Hypoxia-Inducible Factor-1α Is Associated With Sprouting Angiogenesis in the Murine Laser-Induced Choroidal Neovascularization Model

Heldner Andre, Selcuk Tunik, Monica Aronsson, Anders P. Kvanta. St Erik Eye Hospital, Karolinska Institute, Stockholm, Sweden.

Purpose: To investigate the expression and distribution of neoangiogenic molecules and the role of hypoxia during the development of experimental choroidal neovascularization (CNV).

Methods: Lesions were induced on C57Bl6 mice using laser photocoagulation. Animals were euthanized in a timely manner and eyecups were dissected from enucleated eyes. Choroids were immunostained for pericytes, sprouting endothelial cells (EC), or vascular EC. Choroidal neovascularization lesions where analyzed for tissue hypoxia, hypoxia-inducible factors (HIF), and heat-shock proteins (HSP).

Results: Choroidal neovascularization lesions showed a trend of increased cellular recruitment throughout the time-course and the lesions displayed positive staining for angiogenic markers. Both pericytes and sprouting EC displayed a radial progression, while vascular EC displayed a more uniform distribution across the CNV lesions. Furthermore, positive tissue hypoxia staining was observed and associated with expression of HIF-1α and vascular endothelial growth factor (VEGF).

Conclusions: Our data delineate specific temporal windows during CNV initiation, propagation, maturation, and even recovery in experimental CNV. We show that murine CNV undergoes hypoxia-associated sprouting angiogenesis, and demonstrate involvement of pericytes. Moreover, we have shown expression of HIF-1α to the retinal pigment epithelium surrounding the CNV lesions, together with VEGF upregulation, independently of the HSP response induced by the laser thermal insult.

Commercial Relationships: Heldner Andre, None; Selcuk Tunik, None; Monica Aronsson, None; Anders P. Kvanta, None

Program Number: 2121 Poster Board Number: C0028
Presentation Time: 11:00 AM–12:45 PM

Griseofulvin inhibits choroidal neovascularization

Timothy W. Corson, Rania S. Sulaiman, Sameerah Alkhairy, Kamna Gupta, Halesha D. Basavarajappa. Ophthalmology, Indiana University School of Medicine, Indianapolis, IN.

Purpose: Much of the vision loss in the common blinding eye disease wet age-related macular degeneration (AMD) is due to neovascularization of the choroid. No small molecule pharmacotherapies are yet approved for this disease. We previously identified the heme biosynthesis enzyme ferrochelatase as an important mediator of ocular neovascularization; knockdown of ferrochelatase blocked angiogenesis in vitro and in the murine laser-induced choroidal neovascularization (L-CNV) model. Small molecule inhibition of ferrochelatase is thus an appealing approach for impulses neovascularization. Excitingly, the FDA-approved antifungal drug griseofulvin inhibits ferrochelatase as an off-target effect. Hence, here we sought to investigate if griseofulvin can block angiogenesis.

Methods: The anti-angiogenic effects of griseofulvin and its active metabolite, N-methylprotoporphyrin (NMPP), were tested in vitro using proliferation, scratch-wound migration, and tube formation assays with human retinal endothelial cells. The choroidal sprouting assay assessed griseofulvin’s effects on choroidal cells ex vivo. The murine L-CNV model was used to test the in vivo anti-angiogenic potential of griseofulvin, delivered intravitreally or orally. L-CNV was analyzed both in vivo by optical coherence tomography, and ex vivo by confocal microscopy.

Results: Both griseofulvin and NMPP dose-dependently inhibited proliferation, migration, and tube formation of retinal endothelial cells, without obvious toxicity at effective concentrations. Griseofulvin at 50 µM or higher profoundly suppressed choroidal sprouting. In the L-CNV model, griseofulvin dose-dependently decreased choroidal neovascularization. Intravitreally, 50 µM and 100 µM griseofulvin reduced CNV volume by 28 and 67%, respectively. Similarly, 0.5% and 1% (w/w) griseofulvin delivered in food were also efficacious, reducing CNV volume by 33% and 42% (all p<0.05, ANOVA with Tukey’s post hoc tests).

Conclusions: Griseofulvin at clinically achievable concentrations has significant anti-angiogenic potential in the eye. Since oral griseofulvin is already approved for human use and well tolerated during long-term administration, these findings could progress rapidly toward human trials, with potential benefit for the sight of wet AMD patients and applicability to other ocular neovascular diseases.

Commercial Relationships: Timothy W. Corson, US Provisional 62/111,149 (P); Rania S. Sulaiman; Sameerah Alkhairy, None; Kamna Gupta, None; Halesha D. Basavarajappa, US Provisional 62/111,149 (P)
Support: NIH NCATS UL1TR001108, International Retinal Research Foundation, Research to Prevent Blindness, Inc.

Program Number: 2123 Poster Board Number: C0030
Presentation Time: 11:00 AM–12:45 PM

The complement system is dual-hatted, acting in both damage and repair processes in the murine model of choroidal neovascularization

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Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness in the U.S. Polymorphisms in various complement components are associated with increased risk for AMD, and it has been hypothesized that an overactive complement system is partially responsible for the pathology of AMD. While it has been shown previously that complement components C3a and C5a promote choroidal neovascularization, it is important to note these components also play a role in tissue repair. Here we investigated the involvement of complement C3 activation and of C3a-receptor signaling in the development and regression of choroidal neovascularization (CNV).

Methods: Laser-induced photocoagulation was used to trigger CNV in wild type mice (C57BL/6J). CNV lesion size was measured by optical coherence tomography (OCT), performing exams regularly between days 5 and 28 after laser. Activation of C3 was inhibited during the induction phase of CNV (days 0-6) with CR2-Crry, and C3a-receptor engagement was inhibited during the regression phase of CNV (days 6-28) with a C3a-receptor antagonist (N2-(2,2-diphenylethoxy)acetyl)-L-arginine, TFA).

Results: CNV lesion size and subretinal fluid accumulation were significantly reduced in mice treated with CR2-Crry, confirming a role for downstream complement activation products in CNV growth. Interestingly, treatment of WT mice with the C3a-receptor antagonist TFA, significantly reduced repair of the lesion, with TFA-treated mice exhibiting prolonged fluid accumulation and fibrosis.

Commercial Relationships: Timothy W. Corson, None; Sameerah Alkhairy, None; Rania S. Sulaiman, None; Kamna Gupta, None; Halesha D. Basavarajappa, None
Support: NIH 62/111,149 (P)

Program Number: 2122 Poster Board Number: C0029
Presentation Time: 11:00 AM–12:45 PM
Dyslipidemia and Lipoprotein Profile in Age-related Macular Degeneration

**Purpose:** We recently identified a mutation (D442G) at the cholesteryl ester transfer protein (CETP) locus associated with elevated HDL-C and late AMD. We now examine the association between lipoprotein particle size distribution and AMD, and whether CETP D442G mutation may modulate this association.

**Methods:** We performed a case-control study of AMD cases (184 early AMD; 193 exudative AMD) and 289 age and gender-matched controls. Serum was analyzed for lipid biochemistry and lipoprotein particle concentrations, particle size and subfractions with nuclear magnetic resonance spectroscopy. CETP status was determined using Illumina Human OmniExpress or Taqman probe. Serum CETP activity was measured using Elisa.

**Results:** HDL-C was higher in exudative AMD cases (1.4mmol/L) than controls (1.3mmol/L, p=0.027) and early AMD cases (1.3mmol/L, p=0.067). There were marked differences in the lipoprotein particle profile between the exudative AMD group and controls, with higher concentrations of HDL particles (37.2 vs 35.3 µmol/L, p=0.039), and intermediate-density lipoprotein (IDL) particles (155.6 vs 110.7mmol/L, p<0.001), and lower concentrations of VLDL particles (60.6 vs 73.2 mmol/L, p<0.001) and ApoA1 (145.3 vs 155.8, p<0.001). Mean HDL particle size was larger in exudative AMD cases than controls (9.3 vs 9.2 nm, p=0.01). After multivariable adjustment, each unit increase in HDL particles was associated with a 5% increase in exudative AMD risk (p=0.002). Early AMD cases only had few differences in lipoprotein profile compared to controls. CETP activity was not significantly different between the two groups. Adjustment for the presence of CETP D442G polymorphism did not significantly alter the associations; however, there was a statistically significant interaction between CETP D442G polymorphism and HDL-particle number and exudative AMD.

**Conclusions:** Patients with exudative AMD have a distinct lipoprotein profile. These findings were not mediated by the CETP D442G mutation, but suggest more widespread disturbance in lipid metabolism in exudative AMD. Lower ApoA1 could correlate with impaired reverse cholesterol transport by causing impaired lipid carrier capacity. Lipoprotein profile in early AMD cases was largely similar to controls, suggesting that lipid metabolism may affect pathways responsible for progression from early to exudative AMD. Modulation of lipid metabolism may represent a novel means to retard the development of AMD.

**Commercial Relationships:** Gemmy Cheung, None; Alfred Tau Liang Gan, None; Qiao Fan, None; Rajendra S. Apte, None; Shyam Chaurasia, None; Ching-Yu Cheng, None; Tien Y. Wong, None; E Shyong Tai, None

**Support:** National Medical Research Council grant NMRC/ NIG/1003/2009 and Biomedical Research Council grant BMRC 10/1/35/19/671

**Program Number:** 2125 Poster Board Number: C0032

**Presentation Time:** 11:00 AM–12:45 PM

**Step towards the resolution of mystery behind ocular lipofuscin**

**Purpose:** Accumulation of lipofuscin in human retinal pigment epithelium is reported to be one of the causative factors in the pathogenesis of age related Macular Degeneration (ARMD). Therefore, the present study was undertaken to identify and evaluate the levels of various lipofuscin components in normal age matched aging Indian donor eyes.

**Methods:** Donor eyes (Group 4, above 80 yrs; N=36, Group 3, 60-80 yrs; N=60, Group 2, 40-60 yrs ;N=80, Group 1, below 40 yrs; N=44) were collected from the Lions’ International Eye Bank of Arvind Hospital, Madurai with prior approval from the Standing Institute Human Ethics Committee. Macular and peripheral portions were dissected out using 8mm punch and were subjected for extraction. Thereafter the samples were further processed and subjected for analysis. Method was developed for lipofuscin components using Multiple Reaction Monitoring modes of ESI-LC-MS/MS and APCI-LC-MS/MS. Confirmed components were again subjected for relative quantification using single ion monitoring mode for higher sensitivity.

**Results:** This study showed increasing levels of A2GPE and ATRD along with increasing age. In the group 4, 3 & 2, A2GPE levels were found to increase significantly by 41, 39 and 7% respectively than that found in macula of >40 years group (group 1). The levels of ATRD were found to have a statistically significant increase by 49, 73 and 24 % then that of >40 years group. However, for A2DHPE the significant rise was only observed in above 80 years.

