal models*. ILAR J 2009, 50(2): 206-24.
90. Stieger, K., Belbellaa, B., Le Guiner, C., Moullier, P., Rolling, F. *In vivo gene regulation using tetracycline-regulatable systems.* Adv Drug Deliv Rev 2009, 61(7-8): 527-41.
91. Pluta, K., Luce, M.J., Bao, L., Agha-Mohammadi, S., Reiser, J. *Tight control of transgene expression by lentivirus vectors containing second-generation tetracycline-responsive promoters*. J Gene Med 2005, 7(6): 803-17.
92. Jiang, L., Rampalli, S., George, D., Press, C., Bremer, E.G., O'Gorman, M.R., Bohn, M.C. *Tight regulation from a single tet-off rAAV vector as demonstrated by flow cytometry and quantitative, real-time PCR.* Gene Ther 2004, 11(13): 1057-67.
93. Toromanoff, A., Adjali, O., Larcher, T. et al. *Lack of immunotoxicity after regional intravenous (RI) delivery of rAAV to nonhuman primate skeletal muscle*. Mol Ther 2010, 18(1): 151-60.
94. Comes, N., Borrás, T. *Functional delivery of synthetic naked siRNA to the human trabecular meshwork in perfused organ cultures*. Mol Vis 2007, 13: 1363-74.
95. López-Fraga, M., Martínez, T., Jiménez, A. *RNA interference technologies and therapeutics: From basic research to products.* BioDrugs 2009, 23(5): 305-32.
96. Campochiaro, P.A. *Potential applications for RNAi to probe pathogenesis and develop new treatments for ocular disorders.* Gene Ther 2006, 13(6): 559-62.
97. Fedorov, Y., King, A., Anderson, E. et al. *Different delivery methods-different expression profiles*. Nat Methods 2005, 2(4): 241.
98. Lingor, P., Koeberle, P., Kügler, S., Bähr, M. *Down-regulation of poptosis mediators by RNAi inhibits axotomy-induced retinal ganglion cell death*. Brain 2005, 128(Pt. 3): 550-8.
99. Sieving, P.A., Caruso, R.C., Tao, W., Coleman, H.R., Thompson, D.J., Fullmer, K.R., Bush, R.A. *Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants.* Proc Natl Acad Sci U S A 2006, 103(10): 3896-901.
100. Tao, W. *Application of encapsulated cell technology for retinal degenerative diseases*. Expert Opin Biol Ther 2006, 6(7): 717-26.
101. Levin, L.A. *Axonal loss and neuroprotection in optic neuropathies*. Can J Ophthalmol 2007, 42(3): 403-8.
102. Weinreb, R.N. *Glaucoma neuroprotection: What is it? Why is it needed?* Can J Ophthalmol 2007, 42(3): 396-8.
103. Walsh, D.T., Bresciani, L., Saunders, D. et al. *Amyloid beta peptide causes chronic glial cell activation and neurodegeneration after intravitreal injection*. Neuropathol Appl Neurobiol 2005, 31(5): 491.
104. Yoneda, S., Hara, H., Hirata, A., Fukushima, M., Inomata, Y., Tanihara, H. *Vitreous fluid levels of beta-amyloid((1-42)) and tau in patients with retinal diseases.* Jpn J Ophthalmol 2005, 49(2): 106-8.

105. Morgan, J.E. *Retinal ganglion cell shrinkage in glaucoma*. J Glaucoma 2002, 11(4): 365-70.

106. Guo, L., Salt, T.E., Luong, V. et al. *Targeting amyloid-beta in glaucoma treatment*. Proc Natl Acad Sci U S A 2007, 104(33): 13444-9.

107. Chen, H.-S.V., Pellegrini, J.W., Aggarwal, S.W., Lei, S.Z., Warach, S., Jensen, F.E., Lipton, S.A. *Open-channel block of N-methyl-daspartate (NMDA) responses by memantine: Therapeutic advantage against NMDA receptor-mediated neurotoxicity*. J Neurosci 1992, 12(11): 4427-36.

108. Osborne, N.N. *Recent clinical findings with memantine should not mean that the idea of neuroprotection in glaucoma is abandoned*. Acta Ophthalmol 2009, 87(4): 450-4.

109. Levin, L.A., Danesh-Meyer, H.V. *Lost in translation: Bumps in the road between bench and bedside.* JAMA 2010, 303(15): 1533-4.

