283 AMD translational studies and choroidal neovascularization
Monday, May 08, 2017 3:45 PM–5:30 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 2298–2321/B0252–B0275
Organizing Section: Retina

Program Number: 2298 **Poster Board Number:** B0252
Presentation Time: 3:45 PM–5:30 PM
Gene Transfer of Prolyl Hydroxylase Domain 2 Inhibits Hypoxia-inducible Angiogenesis in a Model of Choroidal Neovascularization
Helder Andre, Parviz Mammadzada, Anna Takei, Malena Ekström, Ma Yu, Monica Aronsson, Anders P. Kvanta. Clinical Neurosciences, St Erik Eye Hospital, Stockholm, Sweden.
Purpose: Cellular responses to hypoxia are mediated by the hypoxia-inducible factors (HIF). In normoxia, HIF-α proteins are tightly regulated by a family of dioxygenases, both by proteasomal-mediated degradation and transcriptional inactivation. In hypoxic conditions, the dioxygenases become inactive and allow formation of HIF transcription factor, responsible for the upregulation of a myriad of target genes. In ocular neoangiogenic diseases, such as neovascular age-related macular degeneration (nAMD), the role of ischemia and hypoxia is associated with progression of choroidal neovascularization. Here, we investigate the effects of HIF-regulating proteins on the hypoxia pathway in retinal pigment epithelium (RPE) cells, critically involved in nAMD pathogenesis.
Methods: ARPE-19 cells were transfected with HIF-regulating proteins. In vitro angiogenesis was assayed in human retinal and choroidal endothelial cells. In vivo analysis of the effects of HIF-regulating proteins was determined in mouse models of iris and choroidal induced angiogenesis.
Results: Our previous results have associated HIF expression in RPE cells to CNV progression. Our data indicates that, in ARPE-19 cells, prolyl hydroxylase domain (PHD)2 is the most potent negative-regulator of the HIF pathway. Furthermore, the negative effects of PHD2 on the hypoxia pathway were associated with decreased HIF-1α protein levels, and concomitant decrease in secreted VEGF by these cells. Consequently, ARPE-19 cells stably expressing PHD2 impaired angiogenesis in endothelial cells, both in vitro and in vivo. Gene transfer of PHD2 in vivo resulted in mitigation of HIF-mediated angiogenesis in a mouse model of nAMD.
Conclusions: These results may have implications for the clinical treatment, particularly regarding the use of gene therapy to negatively regulate neoangiogenesis present in nAMD patients.
Commercial Relationships: Helder Andre, None; **Parviz Mammadzada**, None; **Anna Takei**, None; **Malena Ekström**, None; **Ma Yu**, None; **Monica Aronsson**, None; **Anders P. Kvanta**, None

Program Number: 2299 **Poster Board Number:** B0253
Presentation Time: 3:45 PM–5:30 PM
Optimized Dual Protein/miRNA Method to Quantitate Clinical Biomarkers of Ocular Disease
Justine J. Cunningham[1], Maria Burton[1], Gerry Rodrigues[1], Thomas C. Hohman[2]. [1]Allergan, Irvine, CA; [2]Envision, Ocean City, NJ.
Purpose: A key challenge to defining supportive clinical biomarkers that can be correlated with therapeutic benefit in patients undergoing treatment for ocular disease, is the limited volume of clinical ocular samples (≤25-50μL's). Our goal was to develop and optimize a dual protein/RNA extraction method to identify biomarkers of ocular disease that might be correlated with target engagement in the retina/choroid. Advantages of miRNAs are that they: 1) inform about regulatory networks rather than a single target, and 2) require a very small sample (≤5μL).
Methods: Six pairs of human eyes (NDRI, NY) were dissected and tissues processed following either; 1) an RNA-only or 2) a dual protein/RNA extraction method. *RNA Only:* tissues were placed directly in the RNA denaturing buffer and processed according to manufacturer's instructions. *Dual RNA/protein:* tissues were first homogenized in the buffers compatible for downstream protein analysis. A portion of the homogenate was set aside for RNA extraction. Four different RNA isolation kits for small RNAs – PARIS, mirVana, miRVana PARIS (Ambion) and miRNeasy (Qiagen) kits – were tested and compared for optimized isolation and total RNA quality based on A260/280; A230/280 ratios; and RIN values (Bionanalyzer, Agilent Technologies). Total RNA preparations enriched for small RNAs were sent to Qiagen for a miRNome analysis using qRT-PCR SYBR green technology. This analysis was able to detect most miRNAs known to date (2,034 miRNAs).
Results: By using the same homogenization buffer (TER-I) used for downstream protein assays we were able to maintain optimal conditions for protein analyses while extracting high quality RNA for miRNA profiling. We also obtained higher total RNA yield and 'purity' from both retina and choroid when using Ambion's mirVana kit versus Qiagen's miRNeasy kit. All RNA samples submitted to Qiagen for quality control (QC) testing met the criteria for miRNA profiling. We determined the expression levels (CT) of 2,032 miRNAs in retina/choroid tissues.
Conclusions: We developed and optimized a method that would allow us to determine expression levels of miRNAs in normal versus diseased ocular tissues e.g. AMD. Together with determination of target protein levels, this analysis will enhance our understanding of miRNA changes in ocular disease and aid us in identifying potential biomarkers to support target engagement.
Commercial Relationships: Justine J. Cunningham, Allergan (E); **Maria Burton**, Allergan (E); **Gerry Rodrigues**, Allergan (E); **Thomas C. Hohman**, Envision (E)