**Conclusions:** In cadaver macular and peripheral retinal age matched extract the levels of A2GPE, ATRD, A2DHPE ATRDE, monofuran A2E and monoperoxy A2E were quantified and correlated with that of A2E levels.

**Commercial Relationships:** Ankita Kottala, None; Senthilkumari Srinivasan, None; Nabaniya Halder, None; B Jayaram, None; Atul Kumar, None; Thirumurthy Velpandian, None

**Support:** This work was sponsored in part by a Department of Veterans Affairs merit award RX000444, a National Institutes of Health grant EY019320, the Feldberg Endowment as well as an unrestricted grant to MUSC from Research to Prevent Blindness (RPB), New York, NY.

**Program Number:** 2124 Poster Board Number: C0031

**Presentation Time:** 11:00 AM–12:45 PM

**Dodson et al**

**Purpose:** We recently identified a mutation (D442G) at the cholesteryl ester transfer protein (CETP) locus associated with elevated HDL-C and late AMD. We now examine the association between lipoprotein particle size distribution and AMD, and whether CETP D442G mutation may modulate this association.

**Methods:** We performed a case-control study of AMD cases (184 early AMD; 193 exudative AMD) and 289 age and gender-matched controls. Serum was analyzed for lipid biochemistry and lipoprotein particle concentrations, particle size and subfractions with nuclear magnetic resonance spectroscopy. CETP status was determined using Illumina Human OmniExpress or Taqman probe. Serum CETP activity was measured using Elisa.

**Results:** HDL-C was higher in exudative AMD cases (1.4mmol/L) than controls (1.3mmol/L, p=0.027) and early AMD cases (1.3mmol/L, p=0.067). There were marked differences in the lipoprotein particle profile between the exudative AMD group and controls, with higher concentrations of HDL particles (37.2 vs 35.3 µmol/L, p=0.039), and intermediate-density lipoprotein (IDL) particles (155.6 vs 110.7mmol/L, p<0.001), and lower concentrations of VLDL particles (60.6 vs 73.2 mmol/L, p=0=0.001) and ApoA1 (145.3 vs 155.8, p<0.001). Mean HDL particle size was larger in exudative AMD cases than controls (9.3 vs 9.2 nm, p=0.01). After multivariable adjustment, each unit increase in HDL particles was associated with a 5% increase in exudative AMD risk (p=0.002). Early AMD cases only had few differences in lipoprotein profile compared to controls. CETP activity was not significantly different between the two groups. Adjustment for the presence of CETP D442G polymorphism did not significantly alter the associations; however, there was a statistically significant interaction between CETP D442G polymorphism and HDL-particle number and exudative AMD.

**Conclusions:** Patients with exudative AMD have a distinct lipoprotein profile. These findings were not mediated by the CETP D442G mutation, but suggest more widespread disturbance in lipid metabolism in exudative AMD. Lower ApoA1 could correlate with impaired reverse cholesterol transport by causing impaired lipid carrier capacity. Lipoprotein profile in early AMD cases was largely similar to controls, suggesting that lipid metabolism may affect pathways responsible for progression from early to exudative AMD. Modulation of lipid metabolism may represent a novel means to retard the development of AMD.
**Mitochondrial Dysfunction in Experimental Mouse Models of SubRPE Deposit Formation and Reversal by the Mito-Reparative Drug MTP-131**

Scott W. Cousins1, 2, Peter Saloupis1, Mulugu V. Brahmajoti1, Priyatham S. Mettu1, *Ophthalmology, Duke University School of Medicine, Durham, NC; 2Immunology, Duke University School of Medicine, Durham, NC.

**Purpose:** To investigate the role of mitochondrial dysfunction in subRPE deposit formation in mouse models of dry AMD and to assess the response to treatment with MTP-131, a mitochondria-targeting drug that can reverse pre-existing dysfunction.

**Methods:** An acute model of subRPE deposits was generated in young BALB/c or C57BL/6/J mice receiving biweekly subconjunctival injection of the environmental toxin hydroquinone (HQ, 25 μL, 75 mM, administered over 2 or 4 weeks). Chronic subRPE deposits were evaluated in ApoE4 transgenic mice fed high fat + cholesterol (HFC) diet for 4 months. Morphology was assessed by transmission electron microscopy. Fluorescence confocal microscopy of RPE flatmounts was used to assess (1) mitochondrial dysfunction (Flavoprotein autofluorescence (FP-AF) and superoxide); (2) activation of signaling cascades (pHSP25); and (3) biochemical mediators of subRPE deposit formation (actin cytoskeleton disruption and vimentin upregulation). Subcutaneous MTP-131 (3 mg/kg) was given at various times after onset of mitochondrial dysfunction.

**Results:** Acute model: subconjunctival HQ produced frequency-dependent accumulation of subRPE deposits, appearing as precursors to deposits observed in chronic models. Simultaneous with deposits, we observed (1) increased FP-AF and superoxide production, (2) increased pHSP25, and (3) actin aggregate formation. MTP-131 reversed pre-existing HQ-induced mitochondria dysfunction and the associated biochemical responses. Chronic model: in ApoE4 mice fed HFC diet, we observed characteristic subRPE deposits in association with (1) mitochondrial dysfunction (intense FP-AF and high superoxide) and (2) activation of deposit mediators (disrupted actin cytoskeleton and increased vimentin expression). Further, in the ApoE4 model, mitochondrial dysfunction was also reversed by MTP-131 in association with improvement in deposit mediators.

**Conclusions:** In several mouse models of deposits, mitochondrial dysfunction appears to be a trigger of subRPE deposit formation; reversal of dysfunction with MTP-131 also reverses prior activation of signaling cascades and biochemical mediators of deposits. Mitochondrial dysfunction may be a generalized paradigm for dry AMD pathogenesis, and mitochondria-targeting drugs may be therapeutic for dry AMD.

**Commercial Relationships:** Scott W. Cousins, Stealth BioTherapeutics (C), Stealth BioTherapeutics (F); Peter Saloupis, None; Mulugu V. Brahmajoti, None; Priyatham S. Mettu, None

**Support:** Stealth BioTherapeutics (SWC), Research to Prevent Blindness Unrestricted Grant (SWC, PSM), SP30EY005722-28 NEI Core Grant (SWC, PSM), NEI 1K08 EY025325-01 (PSM)

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**Program Number:** 2126
**Poster Board Number:** C0033
**Presentation Time:** 11:00 AM–12:45 PM

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**Purpose:** AMD, a degenerating eye disease leads to vision loss and blindness in the aging population. Advanced AMD is linked to degeneration of the retinal pigment epithelium (RPE), a pigmented monolayer of epithelial cells which helps in the maintenance of photoreceptors health and functions. Previous work suggests that replacing the damaged RPE with an autologous RPE monolayer can provide potential therapy for AMD. Here, we have describe a GLP complaint induced pluripotent stem (iP) cell to RPE differentiation protocol that generates functionally mature RPE patch from patient-specific iP cells.

**Methods:** Viral free, footprint free vectors are used to reprogram patient’s blood cells into GLP-grade iP cells. iP cells are karyotyped and exome sequenced to identify potentially tumorigenic mutations and differentiated under xenofree conditions. Flow cytometry and qRT-PCR based assays are used to determine the efficiency of differentiation and purity of cells using RPE markers TYRP1, CRALBP, and BEST1. RPE patch grown on bio-degradable PLGA (poly (lactic-co-glycolic acid) scaffold is validated using electron microscopy (TEM and SEM), immunofluorescence of RPE markers (Ezrin, Collagen IV, RPE65), electrophysiological measurements, and cytokine secretion for VEGF and PEDF.

**Results:** Karyotypically normal GLP-grade blood cell derived iP cells were free of any potentially pathological oncogenic mutation. Flow cytometry analysis confirmed that more than 95 percent of differentiating cells were positive for BEST1, CRALBP, and TYRP1. TEM images of RPE patches showed complete polarization with extensive apical processes, apically located melanosomes, and basal infoldings. The electrophysiological measurements of iP cell-derived RPE monolayer confirm the intactness of the tissue. The trans-epithelial resistance (TER) for monolayer was 400 ohms. cm2 comparable to the primary human RPE. iP cell derived RPE monolayer secretes cytokines VEGF and PEDF in a polarized fashion with higher VEGF basally and higher PEDF apically.

**Conclusions:** Research grade developmentally guided differentiation protocol was successfully converted into to GLP compliant manufacturing process. This process is reproducible and generates pure RPE cells from patient-specific iP cells. GLP-grade iPSC-RPE monolayers function similar to the native RPE.

**Commercial Relationships:** Fnu Ruchi, None; Vladimir Khristov, None; Balendu Jha, None; Dishita Patel, None; Qin Wan, None; Nathan Hotaling, None; Congxiao Zhang, None; Kapil Bharti, None

**Program Number:** 2127
**Poster Board Number:** C0034
**Presentation Time:** 11:00 AM–12:45 PM

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**Purpose:** Under normal conditions RPE cells are growth-arrested but retain their proliferative capacity. We test the hypothesis that gene transfer of E2F2, a potent transcriptional regulator of cell proliferation, to RPE cells can induce mitosis and increase RPE cell density.

**Methods:** We used non-integrating lentiviral vectors to deliver E2F2 (LNT-E2F2) or hrGFP (LNT-GFP) as a control to either confluent, serum starved ARPE19 cells (MOI 5) or to the RPE of C57Bl/6J mice by subretinal injection (4 x 10^6 infectious particles per eye). Transgene expression was verified by immunofluorescence on

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Results: In vitro, gene transfer of E2F2 to growth-arrested ARPE19 cells induced an increase of Ki67 positive cells (2.3-fold) and uptake of BrdU (3.5-fold) 7 days after transfection. In vivo, 10 days after subretinal injection, LNT-E2F2 caused a 40-fold ±27.2 (median±SD) increase in E2F2 positive RPE cells, and a 10-fold ±4.7 increase in BrdU positive cells in both young (12 weeks) as well as old (18 months) wildtype mice. After LNT-E2F2 treatment, mean RPE cell density increased by 17%±12% compared to control vector treatment (p=0.0011, n=15 eyes per group) and by 14±7% compared to untreated eyes (p=0.0071, n=15 eyes).

We also tested this approach in a mouse model of RPE thinning. After DTA induction, RPEcreERT2/DTA mice showed a 24±10% reduction in central RPE cell density where pathology was strongest (mean±SEM, n=8 eyes, p=0.0231). LNT-E2F2 subretinal injection led to increased BrdU uptake (9-fold ±3.4) and an increase in cell density (37±12%, mean±SEM) in the central RPE (p=0.0458, n=4 eyes per group).

