109 Cytokines; growth factors; Antiangiogenic drugs
Sunday, May 07, 2017 8:30 AM–10:15 AM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 189–211/B0220–B0242
Organizing Section: Physiology/Pharmacology

Program Number: 189 **Poster Board Number:** B0220
Presentation Time: 8:30 AM–10:15 AM
CNTF treatment prevents development of pathological tuft formation in VLDLR -/- mice
Edith Aguilar[1], Felicitas Bucher[1], Mauricio Rosenfeld[1], Susumu Sakimoto[1], Salome Murinello[1], Marin Gantner[2], kevin eade[2], Sophia Diaz-Aguilar[1], Maki Kitano[1], Martin Friedlander[1, 2]. [1]Cell Biology, Scripps Research Institute, Del Mar, CA; [2]The Lowy Medical Research Institute, San Diego, CA.
Purpose: Ciliary neurotrophic factor (CNTF) is one of the best studied neurotrophic agents and is currently in clinical trials for the treatment of retinitis pigmentosa and macular telangiectasia. Recent studies suggest that CNTF provides angio-modulatory effects in ischemic retinopathies. In this study, we tested the effect of CNTF on pathological tuft formation in VLDLR -/- knockout mice and explored the mechanism behind the angio-modulatory effect of CNTF treatment.
Methods: VLDLR -/- knock-out mice received intravitreal injections with recombinant rat CNTF (rrCNTF) at postnatal day 12 (P12) or P19. Intraretinal tuft formation was quantified at P18 or P34. Western blot and qPCR analysis of whole retina lysates were performed to identify downstream signaling pathways activated by CNTF treatment. Immunhistochemical stainings were used to identify CNTF responsive cells. In vitro experiments with primary mouse Müller cells were performed to explore the effect of CNTF on these cells.
Results: Intravitreal injections of rrCNTF at P12 significantly decreased intraretinal tuft formation at P18 and P34 while treatment at later time points (P18) did not reduce the number of intraretinal tufts. Peri-neovascular regions in VLDLR -/- mice showed a decrease in opsin staining in untreated, as well as rrCNTF treated eyes. CNTF treatment strongly induced phosphorylation of signal transducer and activator of transcription 3 (STAT3) 6 hours post injection and increased overall retinal expression levels of STAT3 up to 6 days post injection. Immunhistochemical analysis detected pSTAT3 signals in the inner nuclear layer. In vitro experiments confirmed that Müller cells respond to CNTF treatment with phosphorylation of STAT3.
Conclusions: CNTF treatment shows significant therapeutic potential for retinal neovascular disease. While CNTF treatment effectively prevented intraretinal tuft formation, it could not reduce pre-existing vascular abnormalities. Mechanistic studies showed that CNTF treatment did not prevent localized photoreceptor loss in the peri-neovascular regions of VLDLR -/- retinas. Activation of the Jak/STAT3 signaling pathway following CNTF treatment was detected in retinal Müller, but not photoreceptor, cells suggesting that the anti-angiogenic effect observed in VLDLR -/- is at least partially Müller-cell mediated.
Commercial Relationships: Edith Aguilar, None; **Felicitas Bucher**, None; **Mauricio Rosenfeld**, None; **Susumu Sakimoto**, None; **Salome Murinello**, None; **Marin Gantner**, None; **kevin eade**, None; **Sophia Diaz-Aguilar**, None; **Maki Kitano**, None; **Martin Friedlander**, None
Support: Martin Fiedlander: NEI Grant (EY11254) and the Lowy Medical Research Institute, Felicitas Bucher: German Research Foundation (Bu 3135/1-1)

Program Number: 190 **Poster Board Number:** B0221
Presentation Time: 8:30 AM–10:15 AM
Systemic Effects of AAV.COMPAng1 in the Ins2Akita Diabetic Mouse
Malkit Singh[2, 1], Susie Choi[2], Petra Soininen[2], Zoya Sandhu[2], Daniel Fang[2], Deepti Vashist[2], Austin Bohner[2], Xiaohui Zhang[2], Yuanyuan Wu[2], Lara Carroll[2], Yufeng Huang[3], Hironori Uehara[2]. [1]Ophthalmology, University of Texas Medical Branch / The Houston Methodist Hospital, Galveston, TX; [2]Ophthalmology, Moran Eye Center, University of Utah, Salt Lake City, UT; [3]Internal Medicine - Nephrology, University of Utah, Salt Lake City, UT.
Purpose: Diabetic retinopathy (DR) involves vascular hyperpermeability and capillary dropout. Angiopoietin-1 (Ang1) acting on Tie2 receptor is known to be important in retinal vasculature stabilization; angiopoietin-2, an Ang-1-antagonist, is increased in DR. We previously showed that intravitreal injection of AAV2.COMPAng1, a more stable analogue of Ang1, prevents vascular loss and leakage and preserves visual acuity in type 1 diabetic Ins2Akita mice. AAV10 serotype has been shown to elicit minimal neutralizing immune response in mice and humans; we previously showed that a single intravenous injection of AAV10.COMPAng1 does not transduce the gene in the eye but achieves systemic distribution and sustains therapeutic levels of serum COMPAng1 at least 2 weeks post-injection. We now examine effects of AAV10.COMPAng1, systemically and on visual function, 6 months post-injection.
Methods: We treated 2-month-old Akita with a single tail vein injection of AAV10.COMPAng1, AAV10.GFP, or PBS for controls. At 6 months post-injection, we assessed mice for fasting blood glucose, body weight, hemoglobin A1c (HgbA1c), serum COMPAng1 by ELISA, visual tracking response by OptoMotry, retinal thickness by OCT, and scotopic ERG response. At 6 weeks and 3 months post-AAV10.GFP treatment, we harvested liver, lung, kidney, and retina to examine GFP expression by immunofluorescence staining and semi-quantitative RT-PCR.
Results: Therapeutic levels of serum COMPAng1 were reached as early as 7 days and sustained at 6 months post-injection. At 8 months old, Akita treated with AAV10.COMPAng1 when 2 months old had significant lowering of HgbA1c, fasting blood glucose, and body weight as compared to Akita controls. There was no difference in visual tracking response, retinal thickness, or ERG response. Mice did not show adverse effects or reactions. At 6 weeks and 3 months after AAV10.GFP treatment, we found most GFP expression in liver, followed by lung, then kidney.
Conclusions: Serum COMPAng1 was expressed at therapeutic levels as early as 7 days and sustained for 6 months after a single intravenous treatment. Although AAV10.COMPAng1-treated Akita did not differ from Akita controls on visual function measures, the treated mice, on average, had significantly lower HgbA1c, fasting blood glucose, and body weight as compared to controls. Further studies are required to evaluate the potential of AAV.COMPAng1 as a therapeutic against diabetic end-organ disease.
Commercial Relationships: Malkit Singh, None; **Susie Choi**, None; **Petra Soininen**, None; **Zoya Sandhu**, None; **Daniel Fang**, None; **Deepti Vashist**, None; **Austin Bohner**, None; **Xiaohui Zhang**, None; **Yuanyuan Wu**, None; **Lara Carroll**, None; **Yufeng Huang**, None; **Hironori Uehara**, None
Support: RPB Unrestricted Grant