Program Number: 2300 **Poster Board Number:** B0254
Presentation Time: 3:45 PM–5:30 PM
Frequency of Human Leucocyte Antigen -A, C, B, DRB1, DQB1, and DPB1 Alleles and Haplotypes in Japanese Patients with Neovascular Age-Related Macular Degeneration
Seiji Takagi. Ophthalmology, Kobe City Medical Center General Hospital, Kobe, Japan.
Purpose: In stem cell therapy, the human leucocyte antigen (HLA) plays an important role in graft rejection. Therefore, retinal pigment epithelium (RPE) graft cells, derived from HLA-matched induced pluripotent stem cells (iPSCs), are considered good candidates as sources for allogenic cell therapy in age related macular degeneration (AMD). Moreover, RPE cells derived from iPSCs with the highest frequencies of HLA haplotypes could be used for an even wider spectrum of patients. HLA alleles and haplotypes have been known to be unique in each ethnic group and to have significant positive and negative associations with AMD. However, the HLA alleles and haplotypes in Japanese patients with AMD remain unknown. Therefore, the present cross-sectional observational study investigates the HLA A, C, B, DRB1, DQB1, and DPB1 allele and haplotype frequencies in Japanese patients with AMD.
Methods: Eighty-four consecutive patients with neovascular AMD (76.2 ± 16.5 y, male: 40), who had active exudative lesions and were receiving anti- vascular endothelial growth factor therapy, from September 2015 to January 2016 were included in this study. We determined the frequency of HLA-A,-C,-B, DRB1,-DQB1,-DPB1 allele using next generation sequencing. The haplotype frequencies

were counted directly by segregation, and the match ratio with highest haplotype frequency in the Japanese population (A*24:02, B*52:01, C*12:02, DRB1*15:02, DQB1*06:01, DPB1*09:01), previously reported, was analyzed.
Results: We identified 14 A, 27 B, 16 C, 23 DRB1, 11 DQB1, and 11 DPB1 alleles. The alleles with the highest prevalence at each locus were A*24:02 (29.7%), B*52:01 (15.5%), C*12:02 (16.1%), DRB1*09:01 (19.1%), DQB1*06:01 (23.2%), and DPB1* 05:01 (40.5%). The match ratio with the highest haplotype frequency in the Japanese population and this study group was 9.5%.
Conclusions: The frequency of HLA A, C, B, DRB1, DQB1, and DPB1 alleles and haplotypes in Japanese patients with neovascular AMD is equivalent to that in normal Japanese individuals, as reported. The RPE cells therapy, which is derived from iPSCs with frequent haplotype of HLA, could be options for AMD treatment for large population.
Commercial Relationships: Seiji Takagi, None