Conclusions: This data suggests that E2F2 can induce RPE cell proliferation and increase cell density in vivo, especially when RPE density is reduced. Such in situ regeneration may lead to a new treatment concept for retinal degenerations with RPE loss.

Commercial Relationships: Daniel Kampik, None; Ulrich F. Luhmann, None; Koji M. Nishiguchi, None; Mark Basche, None; Alexander J. Smith, None; Hong Han, None; Jennifer Williams, None; John Greenwood, None; Stephen E. Moss, None; Frank Larkin, None; Robin R. Ali, None

Support: National Institute of Health Research, NIHR BMRC in Ophthalmology Project Grant 038

Program Number: 2129 Poster Board Number: C0036
Presentation Time: 11:00 AM–12:45 PM

COMPLEMENT FACTOR I BIOACTIVITY: A POTENTIAL BIOMARKER FOR ANTI-AMYLOID BETA TREATMENT IN AGE-RELATED MACULAR DEGENERATION/ GEOGRAPHIC ATROPHY

Francisco J. Lopez1, Hong Chen2, Yong-Qing Lin2, Sanjay Kumar1, Megan M. McLaughlin1. 1GSK, King of Prussia, PA; 2Alliance Pharma, Malvern, PA.

Purpose: Complement activation, particularly the alternative pathway, is thought to be central to the pathogenesis of age-related macular degeneration (AMD). Amyloid β (BAM) is one of the components of drusen, a hallmark of AMD, and complement activation by BAM through interaction with complement factor I (CFI) has been proposed. CFI, a serine protease, limits C3 convertase formation in the alternative pathway through its actions on C3b. An anti-BAM monoclonal antibody (mAb), therefore, is expected to overcome the BAM-mediated reduction of CFI activity. Our goal was to establish a quantitative assay to measure CFI bioactivity and the effect of an anti-BAM mAb, for use not only as a biomarker for target engagement and effectiveness of anti-BAM therapy, but also as a bioassay during the mAb’s manufacturing.

Methods: We have established an in vitro cofactor assay that uses ELISA to quantify iC3b production. Additionally, a modified protocol allows for the measurement of the plasma volume needed to convert half of the C3b added in the reaction mix into iC3b (which is defined as 1 μU of CFI activity in the assay). The estimated volume, therefore, indicates the potency of sample CFI for cleaving C3b. By measuring the concentration of CFI protein by ELISA, the specific activity of the protein can be readily determined.

Results: Our studies show that pre-incubation of CFI with BAM significantly reduces the ability of CFI to cleave C3b into iC3b, recapitulating previous studies. BAM-mediated reduction of CFI activity can be blocked by GSK933776, an anti-BAM mAb, which is directed against the N-terminus of BAM. These data provide proof-of-principle for this assay. In addition the assay has been modified to measure CFI bioactivity in plasma samples.

Conclusions: Our data support assays that may provide early evidence of therapeutic benefit for anti-BAM therapies in long AMD/GA clinical trials if BAM-mediated inhibition of CFI bioactivity plays a role in AMD. In addition, these assays are potentially useful to evaluate anti-BAM monoclonal antibody potency during manufacturing and release.

Commercial Relationships: Francisco J. Lopez, Allergan, GSK; Hong Chen, Alliance Pharma; Yong-Qing Lin, Alliance Pharma; Sanjay Kumar, GSK; Megan M. McLaughlin, GSK

Program Number: 2130 Poster Board Number: C0037
Presentation Time: 11:00 AM–12:45 PM

Three-Month Outcome of Ziv-aflibercept for Exudative Age-related Macular Degeneration

Rafic Antonios1, Ahmad M. Mansour2,3, Jay Chhablani2, Rohit Yogi4, Muhammad Younis5, Mona Keaik2, Rola Dakroub1,2, Hasan Chahtie1. 1Ophthalmology, American University of Beirut Medical Center, Beirut, Lebanon; 2Ophthalmology, Rafic Hariri University Hospital, Beirut, Lebanon, Beirut, Lebanon; 3Smt. Kanuri Santhamma Centre for vitreoretinal Diseases, LV Prasad Eye Institute, Hyderabad, India.

Purpose: Aflibercept is an approved therapy for neovascular macular degeneration (AMD) while ziv-aflibercept is approved for oncology and is cost-effective relative to the expensive same molecule aflibercept. In vitro and in vivo studies did not detect toxicity to the retinal pigment epithelium cells using ziv-aflibercept. Our purpose is to ascertain the 3-month safety and efficacy in AMD treated with intravitreal ziv-aflibercept.

Methods: Prospectively, consecutive patients with wet age-related macular degeneration that required aflibercept underwent ziv-aflibercept intravitreal injection of 0.05 ml of compounded ziv-aflibercept (1.25mg) from March 2015 to November 2015 in the Lebanese series and scattered select cases in the Indian cases. Monitoring of best-corrected visual acuity, intraocular inflammation, cataract progression, and retinal structure by spectral domain OCT were carried initially, one week, one month, two months and three months after injections. The study received Institutional Review Board approval and received the registration NCT02486484.

Results: 30 eyes were treated (22 Caucasians, 8 Indians; 16 men, 14 women; 14 right eye and 16 right eye) with mean age of 74.3 years with 11 naive cases and 19 having had prior injections 4 months prior to our treatment. Best-corrected visual acuity improved from baseline logMar 1.08 to 0.74 at 1 week, 0.72 at 1 month, 0.67 at 2 month, and 0.71 at 3 month (p<0.001 for all time periods). CMT in microns decreased from 332.8 to 302.0 at 1 week, 244.8 at 1 month, 225.9 at 2 months and 208.2 at 3 month (p<0.001 for all time periods). There were no signs of intraocular inflammation, or change in lens status throughout the study. Intraocular pressure was unchanged initial and at 3 month in 10 eyes (12.8±2.3 mmHg vs. 12.8±2.2 mmHg). Significance was also present for the Lebanese series and scattered select cases in the Indian cases.

Conclusions: Off label use of ziv-aflibercept improves visual acuity, without ocular toxicity and offers a cheaper alternative to the same molecule aflibercept, especially in the third world.

Commercial Relationships: Rafic Antonios, None; Ahmad M. Mansour, None; Jay Chhablani, None; Rohit Yogi, None.

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LAURA MONJE-FERNANDEZ, María Andreu-Fenoll, Fe, Valencia, Spain; Ophthalmology Department, University of Valencia, Polytechnical Hospital La Fe, Valencia, Spain; 3Ophthalmology Department, University Assisstential Complex of León, León, Spain.

**Purpose:** To analyze different tomographic characteristics and their influence in visual prognosis and need of treatment in patients with myopic choroidal neovascularization (CNV).

**Methods:** We conducted a retrospective, observational study including all newly diagnosed patients with myopic CNV from May 2013 to May 2015, receiving antiangiogenic therapy with intravitreal ranibizumab injections in a pro re nata regime. Demographic data and visual acuity (VA) were collected. Optical coherence tomography (OCT) scans were evaluated analyzing the presence, or absence of a hyperreflective envelope around the neovascular tissue. Statistical analysis was performed with non-parametric test (Kruskal Wallis).

**Results:** Twenty-seven eyes of 27 patients (mean age: 67.66 years +/- 11.42; 6 men: 21 women) were included. Sixteen patients were classified as group 1 (presence of hyperreflective envelope; mean age: 68.27 years +/-11.26; 5 men: 11 women); 5 patients were classified as group 2 (absence of the envelope; mean age: 69.25 years +/- 13.00; 5 women); 6 patients were classified as group 3 (mixed lesion; mean age: 64.72 years +/-12.21; 1 man: 21 women). Mean baseline VA in logMAR was 0.82 (SD 0.95; 0.0-3.0) and final VA 0.42 (SD 0.43; 0.0-1.3); mean follow-up time was 10.66 months (SD 6.37; 0.0-27.37). No statistically significant differences were found between the 3 groups regarding the age (p=0.543), follow-up time (p=0.053), baseline VA (p=0.578), final VA (p=0.267), macular choroidal volume (p=0.904) and central choroidal thickness (p=0.883). Differences were observed in the total number of injections (p=0.01; Group 1: 1.8+/-.1; Group 2: 2.69+/-.1.14; Group 3: 3.33+/-.1.03). A statistically significant improvement between baseline and final VA was found only in Group 1 (p=0.006).

**Conclusions:** We suggest a new anatomic tomographic classification in patients with myopic CNV based on the presence or absence of the hyperreflective envelope around the neovascular tissue. The presence of this sheath was related with a better visual prognosis with less number of intravitreal injections. Further studies are warranted in order to confirm these results.

**Commercial Relationships:** Isabel Pascual-Camps, None; Pablo Hernández-Martinez, None; LAURA MONJE-FERNANDEZ, None; Maria Andreu-Fenoll, None; Rosa D'olz-Marco, None; Roberto Gallego-Pinazo, None.
Differential hypoxic response of human choroidal and retinal endothelial cells proposes tissue heterogeneity of ocular angiogenesis

Parviz Mammadzada1, 2, Johann Gudmundsson1, 2, Anders P. Kvanta1, 2, Helder Andre1, 2

Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden; 1St. Erik Eye Hospital, Stockholm, Sweden.

Purpose: To elaborate molecular differences between choroidal and retinal angiogenesis by generating and comparatively analyzing human primary choroidal and retinal endothelial cell (CEC and REC) lines.

Methods: Human CEC and REC were isolated by positive-selection and were cultured. Characterization was performed by immunostaining for endothelial cell (EC)-specific markers. Total RNA and protein were extracted from normoxic or hypoxic CEC and REC cultures. Quantitative PCR arrays were used to comparatively analyze 133 genes between CEC and REC, and the expression differences were calculated by ΔΔCt method. A total of 57 angiogenesis-related protein expression differences were investigated by western blot and proteome profiler, and were calculated by densitometry.

Results: Primary human CEC and REC lines stained positively for all EC markers and demonstrated high purity with similar staining and morphology. Under normoxia, CEC showed significantly lower expression levels for cell proliferation and vessel maturation genes and higher expression levels for inflammation-related genes when compared to REC. In response to hypoxia, CEC and REC displayed differential regulation for a multitude of angiogenesis-related genes and proteins. Furthermore, within the vascular endothelial growth factor (VEGF) family, CEC showed preferential upregulation for VEGFA while REC upregulated placenta growth factor (PIGF) levels.

Conclusions: Differential normoxic and hypoxic regulation of angiogenesis-related factors by CEC and REC outlines tissue heterogeneity of ocular angiogenesis and suggests that tissue specificity should be considered as a novel treatment modality for successfully overcoming choroidal and retinal angiogenic conditions in the clinic.