Program Number: 191 **Poster Board Number:** B0222
Presentation Time: 8:30 AM–10:15 AM
H-1129 suppresses neovascularization through inhibition of secretion of VEGF and proliferation of vascular endothelial cells
Yoko Yoshida[1, 2], Atsuko Kasai[1, 2], Hiroyoshi Hidaka[1, 2]. [1]D.Western Therapeutics Institute, Inc., Nagoya, Japan; [2]Human Research Promotion and Drug Development, Mie University, Tsu, Japan.
Purpose: We reported that H-1129 bound to Hsp90 specifically in ARVO2012. In this study, we elucidate the mechanism of action of H-1129 against neovascularization. It was investigated that effects of H-1129 on production of vascular endothelial growth factor (VEGF), migration of retinal pigment epithelial cells (RPE cells) and proliferation of vascular endothelial cells (VECs). Effects of H-1129 on hypoxia-inducible factor-1 (HIF-1) were also investigated. HIF-1, a transcription factor regulating expression of VEGF, is stabilized through Hsp90 action (Wu WC et al., 2007).
Methods: H-1129 was discovered, synthesized and patented by D. Western Therapeutics Institute, Inc. (DWTI) laboratory. VEGF concentration in the culture supernatant of ARPE-19 cells following pretreatment with H-1129 was measured by ELISA. Cell migration was assessed by wound healing assay and cell proliferation was assessed by Cell Counting Kit-8 assay. HIF-1 alpha, a subunit of HIF-1, in the cells was analyzed by Western blotting.
Results: H-1129 significantly reduced VEGF secretion from ARPE-19 cells and inhibited migration of ARPE-19 cells. H-1129 also inhibited proliferation of human retinal microvascular endothelial cells (hRMECs) and human umbilical vascular endothelial cells (HUVEC) in the presence of VEGF. Both H-1129 and its main metabolite, H-1129M1, reduced HIF-1 alpha in ARPE-19 cells cultured under hypoxic condition.
Conclusions: H-1129 inhibited cell proliferation of VECs, cell migration of RPE cells and secretion of VEGF from RPE cells. H-1129 and H-1129M1 reduced induction of HIF-1 alpha in RPE cells under hypoxic condition. These results suggest double-action mechanism of H-1129 for suppression of production of VEGF in RPE cells and inhibition of cell proliferation of VECs in the presence of VEGF stimulation. H-1129 has the possibility to become a superior therapeutic drug for choroidal neovascularization.
Commercial Relationships: Yoko Yoshida, D. Western Therapeutics Institute, Inc. (E); **Atsuko Kasai**, D. Western Therapeutics Institute, Inc. (E); **Hiroyoshi Hidaka**, D. Western Therapeutics Institute, Inc. (E)

Program Number: 192 **Poster Board Number:** B0223
Presentation Time: 8:30 AM–10:15 AM
The Potential Roles of IL-33 and TGF-β1 in the Pathogenesis of Stevens - Johnson syndrome/ Toxic Epidermal Necrolysis
Omer Iqbal[1], Charles S. Bouchard[1], makio iwashima[2], Sean Till[2], Ping Bu[1]. [1]Ophthalmology, Loyola University Chicago, Maywood, IL; [2]Loyola University Medical Center, Maywood, IL.
Purpose: The exact mechanism of keratinocyte apoptosis in the pathogenesis of Stevens - Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) characterized by drug-induced mucocutaneous reactions and ocular involvement leading to corneal blindness remains unclear. We tested the hypothesis that increased levels of Interleukin-33 (IL-33) and transforming growth factor beta one (TGF-β1) in the plasma and also their increased expression in the skin of patients with biopsy confirmed SJS/TEN compared to that with lichen planus as controls obtained and archived from a clinical study may play important roles in keratinocyte apoptosis.
Methods: Under a current, Loyola IRB approved protocol clinical study, collected and archived unstained slides (n=8) of skin from patients with biopsy confirmed SJS/TEN and lichen planus as controls (n=6) were used for this study. Immunohistochemical analysis was performed using IL-33 and TGF-β1 antibodies followed by imaging on a DeltaVision microscope. ELISA analysis was used to determine the levels of IL-33 and TGF-β1 expression in multiple citrated plasma samples totaling 78 from SJS/TEN patients (n=14). Statistical analysis was performed using linear regression analysis, ANOVA, and Turkey's *post-hoc* tests on STA-TA software.
Results: Immunofluorescent microscopy of SJS/TEN skin biopsy samples revealed elevated levels of both TGF-β1and IL-33 in the epithelium compared to lichen planus skin biopsy samples as controls. However, ELISA analysis of SJS/TEN patient plasma samples showed no marked elevation of TGF-β1 or IL-33 compared to normal human plasma ($p= 0.41$ and 0.26 respectively). The results of this study may enhance our understanding of the pathogenesis of SJS/TEN and lead to the development of new treatment modalities for this disease.
Conclusions: Our results are consistent with our hypothesis that increased expression of IL-33 and TGF-β1 in the skin of patients with SJS/TEN compared to lichen planus as controls play important roles in keratinocyte apoptosis. However, their corresponding undetectable levels in plasma by the ELISA technique needs to be evaluated in freshly obtained larger number of plasma samples. Further large-scale studies are warranted to validate these results.
Commercial Relationships: Omer Iqbal, None; **Charles S. Bouchard**, None; **makio iwashima**, None; **Sean Till**, None; **Ping Bu**, None
Support: Illinois Society for the Prevention of Blindness

Program Number: 193 **Poster Board Number:** B0224
Presentation Time: 8:30 AM–10:15 AM
The efficacy of aflibercept in treating retinopathy of prematurity in the mouse model of oxygen-induced retinopathy
Sarina M. Amin, Swati Agarwal, W. Clay Smith, Jade G. Guevara. Ophthalmology, University of Florida, Gainesville, FL.
Purpose: Many studies have reported on bevacizumab for the treatment of retinopathy of prematurity (ROP); however, there is a scarcity of literature on the efficacy and safety of aflibercept in the treatment of ROP despite anecdotal evidence of promising outcomes in human neonates. The goal of this study was to assess the efficacy of aflibercept in regression of neovascularization in the mouse model of oxygen-induced retinopathy.
Methods: In this study, 14 mice (n=28 eyes) were included and randomly assigned to a room air control (n=10) or hyperoxic conditions with 75% oxygen (n=18). The mice exposed to hyperoxia were assigned to one of two groups: 0 ng (n=8) or 10 ng (n=10) of aflibercept. Intravitreal injections were administered to hyperoxia groups on day 14 of life. Two days post-injection, eyes were enucleated and retinas were extracted. Samples were stained with anti-collagen IV antibody to highlight vasculature. Flat mount preparations were photographed, and areas of perfusion and non-perfusion were quantified using ImageJ software. Statistical analysis was performed using one-way ANOVA with Bonferroni correction.
Results: A significant difference ($p<0.05$) in vascular and avascular surface areas was noted between the room air control and the hyperoxia groups. Curiously, hyperoxic eyes treated with aflibercept showed increased neovascularization compared to the untreated hyperoxic eyes ($p<0.05$). There was a notable trend towards reduction of avascular surface area in the aflibercept-treated eyes, although the difference did not reach statistical significance ($p=0.06$).
Conclusions: Mice exposed to hyperoxic conditions displayed increased vascularity compared with room air controls. Aflibercept-treated eyes showed more vascularity than non-treated eyes possibly

due to inadequate dosing or to the short time from injection to enucleation not allowing for treatment effect. Although non-significant, a clear trend was noted for reduced avascular surface area in eyes treated with aflibercept compared with the hyperoxic control. This study will be expanded to increase sample size and to include groups treated with higher doses of aflibercept. This study will help clarify the doses of aflibercept associated with the adverse effect of persistent avascular retina, and ultimately this study can help establish aflibercept as a potential treatment alternative in human neonates with ROP.

Commercial Relationships: Sarina M. Amin, None; **Swati Agarwal**, None; **W. Clay Smith**, None; **Jade G. Guevara**, None

Support: Unrestricted grant from the foundation on Research to Prevent Blindness

Program Number: 194 **Poster Board Number:** B0225
Presentation Time: 8:30 AM–10:15 AM

Blockade of Apelin Receptor (APJ) can inhibit developmental retinal vessel outgrowth in pups and promote normal revascularization and reduce pathological neovascularization in OIR model in mice

Eunice Cheung, Panayiotis Stevis, Yonaton Ray, Stanley J. Wiegand, Carl Romano, Ivan Lobov. Regeneron Pharmaceuticals, Tarrytown, NY.

Purpose: During angiogenesis, apelin/apelin receptor (APJ) in combination with VEGF, induces the proliferation and assembly of endothelial cells (H Kidoya, et al. EMBO J, 2008; 27). Much remains to be discovered about the expression of the apelinergic system and precisely how it affects numerous physiological functions. In this study, we evaluate apelin receptor inhibition in normal retinal vascular development and in oxygen retinopathy model (OIR) in mice.

Methods: Study 1: Humanized (Hu) ApelinR mice were systemically (IP) injected with 25 mg/kg and 50 mg/kg Fc (control) and anti-Apelin Receptor (αAR) at P2 and collected at P5;
Study 2: Repeat of Study 1 at the highest dose, analyzed by masked graders.
Study 3: P2 Hu pups were IP injected with 50 mg/kg Fc, αAR or aflibercept and collected at P5;
Study 4: Hu were place in a hyperoxic environment (75% O_2) at P6 and returned to room air at P11. Pups were injected systemically with 50 mg/kg Fc (control), αAR, or aflibercept at P12 and collected at P16;
Study 5: OIR Hu mice were injected intravitreally (IVT) with 5 µg Fc, αAR, or aflibercept at P12 and collected at P16.
All retinas were stained with FITC-labeled Griffonia simplicifolia lectin I (Vector Laboratories).

