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Meeting Report

Highlights from the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2008: eyes on Innovation

Elisa Ferrer
Prous Science, Provenza 388, 08025 Barcelona, Spain

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Abstract

The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) took place in the coastal city of Fort Lauderdale, Florida, on April 27-May 1, 2008, where over 10,000 basic and clinical eye and vision researchers from all over the world gathered to discuss the most innovative techniques, tools and treatments to improve the management of eye diseases. Here, we highlight selected presentations from this year's meeting, with special emphasis on new pharmacological targets, biomarkers and new therapies.

Introduction

The increasing knowledge of the pathophysiology of eye diseases, together with the development of new surgical techniques and procedures and the introduction of biological products in the drug armamentarium, has greatly improved the management of ocular disorders. This year's ARVO meeting featured a series of exciting research presentations that shared in common the innovative nature of the treatments investigated, from small interfering RNA (siRNA) for the treatment of glaucoma and age-related macular degeneration (AMD) to gene therapy for the treatment of rare inherited retinal diseases. In addition to newly discovered pharmacological targets, research on biomarker discovery is opening a new avenue for the diagnosis and treatment of ocular diseases. Here, we highlight a selection of relevant studies presented at this year's meeting, including new findings on pathogenic mechanisms, ocular biomarkers and new therapies.

New targets and mechanisms of disease

Exfoliation syndrome and exfoliative glaucoma

Exfoliation syndrome (XFS) is characterized by excessive production and progressive accumulation of fibrillar extracellular matrix (ECM) material in many ocular tissues, most commonly affecting the anterior chamber. The prevalence of XFS increases with age. Exfoliation material can obstruct the trabecular meshwork (TM), hence raising the intraocular pressure (IOP) and leading to exfoliative glaucoma (XFG), a common, sight-threatening complication of XFS. Several factors have been identified to play a pathogenic role in XFS. Genome-wide association studies conducted at deCODE identified that mutations in the lysyl oxidase-like protein 1 (*LOXL1*) gene were associated with increased susceptibility to XFG (1). *LOXL1* is a member of the lysyl oxidase family of genes that encode proteins essential for the formation of elastin polymers, which are the major constituent of intraocular XFG lesions. Additionally, a pathogenic role for transforming growth factor β (TGF-β), a major modulator of ECM formation, has been proposed. Particularly, increased levels of active and latent TGF-β1 have been found in the aqueous humor of patients with XFG (2), which may prompt the formation of abnormal ECM.

Glaucoma

Research undertaken at Dr. Wordinger's lab has identified the role of bone morphogenetic proteins (BMPs) as inhibitors of TGF-β signaling in glaucoma. TGF-β2 increases the gene expression of connective tissue growth factor (CTGF), plasminogen activator inhibitor 1 (PAI-1), tissue transglutaminase, collagen and fibronectin in the TM. TGF-β alters ECM metabolism, hence causing increased ECM deposition in the TM, which in turn causes a decrease in aqueous humor outflow, raising IOP. In TM, BMP has been found to counterbalance TGF-β2 actions. In glaucomatous TM, BMP action is inhibited by the BMP antagonist gremlin. In fact, BMP, BMP receptors and BMP antagonists are expressed in human TM cells and tissues. In particular, BMP-4 and its receptor are expressed in TM, as are receptor Smads, which are sig-

naling molecules in the BMP signaling pathway downstream of the BMP receptor. BMP-4 has been found to inhibit TGF-β2-stimulated fibronectin expression in TM. The BMP pathway is tightly controlled at several checkpoints, such as blockade of BMP receptor phosphorylation by inhibitory Smads or BMP receptor ubiquitination by SMURF proteins. Gremlin is present in the aqueous humor and in the perfusate medium of perfusion-cultured human eyes. Its expression is increased in glaucomatous TM cells and it has been shown to reversibly increase IOP in perfused cultured eyes. Similarly, BMP-4 also inhibits TGF-β2-stimulated ECM in optic nerve head (ONH) astrocytes. Human normal and glaucomatous ONH astrocytes and lamina cribrosa cells express and secrete gremlin. Gremlin inhibits BMP-4's inhibitory effect on TGF-β2-stimulated fibronectin expression in ONH astrocytes and lamina cribrosa. BMP inhibits the deleterious effects of TGF-β2 signaling. Overexpression of endogenous antagonists such as gremlin blocks the protective effects of BMP-4 (3, 4).

The small Rho GTPase RhoA is known to regulate the actin cytoskeleton and cellular contractility of the TM. RhoA inhibitors are currently under development for the treatment of glaucoma. Researchers at the University of Barcelona have investigated the role of Rac1, another Rho protein family member, in modulating aqueous humor outflow and IOP. Using platelet-derived growth factor (PDGF), a known Rac1 activator, outflow facility in perfused anterior segments was increased by 26%. Rac1 activation by PDGF promoted the formation of lamellipodia in bovine TM cells *in vitro*. Topical PDGF application to rabbit cornea dose-dependently reduced IOP by 20% (EC_{50} = 2.7 nM), likely due to facilitation of aqueous humor outflow. These results indicate that Rac1 may be a therapeutic target for the treatment of ocular hypertension (5).