Commercial Relationships: Parviz Mammadzada, None; Johann Gudmundsson, None; Anders P. Kvanta, None; Helder Andre, None

Program Number: 2135 Poster Board Number: C0042
Presentation Time: 11:00 AM–12:45 PM
Improved visual outcome with early treatment in myopic choroidal neovascularization after intravitreal anti-VEGF and associated prognostic factors

Byung Gil Moon, JOO YONG LEE, June-Gone Kim, Young Hee Yoon, Ophthalmology, Asan Medical Center, Seoul, Korea (the Republic of).

Purpose: To determine the correlation between the duration of myopic choroidal neovascularization (CNV) and visual outcome and to identify the baseline predictors for final visual outcome.

Methods: Treatment naïve patients who were received one or more intravitreal anti-VEGF injection and who were followed for at least 24 months due to myopic CNV, were divided into three groups based on the duration of their CNV (group A: <2, B: 2-8, group C: 8-24 weeks). We evaluated the correlation between the duration of the CNV and the treatment outcomes including the BCVA improvement and the decrease in central retinal thickness (CRT) at six, 12, 24 months and at the time of their final visit. The predictive factors related to their final visual outcome as well as the recurrence of CNV were identified.

Results: In a total 106 eyes of 100 patients; there were 26 eyes in group A, 36 eyes in group B and 44 eyes in group C. The mean baseline BCVA (0.48, 0.50 and 0.74), CRT (339.4, 317.8 and 365.5μm) and the presence of subfoveal hemorrhage (73%, 39% and 39%) differed significantly among the groups (p=0.047, 0.010 and 0.010, respectively). During the study period, the total injection number (3.5, 4.0 and 5.5) and recurrence rates (19%, 25% and 52%) differed significantly among the groups (p=0.021, 0.006). We could not determine the differences of mean change of BCVA and CRT, although the final BCVA (0.16, 0.29 and 0.58) and foveal atrophy (15%, 22% and 52%) differed significantly (p=0.001, p=0.005).

After being controlled by other factors, the duration of CNV was significantly correlated with final BCVA (r=0.318, p=0.002). On multivariate analysis, patient age, duration of CNV, baseline BCVA and CNV size were significantly associated with final BCVA (p<0.001, 0.020, <0.001 and 0.004, respectively). Recurrence was related with duration of CNV, subfoveal choroidal thickness and CNV size (p=0.004, 0.023 and 0.037, respectively).

Conclusions: Initiation of treatment as soon as possible following the onset of myopic CNV decreased recurrence rates and required injection number and consequently improved visual outcome. The CNV size and the duration of CNV were the main prognostic factors of myopic CNV after anti-VEGF injection. These results support the hypothesis that early treatment to obtain better visual outcomes and lower recurrence rates in patients with myopic CNV.

Commercial Relationships: Byung Gil Moon, None; JOO YONG LEE, None; June-Gone Kim, None; Young Hee Yoon, None

Program Number: 2134 Poster Board Number: C0041
Presentation Time: 11:00 AM–12:45 PM
Differential hypoxic response of human choroidal and retinal endothelial cells proposes tissue heterogeneity of ocular angiogenesis

Parviz Mammadzada1, 2, Johann Gudmundsson1, 2, Anders P. Kvanta1, 2, Helder Andre1, 2

Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden; 1St. Erik Eye Hospital, Stockholm, Sweden.

Purpose: To elaborate molecular differences between choroidal and retinal angiogenesis by generating and comparatively analyzing human primary choroidal and retinal endothelial cell (CEC and REC) lines.

Methods: Human CEC and REC were isolated by positive-selection and were cultured. Characterization was performed by immunostaining for endothelial cell (EC)-specific markers. Total RNA and protein were extracted from normoxic or hypoxic CEC and REC cultures. Quantitative PCR arrays were used to comparatively analyze 133 genes between CEC and REC, and the expression differences were calculated by ΔΔCt method. A total of 57 angiogenesis-related protein expression differences were investigated by western blot and proteome profiler, and were calculated by densitometry.

Results: Primary human CEC and REC lines stained positively for all EC markers and demonstrated high purity with similar staining and morphology. Under normoxia, CEC showed significantly lower expression levels for cell proliferation and vessel maturation genes and higher expression levels for inflammation-related genes when compared to REC. In response to hypoxia, CEC and REC displayed differential regulation for a multitude of angiogenesis-related genes and proteins. Furthermore, within the vascular endothelial growth factor (VEGF) family, CEC showed preferential upregulation for VEGFA while REC upregulated placenta growth factor (PIGF) levels.

Conclusions: Differential normoxic and hypoxic regulation of angiogenesis-related factors by CEC and REC outlines tissue heterogeneity of ocular angiogenesis and suggests that tissue specificity should be considered as a novel treatment modality for successfully overcoming choroidal and retinal angiogenic conditions in the clinic.

Commercial Relationships: Parviz Mammadzada, None; Johann Gudmundsson, None; Anders P. Kvanta, None; Helder Andre, None
Purpose: Multicenter evaluation of the clinical characteristics, treatment modalities and outcomes of pediatric choroidal neovascular membranes (CNVM).

Methods: Retrospective case series of all patients 18 years old or less diagnosed with CNVM. Statistical tests were 2-tailed and significance was defined as \( P < 0.05 \). Stata version 9.0 (StataCorp, LP, College Station, TX) was used for statistical analyses.

Results: Forty six eyes of 37 patients (16 Male, 21 Female) with a mean age of 11.1 years were analyzed. CNVM was associated with Best vitelliform macular dystrophy (n=14), idiopathic (n=12), post inflammatory (n=4), coloboma (n=4), optic nerve head drusen (n=3), myopia (n=3), choroidal rupture (n=1), persistent fetal vasculature syndrome (n=1), familial exudative vitreoretinopathy (n=1), and choroidal osteoma (n=1). 75% (n=28) of patients had unilateral disease. Presenting visual acuity ranged from count fingers to 20/25. The most common location was subfoveal (56.5%), followed by peripapillary (30.8%), juxtafoveal (13.0%), and extrafoveal (10.9%) (\( P < 0.001 \)). Fluorescein angiography showed lesions to be classic (90.9%; \( P < 0.001 \)) in the majority of cases. Optical coherence tomography (OCT) showed mainly type 2 CNVM (76.1%; \( P < 0.001 \)).

Initial treatment modalities were anti-vascular endothelial growth factors (VEGF) (n=30; 90.9%) or laser (n=3; 9.1%). Bevacizumab was used predominantly (n=20). Anti-VEGF treatment was used alone (n=25), followed by an alternate anti-VEGF agent (n=3) or in combination with laser (n=1) or PDT (n=2). Nineteen eyes (63.3%) showed regression with anti-VEGF treatment alone with a mean of 2.1 (range 1-10) injections. Recurrences occurred in 7 eyes (21.2%) with an average of 1.1 recurrences per eye. Three of these eyes stabilized with repeat anti-VEGF therapy, while the remainder required photodynamic therapy (PDT), laser or surgery (n=1).

Conclusions: This is the largest reported study on pediatric CNVM. Anti-VEGF therapy was effective with a low recurrence rate.

Commercial Relationships: Bradley Anderson, None; Tapas R. Padhi, None; Ashkan Abbey, None; Yoshihiro Yonekawa, Kimberly A. Drenser, Thrombogenics (S), Synergetics (C), FocusROP (S), Allergan (S); Antonio Capone, Jr., Novartis (C), FocusROP (C), Synergetics (C), Retinal Solutions (C), Allergan (C); Michael T. Trese, None; Cagri G. Besirli, None.

Program Number: 2136 Poster Board Number: C0043
Presentation Time: 11:00 AM–12:45 PM
Multicenter Evaluation of Pediatric Choroidal Neovascular Membrane: Clinical characteristics and treatment outcomes
Bradley Anderson1, Tapas R. Padhi2, Ashkan Abbey3, Yoshihiro Yonekawa4, Kimberly A. Drenser5, Antonio Capone, Jr.3, Michael T. Trese2, Cagri G. Besirli6
1Ophthalmology, Beaumont Hospital, Royal Oak, United Kingdom; 2Massachusetts Eye and Ear Infirmary, Boston, MA; 3Associated Retinal Consultants, Royal Oak, MI; 4University of Michigan, Ann Arbor, MI.

Purpose: Multicenter evaluation of the clinical characteristics, treatment modalities and outcomes of pediatric choroidal neovascular membranes (CNVM).

Methods: Retrospective case series of all patients 18 years old or less diagnosed with CNVM. Statistical tests were 2-tailed and significance was defined as \( P < 0.05 \). Stata version 9.0 (StataCorp, LP, College Station, TX) was used for statistical analyses.

Results: Forty six eyes of 37 patients (16 Male, 21 Female) with a mean age of 11.1 years were analyzed. CNVM was associated with Best vitelliform macular dystrophy (n=14), idiopathic (n=12), post inflammatory (n=4), coloboma (n=4), optic nerve head drusen (n=3), myopia (n=3), choroidal rupture (n=1), persistent fetal vasculature syndrome (n=1), familial exudative vitreoretinopathy (n=1), and choroidal osteoma (n=1). 75% (n=28) of patients had unilateral disease. Presenting visual acuity ranged from count fingers to 20/25. The most common location was subfoveal (56.5%), followed by peripapillary (30.8%), juxtafoveal (13.0%), and extrafoveal (10.9%) (\( P < 0.001 \)). Fluorescein angiography showed lesions to be classic (90.9%; \( P < 0.001 \)) in the majority of cases. Optical coherence tomography (OCT) showed mainly type 2 CNVM (76.1%; \( P < 0.001 \)).

Initial treatment modalities were anti-vascular endothelial growth factors (VEGF) (n=30; 90.9%) or laser (n=3; 9.1%). Bevacizumab was used predominantly (n=20). Anti-VEGF treatment was used alone (n=25), followed by an alternate anti-VEGF agent (n=3) or in combination with laser (n=1) or PDT (n=2). Nineteen eyes (63.3%) showed regression with anti-VEGF treatment alone with a mean of 2.1 (range 1-10) injections. Recurrences occurred in 7 eyes (21.2%) with an average of 1.1 recurrences per eye. Three of these eyes stabilized with repeat anti-VEGF therapy, while the remainder required photodynamic therapy (PDT), laser or surgery (n=1).

Conclusions: This is the largest reported study on pediatric CNVM. Anti-VEGF therapy was effective with a low recurrence rate.

Commercial Relationships: Bradley Anderson, None; Tapas R. Padhi, None; Ashkan Abbey, None; Yoshihiro Yonekawa, Kimberly A. Drenser, Thrombogenics (S), Synergetics (C), FocusROP (S), Allergan (S); Antonio Capone, Jr., Novartis (C), FocusROP (C), Synergetics (C), Retinal Solutions (C), Allergan (C); Michael T. Trese, None; Cagri G. Besirli, None.