Results: By injecting αAR at P2, vascular outgrowth was reduced by 23% (n= 6 eyes/groups, $p < 0.05$) at 25 mg/kg and 35% (n= 6 eyes/group, $p < 0.005$) at 50 mg/kg at P6. Aflibercept administration produced 43% reduction in blood vessel outgrowth at 50 mg/kg. In the OIR model, αAR and aflibercept were able to promote vessel regrowth. When pups were IP injected at OIR P12, αAR and aflibercept significantly reduced avascular areas by 29% and 27% (n= 6 eyes/group, $p < 0.005$) and neovascularizations by 69% and 94% (n= 6 eyes/group, $p < 0.0001$) at P16 respectively. With IVT administration aflibercept reduced vessel regrowth by 30% (n= 4 eyes/group, $p < 0.0001$) and eliminated all neovascularization, while αAR was able to promote vessel regrowth by 30% (n= 4 eyes/group, $p < 0.0001$) and reduce neovascularizations by 60% (n= 4 eyes/group, $p < 0.0001$).

Conclusions: Our data suggest that apelin/APJ is an important mediator of developmental angiogenesis and both pathologic and normal angiogenesis in hypoxia-driven proliferative retinopathy.

Commercial Relationships: Eunice Cheung, Regeneron Pharmaceuticals, Inc. (E); **Panayiotis Stevis**, Regeneron Pharmaceuticals, Inc. (E); **Yonaton Ray**, Regeneron Pharmaceuticals, Inc. (E); **Stanley J. Wiegand**, Regeneron Pharmaceuticals, Inc. (E); **Carl Romano**, Regeneron Pharmaceuticals, Inc. (E); **Ivan Lobov**, Regeneron Pharmaceuticals, Inc. (E)

Program Number: 195 **Poster Board Number:** B0226
Presentation Time: 8:30 AM–10:15 AM

Therapeutic potential of topical administration of H-1129, an isoquinoline sulfonamide derivative, in wet AMD and proliferative diabetic retinopathy

Kengo Sumi[1, 3], Hiroyoshi Hidaka[1, 3], Yoko Yoshida[1, 3], Atsuko Kasai[1, 3], Takashi Izuhara[1, 3], Masahiko Sugimoto[2, 3], Mineo Kondo[2, 3].
[1]D. Western Therapeutics Institute, Inc., Nagoya, Japan; [2]Department of Ophthalmology, Mie University, Tsu, Japan; [3]Human Research Promotion and Drug Development, Mie University, Tsu, Japan.

Purpose: H-1129 is protein kinase and Hsp-90 inhibitor (ARVO 2012). Eye drop administration of H-1129 produced significant intraocular pressure (IOP) decrease in normal human volunteers at Phase I study. H-1129 will be tested in Phase II study after 2017. In this study, the efficacy of H-1129 eye drop against choroidal neovascularization (CNV) in a rat laser-induced model was investigated at effective doses for IOP reduction therapy.

Methods: H-1129 was discovered and synthesized by D. Western Therapeutics Institute, Inc. (DWTI) laboratory. Vascular endothelial growth factor (VEGF) concentration in the culture supernatant of ARPE-19 cells was measured by ELISA following pretreatment with H-1129. Cell migration and proliferation were assessed by wound healing assay with ARPE-19 cells and Cell Counting Kit-8 assay with human retinal microvascular
endothelial cells (hRMEC) or human umbilical vascular endothelial cells (HUVEC), respectively. Effects of H-1129 on CNV were investigated in a rat laser-induced CNV model. After laser photocoagulation, Each of H-1129 eye drops (0.5-2%) was topically administered three times per day for two weeks. Progresses of CNV were examined by choroidal flat mount analysis, and evaluated using Image J software. Following topical administration of 2% H-1129 in rats, its concentration in the posterior segment of the eye was measured.

Results: H-1129 decreased VEGF secretion from ARPE-19 cells by 30% ($p < 0.05$ compared with vehicle) and inhibited migration of them by 51% ($p < 0.01$ compared with vehicle) at doses of 10 µM and 100 µM, respectively. H-1129 also inhibited proliferations of both hRMEC and HUVEC. A significant reduction of CNV by applying H-1129 was observed dose-dependently and 2%H-1129 reduced CNV by 61% ($p < 0.05$ compared with vehicle). During two weeks of topical H-1129 administration in rat, cumulative increases of H-1129 and its metabolite, H-1129M1 were confirmed in the retinal pigment epithelium/choroid.

Conclusions: H-1129 has the possibility to be an agent providing a novel therapeutic strategy for proliferative diabetic retinopathy as well as wet AMD, not just as IOP reducing agent. The mechanism of CNV suppression by H-1129 is based on the dual inhibitions of both retinal vascular endothelial cell proliferation and VEGF secretion from ARPE-19 cells.

Commercial Relationships: Kengo Sumi, D. Western Therapeutics Institute, Inc. (E); **Hiroyoshi Hidaka**, D. Western Therapeutics Institute, Inc. (E); **Yoko Yoshida**, D. Western Therapeutics Institute, Inc. (E); **Atsuko Kasai**, D. Western Therapeutics Institute, Inc.

(E); **Takashi Izuhara**, D. Western Therapeutics Institute, Inc. (E); **Masahiko Sugimoto**, D. Western Therapeutics Institute, Inc. (F); **Mineo Kondo**, D. Western Therapeutics Institute, Inc. (F)

Program Number: 196 **Poster Board Number:** B0227
Presentation Time: 8:30 AM–10:15 AM
The long leap from drug affinity to efficacy: Intervening factors that strongly influence clinical outcomes
Eric Wakshull[1], Eric Day[2], Jihong Yang[2], Xiangdan Wang[2], Sandeep Yadav[2], William Hanley[2]. [1]Bioanalytical Sciences, Genentech, South San Francisco, CA; [2]Genentech, Inc., South San Francisco, CA.

Purpose: The clinical efficacy of drug product is strongly impacted by multiple factors (Fig 1). We review data on the relative binding affinity and potency of 2 VEGF inhibitors used to treat retinal diseases (ranibizumab, RBZ; aflibercept, AFL), and assess their relevance to clinical outcomes.

Methods: A comparative review and analysis of key studies on the affinity and potency of VEGF inhibitors and a sensitivity modeling experiment.[1,2]

Results: RBZ and AFL bind to VEGF under various lab conditions. Using Biacore, Papadopoulos et al[1] reported the affinity (K_D) for AFL binding VEGF as <1 pM while RBZ affinity was 46 pM. However, their RBZ affinity analysis had a major limitation due to rapid dissociation of RBZ from the capture antibody.[2] Further we found that K_D values from Biacore measurements were assay-format dependent.[2] Data from an orthogonal method, analytical ultracentrifugation, did not support AFL having a significantly higher affinity to VEGF relative to RBZ. RBZ and AFL showed similar dose-dependent inhibition of VEGF-induced endothelial cell proliferation (IC_{50} ~0.088±0.032, 0.090±0.009 nM, respectively). A sensitivity analysis of RBZ and AFL clinical data indicates that, assuming the affinities reported[1] and intravitreal half-life of drug are within the reported ranges, there appears to be no improvement in vision gained (Fig 2). This analysis is supported by various clinical trials where the RBZ and AFL doses given provide sufficient inhibition of VEGF and results showed comparable vision gains and dosing intervals for RBZ or AFL.

Conclusions: We have shown that affinity values reported for RBZ by Papadopoulos et al[1] are not reliable.[2] Moreover, both molecules appear equipotent in a relevant cell-based assay. Modeling of literature data show that within the range of reported affinities, dosing and pharmacokinetic considerations suggest comparable clinical outcomes. Thus, in this context, affinity is not an important driver of efficacy. Most importantly, clinical trial data from patients support the comparable efficacy and durability of RBZ and AFL.