AMD

Complement factor H (*CFH*) gene polymorphisms have been identified as a risk factor for the development of AMD (6). A joint study by researchers at German and British universities reported that AMD patients show systemic activation of the alternative complement cascade, and in particular, significantly increased concentrations of factor B, factor D, Ba, C3d and SC5b-9 were found compared to controls, hence indicating that inflammation is an important pathogenic factor for AMD (7). Interestingly, blockade of the alternative complement pathway with CR2-FH, a fusion protein containing complement receptor 2 linked to a complement-inhibitory domain of factor H, significantly reduced choroidal neovascularization (CNV) in mice without a functional alternative complement pathway (*Cfb*$^{-/-}$). Both intraocular and intravenous administration were equally effective (8).

Another study reported that the chemokine receptor CCR3 may be a novel therapeutic target in AMD. CCR3-defective mice did not show CNV following laser injury. Similarly, mice lacking the genes for CCR3 ligands, namely eotaxin-1 (*CCL11*) and eotaxin-2 (*CCL24*), were also protected against laser-induced CNV. Pharmacological blockade of CCR3 in wild-type mice resulted in equivalent effects. Moreover, CCR3 inhibition did not affect macrophage infiltration and reduced choroidal endothelial cell proliferation *in vitro* and *in vivo* (9).

Biomarkers

Uveal melanoma

Uveal melanoma is the most common primary intraocular cancer in adults and affects the uveal tract, including the iris, ciliary body and choroids, and can often lead to metastasis. The liver is the main target for metastatic disease associated with uveal melanoma. A research team headed by Dr. Barak at the Hadassah-Hebrew University Medical Center in Israel explored the changes in serum biomarkers associated with metastatic uveal melanoma before the development of liver metastases and their predictive potential. Osteopontin (OPN), S-100β protein, melanoma-inhibitory activity (MIA) and tissue polypeptide-specific antigen (TPS) were the tumor markers investigated. While tumor marker levels of patients who were disease-free for at least 10 years were comparable to those of controls, patients with metastatic disease exhibited a steady increase in all markers before the detection of liver metastases. The lead time of these biomarkers was between 6 and 12 months. In comparison, the lead time for routine liver function tests was found to be 6 months. Moreover, a significantly larger number of patients had abnormal markers before having abnormal liver function values (70% *vs.* 50%). These results suggest that changes in biomarker levels could provide improved early diagnosis of liver metastases in uveal melanoma (10, 11).

High-throughput proteomics is currently applied to many types of tumors. Using two-dimensional electrophoresis and mass spectrometry, scientists from the Tongren Hospital of Capital Medical University in Beijing, China, established a protein database for uveal melanoma that may include potential biomarker candidates. Among the 273 proteins identified from uveal melanoma, researchers highlighted proteins from the peroxiredoxin family, particularly type II, cell cycle proteins such as cyclin G, a p53 target, annexins, which are also overexpressed in other cancers, and molecular chaperones (heat shock protein 10 [Hsp10], chaperonin-containing TCP-1) (12).

Researchers at the University of Pennsylvania and Thomas Jefferson University, U.S.A., reported that a whole-genome single nucleotide polymorphism (SNP)-based assay on a series of 100 fine-needle aspiration biopsy samples of small uveal melanoma may be a feasible method to detect prognostic markers. Monosomy for chromosome 3, which is known to predict significantly reduced disease-free survival, was identified in 37% of samples (13).

Glaucoma

Diadenosine tetraphosphate (Ap_4A) and pentaphosphate (Ap_5A) are natural nucleotides that have been found in the aqueous humor of several species and that exert opposing effects on IOP: while Ap_4A decreases IOP after topical application, Ap_5A increases it (14). In addition, studies in normotensive rabbits suggested a role for naturally occurring Ap_4A in the control of IOP (15). A study headed by Dr. Castany at the Hospital de la Esperança in Barcelona, Spain, detected Ap_4A and Ap_5A for the first time in the aqueous humor of subjects with and without chronic open-angle glaucoma. Interestingly, glaucomatous aqueous humor samples exhibited a 6-fold increase in Ap_4A levels, whereas Ap_5A was no different from controls. The potential use of Ap_4A as a glaucoma biomarker remains to be established (16).

Dry eye

Recent findings by Peral *et al*. identified consistently higher levels of Ap_4A, as well as Ap_5A, in tears of patients with dry eye than in subjects with normal tear production (17). Further studies in patients with Sjögren's syndrome and congenital aniridia revealed that the Ap_4A concentration in tears was markedly higher than in individuals with normal tear production. Among patients with Sjögren's syndrome, those with lower tear production presented higher Ap_4A levels. Interestingly, ocular dryness in aniridia was correlated with age and concomitant increases in Ap_4A (18).

AMD

Macrophage recruitment has been proposed as a cause of progression from dry AMD to neovascular AMD. Researchers at Duke University, U.S.A., analyzed the gene expression of circulating monocytes of patients with dry and neovascular AMD in order to identify gene markers associated with AMD severity. All genes examined showed at least 5-15-fold increased mRNA expression. Interestingly, cyclooxygenase-2 (COX-2) showed a potentially high predictive value, as patients in the highest tertile of expression were three times more likely to have neovascular AMD than those in the lowest tertile (19).