Program Number: 2301 **Poster Board Number:** B0255
Presentation Time: 3:45 PM–5:30 PM
Qualification of a Multiplex Panel to Analyze Complement Proteins in Human Ocular Tissues
Peter Baciu, Maria Burton, Jae Yang, Justine J. Cunningham. Biology, Allergan, Inc, Irvine, CA.
Purpose: Genetic dysregulation of the complement cascade has been linked to an increased risk for the development of age related macular degeneration (AMD). The complexity of the complement cascade together with the lack of multiplex tools, makes it difficult to elucidate the key interacting proteins that underlie the pathogenesis of AMD. Our goal was to qualify a multiplex complement panel as a tool for future determination of complement proteins associated with ocular disease in donor eyes.
Methods: Six pairs of human eyes (NDRI, NY) from a random population sample, were dissected and tissues processed following our previously optimized protocol. Briefly, vitreous samples were diluted 1:1 with PBS + protease inhibitors, passed through 23/25G needles, centrifuged (12,500G/15 mins/4°C), and the supernatant passed over a 0.45µm PVDF Durapore column. Retina/choroid were separated, homogenized in TER-I lysis buffer (Life Technologies) + protease inhibitors. Homogenates were rested (1hr/4°C), centrifuged (12,500G/15 mins/4°C), and supernatant collected. Pooled normal human serum from Quidel (San Diego, CA), and complement proteins from CompTech (Tyler, TX) and Quidel, were used to benchmark the assay and to assess assay specificity, accuracy, linearity and matrix effects. Multiplex complement panels were purchased from EMD Millipore (Billerica, MA).
Results: No matrix interference and good standard recovery (≥80%) was observed in the buffers used for tissue homogenization. Good spike recovery (CV ±20%) and assay linearity was observed for purified proteins (C5, C3, FD, FB, FP, FI, FH, MBL). Serum levels of the complement proteins approximated values from the literature and showed corresponding changes upon serum activation. Within the ocular tissues FB, FD and C3 showed similar levels to those reported in the literature. Ocular levels of C5, FP, FH and MBL were consistent with local synthesis. Six proteins – C1q, C2, C4, C4a, C5a, and C9 – did not meet our assay criteria for specificity, accuracy, linearity and matrix effects.
Conclusions: We qualified a multiplex complement panel for use in ocular tissues and provide the first report for levels of C5, C3, FD, FB, FP, FI, FH, MBL in donor human eyes.
Commercial Relationships: Peter Baciu, Allergan (E); **Maria Burton**, Alllergan (E); **Jae Yang**, Allergan (E); **Justine J. Cunningham**, Allergan (E)

Support: None

Program Number: 2303 **Poster Board Number:** B0257
Presentation Time: 3:45 PM–5:30 PM
Laser-induced Choroidal Neovascularization in the Yucatan Minipig – Characterization of a Novel Model of Neovascular Age-Related Macular Degeneration
Ryan F. Boyd[1], Douglas B. Snider[2], Krishna Yekkala[2], Betsy M. Geddings[1], Victoria J. Stevenson[1], Ashley Sparkes[3], Alexander Quiambao[3], Didier J. Nuno[3], Rafal Farjo[3], Thomas S. Vihtelic[1], Joshua T. Bartoe[1]. [1]Ophthalmology Services, MPI Research, Inc, Mattawan, MI; [2]Anatomic Pathology, MPI Research, Inc, Mattawan, MI; [3]EyeCRO, Oklahoma City, OK.
Purpose: Historically, large animal models of neovascular age-related macular degeneration have been unpredictable, with only 70% of laser-induced choroidal neovascularization (CNV) lesions in non-human primates (NHP) considered clinically relevant. Furthermore, only up to 40% of these CNV lesions are considered ideal, exhibiting Grade IV leakage on fluorescein angiography. This inefficiency leads to excess animal use and high study cost. Previous swine CNV models displayed extensive retinal damage and only minimal choroidal involvement when neovascularization was present. We aimed to create a reproducible, predictable swine model of laser-induced CNV improving efficiency and lowering cost compared to available NHP CNV models.
Methods: Yucatan minipigs were used to optimize laser induction of CNV. Bilaterally, six lesions were created using a 532nm green argon laser under direct visualization with a slit lamp and condensing lens. Follow-up examinations included optical coherence tomography (OCT) and fluorescein angiography (FA) at five day intervals, followed by histopathology at 15 days post-operatively.
Results: Lesions were reliably produced exhibiting rupture of Bruch's membrane as demonstrated by a focal region of discontinuity of the hyperreflective line representing the retinal pigment epithelium/Bruch's membrane complex on OCT. These regions showed ingrowth of hyperreflective tissue into the outer retinal layers, as well as hyperfluorescence on FA, both suggestive of fibrovascular proliferation from the choroid into the retina. Histopathological characterization of the lesions supported the *in vivo* findings with moderate to marked subretinal fibrosis and neovascularization extending from the choroid into the outer nuclear layer. The inner retina was generally unaffected.
Conclusions: The Yucatan minipig is an excellent alternative to other large animal models of neovascular age-related macular degeneration. Similar globe size and retinal anatomy to human patients makes the swine model ideal for characterizing novel investigational molecules targeting proliferative posterior segment disease.
Commercial Relationships: Ryan F. Boyd, None; **Douglas B. Snider**, None; **Krishna Yekkala**, None; **Betsy M. Geddings**, None; **Victoria J. Stevenson**, None; **Ashley Sparkes**; **Alexander Quiambao**, None; **Didier J. Nuno**, None; **Rafal Farjo**, None; **Thomas S. Vihtelic**, None; **Joshua T. Bartoe**, None