Program Number: 2137 Poster Board Number: C0044
Presentation Time: 11:00 AM–12:45 PM
OCT Angiography for Diagnosis and Possible Guided Laser Therapy of Choroidal Neovascular Membranes
Amy Patel. Ophthalmology, University of California, Irvine, Irvine, CA.

Purpose: Choroidal neovascular membranes (CNVM) are associated with macular edema or subretinal fluid on traditional optical computed tomography (OCT), and vasculature dye leakage on indocyanine green (ICG) angiography. We hypothesize that OCT angiography (OCTA) can be an alternative, novel imaging technique that is also effective for diagnosing CNVM. Our aim is to describe and validate OCTA imaging for identifying CNVM and to superimpose OCTA on live fundus imaging as a potential platform for pre-programmed, targeted retinal laser treatment.

Methods: This is a case series in which ICG angiography, traditional OCT, and OCTA (AngioVue System by Optovue) imaging was performed on two patients with CNVM. Imaging was done prior to anti-VEGF treatment and 4 weeks after treatment. The feasibility of superimposing AngioVue images on live fundus imaging in a retina navigation laser treatment system (Navilas) was also evaluated.

Results: Non-invasive OCTA consistently identified choroidal anomalies even with no evidence of subretinal fluid or macular edema on traditional OCT, or leakage on ICG. Choroidal anomalies were typically subfoveal or juxtafoveal. Anomalies were common sites of CNVM based on common finding on ICG or subretinal fluid recurrences. Furthermore, these OCTA images could be superimposed to live fundus imaging.

Conclusions: OCTA can reliably diagnose CNVM and be hybridized to a preprogrammed retinal laser treatment system. This may act as a platform for potential novel, targeted microsecond laser treatment of CNVM in the future.

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Program Number: 2138 Poster Board Number: C0045
Presentation Time: 11:00 AM–12:45 PM
Analysis of Choroidal Neovascularization in myopic eyes using OCT Angiography
Jean-François Korobelnik, Yona GEISMAR, Marie-Noelle Delyfer, Marie B. Rougier. Ophthalmology, CHU de Bordeaux, Bordeaux, France.

Purpose: To analyze the benefit of optical coherence tomography angiography (OCTA) for the diagnosis of myopic choroidal neovascularization (CNV) and discuss the clinical utility of this method as compared to fluorescein angiography

Methods: Eleven eyes of 11 consecutive highly myopic patients with suspected diagnosis of CNV underwent retinal imaging with OCTA (Carl Zeiss Meditec®, Dublin, CA) between May 2015 and October 2015. Fluorescein angiography (FA, Spectralis®, Heidelberg Engineering, Germany) was performed to further validate the diagnosis of myopic CNV.

Results: In 9 eyes out of 11 patients, CNV was visualized on OCTA. 2 groups were constituted. In all eyes with CNV, the diagnosis was confirmed using OCTA. In Group 1, 9 patients with myopic CNV either naive or previously were treated with at least 1 intravitreal injection of anti-VEGF. In Group 2, 2 eyes did not show any CNV, either with FA, or with OCT A. In these two patients, final diagnosis was subretinal hemorrhage related to lacquer cracks.

Conclusions: OCTA easily shows the choroidal neovascular network of myopic CNV. Further larger studies are required to confirm our data and state whether FA is still required in this indication.

Commercial Relationships: Amy Patel, None

Program Number: 2139 Poster Board Number: C0046
Presentation Time: 11:00 AM–12:45 PM
Investigating the laser model of experimental choroidal neovascularization in Long Evans rats
Selwyn M. Prea1, 2, Guei-Sheung Liu1, Gregory J. Dusting1, Algis J. Vingrys1, Bang V. Bui2. 1Optometry and Vision Sciences, University of Melbourne, Parkville, VIC, Australia; 2Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, East Melbourne, VIC, Australia.

Purpose: The aim of this study was to determine the minimum laser energy required to successfully breach Bruch’s membrane (BM) in Long Evans rats and to assay the inflammatory and angiogenic changes following laser application.

Methods: Adult (30 weeks) Long Evans rats were anaesthetised (60:5 mg/kg ketamine:xylazine) and six laser burns (Nd:YAG) of varying energy (2.0 to 10.2 J/mm²) were delivered to the posterior pole of each eye (n = 4 rats). A dose response was established for breach as a function of laser energy. Spectral domain-optical coherence tomography (SD-OCT) was performed at 5 min, 14 days and 28 days after laser and compared against histological findings made at 28 days. Changes in mRNA with qPCR were determined for tumour necrosis factor-alpha (TNF-α) and vascular endothelial growth factor-A (VEGF-A) 2 days after high dose (5.10 J/mm²) laser (n = 6 eyes) that invoked breach of BM. Rats were sacrificed at 28 days and choroidal wholemounts were stained with isoclin B4 and von Willebrand Factor. Confocal microscopy was used to determine if a choroidal neovascular (CNV) membrane was present with IB4 and vWF staining. All group data are shown as mean [95% CLs].

Results: The dose response found that the amount of laser energy required to breach BM (95% success) was 3.12 J/mm² [3.01, 3.29]. Rupture of BM with high laser energy (5.10 J/mm²) resulted in immediate ablation of the retinal pigment epithelium viewed with SD-OCT at 5 mins. CNV was not observed in these lesions at 28 days although macrophages were present at 10 µm above the choriocapillaris. Elevated levels of TNF-α mRNA were detected in the retina (9.7 fold increase, p<0.001) and choroid (2 fold increase, p<0.05) at 2 days post laser. However, a downregulation of VEGF-A mRNA was found in the retina (1.2 fold decrease, p<0.05) and choroid (1.6 fold decrease, p=0.01) 2 days after laser.

Conclusions: The minimum energy to successfully breach BM in Long Evans rats was 3.12 J/mm² [3.01, 3.29] which was visualised by SD-OCT. In the presence of breach, we found upregulation in TNF-α and downregulation of VEGF-A.

Commercial Relationships: Selwyn M. Prea, None; Guei-Sheung Liu, None; Gregory J. Dusting, None; Algis J. Vingrys, None; Bang V. Bui, None

Program Number: 2140 Poster Board Number: C0047
Presentation Time: 11:00 AM–12:45 PM
Ranibizumab in patients with myopic choroidal neovascularization: latest results from the third interim analysis of the LUMINOUS™ study
Robin D. Hamilton. Moorfields Eye Hospital, London, United Kingdom.

Purpose: LUMINOUS™ (NCT01318941), the largest prospective observational trial in medical retina, is designed to evaluate the long-term safety, effectiveness, treatment patterns, and health-related quality-of-life associated with ranibizumab treatment in clinical practice across all licensed indications. As of June 2015, the study has enrolled 30,514 patients, spanning 494 sites across 43 countries. Here we describe the results from the third interim analysis from the cohort of 285 patients with myopic choroidal neovascularization (mCNV), recruited prior to March 2015.

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Methods: Patients were treated according to the local product label, and the dataset was analyzed by prior treatment status of the primary treated eye (treatment naïve, T1; prior ranibizumab treated, T2; or other prior ocular treated, T3).

Results: In total, 29,055 patients were recruited prior to March 2015, of whom 285 had mCNV: 95 (33.4%) were T1, 166 (58.2%) were T2, and 24 (8.4%) were T3. Overall, the mean age of patients with mCNV was 58.2 years, 71.0% were female, 87.4% were Caucasian, and 7.4% were Asian. Mean baseline visual acuity (VA) was higher in T2 (58.2) than both T1 (49.8) and T3 (47.1). The baseline characteristics of patients with mCNV in this real-world study were comparable to those of the phase III RADIANCE study (NCT01217944) (Table). Median time from diagnosis of mCNV to first ranibizumab treatment/study entry was 0.01, 1.19, and 1.37 years for T1, T2, and T3, respectively. Baseline comorbidities included hypertension (33.7% of patients), obesity (14.0%), diabetes (8.8%), history of myocardial infarction (3.5%), and history of stroke (2.5%).

Conclusions: LUMINOUS™ has included patients with mCNV from 23 countries across Europe, Asia, South America, and Canada; many of these are currently under-represented in randomized controlled trials. The baseline characteristics of patients with mCNV in LUMINOUS™ are similar to those enrolled in the phase III RADIANCE study, supporting the generalizability of the results of this registration study to other mCNV populations. At baseline, patients previously treated with ranibizumab had better VA than those who were treatment naïve or who had previously received other ocular treatments. Future follow-up data from the mCNV cohort will provide long-term evidence on ranibizumab treatment outcomes in real-world clinical practice.

**Demographics and baseline ocular characteristics**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>T1 (n = 285)</th>
<th>T2 (n = 285)</th>
<th>T3 (n = 285)</th>
<th>Total (n = 305)</th>
<th>Total (n = 305)</th>
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</thead>
<tbody>
<tr>
<td>Mean (SD) age, years</td>
<td>58.7</td>
<td>56.0</td>
<td>58.2</td>
<td>57.5</td>
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<tr>
<td>Gender, Male, %</td>
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<td>54</td>
<td>30</td>
<td>40</td>
<td>25</td>
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<tr>
<td>Race, Caucasian, %</td>
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<td>98.1</td>
<td>95.8</td>
<td>95.4</td>
<td>56.5</td>
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<tr>
<td>Mean (SD) VA, letter score</td>
<td>96.2</td>
<td>96.2</td>
<td>96.2</td>
<td>96.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Mean (SD) CRT, µm</td>
<td>311.5</td>
<td>112.8</td>
<td>275.4</td>
<td>130.2</td>
<td>113.23</td>
</tr>
</tbody>
</table>

*In the RADIANCE study, ranibizumab treatment was guided by visual acuity stabilization criteria and disease activity criteria.*

**Commercial Relationships:** Robin D. Hamilton, Novartis (C), Ellex (R), Novartis (R), Bayer (C), Bayer (R), Novartis (F), Ellex (C), Allergan (R), Bayer (F), Allergan (C)

**Clinical Trial:** NCT01318941

**Program Number:** 2141 Poster Board Number: C0048

**Presentation Time:** 11:00 AM–12:45 PM

**Long-term outcomes of anti-vascular endothelial growth factor treatment in East-Asian patients with myopic choroidal neovascularization**

**Wayne Macfadden**, 1 Adrian Skelly, 1

**1Ophthalmology, Novartis Pharma AG, Basel, Switzerland; 2Ophthalmology, Novartis Ireland Limited, Dublin, Ireland.

**Purpose:** To assess the long-term effectiveness and safety of anti-vascular endothelial growth factor (anti-VEGF) therapies in East-Asian patients treated for visual impairment due to myopic choroidal neovascularization (mCNV).