Another example of a genetic biomarker in AMD was provided by researchers at the Catholic University of Korea, who demonstrated that the *LOC387715* gene (particularly the A69S-SNP variant) appears to be a more sensitive genetic indicator for AMD in Korean patients than the *CFH* gene, which is a recognized genetic factor predisposing to AMD (20).

New therapies

Corneal transplantation

The avascular and alymphatic nature of the cornea prompted investigators led by a group from the University of Kentucky, U.S.A., to search for an endogenous lymphatic inhibitor. AA21127 is a secreted protein that was found to be constitutively expressed in corneal epithelium but not in the conjunctiva, which is densely populated with lymphatic vessels. AA21127 mRNA and protein were cloned from a mouse corneal cDNA library. The expression of AA21127 mRNA and protein was upregulated by suture injury. Conditional ablation of AA21127 corneal expression in mice resulted in increased corneal lymphatic vessel density. AA21127 conditional knockouts also showed enhanced suture-induced lymphangiogenesis, which could be attenuated (70%) by enforced AA21127 expression via intracorneal injection of naked plasmid. In a mouse model of corneal transplantion, exogenous administration of recombinant AA21127 before corneal transplantation into recipient corneas increased allograft survival by suppressing reparative lymphangiogenesis (21).

In a murine model of high-risk keratoplasty, treatment with JSM-6427 (23 mg/kg/day via s.c. osmotic pumps) before and after keratoplasty significantly improved graft clarity by doubling the number of clear grafts compared to the high-risk keratoplasty control group. This effect was due to lymphangiogenesis inhibition, hence improving corneal graft survival (22). Updated results for Jerini's open-label, first-in-human phase I clinical study indicated, in addition to previously reported improvements in best-corrected visual acuity (BCVA), no dose-limiting toxicity or serious adverse events. The maximum tolerated dose of JSM-6427 has not yet been reached. Jerini is also developing a 6-month sustained-release formulation which will reduce treatment burden (23).

Glaucoma

Inspire Pharmaceuticals has described a novel Rho kinase (ROCK) inhibitor, INS-117548, which may be useful for the treatment of glaucoma and ocular hypertension. Using commercially available IMAP technology, scientists showed that INS-117548 potently inhibited both ROCK-1 and ROCK-2 isozymes (K_i = 17 and 5.8 nM, respectively) in the presence of 10 μM ATP. Following instillation at 10 mM, INS-117548 reduced IOP in normotensive monkeys. Ocular tolerability after acute exposure at 5.6 mM was comparable to approved glaucoma therapies (*i.e.*, the prostanoid agonists latanoprost and travoprost). Ocular tolerance improved following repeated instillation at concentrations ranging from 0.8 to 8 mM t.i.d. for the first 2 days and b.i.d. on the third day of treatment (24).

SYL-04003 is a novel RNA interference (RNAi) technology targeting carbonic anhydrase IV (CA IV) developed by Sylentis in collaboration with researchers at the Universidad Complutense of Madrid, Spain. Topical instillation of SYL-04003 in rabbits reduced IOP by 14.47%, comparable to latanoprost (23%). When administered daily for 4 consecutive days at 20 nmol/day followed by weekly application at the same dose for 3 weeks, the IOP-lowering effect of SYL-04003 persisted for 1 month with no desensitization. SYL-04003 was well tolerated,

with no adverse effects observed. The IOP reduction correlated with a decrease in CA IV mRNA levels in the ciliary body in treated animals. Preliminary acute toxicology studies have shown an LD_{50} of > 175 mg/kg. The lack of toxicity and prolonged IOP decrease elicited by SYL-04003 make it a promising approach for the treatment of ocular hypertension and open-angle glaucoma compared to commercially available drugs (25). This compound has also been described in the patent literature (WO 2006021817).

Scientists at the Chinese University of Hong Kong reported on an innovative method that may be developed to treat inherited juvenile-onset open-angle glaucoma caused by mutations in the myocilin gene (*MYOC*). Glaucoma-associated mutants were transfected into human TM cells and were found to be retained in the endoplasmic reticulum (ER), detergent-resistant and unable to be degraded by the ubiquitin-proteasome pathway, hence indicating protein misfolding, compared to wild-type proteins. Using 4-phenylbutyric acid and trimethylamine *N*-oxide, this research team corrected the mutant phenotype, as evidenced by a concentration-dependent increase in mutant myocilin secretion and improved subcellular trafficking. In addition, treatment rescued human TM cells from apoptosis. Thus, these compounds acted as chemical chaperones correcting misfolding of mutant myocilin (26).