Program Number: 2304 **Poster Board Number:** B0258
Presentation Time: 3:45 PM–5:30 PM
Synergistic attenuation of choroidal neovascularization by dietary intake of ω-3 long-chain polyunsaturated fatty acids and lutein in mice
Ryoji Yanai[1], Shang Chen[1], Sho-hei Uchi[1], Tomoaki Nanri[2], Kazuhiro Kimura[1]. [1]Ophthalmology, Yamaguchi Univ Grad Sch of Med, Ube City, Japan; [2]SANTEN PHARMACEUTICAL CO., LTD., Osaka, Japan.
Purpose: Dietary ω-3 long-chain polyunsaturated fatty acids (LCPUFAs) have been found to protect against age-related macular degeneration (AMD), the primary cause of blindness in elderly individuals of industrialized countries. Lutein is a major carotenoid in the macula and retina, where it protects against the adverse effects of photochemical reactions as a result of its antioxidant properties. Although lutein also protects against AMD, its effects in combination with ω-3 LCPUFAs have remained unknown. We therefore examined the effects of dietary intake of both ω-3 LCPUFAs and lutein in a mouse model of AMD.
Methods: C57BL/6 mice were fed a diet enriched in or free of ω-3 LCPUFAs beginning 2 weeks before, and either containing lutein (2.5 mg/kg per day) or not beginning 1 week before, induction of choroidal neovascularization (CNV) by laser photocoagulation. The area of CNV was evaluated in choroidal flat-mount preparations 7 days after photocoagulation. Primary eicosanoids and their metabolites were analyzed by liquid chromatography and tandem mass spectrometry. Expression of cytokines and chemokines in the retina or choroid was examined by RT-PCR analysis and with the use of a multiplex assay system, and that of NADPH oxidase 4 (Nox4) by immunoblot analysis.
Results: The area of CNV was significantly reduced in mice fed ω-3 LCPUFAs or lutein compared with the control diet, and it was synergistically reduced in those fed both ω-3 LCPUFAs and lutein. The serum concentrations of the principal ω-3 LCPUFAs and lutein were significantly increased in mice fed the corresponding diets. The expression of cytokines and chemokines was reduced at the mRNA and protein levels in the retina or choroid of ω-3 LCPUFA–fed mice, lutein-fed mice, and mice fed both dietary components, but no synergistic effect was apparent. The abundance of Nox4 in the retina was significantly reduced in mice fed ω-3 LCPUFAs and synergistically reduced in those receiving both ω-3 LCPUFAs and lutein.
Conclusions: Dietary intake of ω-3 LCPUFAs and lutein attenuated the development of CNV in a synergistic manner, with this effect being accompanied by down-regulation of Nox4, a major source of reactive oxygen species in the retina. Dietary supplementation with both ω-3 LCPUFAs and lutein warrants further study as a means to protect against AMD.
Commercial Relationships: Ryoji Yanai, Sanren Phrmacological Co. ltd (F); **Shang Chen**, None; **Sho-hei Uchi**, None; **Tomoaki Nanri**, Sanren Phrmacological Co. ltd (E); **Kazuhiro Kimura**, Sanren Phrmacological Co. ltd (F)
Support: A grant (15K10869) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan

Program Number: 2305 **Poster Board Number:** B0259
Presentation Time: 3:45 PM–5:30 PM
Altered levels of aqueous humour soluble factors are associated with therapy-mediated resolution of macular edema in patients with Choroidal Neovascular Membranes
Santosh G. Gadde[1], Priyanka Chevour[2], Shivani Sinha[1], Rohit Shetty[3], Swaminathan Sethu[2], Arkasubhra Ghosh[2]. [1]Vitreo Retina, Narayana Nethralaya, Bangalore, India; [2]GROW LAB, NARAYANA NETHRALAYA, BANGALORE, India; [3]CORNEA AND REFRACTIVE SURGERY, NARAYANA NETHRALAYA, BANGALORE, India.
Purpose: Choroidal neovascularization membranes (CNVM), a progressive, advanced form of age-related macular degeneration results in vision loss. Varying response to intra-vitreal injection therapy has been observed. Hence, this study aims to determine the association between the levels of various soluble secreted factors in the aqueous humour and response of macular edema to therapy in CNVM patients.

Methods: Aqueous humour samples were collected from CNVM patients (n=31) undergoing intravitreal anti-VEGF after obtaining informed written consent and prior study approval by the Institutional Ethics Committee. The study subjects included CNVM patients (n=31) undergoing intravitreal anti-VEGF. Routine investigations including OCT imaging along with intravitreal injections were performed during visits. The subjects were then grouped as resolving edema (n=18) and persistent edema (n=8) based on the reduction in edema volume (analysed using MATLAB image processing) between two visits. Cytometric bead array was used for simultaneous quantification of IL-2, IL-4, IL-8, IL-17F, MCP-1, MIP-1β, CXCL9, CXCL10, CXCL11, Fractalkine, CD62L, CD62P, ICAM1, VCAM, Angiogenin and VEGF in aqueous humour collected during first visit.
Results: VEGF levels in aqueous humour was significantly (p=0.03) lower in the persistent edema group (66±13 pg/ml) compared to resolving edema group (249±92 pg/ml). A significant increase in the levels of ICAM1, VCAM, CD62L and CXCL10 were observed in the aqueous humour of those patients with persistent edema compared to those with resolving edema. A positive correlation was observed between difference in edema volume (between visits) and ICAM1 (r=0.550; p=0.003), VCAM (r=0.455; p=0.01), CD62L (r=0.537; p=0.004) and IL-2 (r=0.424; p=0.03). These observations suggest the possible role of soluble cell adhesion molecules in the response of macular edema to therapy in CNVM patients. In addition, the decreasing trend in central subfoveal thickness was greater in those with resolving edema compared to persistent edema.
Conclusions: The findings demonstrate a unique set of soluble factors in aqueous humour which may influence or indicate the status of therapy-mediated reduction of macular edema in CNVM patients.
Commercial Relationships: Santosh G. Gadde, None; **Priyanka Chevour**, None; **Shivani Sinha**, None; **Rohit Shetty**, None; **Swaminathan Sethu**, None; **Arkasubhra Ghosh**, None
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Program Number: 2306 **Poster Board Number:** B0260
Presentation Time: 3:45 PM–5:30 PM
Subretinal Lipid Hydroperoxide Induced-Oxidative Stress and Retinal Degeneration Results in a Rat Model of Age-Related Macular Degeneration
Soo-Young Kim, Siva Pramodh Kambhampati, Imran A. Bhutto, Gerard A. Lutty, Kannan Rangaramanujam. Center for Nanomedicine, Opthalamology, Wilmer Eye Institute, Johns Hopkins Univ, Baltimore, MD.
Purpose: In the pathogenesis of AMD, chronic oxidative stress and inflammation have been shown to be key events in early AMD, leading to degeneration and angiogenesis (CNV) in later stages. We have previously reported that subretinal injection of lipid hydroperoxide, 13(S)-hydroperoxy-9Z, 11E-octadecadienoic acid (HpODE) causes inflammation and CNV but the retinal oxidative stress and degeneration has not been explored. In this study, we investigate the involvement of oxidative stress and retinal degeneration in disease progression.
Methods: HpODE was administered via subretinal injection in Sprague Dawley rats on day 0 and the time course of oxidative stress and retinal degeneration was studied qualitatively using immunohistochemistry (flatmount and crossections), and quantitatively via qPCR and western blot on day 2, 5 12 and 20.
Results: Subretinal lipid provoked oxidative stress and damage in retinal tissue and retinal pigment epithelium (RPE) with accumulated autofluoresent material in subretina. RPE degeneration and atrophy was detected early at day 5 followed by neural tissue degeneration at day 12 with robust TUNEL positive cells. Oxidative stress marker, 8-OHdG, was demonstrated in RPE cells at day 2 and oxidative damage (4-HNE, MDA, NT and 8-OHdG) increased in retinal tissue from day 5 to 12. Retinal inflammation was confirmed by subretinal microglial/macrophage accumulation and Müller glial activation in the lipid injected area was present at day 5. Western blot and RT-qPCR confirmed an increase of pro-apoptotic Bax. We further confirmed an increase in NLRP3 inflammasome and pro-inflammatory cytokine TNFa and a decrease of anti-oxidative Nrf2, Sod1 and Sod2 in RPE/Choroid tissue.
Conclusions: Understanding the roles of oxidative stress and inflammation in AMD progression may give insights for developing targeted therapies for early and intermediate stages of AMD. This model is a relevant experimental model for nonexudative (dry) AMD as well with the previously report as an exudative (wet) AMD, and would be useful to study molecular pathology of oxidative damage in retina diseases and protective effect of antioxidant and anti-inflammatory substances.
Commercial Relationships: Soo-Young Kim, None; **Siva Pramodh Kambhampati**, None; **Imran A. Bhutto**, None; **Gerard A. Lutty**, None; **Kannan Rangaramanujam**, Orpheris (I)
Support: NEI EY025304-01, Altsheler Durell Grant and Research to Prevent Blindness