110. Danesh-Meyer, H.V., Levin, L.A. *Neuroprotection: Extrapolating from neurologic diseases to the eye*. Am J Ophthalmol 2009, 148(2): 186-91.

111. Danesh-Meyer, H.V. *Neuroprotection in glaucoma: Recent and future directions*. Curr Opin Ophthalmol 2011, 22(2): 78-86.

112. Weinreb, R.W., Kaufman, P.L. *The Glaucoma Research Community and FDA Look to the Future: A Report from the NEI/FDA CDER Glaucoma Clinical Trial Design and Endpoints Symposium*. IOVS 2009, 50: 1497-505.

113. Gupta, N., Greenberg, G., de Tilly, L.N., Gray, B., Polemidiotis, M., Yücel, Y.H. *Atrophy of the lateral geniculate nucleus in human glaucoma detected by magnetic resonance imaging*. Br J Ophthalmol 2009, 93(1): 56-60.

114. Yücel, Y.H., Zhang, Q., Weinreb, R.N., Kaufman, P.L., Gupta, N. *Atrophy of relay neurons in magno- and parvocellular layers in the lateral geniculate nucleus in experimental glaucoma*. Invest Ophthalmol Vis Sci 2001, 42(13): 3216-22.

115. Gupta, N., Ang, L.C., Noël de Tilly, L., Bidaisee, L., Yücel, Y.H. *Human glaucoma and neural degeneration in intracranial optic nerve, lateral geniculate nucleus, and visual cortex*. Br J Ophthalmol 2006, 90(6): 674-8.

116. Osborne, N.N., Lascaratos, G., Bron, A.J., Chidlow, G., Wood, J.P. *A hypothesis to suggest that light is a risk factor in glaucoma and the mitochondrial optic neuropathies*. Br J Ophthalmol 2006, 90(2): 237-41.

117. Weber, A.J., Viswanáthan, S., Ramanathan, C., Harman, C.D. *Combined application of BDNF to the eye and brain enhances ganglion cell survival and function in the cat after optic nerve injury.* Invest Ophthalmol Vis Sci 2010, 51(1): 327-34.

118. Martin, K.R., Quigley, H.A., Zack, D.J. et al. *Gene therapy with brain-derived neurotrophic factor as a protection: Retinal ganglion cells in a rat glaucoma model.* Invest Ophthalmol Vis Sci 2003, 44(10): 4357-65.

119. Johnson, T.V., Bull, N.D., Martin, K.R. *Neurotrophic factor delivery as a protective treatment for glaucoma*. Exp Eye Res 2010, Epub ahead of print.

120. Saylor, M., McLoon, L.K., Harrison, A.R., Lee, M.S. *Experimental and clinical evidence for brimonidine as an optic nerve and retinal neuroprotective agent: An evidence-based review*. Arch Ophthalmol 2009, 127(4): 402-6.

121. Dong, C.J., Guo, Y., Agey, P., Wheeler, L., Hare, W.A. *Alpha2 adrenergic modulation of NMDA receptor function as a major mechanism of RGC protection in experimental glaucoma and retinal excitotoxicity.* Invest Ophthalmol Vis Sci 2008, 49(10): 4515-22.

122. Kuno, N., Fujii, S. *Biodegradable intraocular therapies for retinal disorders: Progress to date*. Drugs Aging 2010, 27(2): 117-34.

123. *Effect of alphagan on retinal blood flow autoregulation and motion detection in patients with normal pressure glaucoma (NCT01105065).* ClinicalTrials.gov Web site, April 13, 2011.

124. Kaeser, P., Orgül, S., Zawinka, C., Reinhard, G., Flammer, J. *Influence of change in body position on choroidal blood flow in normal subjects.* Br J Ophthalmol 2005, 89(10): 1302-5.

125. Galambos, P., Vafiadis, J., Vilchez, S.E. et al. *Compromised autoregulatory control of ocular hemodynamics in glaucoma patients after postural change*. Ophthalmology 2006, 113(10): 1832-6.

126. Feke, G.T., Pasquale, L.R. *Retinal blood flow response to posture change in glaucoma patients compared with healthy subjects.* Ophthalmology 2008, 115(2): 246-52.

127. Yin, H., Chen, L., Chen, X., Liu, X. *Soluble amyloid beta oligomers may contribute to apoptosis of retinal ganglion cells in glaucoma*. Med Hypotheses 2008, 71(1): 77-80.