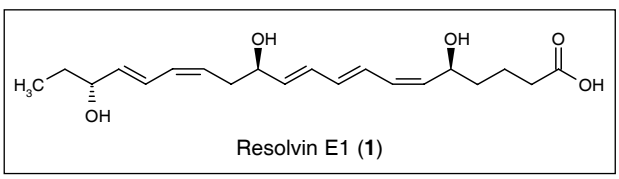
Dry eye

Researchers at Resolvyx Pharmaceuticals have developed synthetic analogues of resolvin E1 (**1**), an endogenous mediator derived from the omega-3 polyunsaturated fatty acid eicosapentaenoic acid that plays a role in the resolution of inflammation and tissue protection. RX-10005 (**2**) and RX-10065 are methyl ester prodrugs of resolvin E1 and its synthetic analogue RX-10008. Following 2-4 days of topical treatment at a concentration 0.001% in a mouse model of dry eye, both compounds significantly increased tear secretion and prevented loss of superficial epithelial cells. Moreover, the expression of α-smooth muscle actin (present in myofibroblasts) and COX-2 was significantly decreased following treatment. These latter results indicate that resolvin E1 analogues can prevent keratinocyte transformation to a myofibroblast phenotype and reduce the formation of proinflammatory prostaglandins (27). Further results showed that both compounds also protected against corneal goblet cell loss and reduced the degree of corneal epithelial barrier disruption in mice exposed to desiccating conditions (28). Resolvin E1, as well as its analogues, significantly prevented hypertonicity-induced cytokine release *in vitro*, simulating tear film hypertonicity occurring in dry eye syndrome, in a concentration-dependent manner (29). Pharmacokinetic studies in rabbits have shown that the resolvin analogues enhance eye tissue penetration and may also have unique pharmacokinetic properties that would make them suitable as eye drops for the treatment of retinal diseases (30). Other experiments have suggested that the resolvins could be novel therapies for improving corneal wound healing and treating AMD (31, 32). Resolvin E1 analogues are expected to progress to clinical evaluation shortly.

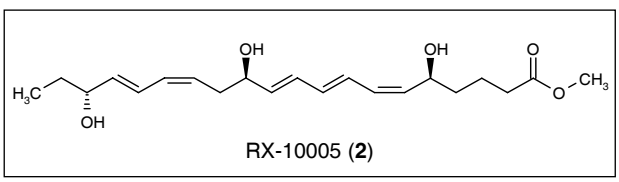
Lux Biosciences has developed a novel formulation of the calcineurin inhibitor voclosporin (**3**) consisting of mixed micelles formed by octoxynol-40 and vitamin E TPGS, a surfactant-like amphiphilic molecule, known as LX-211. LX-211, which contains 0.2% voclosporin and has a particle size of approximately 12.5 nm, was selected from among different micellar formulations due to its good tolerability in ocular irritability tests in rabbits. In dogs with naturally occurring keratoconjunctivitis sicca, twice-daily application of LX-211 resulted in significantly greater tear production as measured by the Schirmer tear test (STT) compared to expected STT values without therapy. Further preclinical safety studies are currently ongoing (33).

AMD

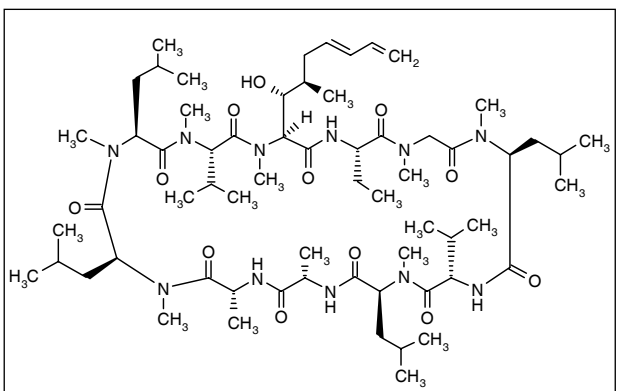
Resolvin E1 and its synthetic analogue RX-10008 reduced the amount of retinal leakage in laser-induced CNV lesions in mice. Resolvin E1 demonstrated a faster onset of action and it was also found to be more potent than RX-10008, as it reduced lesion size by 70% whereas this remained unchanged with RX-10008 treatment and increased in controls. Further development of resolvin E1 for the treatment of AMD is warranted (31).



Resolvin E1 (**1**)



RX-10005 (**2**)

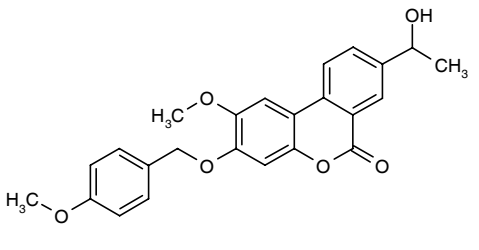

Voclosporin (**3**)

Research at Regeneron has led to the development of a novel angiopoietin-2 (ANG-2)-neutralizing antibody, RS-777, which has shown benefit in experimental CNV models. ANG-2 binds to the TIE-2 receptor, which is upregulated in many forms of pathological neovascularization. Ten days following matrigel-induced CNV in rats, treatment with RS-777 (25 mg/kg s.c.) caused a 33% reduction of established CNV on day 20, in addition to blocking further neovascularization. Furthermore, RS-777 decreased cellular proliferation and subretinal fibrosis, collagen components and leukocyte infiltration in CNV lesions. These findings indicate a role for ANG-2 in the pathogenesis of CNV (34).