Program Number: 2307 **Poster Board Number:** B0261
Presentation Time: 3:45 PM–5:30 PM
Scaffold and Cell Engineering of Artificial Bruch s Membrane
Montserrat Perez[1], Abigail Torres[1], Uriel Hernandez[1], Cristina Velasquillo[2], Phaedra Silva[2], Roberto Gonzalez[1], Ana M. Lopez-Colome[3], Edith Maldonado[3], Shay Soker[4], Hugo Quiroz[1], LUIS F F. HERNANDEZ[1]. [1]Association to prevent blindness, Mexico, Mexico; [2]Research INR, Mexico, Mexico; [3]Neuroscience IFC-UNAM, MEXICO, Mexico; [4]WFIRM, WAKE, NC.
Purpose: Our main goals are to design and create and improve functional and artificial Bruch s membranes through established bioengineering techniques.
Methods: We fabricated novel ultrathin 3-dimensional(3D) nanofibrous membranes from Polycaprolactone, and Polycaprolactone/5% gelatine and Polycaprolactone/30% gelatin by an advanced clinical-grade needle-free-electrospinning process. The nanofibrillar 3D networks highly mimicked the fibrillar architecture of the native inner collagenous layer of human BM. ARPE-19 cells (n 15,000) were plated onto the surface and the RPE reattachment, apoptosis, and proliferation ratios were determined on the modified surfaces. Cells were cultured up to 17 days to determine the surface coverage. Ultrastructure of the modified Bruchs membrane and RPE morphology were studied with transmission and scanning electron microscopy. Triplicate wells were used to calculate the average reattachment, apoptosis, and proliferation ratios and the final fate of RPE cells seeded
onto each substrate. MTT assays were performed in order to evaluate ARPE cell viability and citotoxicity of the membranes. SEM studies were performed in duplicate only.
Results: Data from all experiments were pooled and expressed as the mean SD. The reattachment, apoptosis, and proliferation ratios and surface coverage on different substrates and RPE cell populations were analyzed in pairs by the Dunn's multiple comparison test 28. A confidence level of P 0.05 was considered to be statistically significant. ARPE19 cells grown on our nanofibrous membranes in a similar mannes to native human RPE. They exhibited a correctly orientated monolayer with polygonal cell shape and abundant sheet-like microvilli on their apical surfaces after several days of culture. ARPE 19 cells built tight junctions and expressed RPE65 protein.