**Methods:** Post-RADIANCE was a non-interventional, observational, 36-month retrospective multicenter chart review conducted in a cohort of East-Asian patients with mCNV who completed the RADIANCE study (NCT01217944), had at least one follow-up visit after RADIANCE trial participation and whose medical records were available. Patients were excluded if they had participated in an interventional study after the RADIANCE final visit. Demographic and baseline clinical characteristics of patients included in the Post-RADIANCE study were retrieved from the RADIANCE study database. The primary outcome was mean best-corrected visual acuity (BCVA; letters) change (±standard deviation [SD]) from the baseline visit of the RADIANCE trial (Month 0) to each follow-up visit during the Post-RADIANCE observation period (Month 13–48).

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Secondary objectives were assessed during Post-RADIANCE observation period. These included mCNV recurrence in the study eye, treatment administered in the study eye, and ocular adverse events (AEs).

**Results:** Forty-one patients were included in the study. At baseline, patients had a mean age of 55.1 (±13.5) years, majority were female (33 [80.5%]), and had a mean BCVA of 56.5 (±12.1). Mean changes in BCVA (±SD) from baseline to Month 12 and Month 48 were +14.3 (±11.4) (n=40; p<0.0001) and +16.3(±18.7) (n=16; p=0.0034), respectively. During the 36-month follow-up period, 7 (17.1%) patients required additional anti-VEGF treatments, 7 (17.1%) patients had mCNV recurrence and 4 (9.8%) patients had a second recurrence, and 5 (12.2%) experienced an AE with none considered to be serious or related to anti-VEGF treatment.

**Conclusions:** BCVA gained at the end of the RADIANCE study was sustained over an additional 36 months of follow-up in East-Asian patients, during which time most patients did not require any further anti-VEGF treatment and no new safety concerns were observed.

**Commercial Relationships:** Wayne Macfadden, Novartis Pharma AG, Basel, Switzerland; Adrian Skelly, Novartis Ireland Limited, Dublin, Ireland

**Program Number:** 2143 **Poster Board Number:** C0050 **Presentation Time:** 11:00 AM–12:45 PM

**Novel organotypic culture model of pig choroid-scleral explant as a therapeutic screening tool**

**Authors:** Chunhua Jiao, Shemin Zeng, Michael A. Schelling, Ryson Stuart, Robert F. Mullins, Elliott H. Sohn

**Purpose:** To establish a pig organotypic choroid-scleral explant culture system for use as an ex-vivo choroid tubulogenesis screening tool to study novel potential pharmaceutical therapies.

**Methods:** 2mm explants of choroid/sclera without the RPE were obtained from adult porcine eyes and cultured on three-dimensional collagen matrix. After 2 days of culture in medium with 10% fetal bovine serum and 1 day of serum starvation in 4% serum medium, the explants were further cultured in the presence or absence of varying concentrations of VEGF (5ng/ml, 10ng/ml, 50ng/ml), complement 5a (10ug/ml, 50ug/ml), or tamoxifen (10ug/ml, 20ug/ml) for an additional 48 and 72 hour period. Vascular sprouting from the choroid-scleral complex was visualized using B. simplicifolia isoelectin B4 (BSI-B4) and anti-CD31 antibody using immunofluorescence and confocal microscopy. Morphology of sprouting was assessed on toluidine blue stained semithin sections using light microscopy. Density of vascular elements in each condition was quantified using ImageJ software. Comparisons of controls and each treatment group were performed by one-way ANOVA using SPSS. Results were expressed as mean ± SEM. P<0.05 was considered statistically significant.

**Results:** Choroidal endothelial cells began to proliferate and migrate out of the explant after 24 hours; tubulogenesis began at 48 hours, with a marked abundance of vascular tubes by 72 hours. Cells comprising the tubes were labeled with endothelial cell markers BSI-B4 and CD31 antibody. Explants treated with VEGF showed dose dependence and 50ng/ml of VEGF (n=5) had a statistically significant increase in vascular area compared to controls (n=9, p=0.038) after 48 hours of culture; a VEGF directed antibody inhibited this effect of VEGF. In addition, after 72 hours of incubation, 50ug/ml C5a induced tubulogenesis; tamoxifen (10ug/ml, n=4; 20ug/ml, n=2) on the other hand significantly reduced vessel formation compared to control (n=6, p=0.007) in porcine choroid-scleral explant.

**Conclusions:** This adult pig choroid-sclera explant culture model is a short-term organotypic culture system that offers an opportunity to evaluate pro- and anti-angiogenic effects of pharmacologic compounds on choroid biology.

**Commercial Relationships:** Chunhua Jiao; Shemin Zeng, None; Michael A. Schelling, None; Ryson Stuart, None; Robert F. Mullins, None; Elliott H. Sohn, None

**Program Number:** 2144 **Poster Board Number:** C0051 **Presentation Time:** 11:00 AM–12:45 PM

**Functional and anatomical outcomes of anti-VEGF therapy for CNV due to angioid streaks: 4 and a half years of follow up**

**Authors:** May Cadena-Torres, M. Guadalupe Martinez-Serrano, Abelardo Rodriguez-Reyes, Vianney Cortés-González, Virgilio Morales-Canton, Raul Velez-Montoya, Guillermo Salcedo.

**Purpose:** Intravitreal antiangiogenics are the first-line therapy for choroidal neovascularization (CNV) of any cause. CNV can be recurrent and lead to progressive visual loss, as an important complication of angioid streaks. We performed a retrospective case series to describe the clinical course, angiographic and tomographic findings of CNV due to angioid streaks (AS), and its response to intravitreal anti-vascular endotelial growth factor (VEGF).

**Methods:** This study included 14 eyes of 7 patients with CNV, secondary to AS who underwent intravitreal anti-VEGF, followed up from January 2005 to October 2015. Ophthalmic evaluation included best corrected visual acuity (BCVA), slit lamp biomicroscopic examination, optical coherence tomography (OCT) and fluorescein angiography before and after treatment.

**Results:** There were 3 males (42.9%) and 4 females (57.1%). The mean age of presentation was 53.2 months (range 44-71 months). At first visit 9 eyes had CNV which was subfoveal in 88.8% and extrafoveal in 11.1%. Atrophic changes and fibrosis were present in 2 and 6 cases respectively at initial examination, and there were 4 and 10 eyes with atrophy and fibrosis at the end of the study. During the mean follow-up period of 53.85 months, eyes included received an average of 4.42 intravitreal injections (range 1-11). Of 5 patients (71.43%, 95%CI: 29.04%-96.33%) with initial unilateral active CNV, 4 (57.14%, 95%CI: 18.4%-90.1%) continued with activity through last visit. 2 patients (28.57%, 95%CI: 3.66%-70.95%) presented initially with bilateral active CNV and at last visit and there were 3 patients in this group (95%CI:18.4%-90.1%), so the risk of developing CNV in the fellow eye is 1.875 (OR:1.875; 95%CI: 0.128-30.60). The mean BCVA in eyes with initial CNV was 0.87 logMAR and at last visit it was 0.69 logMAR (p=0.8946). The initial mean central retinal thickness (CRT) in patients with initial CNV was 359 and at last visit it was 354 (p=0.9296). The mean BCVA in eyes without initial CNV was 0.09 logMAR and at last visit it was 0.69 logMAR (p<=0.8946). The mean CRT in patients without a initial mean central retinal thickness was 218 and at last visit it was 257 (p=0.5476).

**Conclusions:** Antiangiogenic therapy for CNV in AS is useful to inactivate the lesion, however it did not improve BCVA, nor CRT. The fellow eye has 1.85 times the risk to develop CNV at 1 year, presenting bad visual prognosis.

**Commercial Relationships:** May Cadena-Torres, None; M. Guadalupe Martinez-Serrano, Abelardo Rodriguez-Reyes, Vianney Cortés-González, Virgilio Morales-Canton, Raul Velez-Montoya, Guillermo Salcedo, None
**Program Number: 2145 Poster Board Number: C0052**
**Presentation Time: 11:00 AM–12:45 PM**

**The use of anti-vascular endothelial growth factor therapies in the macular telangiectasia associated with choroidal neovascularization**

*Musa Abdelaziz*, Mahdi Rostamizadeh, Jerome Schartman,1,2 Hernando Zegarra,2,3 Z Nicholas Zakov2, Michael Novak2, Scott Pendergast2, Joseph Coney2, Lawrence J. Singerman2, David Miller.1 Ophthalmology, University Hospitals, Cleveland Heights, OH; 2 Retina Associates of Cleveland, Cleveland, OH.

**Purpose:** There are a limited number of case studies evaluating the use of anti-vascular endothelial growth factor (VEGF) therapies in the treatment of macular telangiectasia associated with choroidal neovascularization (CNV), with the largest having a sample size of six. We performed a retrospective, observational clinical study to assess the efficacy and visual outcomes of anti-VEGF therapies in the treatment of macular telangiectasia associated with CNV.

**Methods:** After approval was obtained from Retina Associates of Cleveland institutional review board, electronic medical charts were searched based on international classification of disease codes 9 and 10 for retinal telangiectasia and intravitreal injections, resulting in a total of 101 subjects. After accounting for duplicates, excluding non-macular telangiectasia subjects, and excluding subjects treated for age related macular degeneration, a total of 22 eyes from 20 subjects remained. The gender, age, visual acuity (VA) at diagnosis and at last visit, number and type of intravitreal injection, and duration of follow up were recorded.

**Results:** The average age of subjects included were 71 years of age, with 13 out of 20 subjects being female (65%). 8 patients were treated with ranibizumab, 16 with bevacizumab and 3 with aflibercept. The mean numbers of injections were three. The mean follow up was 553 days. The average initial VA was 20/200 and average final VA was 20/100-1. The average improvement in ETDRS letters was 14. Overall, only 1 eye had a decreased in VA from baseline, 5 eyes (23%) had no change in VA, and 11 eyes (50%) had an improvement of ≥20 letters.

**Conclusions:** In this study, it appears that anti-VEGF is effective in preserving and improving vision in macular telangiectasia associated with CNV with a limited number of intravitreal injections. A multicenter prospective study would further delineate the role of anti-VEGF therapy in macular telangiectasia associated with CNV.

**Commercial Relationships:** Musa Abdelaziz; Mahdi Rostamizadeh, None; Jerome Schartman, None; Hernando Zegarra, None; Z Nicholas Zakov, None; Michael Novak, None; Scott Pendergast, None; Joseph Coney, None; Lawrence J. Singerman, None; David Miller, None; Genentech (R); Lawrence J. Singerman, None; David Miller, None; Retina Associates of Cleveland, Cleveland, OH.