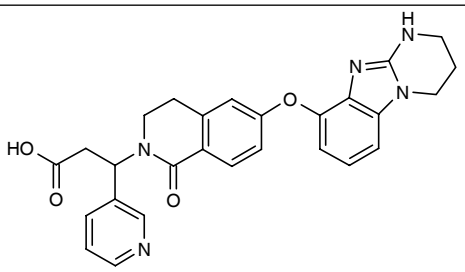
Researchers at the Katholieke Universiteit Leuven in Belgium reported an alternative antiangiogenic approach for the treatment of neovascular AMD which may prevent complications associated with classical therapies targeting vascular endothelial growth factor (VEGF). Blockade of placental growth factor (PlGF) has been demonstrated to reduce laser-induced CNV, as shown by studies in PlGF knockout mice. Similarly, treatment of wild-type mice with an anti-PlGF antibody (6, 12, 25 or 50 mg/kg) significantly and dose-dependently decreased neovascular growth following laser treatment as effectively as anti-VEGFR-2 antibody therapy. The most effective dose was 25 mg/kg. Combination of anti-PlGF (25 mg/kg) and anti-VEGFR-2 (12.5 mg/kg) treatments had synergistic effects. Moreover, blocking PlGF resulted in a significant reduction in the number of inflammatory cells at CNV lesions, whereas anti-VEGFR-2 treatment did not show antiinflammatory effects. In addition, anti-PlGF therapy did not negatively affect retinal ganglion cells when tested in BL/6 mice, which are prone to developing retinal degeneration (35).

Palomid-529 (P-529, [**4**]) is a nonsteroidal small molecule with antiproliferative and antiangiogenic activity currently being developed at Paloma Pharmaceuticals for the treatment of cancer and diabetic retinopathy. P-529 is known to block both VEGF- and basic fibroblast growth factor (bFGF)-induced endothelial cell proliferation *in vitro* (IC_{50} = 0.02 and 0.2 μM, respectively). It also inhibited VEGF-induced permeability in a mouse ear model of angiogenesis, where mice were pretreated with P-529 (200 mg/kg i.p.) 24 h before an intradermal injection of an adenovirus expressing mouse VEGF-A164 in the ear. The mechanism of action of P-529 may involve the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) prosurvival pathway common to many growth factors, as P-529 (10 μM) inhibited both endogenous and VEGF-stimulated Akt phosphorylation in human dermal microvascular endothelial cells. Further experiments showed that P-529 is a dual inhibitor of the TOR complexes TORC-1 and TORC-2. In a murine model of retinopathy of prematurity (ROP), P-529 (1 mg/day i.p. for 5 days in 7-day-old mouse pups) significantly blocked neovascular growth by 75%, allowing normal blood vessel growth. P-529 also suppressed laser-induced retinal neovascularization. Subretinal neovascularization induced by subretinal bFGF/VEGF pellets was completely and persistently (28 days) abolished by P-529 (36). P-529 has also been shown to decrease cell proliferation induced by retinal detachment (proliferative vitreoretinopathy) by reducing Müller cell proliferation and glial scar formation when given intravitreally (600 μg) immediately after retinal detachment in rabbits (37). Phase I studies are expected to begin by the end of 2008.

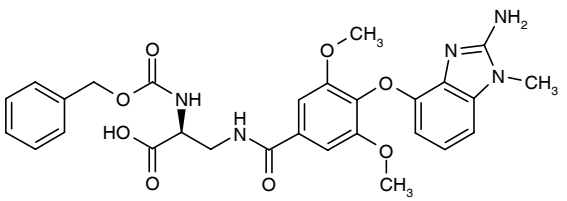
Researchers at Allergan, in collaboration with Pharmacopeia, have investigated a novel approach for reducing ocular neovascularization involving new integrin $\alpha_v\beta_3$ antagonists. Three compounds, namely PS-680648 (**5**), PS-780035 (**6**) and PS-388023 (**7**), demonstrated comparable antagonist activity against



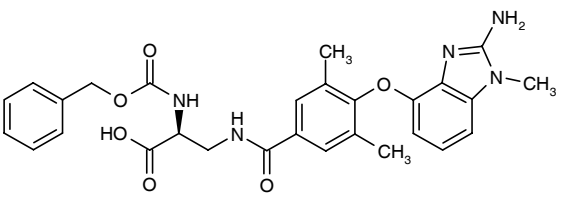
Palomid-529 (**4**)



PS-680648 (**5**)



PS-780035 (**6**)



PS-388023 (**7**)

$\alpha_v\beta_3$, with respective IC_{50} values of 0.0014, 0.0007 and 0.0006 μM. Despite these results, only PS-388023 and PS-680648 inhibited the neovascular response in a rat model of ROP by more than 90%, whereas PS-780035 failed to block neovascular vessel growth in the laser-induced CNV model in rats. PS-388023 also blocked laser-induced CNV in rats. These results were correlated with the ability of each compound to induce talin recruitment, a surrogate marker of integrin ligation. The active compounds induced a 6-7-fold increase in talin recruitment. Therefore, potentially useful $\alpha_v\beta_3$ antagonists should not only block ligand/receptor interaction but also act downstream of the integrin receptor (38).