MTT assays demostrate that our artificial membrane is not toxic for ARPE19 cells.
Conclusions: Age-related changes that impair RPE repopulation of Bruch s membrane can be significantly reversed by combined cleaning and ECM protein coating of the ICL. Our artificial membranes may imitate the natural BM to such extent that they allow for the engineering of in vivo-like human RPE monolayer and maintaining its biofunctional characteristics. Such artificial membranes may be a promising vehicle for a functional RPE cell monolayer implantation in the subretinal space in patients with AMD or SD.
Commercial Relationships: Montserrat Perez, None; **Abigail Torres**, None; **Uriel Hernandez**, None; **Cristina Velasquillo**, None; **Phaedra Silva**, None; **Roberto Gonzalez**, None; **Ana M. Lopez-Colome**, None; **Edith Maldonado**, None; **Shay Soker**, None; **Hugo Quiroz**, None; **LUIS F F. HERNANDEZ**, None

Program Number: 2308 **Poster Board Number:** B0262
Presentation Time: 3:45 PM–5:30 PM
Switching to aflibercept from ranibizumab in choroidal neovascularization related to angioid streaks: a multicenter real life study
Rim Sekfali[1], gerard mimoun[1,2], Vittorio Capuano[1], Salomon Y. Cohen[1,3], Camille JUNG[4], OUBRAHAM-MEBROUKINE Hassiba[1,5], Giuseppe Querques[1,6], Eric H SOUIED[1]. [1]Department of Ophthalmology, Centre Hospitalier Intercommunal de Créteil, Créteil, France; [2]Centre Ophtalmologique d'Imagerie de l'Ecole miliataire, Paris, France; [3]Centre d'Imagerie et de Laser, Paris, France; [4]Centre de Recherche Clinique- Centre de Ressources Biologiques, Hôpital Intercommunal de Créteil, Créteil, France; [5]Centre Ophtalmologique OPHTA45, Montargis, France; [6]Departement of ophthalmology, IRCCS Ospedale San Raffaele, University Vita-Salute, Milan, Italy.
Purpose: To evaluate the efficacy and the safety of Pro Re Nata (PRN) intravitreal aflibercept (Eylea; VEGF trap-Eye, Regeneron, Tarrytown and Bayer HealthCare, Berlin, Germany) in patients with refractory or recurrent choroidal neovascularization (CNV) secondary to angioid streaks (AS), previously treated by ranibizumab (Lucentis, Novartis Pharma SAS, Huningue, France; 0.5 mg in 0.05 m).
Methods: Retrospective, multicenter study (n=3). Complete ophthalmic examination, best corrected visual acuity (BCVA), fluorescein angiography (FA), indocyanine green angiography (ICGA), central macular thickness (CMT) on spectral domain optical coherence tomography (SD-OCT), were collected. Patients were recorded into "refractory" (persistent exudation despite six monthly injections of ranibizumab) and "recurrent" groups (exudation suppressed, but requiring frequent injections with at least two recurrences during the last year before the switch).
Results: Twenty eyes of 16 patients (10 female, 67.3 ± 11.7 years old) were included. Mean follow up was 28.4 ± 8,7 months (range 13-41 months). Mean BVCA at baseline was 0.25 ± 0.33 logMAR and 0.35 ± 0.45 log MAR at the last visit (p 0.17). CMT was 321.8 ± 169.21 µm at baseline and 251.5 ± 63.34 µm at last follow up (p 0.067). Mean number of intravitreal injections of aflibercept was 12.27 ± 6.03. Four eyes were classified as "refractory" and 16 as "recurrent". No adverse effect was observed.
Conclusions: Switching patients with CNV secondary to AS to aflibercept in a PRN schedule provided a stabilized vision and improved anatomic outcomes.
Commercial Relationships: Rim Sekfali, None; **gerard mimoun**, None; **Vittorio Capuano**, None; **Salomon Y. Cohen**, Thea (C), Novartis (C), Bayer (C), Allergan (C), Alcon (C); **Camille JUNG**, None; **OUBRAHAM-MEBROUKINE Hassiba**, None; **Giuseppe Querques**, Novartis (C), Bausch and Lomb (C), Zeiss (C), Heidelberg (C), Bayer (C), Allergan (C), Almeria sciences (C); **Eric H SOUIED**, Novartis (C), Thea (C), Bayer (C), Allergan (C)