1. Papadopoulos et al. Angiogenesis 2012;15:171-85.
2. Yang et al. Mol Pharmaceutics 2014;11:3421-30.

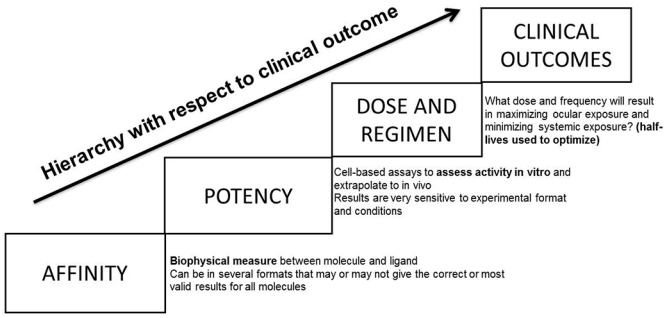


Figure 1. Putting molecular characteristics and clinical outcomes into perspective

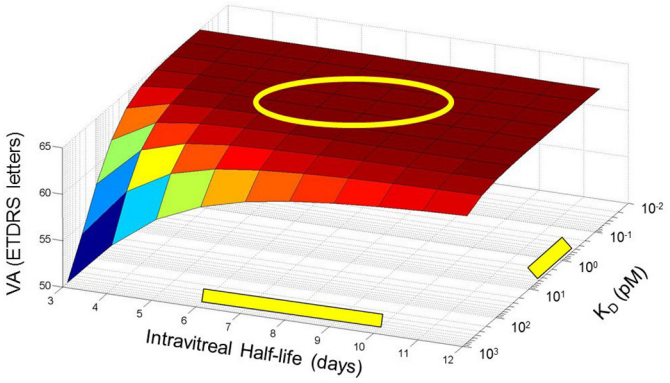


Figure 2. Results of sensitivity analysis using RBZ and AFL data

Commercial Relationships: Eric Wakshull, Genentech, Inc. (E); **Eric Day**, Genentech, Inc. (E); **Jihong Yang**, Genentech, Inc. (E); **Xiangdan Wang**, Genentech, Inc. (E); **Sandeep Yadav**, Genentech, Inc. (E); **William Hanley**, Genentech, Inc. (E)
Support: Genentech, Inc., South San Francisco, CA, provided support for the study and participated in the study design; conducting the study; and data collection, management, and interpretation

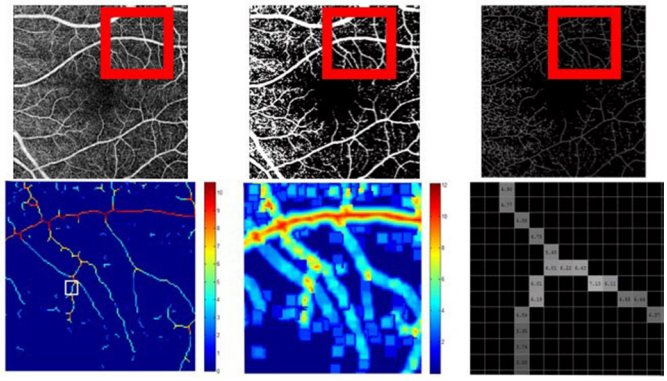
Program Number: 197 **Poster Board Number:** B0228
Presentation Time: 8:30 AM–10:15 AM
Aflibercept therapy can lead to vascular abnormalities
Adam Wylegala, Filip Wylegala, Edward Wylegala. Ophtalmology, Railway Hospital, Katowice, Katowice, Poland.

Purpose: Purpose: Recent studies do not support the hypothesis of vascular normalization in eyes receiving intravitreous anti–vascular endothelial growth factor (VEGF). We wanted to test whether anti-VEGF treatment affects biometric parameters of vascular plexus of the retina.

Methods: The study was conducted in Department of Ophthalmology Silesian Medical University in Katowice.
The mean age of 16 women and 8 men, was 71 (Standard Deviation ± 13,8). All participant were examined using DRI OCT Triton (Topcon, Tokyo, Japan) by a single trained technician. We have measured proportions of radius of the parent vessel in superficial retinal plexus to the sum of the radii of the daughter branches. The same ratios was used to measure length of the vessels. Furthermore, we measured vascular density. The images were letter altered to binary colours and skeletonized. Subsequently Stahler analysis was performed (Fig.1). We letter conducted meassurements using semi-automatic algorithm. Vessel parameters were calculated using ImageJ software with NeuronJ plugin. While statistical analysis was conducted in Statistica (Statsoft).

Results: The ratio of the lengths of the branches were 1,50 in healthy, 1,52 in AMD patients and 1,41 in AMD treated eyes. Ratio of radii was statistically significant (p=0,001) and it was 1,03; 1,48 and 1,57 in healthy, AMD and AMD treated patients respectively. Vascular Density of the superficial plexus was 2,05; 2,1 and 1,99 in AMD, patients undergoing treatment and in controls respectively.

Conclusions: Anti-VEGF treatment as well as AMD can lead to alteration of vessel radii, thus creating disturbances in blood flow.



Commercial Relationships: Adam Wylegala, None; **Filip Wylegala**, None; **Edward Wylegala**, None

Program Number: 198 **Poster Board Number:** B0229
Presentation Time: 8:30 AM–10:15 AM
Panobinostat reduces neovascularization in an alkali-induced corneal injury model
Jianping Chen, Shaobo Su. Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.
Purpose: Histone deacetylases (HDACs) plays a causative role in cancer and central nervous diseases by modification of histone and non-histone protein. Downregulation of HDAC expression by HDAC inhibitors (HDACis) restores or increases the level of histone acetylation and shows the potential approach to treat tumorigenesis and angiogenesis. In this study, we examined the effect of panobinostat, a non-selective HDACis, on inflammatory corneal angiogenesis.
Methods: Corneal inflammation was induced with 1 N NaOH. Panobinostat was applied topically to the injury corneas twice a day for 7 days. Eyes were examined with a slit lamp (Zeiss, Germany) 7 and 14 days after alkali injury. Mice were sacrificed and the corneas were removed from both eyes for total RNA extraction or histological analysis. In vitro, the effect of panobinostat on migration, proliferation and tube formation by human microvascular endothelial cells (HEMC-1) was examined. Total RNA of corneas and HEMC-1 were performed by real time PCR to examine the expression of pro/anti-angiogenic factors. Experiments were repeated at least 3 times.
Results: Topical application of panobinostat to the injured corneas attenuated corneal neovascularization (CNV) in an alkali-induced corneal injury model. In addition, in vivo treatment with panobinostat downregulated the expression of the pro-angiogenic factors vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF-β1), and epidermal growth factor (EGF), but upregulated the expression of the anti-angiogenic factors thrombospondin (TSP)-1, TSP-2, and ADAMTS-1 in the injured corneas. Furthermore, panobinostat inhibited the expression of pro-angiogenic factors, migration, proliferation, and tube formation by human microvascular endothelial cells (HEMC-1) in vitro.
Conclusions: We found that topical application of panobinostat attenuated CNV by inhibition of the expression of the pro-angiogenic factors. The data suggest that panobinostat has therapeutic potential for angiogenesis-associated diseases such as CNV.
Commercial Relationships: Jianping Chen; **Shaobo Su**, None
Support: 31471122 from the National Natural Science Foundation of China