Based on the known involvement of the growth hormone (GH) axis in ocular neovascular disease, researchers at Merck & Co. are developing somatostatin receptor sst_2 antagonists with the aim of blocking GH release, thus preventing neovascularization. In a rat model where GH was increased using a GH secretagogue, both sst_2 agonist A and sst_2 agonist B significantly reduced GH levels by 85% compared to vehicle controls. Although when tested in a rat model of ROP systemic delivery of sst_2 agonist A (10 mg/kg s.c. t.i.d.) failed to decrease retinal neovascularization area, intravitreal administration of sst_2 agonist B (15 μg every 4 days) significantly reduced lesion size by 50% compared to controls in a rat laser-induced CNV model (39).

OC-10X is a new pan-tubulin inhibitor developed by OcuCure Therapeutics and Eastern Virginia Medical School which has shown activity in murine CNV models. Initial *in vitro* experiments demonstrated inhibition of bovine and human retinal endothelial cell growth at 3 μM in the presence or absence of VEGF, with half-maximal activity compared to the pan-tubulin inhibitor combretastatin. Topically applied single doses of OC-10X entered the rat retina and reached concentrations that would inhibit endothelial cell proliferation according to *in vitro* studies. Intravenous, intravitreal or topical OC-10X administration was well tolerated, with no ocular or systemic toxicity in rats. At therapeutic doses (1% OC-10X in Tocrisolve, t.i.d.), chronic topical administration for 14 days showed penetration through the cornea and sclera to reach the retina, with no accumulation in other organs and no evidence of toxicity (40).

Additionally, 1% OC-10X topical treatment for 4 days before laser CNV induction and 6 weeks later reduced CNV by 44% in the rat model of CNV. Similarly, a 35% regression of blood vessels was observed when rats were treated only after laser-induced CNV for a total of 6 weeks (angiolytic model). Topical treatment for up to 6 weeks was well tolerated (41).

In other experiments in AMD rats, no evidence of retinal toxicity was observed in 46-day toxicity studies with 1% topical OC-10X, as multifocal electroretinogram assessments did not show functional deficits (42).

Charlesson researchers have developed a series of small-molecule STAT3 (signal transducer and activator of transcription 3) inhibitors to target pathological angiogenesis and inflammation occurring in ocular disorders such as AMD, diabetic retinopathy and diabetic macular edema. CLT-005 was investigated in cultured primary bovine retinal capillary endothelial cells and pericytes, where it concentration-dependently decreased retinal cell viability, with minor adverse effects on pericytes, at concentrations ranging from 5 to 40 μM. In streptozotocin (STZ)-induced diabetic rats, intravitreally administered CLT-005 (5 μg or 50 ng) reduced retinal mRNA expression of proinflammatory, proangiogenic and pro-proliferative genes, such as *Tnf*, *Il-6* and the TGF-β1 gene. Moreover, *Icam1* and *Vegf* expression levels were also significantly and dose-dependently attenuated by intravitreal CLT-005 at 10 and 30 mM. Retinal permeability was also reduced by intravitreal administration of CLT-005 (1 or 5 μg) in STZ-diabetic rats. A CLT-005 nanoparticle formulation (5 μg intravitreally) provided sustained suppression of the proinflammatory chemokine MCP-1 (monocyte chemotactic protein 1). Further experiments in a mouse model of CNV are currently under way (43).

CLT-003 (0.5-1 μg/eye intravitreally) is a novel analogue of thalidomide that dose-dependently reduced retinal vascular leakage in a model of oxygen-induced retinopathy in STZ-diabetic rats and in a mouse AMD model. Similarly to CLT-005, CLT-003 also downregulated *Vegf* and *Icam1* retinal expression. CLT-003 did not induce morphological or functional abnormalities in ocular tissues according to histological and electroretinogram study results (44).

Charlesson further reported *in vitro* results for CLT-006-001, a new combretastatin A4 analogue, which inhibited the growth of cultured bovine retinal endothelial cells (IC_{50} = 14.81 nM) without affecting neighboring pericytes. It also blocked tubulin polymerization (IC_{50} = 4.5 nM) and significantly reduced TNF-α-stimulated generation of reactive oxygen species (ROS), a main risk factor in diabetic retinopathy, in a human adult retinal pigment epithelial cell line at 2.5 nM (45).

Dr. Kleinman at the University of Kentucky, U.S.A., described how targeted and nontargeted siRNAs share a common mechanism of suppression of CNV via activation of Toll-like receptor 3 (TLR3), thus suggesting that CNV inhibition is a siRNA-class effect. Nontargeted siRNAs comprised molecules targeting nonmammalian genes, while targeted siRNAs inhibited VEGFR-1 or VEGF-A. Treatment with either type of siRNA reduced laser-induced CNV in wild-type mice, but not in TLR3-deficient mice. A minimum of 21 nucleotides was required for siRNA to suppress CNV, as this appears to be the length required to bridge functional residues on TLR3 (2:1 TLR3–RNA complexes). These results suggest that current investigational siRNA-based therapies, such as bevasiranib and AGN-211745 (Sirna-027), suppress CNV by means of TLR3 activation and not by target knockdown (46, 47). Opko is currently conducting phase III studies evaluating bevasiranib, a naked VEGF-A siRNA, for the treatment of AMD. Similarly, AGN-211745, an siRNA targeting VEGFR-1, is undergoing phase II development at Allergan and Sirna Therapeutics.