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**Program Number: 2146 Poster Board Number: C0053**
**Presentation Time: 11:00 AM–12:45 PM**

**Optical coherence tomography findings predictive of choroidal neovascularization (CNV) activity in pathologic myopia: correlation with fluorescein angiography in Korean patients**

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**Purpose:** Evaluating disease activity is essential for determining the proper treatment of myopic choroidal neovascularization (CNV). While fluorescein angiography (FA) is the most sensitive tool for CNV detection, its invasiveness limits frequent follow-up examination; furthermore, presence of lacquer crack or subretinal hemorrhage can interrupt proper visualization. Optical coherence tomography (OCT) is a helpful diagnostic tool; however, the correlation between OCT findings and disease activity remains unclear. Therefore, the aim of this study is to evaluate the OCT findings for CNV in assessment of disease activity in association with FA findings.

**Methods:** This was a retrospective study including 30 patients with myopic CNV who were treated with intravitreal anti-VEGF (bevacizumab) between January 2013 and July 2015. All patients underwent OCT and FA examination before and 1 month after intravitreal anti-VEGF treatment for active myopic CNV. We analyzed potential OCT features suggestive of the disease activity as follows: presence of intraretinal cysts, subretinal fluid, CNV height, and fuzziness of CNV border on OCT. CNV network area was also measured on FA. Logistic regression analysis was performed to evaluate the correlation between CNV parameters seen on OCT with leakage degree on FA.

**Results:** After intravitreal anti-VEGF injection, OCT revealed statistically meaningful improvement of intraretinal cysts in 10 eyes (21.73%), subretinal fluid in 9 eyes (19.57%) and fuzziness of CNV border in 21 eyes (45.65%) (p < 0.006, 0.007, < 0.001). The height of CNV lesion decreased significantly from 146.60 ± 68.33 μm to 118.02 ± 55.73 μm (p = 0.001). FA showed statistically significant reduction of CNV network area from 0.73 ± 0.97 mm² to 0.50 ± 0.89 mm² (p = 0.003). Fifteen eyes (32.61%) showed remarkable resolution of active leakage on FA (p = 0.001). Fuzzy border seen on OCT revealed the most significant correlation with leakage on FA (p = 0.009, R² = 0.324).

**Conclusions:** Our study demonstrated that the fuzzy border of CNV is the most common visible finding on SD-OCT and showed the most prominent improvement after intravitreal anti-VEGF injection. Furthermore, it showed the strongest correlation with changes of CNV leakage on FA. Thus, fuzzy CNV border can be considered the most important feature for assessment of CNV activity.

**Commercial Relationships:** Min Kim, None; Dong Hyeon Lee, None; Eunyoung Choi, None; Suk Ho Byeon, None; Hyoun Jun Koh, None; Sung Soo Kim, None; Sung Chul Lee, None.
The area of CNV was measured one week after laser treatment (1, 3, 5, and 9 weeks after microparticle injection). A pharmacokinetic study was also conducted using normal C57BL/6 mice and the drug levels in different ocular tissues were determined by HPLC-MS at various time points following IVT injection of the microparticles.

**Results:** The microparticles had drug loading of 3.4% (by weight) and a mean diameter of about 13 µm. A statistically significant reduction in the area of CNV was observed at each time point through 9 weeks in microparticle-injected eyes compared with corresponding controls. The mean area of CNV (mm² x 10²) in microparticle-injected eyes versus controls was: 1 week, 5.0±0.9 vs 8.0±1.0; 3 weeks, 10.6±1.2 vs 15.3±1.5; 5 weeks, 8.6±1.5 vs 14.5±1.7; and 9 weeks, 7.9±3.7 vs 22.5±5.4.

**Conclusions:** IVT injection of sunitinib microparticles provides sustained suppression of CNV and may provide a durable new treatment for neovascular AMD.

**Commercial Relationships:** Raquel Formica, None; Jie Fu, None; Ji-kui Shen, None; Ming Yang, Graybug, Inc; Yun Yu, Graybug, Inc; Joshua Kays, Graybug, Inc; Yanfei Liu, None; Ward M. Peterson, Graybug, Inc; Jeffrey Cleland, Justin Hanes, Graybug, Inc (I); Peter A. Campochiaro, Graybug, Inc (I)

**Support:** SBIR Grant R43EY024827

**Program Number:** 2148 **Poster Board Number:** C0055 **Presentation Time:** 11:00 AM–12:45 PM

**Optical Coherence tomographic Angiography features in neovascularization secondary to angioid streaks**

**Thibaut Chapron**

**Alexandra Miere**

**Ala Elameen**

**Eric H. Souied**

**Paris Descartes University, Paris, France; ophthalmology, Centre Inter Communal Créteil, Créteil, France; Paris Est University Créteil Val de Marne, Créteil, France.

**Purpose:** Optical coherence tomography angiography (OCT-A) is a new non-invasive technique allowing us imaging retinal microvasculature. We performed a prospective observational study in order to detect the presence of neovascularization (CNV) secondary to angioid streaks and to describe their morphological features on OCT-A.

**Methods:** We analyzed consecutive patients affected with CNV secondary to angioid streaks. All patients underwent a complete ophthalmological examination including best-corrected visual acuity (BCVA), fluorescein angiography (FA), indocyanine green angiography (ICG) and spectral domain optical coherent tomography (SD-OCT; Heidelberg Engineering, Heidelberg; Germany). Presence and activity of CNV was determined on FA, ICG and SD-OCT imaging by two observers. Two others observers, blind from the activity in standard imaging, evaluated OCT-A images to identify the presence of CNV and to describe the features of the lesion. OCT-A was performed using the RTVue XR Avanti (Optovue Inc). We proposed four types of CNV features on OCT-A: “sea fan” characterized by a dense vascular network, “interlacing vessels” characterized by a tortuous network with many hyperdense vascular ramifications without significant dead space, “loop” characterized by a rarified curved and tortuous vascular network, and “dead tree” characterized by linear vascular network with dead spaces between.

**Results:** 32 eyes of 18 consecutive patients with neovascularization secondary to angioid streaks were prospectively included. Median age was 60 years-old (range 40-71). In FA, ICG and SD-OCT, CNV were classified as active lesions in 13/32 eyes at the time of imaging and 12/32 eyes were not injected in the last 6 months. OCT-A showed the presence of CNV in 28/32 (87.5%) eyes. Finally, 4 CNV lesions were classified as “seafan”, 6 as “interlacing vessels”, 3 as “loop”, 4 as “dead tree”, and 11 CNV showed associated forms.

**Conclusions:** OCT-A has the ability to detect 87.5% CNV secondary to angioid streaks. Classic exudative signs are rare in SD-OCT. OCT-A does not appear yet to be sufficient alone to determine if CNV is active or not but could be useful in association with SD-OCT in order to show CNV modifications during follow-up.

**Commercial Relationships:** thibaut Chapron, None; Gerard Mimoun, None; Mayer Srour, None; Alexandra Miere, None; Ala Elameen, None; Oudy Semoun, None; Eric H. Souied, None

**Program Number:** 2149 **Poster Board Number:** C0056 **Presentation Time:** 11:00 AM–12:45 PM

**Anti-vegf Therapy Versus Pdt in the Treatment of Cvn Secondary to Crsc**

**Giulia Caminiti, Riccardo Saldi, Giovanni M. Satta, Beatrice Lobina, Enrico Peiretti.** Department of Ophthalmology, University Of Cagliari, Cagliari, Italy.

**Purpose:** To describe a consecutive series of patients affected by chronic central serous chorioretinopathy (CRSC) complicated with choroidal neovascularization (CNV) treated with intravitreal injection (IVT) of anti-VEGF or photodynamic therapy (PDT).

**Methods:** 40 eyes of 34 consecutive patients have been followed for a minimum of 1 year and maximum of 7 years with an history of CRSC complicated with CNV. All the patients were evaluated with fluorescein, indocyanine angiography (FA, ICG) and OCT scan that revealed polypoidal lesions in 67% of the CNV. Patients were classified in 3 groups: the first group included 18 eyes treated with photodynamic therapy (PDT), the second group comprised 19 eyes treated with intravitreal injection (IVT) of anti-VEGF (Bevacizumb) and the third group involved 3 eyes treated with combination therapy (PDT + IVT). Each patient performed FA, ICG and OCT at the baseline and during the follow-up depending on the physician discretion. The fixed schedule for the follow-up was every 3 months for PDT, after 1,2 and 3 months for the IVT patients with a chance to extend the fixed visit to a maximum of 6 months. The third group had a follow-up similar to the first group.

**Results:** After 1 year of follow-up, the first group (only PDT) showed that 64% of the eyes had an improvement of best corrected visual acuity (BCVA), the 21% had stabilization and the remaining 15% had a worsening of VA, with a mean of 1.5 PDT sessions. In the second group of patients (IVT of anti-VEGF) 47% of the eyes had an improvement of BCVA, 33% had stabilization and 20% showed a reduction of VA, the mean average of IVT was 3.3. In third group of patients (PDT + IVT) 33% increased his BCVA, 33% no change and 33% had a reduction of BCVA, each patient performed a mean of 1.5 sessions of combination therapy.

**Conclusions:** Similarly, IVT of anti-VEGF and Photodynamic therapy as well, demonstrated to be efficacy in maintaining or improving BCVA in more than 65% of patients with CRSC complicated with CNV.

**Commercial Relationships:** Giulia Caminiti, None; Riccardo Saldi, Giovanni M. Satta, None; Beatrice Lobina, None; Enrico Peiretti, None

**Program Number:** 2150 **Poster Board Number:** C0057 **Presentation Time:** 11:00 AM–12:45 PM

**Usefulness of Optical Coherence Tomography Angiography in Myopic Choroidal Neovascularization**


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**Purpose:** To assess the ability of optical coherence tomography angiography (OCT-angiography) to detect choroidal neovascularization secondary to pathologic myopia in patients with contraindication for fluorescein angiography.

**Methods:** Three women (55, 85, and 93 years old, respectively) with pathologic myopia and suspected choroidal neovascularization in one eye participated in this study. Vision decrease and metamorphopsia were the symptoms in all cases. Fluorescein angiography was contraindicated due to allergy in two cases and due to advanced age in one case. The patients were evaluated by OCT, en-face OCT, and OCT-angiography (XR-Avanti; Optovue). Split spectrum amplitude decorrelation angiography algorithm was used to detect the blood flow within the tissue. Scan protocols covering a 3 mm x 3 mm area and a 8 mm x 8 mm area were used.