Program Number: 199 **Poster Board Number:** B0230
Presentation Time: 8:30 AM–10:15 AM
RGX-314, an AAV8 expressing an anti-VEGF protein, strongly suppresses subretinal neovascularization and vascular leakage in mouse models
Ji-kui Shen[1], yuanyuan liu[1], Seth D. Fortmann[1], Stephen Yoo[3], Karen Kozarsky[2], Jiangxia Wang[1], Peter A. Campochiaro[1]. [1]Ophthalmology, Johns Hopkins Wilmer Eye Inst, Baltimore, MD; [2]REGENXBIO Inc, Rockville, MD; [3]REGENXBIO Inc, Rockville, MD.
Purpose: To test the efficacy of RGX-314, an AAV serotype 8 expressing an anti-VEGF Fab, in transgenic mouse models expressing human VEGF
Methods: Transgenic mice in which the rhodopsin promoter drives expression of $VEGF_{165}$ in photoreceptors (rho/VEGF mice) had a subretinal injection of RGX-314 with doses ranging from $3x10^6$-$1x10^{10}$ genome copies (GC), $1x10^{10}$ GC of null vector, or PBS in one eye (n=10 per group) at P14. At P21, the area of subretinal neovascularization (SNV) per eye was measured. Double transgenic mice with doxycycline (DOX)-inducible expression of $VEGF_{165}$ in photoreceptors (Tet/opsin/VEGF mice) had a subretinal injection of $1x10^8$-$1x10^{10}$ GC of RGX-314 in one eye and no injection in the fellow eye or $1x10^{10}$ GC of null vector in one eye and PBS in the fellow eye. Ten days after injection, 2mg/ml of DOX was added to drinking water and after 4 days fundus photos were graded for presence of total, partial, or no retinal detachment (RD). RGX-314 transgene product levels were measured one week after subretinal injection of $1x10^8$-$1x10^{10}$ GC of RGX-314 in adult mice by ELISA analyses of eye homogenates.
Results: Compared to eyes of rho/VEGF mice injected null vector, those injected with $\geq 1x10^7$ GC of RGX-314 had significant reduction in mean area of SNV, with modest reduction in eyes injected with $\leq 3x10^7$ and >50% reduction in eyes injected with $\geq 1x10^8$ GC. Eyes injected with $3x10^9$ or $1x10^{10}$ GC had almost complete elimination of SNV ($p<0.01$). In Tet/opsin/VEGF mice, compared to the null vector group in which 100% of eyes had total RD, there was significant reduction in exudative RD in eyes injected with $\geq 3x10^8$ GC of RGX-314 and reduction of total detachments by 70-80% in eyes injected with $3x10^9$ or $1x10^{10}$ GC ($p<0.01$). The majority of eyes injected with $\leq 1x10^9$ GC of RGX-314 had protein levels below the limit of detection, but all eyes injected with $3x10^9$ or $1x10^{10}$ GC had detectable levels with mean level per eye 342.7ng and 286.2ng.
Conclusions: Gene therapy by subretinal injection of RGX-314 caused dose dependent suppression of SNV in rho/VEGF mice with near complete suppression with doses of $3x10^9$ or $1x10^{10}$ GC. These same doses showed robust protein product expression and markedly reduced total exudative RD in Tet/opsin/VEGF mice.
Commercial Relationships: Ji-kui Shen; **yuanyuan liu**, None; **Seth D. Fortmann**, None; **Stephen Yoo**, REGENXBIO Inc (E); **Karen Kozarsky**, REGENXBIO Inc (E); **Jiangxia Wang**, None; **Peter A. Campochiaro**, REGENXBIO Inc (C)

Program Number: 200 **Poster Board Number:** B0231
Presentation Time: 8:30 AM–10:15 AM
Magnolol reduces retinal neovascularization in the OIR model and protects HUVECs under hypoxia via HIF-1a/VEGF pathway
Xiaoling Liang, Boyu Yang, Yue Xu, Yaguang Hu, Xi Lu, Zhengjie Xu. Zhongshan University; State Key Laboratory, Guangzhou, China.
Purpose: To investigate whether Magnolol may reduce retinal neovascularization (RNV) in an oxygen-induced retinopathy (OIR) model and protect human umbilical vein endothelial cells (HUVECs) under hypoxia.

Methods: The neonatal C57BL/6J mice were exposed to 75% O2 from postnatal day (P) 7 to P12 and subsequently returned to room air. Mice were injected with 25mg/kg Magnolol intraperitoneally once a day from P12 to P17, then retinas were harvested and flat-mounted at P17 to assess the retinal vessels. To further explore the protective effect of Magnolol (20μM) on the HUVECs in vitro, cobalt chloride (CoCl2), a hypoxia-mimicking agent, was used to induce the hypoxia model. HUVECs were incubated with VEGF and CoCl2 in the presence or absence of Magnolol to perform the capillary-like tube formation assay. HUVECs migration was evaluated with wound healing assay. To clarify the molecular mechanisms of Magnolol, we observed the level of hypoxia inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF), which were assessed with immunofluorescence staining, RT-PCR and western blotting.
Results: Magnolol significantly inhibited angiogenesis in vivo and in vitro evidenced by the reduction of RNV in OIR model and by the attenuation of hypoxia and VEGF-induced capillary tube-like structure formation of HUVECs, respectively. In addition, Magnolol inhibited hypoxia-induced HUVECs migration. In hypoxia, the expression of HIF-1α and VEGF in HUVECs were increased significantly. However, decreased levels of HIF-1α and VEGF were detected under hypoxia after Magnolol treatment.
Conclusions: These findings indicated that the anti-angiogenic activity of Magnolol is mediated by suppressing HIF-1a/VEGF-dependent pathways, and suggested that Magnolol might have potential for the treatment of pathological retinal angiogenesis.
Commercial Relationships: Xiaoling Liang, None; **Boyu Yang**, None; **Yue Xu**, None; **Yaguang Hu**, None; **Xi Lu**, None; **Zhengjie Xu**, None

Program Number: 201 **Poster Board Number:** B0232
Presentation Time: 8:30 AM–10:15 AM
Multifunctional small molecule TLR4 antagonist for treating ocular neovascularization
Suchismita Acharya[1, 3], Santosh K. Panda[3], Jiyang Cai[2], Dorota L. Stankowska[1]. [1]North Tx Eye Research Institute, UNT health Science Center, Fort Worth, TX; [2]Ophthalmology, University of Texas Medical Branch, Galveston, TX; [3]AyuVis Research LLC, Dallas, TX.
Purpose: The multifactorial pathological challenge of ocular neovascularization is difficult to address so far only by anti-VEGF therapy. We have tested our hypothesis that, a novel class of natural product derived compound with toll like receptor 4 (TLR4) antagonist activity can ameliorate the hyper-inflammation produced by macrophage/macroglia over activation as well as decrease choroidal neovascularization (CNV) size in mice.
Methods: *Inhibition of cytokines:* Mouse bone marrow derived macrophages were treated with high mobility group box1 (HMGB1, 100 ng/mL), an endogenous TLR4 ligand for 8 hours, with or without 100 mg/mL of test compounds. The mRNA levels of TNF-a, iNOS were measured by real-time RT-PCR, and normalized to the control cells. *Inhibition of VEGF production:* ARPE-19 cells were treated either with 100ng/mL or without HMGB1 along with the compounds (50μg/mL) for 24 h. Supernatants were collected and assayed using human VEGF ELISA kit according to manufacturer's instructions. Experiments were repeated three times and one way ANOVA was used for statistical analysis. *Inhibition of CNV*: Laser CNV was induced in C57BL/6 mice (male, 10-12 weeks, n = 5). Each eye received 4 laser burns. The compounds (200 μg/mL) or BSS (vehicle) were administered by IP injection once before and once daily up to 10 days following laser injury. Fundus fluorescein angiography and optical coherence tomography was used to visualize the CNV lesions. RPE/choroid/sclera flat mounts were prepared and stained with both FITC-conjugated isolectin B4 and anti-ICAM-2 antibody to quantitatively measure the size of CNV lesion.
Results: Compound treatment significantly ($p<0.05$) decreased TNF-a, iNOS level in macrophages compared to HMGB1 (control). Compound C-Heptaose significantly decrease the production of VEGF (118.75±8.18ng/mL) in ARPE-19 cells as compared to HMGB1 (185.42±18.5 ng/mL) and was comparable to untreated control (108.04±16.15 ng/mL). Intraperitoneal injections of C-Heptaose reduced the average size of CNV lesions to about 50% ($p<0.05$, $n = 2$) of those in control mice treated with vehicle only in the mouse model.
Conclusions: Our results are consistent with our hypothesis that this novel class of compounds will decrease inflammation and neovascularization. Further structure optimization of the lead compound and TLR4 dependent and independent mechanistic investigation are underway.
Commercial Relationships: Suchismita Acharya, AyuVis Research (F), AyuVis Research (P), AyuVis Research (E); **Santosh K. Panda**, AyuVis Research (P); **Jiyang Cai**, None; **Dorota L. Stankowska**, None