Update on clinical studies for AMD

Two years after its launch in 2006, results from phase III clinical trials with the anti-VEGF therapy ranibizumab (Lucentis®; Genentech, Novartis Ophthalmics) are still being analyzed in order to identify the best treatment regimen to be used in routine clinical practice.

The effects of ranibizumab on key anatomical outcomes in patients with AMD were examined using 2-year results from the phase IIIb PIER clinical study. In this 2-year, multicenter, double-blind trial, 184 patients were randomized to receive 0.3 or 0.5 mg ranibizumab or sham injection monthly for 3 doses and then quarterly for 2 years. Mean change in total area of CNV, total area of lesions and total area of CNV leakage (classic CNV) from baseline were measured. Ranibizumab groups achieved significant decreases in these three key anatomical outcomes compared to sham injection at month 12, which were maintained at month 24. Further subgroup analysis of patients who had lost less than 15 letters and those who had lost 15 letters or more by the end of the study failed to observe any correlation between lesion anatomical changes and visual acuity. Total and classic CNV and total lesion area did not significantly differ in these two subgroups of patients (48).

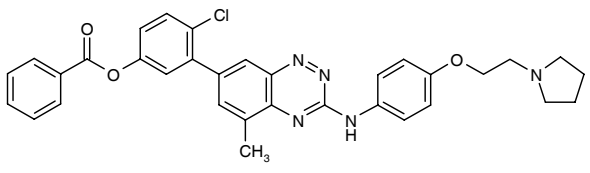
Additional analysis from the PIER study evaluated whether less frequent ranibizumab prevents loss of visual acuity in patients with neovascular AMD. The study analyzed the association between anatomical changes assessed by optical coherence tomography (OCT) and visual acuity outcomes at months 12 and 24. At 3 months, qualitative OCT scan evaluation demonstrated that patients treated with ranibizumab injections (0.3 or 0.5 mg monthly for 3 months and quarterly during study year 1) were more likely to show less retinal fluid (edema) than sham-treated patients when evaluated via OCT scans. Those patients who maintained anatomically normal scans by month 5 also showed better visual acuity outcomes by year 1. However, the correlation between quantitative OCT results (change in central foveal thickness) and visual acuity outcomes was limited, indicating that quantitative OCT alone may not be sufficient to guide retreatment or predict visual acuity outcomes. The use of qualitative OCT as a biomarker for anatomical effects and visual acuity in clinical trials requires further validation (49).

Joint analysis of 2-year data from the MARINA, ANCHOR and PIER phase III clinical trials by researchers at the University of Berlin compared the effect of monthly *versus* quarterly ranibizumab injections on visual acuity in patients with neovascular AMD. The proportion of patients who improved BCVA by gaining 15 letters or more was 33% and 41%, respectively, after 2 years of monthly ranibizumab 0.5 mg treatment in the MARINA and ANCHOR studies, whereas it was only 8% following quarterly administration in the PIER trial. Similarly, at least 6% of patients in the MARINA and ANCHOR trials gained more than 30 letters of visual acuity at year 1, compared to none of the patients in the PIER trial. On both the monthly and quarterly dosing schedules, the proportion of visual acuity gain was maintained over 2 years of ranibizumab treatment in all three studies (50).

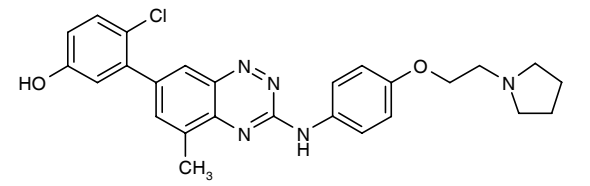
Further analysis of data from the MARINA and ANCHOR trials, in which ranibizumab demonstrated overall improvement in visual acuity from baseline in wet AMD patients, has provided new insight into the causes of vision loss in patients who were resistant to ranibizumab therapy. In those cases, visual acuity loss was not associated with growth of the CNV leakage area, but rather appeared to be due to progressive atrophy of photoreceptors and retinal pigment epithelium layer abnormalities in the central macula (51).

Researchers from Zurich University further compared subsets of data from the ANCHOR and MARINA studies and data from routine clinical use to evaluate the optimized treatment scheme of ranibizumab in AMD patients. The best BCVA values in these trials were obtained following the first injection. BCVA gain was found to stabilize after the third injection. Over 430 patients treated in the University Eye Clinic in Zurich received a ranibizumab dosing regimen consisting of monthly injections for the first 3 months, which were repeated every 5.4 and 5.6 weeks in patients presenting with classic and occult CNV, respectively, for up to 1 year. This treatment schedule correlated with a gain of 3.5 (classic) and 2.9 (occult) letters after 1 year of treatment, BCVA values which are lower than those achieved in pivotal studies, suggesting that patients were undertreated. According to these researchers, the optimized ranibizumab treatment scheme should include treatment initiation with three monthly doses. Shorter patient follow-up may avoid underdosing (52).