**Results:** Imaging with OCT and en-face OCT showed, in all cases, a hyperreflective lesion at the level of the retinal pigment epithelium (RPE) and/or the outer retina with minimal or no fluid, making diagnosis of choroidal neovascularization doubtful. In contrast, OCT-angiography clearly showed, in all cases, a distinct neovascular network at the level of the choriocapillaris adjacent to the RPE. This led to efficient regression of the choroidal neovascularization by intravitreal anti-VEGF therapy with visual acuity improvement in all cases.

**Conclusions:** OCT-angiography is a useful noninvasive imaging technique to detect choroidal neovascularization secondary to pathologic myopia in cases with contraindication for fluorescein angiography. This technique provides detailed images of distinct vascular networks in various depths, enabling accurate diagnosis of choroidal neovascularization and good follow-up after treatment.

**Commercial Relationships:** Ioannis K. Petropoulos, None; Pietro Roberti, None; Michel A. Matter, Philippe M. Desmangles, None

**Program Number:** 2151 Poster Board Number: C0058
**Presentation Time:** 11:00 AM–12:45 PM

**Frequency, phenotype and progression of geographic atrophy associated with pseudoxanthoma elasticum, a model disease for a diseased Bruch’s membran**

*Peter Charbel Issa*,1 Martin Gliem1, Philipp L. Mueller1, Johannes Birtel2, Doris Hendig2, Frank G. Holz1, 1Department of Ophthalmology, University of Bonn, Bonn, Germany; 2Institute for Laboratory and Transfusion Medicine, Heart and Diabetes Center North Rhine–Westphalia, University Hospital of the Ruhr University of Bochum, Bad Oeynhausen, Germany.

**Purpose:** To investigate the characteristics of geographic atrophy (GA) associated with Pseudoxanthoma elasticum (PXE).

**Methods:** For this retrospective analysis, patients with PXE were identified from a database of a German national referral center for PXE. Diagnosis of PXE was confirmed in each patient by genetic testing and/or skin biopsy. The prevalence and the phenotype of GA was investigated using multimodal imaging including fundus autofluorescence (AF), infrared reflectance (IR) and spectral domain optical coherence tomoscopy (SD-OCT) imaging. Areas of GA were quantified based on AF images. For estimation of GA progression longitudinal AF recordings were analyzed. Exclusion criteria included insufficient image quality and additional retinal pathologies unrelated to PXE.

**Results:** 137 patients with a mean age of 52 years (range: 14-89 years) were included in the study. Of the 273 eyes eligible for further analysis, 90 (32%) had GA and 20 (7%) of those showed no signs of concurrent choroidal neovascularization (CNV). The youngest patient with GA was 36 years old. The frequency of GA with and without signs for an additional CNV increased with age, reaching 75% in those older than 70 years of age. Phenotypically most patients had multimodal GA with enhanced fundus AF surrounding the border zone of the atrophic patches. Early cases were characterized by pattern dystrophy like changes (100%), reticular pseudodrusen (82%) and subfoveal choroidal thinning (155µm ± 75µm SD). The most severely affected patients revealed multifocal and widespread choriorotinal atrophy expanding beyond the vascular arcades and nasal to the optic disc. GA progression was measured in 22 eyes of 16 patients (mean age 54.5 ± 2.6, 95% CI: 37-62 years). Yearly expansion rate of the GA area was 3.1 ± 0.9 µm/year (range: 1.0-5.4) in eyes without signs for an associated CNV (n=8) and 1.72 ± 0.7 µm/year (range: 0.6-5.1) in eyes with currently or previously active CNV (n=14).

**Conclusions:** GA is a common finding in PXE patients with early onset and fast progression leading to vision loss independent from the presence of secondary CNV. These findings underline the importance of BM for retinal integrity and suggest a possible pathogenetic contribution of Bruch’s membrane for development of GA in age-related macular degeneration.

**Commercial Relationships:** Peter Charbel Issa, Heidelberg Engineering (F); Martin Gliem, Heidelberg Engineering (F); Philipp L. Mueller, Heidelberg Engineering (F); Johannes Birtel, Heidelberg Engineering (F); Doris Hendig, None; Frank G. Holz, Heidelberg Engineering (F), Heidelberg Engineering (R), Heidelberg Engineering (C)

**Support:** ProRetina Germany

**Program Number:** 2152 Poster Board Number: C0059
**Presentation Time:** 11:00 AM–12:45 PM

**Effect of a new dietary supplement formula on the activity and expression of pro-inflammatory genes in vitro**

*Francesco Giuliano*,1 Manuela Santonocito1, Luca R. La Rosa1, Cristina Zappulla1, Santa Viola1, Marcello Santonocito2, 1Research and Development, S.I.F.I. (Società Industria Farmaceutica Italiana) S.p.A., Lavinia - Aci Sant’Antonio, Italy; 2Ophthalmology Unit, Di Stefano Velona Private Hospital S.r.l., Catania, Italy.

**Purpose:** Wet Age-related Macular Degeneration and Diabetic Macular Edema are characterized by chronic inflammation and high levels of Vascular Endothelial Growth Factor (VEGF). Since anti-VEGF agents do not possess a direct effect on inflammation, we set out to investigate the potential anti-inflammatory activity of a nutritional supplement formula codenamed AVS (SIFI S.p.A.) as a candidate support to anti-VEGF therapies. To this end, we exposed stimulated J774.2 and A549 cells to AVS in order to evaluate the effect exerted upon expression and/or activity of interleukine-1beta (IL-1β) and inducible nitric oxide synthase (iNOS) or cyclooxygenase-2 (COX-2), respectively.

**Methods:** J774.2 and A549 cells grown to sub-confluence were pre-treated (2 h) with AVS (1.9 mg/ml) and then stimulated with LPS (1 µg/ml) or IL-1β (10 ng/ml), respectively. Following overnight incubation, the medium was collected and nitrites or prostaglandin E 2 (PGE 2 ) production were determined. Total RNA was also extracted to assess the expression of IL-1β, iNOS and COX-2 mRNAs by Real Time RT-PCR. Data represent the average of 8 replicates from 4 different experiments. Statistically relevant differences were sought by unpaired t-test.

**Results:** LPS treatment induced the accumulation of ~70 µmol/l nitrites in the J774.2 cell culture medium. Treatment with AVS inhibited the accumulation of nitrites by 89% compared to control (p≤0.001). Consistently, AVS produced a significant (p≤0.0001) inhibition of iNOS and IL-1β mRNA expression by 670-fold (99%) and 1100-fold (96%), respectively. COX-2 mRNA expression was not affected by AVS treatment in IL-1β-stimulated A549 cells. However,
AVS effectively inhibited PGE_2 accumulation by 99% compared to control (p<0.001).

**Conclusions:** The specific composition of AVS was shown to be endowed with anti-inflammatory properties. Indeed, AVS was effective in inhibiting nitrites accumulation in J774.2 cultures, most likely by inhibiting the expression of iNOS and IL-1β genes. Interestingly, AVS effectively inhibited the synthesis of PGE_2 in A549 cells while unable to inhibit COX-2 expression. These findings suggest that AVS is able to act at multiple levels modulating the expression and/or the activity of important pro-inflammatory genes. Therefore, the AVS formula may well prove a useful anti-inflammatory aid in patients undergoing standard anti-VEGF therapy.


**Program Number:** 2153 **Poster Board Number:** C0060
**Presentation Time:** 11:00 AM–12:45 PM

**Survey of bilateral intravitreal injection practices**

**Purpose:** The frequency with which bilateral same-day intravitreal anti-VEGF injections (BSI) are performed is not well known. Furthermore, special techniques for BSI to reduce risk of complications are not universally agreed upon or standardized. We sought to determine present opinion among retina specialists of BSI, how frequently BSI are performed, and what measures are most frequently employed to prevent complications.

**Methods:** An online survey was distributed to retina specialists of the American Society of Retina Specialists and Dallas Academy of Ophthalmology via an online link. A total of 84 respondents completed the survey between March 2 and May 8, 2015.

**Results:** Of the 82 physicians who perform frequent injections, 66 (80.4%) report performing BSI, with 53 (64.6%) performing at least monthly BSI. While a significant majority of respondents had cared for patients with unilateral endophthalmitis from intravitreal injection (69/82, 84.2%), a more significant majority had not cared for a patient with bilateral endophthalmitis (81/82, 98.8%). Of those who reported using bevacizumab, 66.2% (51/77) reported using a compounding pharmacy accredited by the Pharmacy Compounding Accreditation Board (PCAB). Nearly half of the respondents (30/66, 45.5%) reported not taking any special measures with regards to lot, batch or vial number because the issue had never been raised (19/66, 28.8%) or because of a lack of resources (11/66, 16.7%). A majority of respondents (54/82, 66%) felt that precautions related to lot and batch number were good but not necessary because the risk of bilateral complications is minimal.

**Conclusions:** BSI are performed frequently among retina specialists and by a higher percentage of retina specialists than previously documented through survey. Despite recent outbreaks linked to compounding of bevacizumab, there remains little consensus in practice about measures used to reduce the risk of bilateral complications, including whether precautions related to medicine lot, batch or vial number are important.
drug above the efficacious levels (> 90 ng/g) for approximately 3 months in humans.

**Conclusions:** Following intravitreal administration of Brimo DDS to rabbits and monkeys, targeted delivery of brimonidine to the retina was observed. Model predictions based on non-clinical data were used to inform the dosing frequency in the human clinical trial.

<table>
<thead>
<tr>
<th>Species</th>
<th>Matrix</th>
<th>$C_{\text{max}}$ (ng/mL or ng/g)</th>
<th>AUC (ng<em>day/mL or ng</em>day/g)</th>
<th>AUC Interval (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>Vitreous</td>
<td>25400 ± 31600 ± 600000 ± 400000</td>
<td>0 - 60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retina – Central Punch</td>
<td>1830 ± 2240</td>
<td>26700 ± 13100</td>
<td>0 - 30</td>
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<tr>
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<td>Aqueous Humor</td>
<td>8.46 ± 8.13</td>
<td>92.4 ± 32.7</td>
<td>0 - 30</td>
</tr>
<tr>
<td>Monkey</td>
<td>Vitreous</td>
<td>10900 ± 1140 ± 550000 ± 152000</td>
<td>0 - 150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retina – Macula Punch</td>
<td>715 ± 605</td>
<td>40200 ± 12800</td>
<td>0 - 120</td>
</tr>
<tr>
<td></td>
<td>Aqueous Humor</td>
<td>253 ± 307</td>
<td>14400 ± 5550</td>
<td>0 - 150</td>
</tr>
</tbody>
</table>

Table1: Pharmacokinetic parameters in ocular tissues after intravitreal injection of Brimo DDS

**Commercial Relationships:** Mitalee Tamhane, Allergan plc; Michael R. Robinson, Allergan plc; Mayssa Attar, Allergan plc