Program Number: 202 **Poster Board Number:** B0233
Presentation Time: 8:30 AM–10:15 AM
Anti-VEGF induced nephropathy following intravitreal administration: a potential toxicity
Deepak Mangla[1], Manjot Gill[1], Susan E. Quaggin[2]. [1]Department of Ophthalmology, Northwestern University, Chicago, IL; [2]Northwestern Department of Medicine, Feinberg Cardiovascular Institute, Chicago, IL.
Purpose: Anti-VEGF induced kidney injury has rarely been described in the ophthalmology literature. Consideration of this potential toxicity will become increasingly important as the use and indications of anti-VEGF treatment are broadened.
Methods: This is a case report and review of the literature. We present a patient with neovascular AMD receiving anti-VEGF injections who developed declining renal function. We reviewed both the opthalmology literature and nephrology literature for renal injury secondary to alterations in systemic VEGF levels.
Results: After extensive work-up to exclude other etiologies for renal failure including renal biopsy, injections were halted temporarily per the nephrology service. A renal biopsy showed chronic tubule-interstitial disease.
Conclusions: The nephrology team believed the renal injury to be secondary to ocular anti-VEGF injections. Studies have shown that serum VEGF levels are depressed following intraocular anti-VEGF injection, however the extent is dictated by each drug's pharmacokinetics. Ophthalmologists should be aware of this entity as patients may require pretreatment counseling particularly for those with pre-existing renal disease. Close monitoring of the patient may be required in order to assess renal function before and after the intravitreal administration of anti-VEGF.
Commercial Relationships: Deepak Mangla, None; **Manjot Gill**, None; **Susan E. Quaggin**, None

Program Number: 203 **Poster Board Number:** B0234
Presentation Time: 8:30 AM–10:15 AM
The effect of nicotinamide phosphoribosyl transferase inhibitor JSNMPT-029 on corneal neovascularization
Jiayi Jin, Shaobo Su. Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.
Purpose: Many diseases, such as heart failure, tumor occurrence,Huntington's disease are associated with aberrant energy metabolism. Nicotinamide phosphoribosyl transferase (NAMPT) is a

rate limiting enzyme catalyzing the synthesis of cellular nicotinamide adenine dinucleotide (NAD+), which is required for ATP production. In this study, we examined the effect of JSNMPT-029, an inhibitor of NAMPT, on inflammatory corneal angiogenesis.
Methods: Alkali-induced injured corneas of mice were topically applied with JSNMPT-029 twice a day. Eyes were examined with a slit lamp 7 and 14 days after alkali injury. Mice were scarified and the corneas were harvested for total RNA extraction for real time PCR or for histological analysis with 10% neutral formalin fixation. In vitro, the effect of JSNMPT-029 on migration, proliferation and tube formation by human umbilical vein endothelial cells (HUVECs) were examined. The expression of pro/anti-angiogenenic factor in corneas and HUVECs were examined by real time PCR.
Results: Topical application of JSNMPT-029 to the injured corneas attenuated corneal neovascularization (CNV) with down-regulation of the expression of the pro-angiogenic factors VEGF, b-FGF, TGFβ1 and EGF but up-regulation of the expression of the anti-angiogenic factors Tsp-1, Tsp-2 and ADAMTS-1 in the injured corneas. Furthermore, JSNMPT-029 inhibited the expression of pro-angiogenic factors, migration, proliferation and tube formation by human umbilical vein endothelial cells (HUVECs) *in vitro*.
Conclusions: Topical application of JSNMPT-029 to the injured corneas attenuated CNV by inhibition of the expression of the pro-angiogenic factors but promotion of the expression of the anti-angiogenic factors in the injured corneas. The data suggest that JSNMPT-029 has therapeutic potential for angiogenesis- associated diseases such as CNV.
Commercial Relationships: Jiayi Jin, None; **Shaobo Su**, None
Support: 31471122 from the National Natural Science Foundation of China

Program Number: 204 **Poster Board Number:** B0235
Presentation Time: 8:30 AM–10:15 AM
Effects of anti-angiogenic drugs on expression patterns of genes involved in different AMD pathogenetic pathways
Mohamed Mohamed[1, 2], M. Tarek Moustafa[1, 2], Shari R. Atilano[1], Abdul Sami Memon[1], Mohamed Riazi[1], Sean W. Tsao[1], Cristina M. Kenney[1], Baruch D. Kuppermann[1]. [1]Gavin Herbert Eye Institute, University of California Irvine, Irvine, CA; [2]Ophthalmology Department, University of Minia, Minia, Egypt.
Purpose: Age-related macular degeneration (AMD) is a highly heritable neurodegenerative disease with at least 19 identified risk loci to date. This study examines effects of anti-VEGF drugs on the expression of genes involved in different AMD pathogenetic pathways in immortalized human RPE cells (ARPE-19) *in vitro*.
Methods: ARPE-19 cells were treated for 24 h with ranibizumab, bevacizumab, or aflibercept in 1X and 2X concentrations of the clinical intravitreal dose. Untreated cells were used as controls. RNA was isolated and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed in triplicate using primers for angiogenesis (*VEGFA* and *HIF1A*), apoptosis (*BAX* and *BCL2L13*), inflammation (*IL18* and *IL1B*) and oxidative stress (*GPX3* and *SOD2*). ΔΔCt (differences in cycle thresholds) was obtained and folds calculated using the formula 2^ΔΔCt. Unpaired t test was used for statistical analysis.
Results: Aflibercept-treated cells significantly overexpressed *GPX3* and *IL1B* at 1X concentration, and *SOD2*, *BAX*, *GPX3*, and *BCL2L13* at 2X concentration compared to untreated cultures. Ranibizumab-treated cells significantly overexpressed *SOD2*, *BAX*, *GPX3*, and *BCL2L13* at 1X concentration, and *HIF1A*, *SOD2*, *BAX*, *GPX3*, and *BCL2L13* at 2X concentration. Bevacizumab-treated cells significantly overexpressed *VEGFA*, *HIF1A*, *SOD2*, *BAX*, *GPX3*, and *BCL2L13* at 1X concentration, and *VEGFA*, *SOD2*, *BAX*, *GPX3*, *IL1B*, and *BCL2L13* (Table 1).
Conclusions: Our results show that anti-VEGF drugs can alter expression of angiogenesis, apoptosis and inflammation genes, which are important pathways involved in AMD pathogenesis. Our findings suggest that in addition to binding vascular endothelial growth factor (VEGF) and blocking receptor interactions for angiogenesis inhibition, these drugs have broader mechanisms of action. That may help us understand patient's variability in response to anti-VEGF drugs.

		AFLIBERCEPT				RANIBIZUMAB				BEVACIZUMAB			
		1X		2X		1X		2X		1X		2X	
	Gene	Fold	p	Fold	p	Fold	p	Fold	p	Fold	p	Fold	p
Angiogenesis	VEGF-A	1.03	0.83	1.22	0.18	1.25	0.14	1.31	0.08	1.39	**0.04**	1.81	**0.005**
	HIF1A	0.95	0.62	1.28	0.09	1.01	0.94	1.42	**0.04**	1.59	**0.02**	1.14	0.54
Pro-apoptosis	BAX	0.92	0.11	2.16	**< 0.0001**	1.88	**< 0.0001**	1.81	**0.0008**	2.04	**< 0.0001**	1.88	**0.0004**
	BCL2L13	1.10	0.25	1.73	**0.0004**	2.03	**< 0.0001**	1.94	**0.0004**	1.87	**0.0005**	2.10	**0.0002**
Inflammation	IL1β	1.23	**0.05**	0.99	0.87	1.07	0.49	0.91	0.42	0.93	0.38	1.59	**0.009**
	IL18	0.94	0.60	1.06	0.57	0.98	0.88	0.87	0.24	0.98	0.84	0.93	0.51
Oxidative stress	GPX3	**1.37**	**0.02**	**2.34**	**< 0.0001**	**2.36**	**< 0.0001**	**2.17**	**0.0008**	**2.30**	**< 0.0001**	**2.03**	**0.01**
	SOD2	0.89	0.06	2.63	**< 0.0001**	2.38	**< 0.0001**	1.91	**0.0002**	1.95	**0.0002**	1.84	**< 0.0001**

Table 1. Differences in gene expression after treatment with anti-VEGF drugs. Folds > 1 denote gene upregulation and folds < 1 gene downregulation compared to untreated.
Commercial Relationships: Mohamed Mohamed, None; **M. Tarek Moustafa**, None; **Shari R. Atilano**, None; **Abdul Sami Memon**, None; **Mohamed Riazi**, None; **Sean W. Tsao**, None; **Cristina M. Kenney**, None; **Baruch D. Kuppermann**, Regeneron (F), Ophthotech (C), Genentech (R), Alcon (C), Allergan (F), Allergan (R), Regeneron (C), Regeneron (R), Catalyst (C), Genentech (F), Novartis (C), GSK (F), Novartis (R), Genentech (C), Alcon (F), Ophthotech (F), Apellis (F), Allergan (C)
Support: Funding Supported by Discovery Eye Foundation, Polly and Michael Smith, Iris and B. Gerald Cantor Foundation, Max Factor Family Foundation. Supported by an RPB Unrestricted Grant.