128. Wax, M.B., Tezel, G. *Immunoregulation of retinal ganglion cell fate in glaucoma*. Exp Eye Res 2009, 88(4): 825-30.

129. Reichelt, J., Joachim, S.C., Pfeiffer, N., Grus, F.H. *Analysis of autoantibodies against human retinal antigens in sera of patients with glaucoma and ocular hypertension*. Curr Eye Res 2008, 33(3): 253-61.

130. *Effect of glaucoma educators on adherence to prescribed therapeutic regimens in glaucoma patients (NCT00454922).* ClinicalTrials.gov Web site, April 13, 2011.

131. *Value of an intervention to enhance adherence in glaucoma patients (NCT00756184).* ClinicalTrials.gov Web site, April 13, 2011.

132. McKinnon, S.J., Goldberg, L.D., Peeples, P., Walt, J.G., Bramley, T.J. *Current management of glaucoma and the need for complete therapy.* Am J Manag Care 2008, 14(1, Suppl.): S20-7.

133. Rotchford, A.P., Murphy, K.M. *Compliance with timolol treatment in glaucoma*. Eye 1998, 12: 234-6.

134. Gurwitz, J.H., Glynn, R.J., Monane, M., Everitt, D.E., Gilden, D., Smith, N., Avorn, J. *Treatment for glaucoma: Adherence by the elderly.* Am J Public Health 1993, 83(5): 711-6.

135. Patel, S.C., Spaeth, G.L. *Compliance in patients prescribed eyedrops for glaucoma*. Ophthalmic Surg 1995, 26(3): 233-6.

136. Nordstrom, B.L., Friedman, D.S., Mozaffari, E., Quigley, H.A., Walker, A.M. *Persistence and adherence with topical glaucoma therapy*. Am J Ophthalmol 2005, 140(4): 598-606.

137. Tsai, J.C. *Medication adherence in glaucoma: Approaches for optimizing patient compliance.* Curr Opin Ophthalmol 2006, 17: 190-5.

138. Tsai, J.C. *A comprehensive perspective on patient adherence to topical glaucoma therapy.* Ophthalmology 2009, 116(11, Suppl.): S30-6.

139. Budenz, D.L. *A clinician's guide to the assessment and management of nonadherence in glaucoma*. Ophthalmology 2009, 116(11, Suppl.): S43-7.

140. Medeiros, F.A., Weinreb, R.N., Zangwill, L.M., Alencar, L.M., Sample, P.A., Vasile, C., Bowd, C. *Long-term intraocular pressure fluctuations and risk of conversion from ocular hypertension to glaucoma*. Ophthalmology 2008, 115(6): 934-40.

141. Barkana, Y., Anis, S., Liebmann, J., Tello, C., Ritch, R. *Clinical utility of intraocular pressure monitoring outside of normal office hours in patients with glaucoma.* Arch Ophthalmol 2006, 124(6): 793-7.

142. Hughes, E., Spry, P., Diamond, J. *24-Hour monitoring of intraocular pressure in glaucoma management: A retrospective review*. J Glaucoma 2003, 12(3): 232-6.

143. Baltmr, A., Duggan, J., Nizari, S., Salt, T.E., Cordeiro, F.M. *Neuroprotection in glaucoma - Is there a future role?* Exp Eye Res 2010, 91(5): 554-66.

144. Medeiros, F.A., Alencar, L.M., Zangwill, L.M., Bowd, C., Vizzeri, G., Sample, P.A., Weinreb, R.N. *Detection of progressive retinal nerve fiber layer loss in glaucoma using scanning laser polarimetry with variable corneal compensation.* Invest Ophthalmol Vis Sci 2009, 50(4): 1675-81.

145. Chauhan, B.C., Nicolela, M.T., Artes, P.H. *Incidence and rates of visual field progression after longitudinally measured optic disc change in glaucoma*. Ophthalmology 2009, 116(11): 2110-8.

146. Leung, C.K., Cheung, C.Y., Weinreb, R.N. et al. *Evaluation of retinal nerve fiber layer progression in glaucoma: A study on optical coherence tomography guided progression analysis*. Invest Ophthalmol Vis Sci 2010, 51(1): 217-22.

147. Leung, C.K., Lam, S., Weinreb, R.N. et al. *Retinal nerve fiber layer imaging with spectral-domain optical coherence tomography: Analysis of the retinal nerve fiber layer map for glaucoma detection.* Ophthalmology 2010, 117(9): 1684-91.