TG-100801 (**8**) is TargeGen's VEGFR inhibitor produg currently under development as a topical treatment for AMD. Upon ocular esterase cleavage, TG-100801 releases TG-100572 (**9**), its active metabolite that inhibits multiple kinases known to regulate vascular permeability, such as VEGFR, Src and PDGFR. Results from a randomized, double-blind, parallel-group, dose-escalation phase I clinical study showed good tolerability, with adverse events



TG-100801 (**8**)



TG-100572 (**9**)

limited to ocular discomfort (burning, tearing) and no serious adverse events. In addition, no clinically significant changes in IOP were detected. The absence of electrocardiographic signs or liver enzyme alterations indicated no systemic drug effect. Early results from a phase IIa trial initiated last year have reported decreased retinal edema and subretinal fluid with TG-100801 treatment. However, the detection of corneal deposits prompted early trial discontinuation after 7 patients had been recruited. No adverse events affecting vision have been reported. TargeGen is now evaluating the development of an alternative formulation and investigating other kinase inhibitors with improved corneal properties (53).

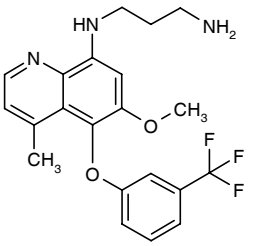
Diabetic retinopathy

Researchers at Kansas State University, U.S.A., reported the synthesis of PQ-1 (**10**), the first member of a class of substituted quinolines, which has shown potential for the treatment of disorders involving retinal ischemia, such as diabetic retinopathy. PQ-1 (10 μM) showed gap junction-inhibitory activity via inhibition of dye transfer in the R28 retinal precursor cell line. In a model of cobalt chloride-induced hypoxia *in vitro*, pretreatment with PQ-1 (10 μM) prevented activation of hypoxia-inducible factor HIF-α and caspase-3, suggesting that gap junction inhibition may protect against hypoxia-induced apoptosis. Further experiments in mice showed that severe damage to the photoreceptor cell layer caused by chronic cobalt chloride exposure for 1 week was attenuated by intravitreal pretreatment with PQ-1 (200 μM). PQ-1 appeared to selectively target connexin-43, a gap junction protein known to be involved in retinal pigment epithelial cell differentiation (54).

Reactive oxygen species (ROS) play a role in the pathogenesis of certain ocular disorders, such as AMD and diabetic retinopathy, where oxidized phospholipids have been found to accumulate in the retina and retinal pigment epithelium. Sirion researchers have developed two different classes of modulators of oxidative stress. SIR-1032 is representative of class I compounds, which are hydrolyzable prodrugs characterized by the presence of a cleavable hydrophilic moiety. SIR-1032 was cleaved by corneal esterases in mouse ocular anterior segments *in vitro* and did not show lens toxicity *in vitro*. SIR-1032 significantly reduced light-mediated oxidative stress (lipid peroxidation) in retinal explants after 2 days of intense light exposure. Sirion also developed a second class of nonhydrolyzable antioxidants, such as SIR-1076, which reduced hydrogen peroxide-induced oxidation in retina homogenates. Both compounds significantly suppressed the development of oxidative stress markers, such as lipid hydroperoxides, in superoxide dismutase 1 (SOD1)-deficient mice (55).

Other retinal disorders

Leber congenital amaurosis (LCA) refers to a group of recessively inherited, severe photoreceptor dystrophies



PQ-1 (**10**)

associated with poor vision at birth that progresses to blindness in early adulthood. LCA is caused by mutations in the gene encoding the retinal pigment epithelium-specific 65-kDa protein (*RPE65*), an essential component of the visual cycle. A collaborative study presented by researchers from Moorfields Eye Hospital and Targeted Genetics during the recent ARVO annual meeting reported the effects of subretinal injections of recombinant adeno-associated virus vector 2/2 expressing *RPE65* complementary DNA under the control of a human *RPE65* promoter (rAAV2/2.hRPE65p.hRPE65) developed by Targeted Genetics. The study included 3 young adult patients who had little or no vision in low light from an early age but retained some limited visual function in good lighting conditions. No complications were associated with subretinal injection of the vector and no serious adverse events were reported. Neither systemic dissemination of the vector nor immune responses to the vector capsid or RPE5 protein were detected. Although no clinically significant changes in visual acuity or peripheral visual fields (Goldmann perimetry) were observed, 1 patient showed significantly improved visual function on microperimetry and on dark-adapted perimetry and in a subjective test of visual mobility (56, 57).

Jerini researchers reported preclinical data on JSM-6427 (also see above), an intravitreally administered integrin $\alpha_5\beta_1$ inhibitor currently in development for the treatment of AMD. In addition to inducing regression of neovascular growth in models of CNV, JSM-6427 may be potentially useful in proliferative vitreoretinopathy. *In vitro*, JSM-6427 blocked human retinal pigment epithelial cell migration and proliferation induced by growth factors (58). Moreover, retinal proliferation following experimental retinal detachment in rabbits was inhibited by intravitreal JSM-6427 treatment (1 mg) given immediately after detachment. JSM-6427 also attenuated glial scar formation (59).

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