Program Number: 205 **Poster Board Number:** B0236
Presentation Time: 8:30 AM–10:15 AM
Ciliary neurotrophic factor in patients with age-related cataract
Alexander A. Shpak[1], Alla B. Guekht[2], Tatiana Druzhkova[2], Ksenia Kozlova[3], Natalia Gulyaeva[3]. [1]Clin & Functional Diag, S Fyodorov Eye Microsurg Federal State Institution, Moscow, Russian Federation; [2]Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russian Federation; [3]Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russian Federation.
Purpose: Analysis of the ciliary neurotrophic factor (CNTF) content in the eye may be of great importance for clarification of the pathogenesis of different ophthalmological diseases and evaluation of the efficacy of treatment. However, in practice this analysis is impossible except for during surgical interventions. The aim of the study was to measure and compare CNTF levels in the aqueous humor, tears, and blood serum in patients with age-related cataract.
Methods: Fifty-five patients (55 eyes) operated for age-related cataract were examined. Exclusion criteria were any serious ophthalmic or somatic pathology, high refractive errors. Mean age of patients was 68.7±7.9 years (range 53-85 years); there were 16

men and 39 women. Collection of stimulated tears was performed by a pipette on the day preceding surgery; the aqueous humor of the anterior chamber and the blood were sampled during the phacoemulsification of a cataract. The concentration of CNTF was measured in the studied biological media by an enzyme immunoassay (EIA) using a Quantikine Elisa test system (R&D Systems, USA) on a ChemWell 2910 automatic analyzer (Awareness Technology Inc., USA).
Results: On average, the concentration of CNTF was 55.8±25.0 pg/mL in aqueous humor, 38.6±17.6 pg/mL in tears, and 5.76±5.39 pg/mL in serum. The concentration of CNTF in the aqueous humor correlated well with its content in the tears (Pearson's correlation coefficient was 0.68, P<0.000) and was higher on average by a factor of 1.50±0.51. The CNTF concentration in serum did not show significant correlation with the CNTF level in aqueous humor or tears.
Conclusions: In patients with age-related cataract CNTF concentration in the aqueous humor significantly correlated with its content in the tears being higher on average by a factor of 1.50±0.51. The CNTF concentration in serum was much lower and did not show any correlation with the CNTF level in aqueous humor or tears. These data may serve as a basis for approximate evaluation of CNTF concentrations in the aqueous humor using the results of tears analysis.
Commercial Relationships: Alexander A. Shpak, None; **Alla B. Guekht**, None; **Tatiana Druzhkova**, None; **Ksenia Kozlova**, None; **Natalia Gulyaeva**, None

Program Number: 206 **Poster Board Number:** B0237
Presentation Time: 8:30 AM–10:15 AM
Surface analytical studies of growth factor coupling to collagen by copper-free click chemistry
Hyun Jong Lee[1, 2], Gabriella Fernandes-Cunha[1], Won-Gun Koh[2], Jeffrey L. Goldberg[1], David Myung[1]. [1]Byers Eye Institute, Stanford University, Palo Alto, CA; [2]Chemical and Biomolecular Engineering, Yonsei University, Seoul, Korea (the Republic of).
Purpose: Direct immobilization of growth factors onto the surface of ocular wounded tissue via biocompatible chemical reactions may improve their therapeutic potential. Given the continuous fluid turnover of the ocular environment, anchoring of growth factors would increase their residence time compared to topical application. To achieve direct immobilization, we utilized strain-promoted alkyne-azide cycloaddition (SPAAC) which is a bio-orthogonal form of copper-free click chemistry, a promising tool for binding growth factors onto a tissue surface due to its specificity, versatility, and biocompatibility.
Methods: N-Hydroxysuccinimide ester (NHS ester) reaction was used to conjugate azide groups onto epidermal growth factor (EGF) or fluorescein isothiocyanate (FITC)-labeled EGF, depending on the experiment being conducted. In all cases, EGF was conjugated with azide-NHS ester and then purified by dialysis. Collagen-coated polystyrene, glass, and gold were used depending on the surface analysis technique being used. Both bicyclononyne (BCN) and dibenzocyclooctyne (DBCO) moieties were applied to introduce alkyne groups to collagen. We attached the azide-EGF onto the alkyne-modified collagen surfaces via SPAAC and verified the binding via fluorescence microscopy, ellipsometry, surface plasmon resonance (SPR), and enzyme-linked immunosorbent assay (ELISA).
Results: When the FITC conjugated Azide-EGFs (FITC-Azide-EGF) was applied, growth factor binding yielded surfaces with significantly higher green fluorescence compared to controls. EGF binding on collagen surfaces by physical adsorption and SPAAC were measured real-time by observation of increasing thicknesses and response units in ellipsometry and SPR, respectively. Chemical coupling of EGF via SPAAC yielded significantly greater ligand binding and resistance to dissociation compared to physisorption. ELISA assays showed that the EGF surface concentration could be modulated by varying the concentration of applied EGF as well as SPAAC reaction time.
Conclusions: Our results showed that SPAAC could be used to controllably and efficiently bind EGF to collagen surfaces. This method may provide a biocompatible way to immobilize therapeutic biomolecules to tissues *in situ* to promote ocular wound healing.
Commercial Relationships: Hyun Jong Lee, None; **Gabriella Fernandes-Cunha**, None; **Won-Gun Koh**, None; **Jeffrey L. Goldberg**, None; **David Myung**, None
Support: Korean Health Technology R&D Project through the Korean Health Industry Development Institute (HI15C1744)

Program Number: 207 **Poster Board Number:** B0238
Presentation Time: 8:30 AM–10:15 AM
Elevated Levels of TGFβ2 and sFRP1 in Aqueous Humor of Glaucomatous Patients
tao guo[1], Li Guo[1], Yuan Deng[1], Iok-Hou Pang[2], Xianqun Fan[1]. [1]Ophthalmology, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, China; [2]North Texas Eye Research Institute, Fort Worth, TX.
Purpose: To assess levels of transforming growth factor β2 (TGFβ2) and secreted frizzled-related protein 1 (sFRP1) in the aqueous humor(AH) of eyes with different types of glaucoma.
Methods: AH samples were collected from 36 patients (36 eyes). Those samples, which were divided into six groups averagely, respectively came from eyes with primary open angle glaucoma (POAG), chronic angle closure glaucoma (CACG), primary angle closure suspects (PACS), acute angle-closure glaucoma (AACG) with high intraocular pressure (IOP), AACG with low IOP and cataract serving as controls. Patients undergoing cataract or glaucoma surgery without associated significant intraocular pathology were selected for this study. At the time of surgery, a small amount of aqueous was withdrawn. The concentration of TGFβ2 and sFRP1 were measured by enzyme-linked immunosorbent assay.
Results: The mean concentration (± standard error) of TGFβ2 in the AH of eyes with POAG (457.18±122.79 pg/ml) was significantly higher than that in eyes with controls (292.83±80.45 pg/ml) (P=0.02). Levels of sFRP1 in AH of AACG with high IOP eyes(2.18±0.73 ng/ml) were found to be elevated when comparing to those of controls(1.37±0.18 ng/ml)(P=0.04). There was no significant change in concentration of TGFβ2 between eyes with controls and CACG (339.42±129.44 pg/ml), PACS(313.79±151.39 pg/ml), AACG with high IOP(278.96±166.88 pg/ml), AACG with low IOP(329.91±127.63 pg/ml), respectively. The mean concentration of sFRP1 in the AH of eyes with POAG (1.43±0.66 ng/ml), CACG(1.51±0.59 ng/ml),PACS(1.75±0.56 ng/ml),AACG with low IOP(1.84±1.12 ng/ml),were not significantly different from that in eyes with controls. No correlation between TGFβ2 or sFRP1 levels and age, gender, previous treatment or type of surgery was found.
Conclusions: Our results indicate that elevated levels of TGFβ2 may play an important role in the pathogenesis of POAG, while that of sFRP1 may correlate with the progression of AACG.
Commercial Relationships: tao guo, None; **Li Guo**, None; **Yuan Deng**, None; **Iok-Hou Pang**, None; **Xianqun Fan**, None
Support: Shanghai Municipality Commission for Science and Technology(15411970000)

Program Number: 208 **Poster Board Number:** B0239
Presentation Time: 8:30 AM–10:15 AM
The Effect of Interleukin 38 on Angiogenesis in a Model of Oxygen-induced Retinopathy
Jing Zhang, Shaobo Su. Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.
Purpose: Interleukin 38 (IL-38) is a novel identified cytokine of IL-1 family in which some members are important in inflammation and angiogenesis. However, the role of IL-38 in angiogenesis is unknown. The aim of the present study is to explore the effect of IL-38 on angiogenesis.
Methods: Mouse oxygen-induced retinopathy (OIR) model was induced of by exposure of hyperoxia (75% oxygen) to C57BL/6J mice from postnatal day 7 (P7) to P12 and then returned to room air. The mice were intravitreal injection with 1μl of IL-38 dissolved at different concentrations (0, 5, or 25 ng/μl) at P12. At P17, neovascular region (tufts) and avascular area were analyzed. In addition, the effect of IL-38 on the migration, proliferation and tube formation were examined by human umbilical vein endothelial cells (HUVECs) in vitro.
Results: In vitro, IL-38 was expressed in endothelial cells and was down-regulated under hypoxic condition. IL-38 reduced the proliferation, migration and tube formation of endothelial cells in a dose-dependent manner. In vivo, IL-38 was expressed in mouse retina. Neonatal mice administrated with IL-38 in a mouse model of oxygen-induced retinopathy (OIR) showed that retina neovascularization was reduced.
Conclusions: Our findings suggest that IL-38 is an antiangiogenic cytokine in pathophysiological settings.
Commercial Relationships: Jing Zhang, None; **Shaobo Su**, None
Support: 31471122 from the National Natural Science Foundation of China

Program Number: 209 **Poster Board Number:** B0240
Presentation Time: 8:30 AM–10:15 AM
An increased expression of IL-17 and Foxp3 is observed in contrast to other Th genes in primary pterygium
JOSE NAVARRO-PARTIDA[1], Carlos D. Diaz-Palomera[2], RAMSES ROSALES DIAZ[2], ABRIL B. MARTINEZ-RIZO[2], Adolfo D. Rodriguez-Carrizalez[3], ARTURO SANTOS[1].
[1]DIVISION DE BIOTECNOLOGIA Y SALUD. CAMPUS GUADALAJARA., TECNOLOGICO DE MONTERREY, ZAPOPAN, Mexico; [2]UNIDAD ACADEMICA DE MEDICINA, UNIVERSIDAD AUTONOMA DE NAYARIT, TEPIC, Mexico; [3]CENTRO UNIVERSITARIO DE CIENCIAS DE LA SALUD, UNIVERSIDAD DE GUADALAJARA, GUADALAJARA, Mexico.
Purpose: An increasing number of pro-inflammatory cytokines, angiogenic and fibrogenic growth factors and their receptors have been related to pterygium (Pt). T helper cells $CD4^+$ (Th) are an important constituent on Pt tissues, however The effect of Th cytokines on Pt pathogenesis has been poorly studied. To date, only interleukin 4 (IL-4) from Th cells has been linked to pterygium recurrence. Therefore, this study was undertaken to evaluate the expression of Th cytokines and Th transcription factors in primary Pt.
Methods: Pterygia heads were collected from 28 eyes of 28 Mexican patients undergoing primary pterygium excision with conjunctival autografting. Conjunctival biopsies from 8 patients undergoing cataract surgery were used as control group. Gene expression of the Th citokines; interferon gamma (IFN-γ), IL-13, IL-17, IL-10, as well as the gene expression of the Th transcription factors; T-bet, GATA3, Foxp3 and RORγt were analyzed by real time RT-PCR using TaqMan assays. The $2^{-\Delta\Delta CT}$ method was used for data analysis.
Results: The expression of T-bet, GATA3, RORγt, IFN-γ, IL-13 and IL-10 were downregulated in Pt. Interestingly, the lowest transcript expression for IL-13 and GATA3 were reached at maximum corneal invasion. The expressions of IL-17 and Foxp3 were increased up to 6 fold changes in Pt tissues ($P< 0.0001$), and occurred for the latter gene in a Pt size and Pt extension dependent manner.
Conclusions: The increased expression of IL-17 and Foxp3 in Pt specimens suggest the presence of $IL\text{-}17^+Foxp3^+$ T cells, a subset of Th population which suppress T cell proliferation and promotes tumor progression. Therefore, $IL17^+Foxp3^+$ T cells could be a therapeutic target to prevent pterygium growth.
Commercial Relationships: JOSE NAVARRO-PARTIDA, None; **Carlos D. Diaz-Palomera**, None; **RAMSES ROSALES DIAZ**, None; **ABRIL B. MARTINEZ-RIZO**, None; **Adolfo D. Rodriguez-Carrizalez**, None; **ARTURO SANTOS**, None

Program Number: 210 **Poster Board Number:** B0241
Presentation Time: 8:30 AM–10:15 AM
Quantification of aflibercept and ranibizumab efficacy in DL-2-aminoadipic acid (DLAAA)-induced retinal neovascularization and vascular leakage in nonhuman primates
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Purpose: To define the optimal strategy for grading and quantifying the retinal neovascularization and leakage induced by intravitreal (IVT) injection of DLAAA in the green monkey and to further validate the model by evaluating the comparative magnitude of response to IVT aflibercept and ranibizumab treatments.
Methods: DLAAA was administered intravitreally at one dose of 5 mg in adult St. Kitts green monkeys (*Chlorocebus sabaeus*). Weekly ophthalmic examinations including slit lamp biomicroscopy, color fundus photography, fluorescein angiography and optical coherence tomography were conducted up to 18 weeks. Aflibercept, ranibizumab or normal saline were injected intravitreally at 8 weeks post-DLAAA following randomization of eyes to treatment on the bases of week 8 leakage. The area and fluorescence intensity of retinal vascular leakage were quantified from the 1 and 3 minute angiograms by Amira software (Materials & Structural Analysis, Hillsboro, OR).
Results: Both aflibercept and ranibizumab treatments attenuated the area and fluorescence intensity of retinal vascular leakage, starting from 1 week, peaking around 4 weeks, with gradual reoccurrence of leakage to a level less than the pre-treatment condition at 10 weeks post-administration. Treatment with aflibercept resulted in a 75% reduction in average leakage area in 3 minute angiograms while a 50% reduction was observed in response to ranibizumab. The changes in area and intensity were quite similar between 1 and 3 minute angiograms, however, a complete inhibition of leakage was only observed at the 1 minute angiogram interval in eyes treated with aflibercept.
Conclusions: Measurement of the area and fluorescence intensity of DLAAA-induced neovascularization and leakage using Amira software provides an additional reproducible method for quantitative analysis of retinal vascular leakage. Both aflibercept and ranibizumab treatments significantly reduced retinal vascular leakage, with aflibercept exhibiting a more pronounced effect. Reference to the established behaviour of these control articles in the DLAAA test system and application of these quantitative methods will allow more robust screening of candidate anti-angiogenic compounds.

Commercial Relationships: Wenzheng Hu, RxGen (E); **Donnicia James**, RxGen (E); **Anish Kurian**, RxGen (E); **Jordan Attwood**, RxGen (E); **Cyrene Phipps**; **Akeem Browne**, RxGen (E); **Vernard Woodley**, RxGen (E); **Akeba Matthew**, RxGen (E); **Alex Lewis**, RxGen (E); **Robin J. Goody**, RxGen (E); **Matthew S. Lawrence**, RxGen (E)

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Comparison of pain and comfort for intravitreal administration of anti-VEGF agents using blepharostat vs non blepharostat techniques

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Purpose: Compare patients pain and comfort on both techniques with blepharostat and non blepharostat, to choose the comfortable, safe and adequate technique for the patient intravitreal administration of anti-VEGF.

Methods: Patients eligible for intravitreal administration of anti-VEGF agents due to various types of retinal diseases were interrogated to assess pain and comfort, using blepharostat vs non-blepharostat with appropriate aseptic techniques and Antisepsis prior iodine instillation 5%. To evaluate pain, we are using the numerical scale of pain score 0-10 (0 = no pain - 5 = moderate pain, 10 = worst pain). In addition, signs of cataract, inflammation or infection were sought immediately and at 28 days

Results: Intravitreal administration without blepharostat was less painful and more comfortable for patients, also in both groups were not found signs of cataract secondary to intravitreal administration, infectious or inflammatory signs.

Conclusions: Adequate intravitreal administration of anti-VEGF technique without blepharostat is less painful, more comfortable and safe for the patient than using a blepharostat technique.

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