Program Number: 2309 **Poster Board Number:** B0263
Presentation Time: 3:45 PM–5:30 PM
Improving diagnostic findings with OCT angiography
Taha Soomro, James Talks. Ophthalmology, Royal Victoria Infirmary, Newcastle-upon-tyne, United Kingdom.
Purpose: OCT angiography (OCTA) can show choroidal neovascularization (CNV) in some cases compared to fundus fluorescein angiography (FFA) but does it just replicate FFA findings or can it add to a multimodal imaging approach?
We reviewed the images obtained from a Heidelberg OCTA (beta version), compared to those from OCT, FFA and indocyanine green angiography (ICG), in cases being assessed for CNV.
Methods: A random sample of patients having OCT, FFA and ICG were imaged with OCTA, (Heidelberg Spectralis OCTA beta). The OCTA images were assessed for detection of a vascular network and compared to the OCT, FFA and ICG images. Standard diagnostic criteria were used to classify classic and occult imaging findings. Manual segmentation and assessment through the enface layers of the OCTA were correlated with the cross sectional OCT findings and allowance made for projection artefacts and areas of loss of RPE. In cases where a CNV was seen on OCTA but not clearly defined on FFA a repeat OCTA was performed after antiVEGF treatment to check for response.
Results: 49 patients were imaged, 36 had confirmed CNV on FFA/ICG. 19 of these patients had CNV on OCTA. A vascular network was seen with OCTA, compared with FFA in 11/12 (92%) classic lesions, 2/10 (20%) occult lesions, 2/4 (50%) RAP lesions, 2/6 (33%) polyps, 0/2 (0%) peripapillary CNV and 2/2 (100%) myopic CNV. Overall OCTA sensitivity was 56% (95% CI 39-73%) and specificity 92% (95% CI 78-107%). In subgroup analysis for classic neovascular AMD the sensitivity was 92% (95% CI 76-107%) and specificity was 100% (95% CI 100-100%). The neovascular network was more clearly defined on OCTA compared to FFA/ICG, as the availability of early shots varied and FFA leakage obscured the vascular pattern. In two cases OCT showed subretinal fluid with subtle RPE thickening where it was uncertain if the diagnosis was CSR or CNV. In both cases FFA showed a non-specific leak, which could have been occult CNV or CSR, ICG was more suggestive of a CNV and OCTA showed a CNV. Both cases responded to antiVEGF therapy with decrease of the subretinal fluid. Repeat OCTA showed shrinkage of the CNV confirming we had not seen a projection artefact or window defect.
Conclusions: This OCTA system can detect CNV, particularly type 2, classic CNV, often providing a clearer image of the network than seen in FFA. In some cases it can detect a CNV not seen on FFA, which can alter a patient's management.
Commercial Relationships: Taha Soomro, Heidelberg Engineering (R), Bayer (C), Bayer (R), Bayer (F); **James Talks**, Heidelberg Engineering (R), Bayer (C), Bayer (R), Bayer (F)

Program Number: 2310 **Poster Board Number:** B0264
Presentation Time: 3:45 PM–5:30 PM
Correlation of Fundus Autofluorescence and Histology of Laser-induced Choroidal Neovascularization in the Rat
Min Zhao[1,2], Wankun Xie[1,2], Shu-Huai Tsai[2], Brent A. Rocke[1,3], Travis W. Hein[1,2], Lih Kuo[1,2], Robert H. Rosa[1,2]. [1]Ophthalmic Vascular Research Program, Department of Ophthalmology, Baylor Scott & White Eye Institute, Temple, TX; [2]Departments of Surgery and Medical Physiology, Texas A&M University Heath Science Center, Temple, TX; [3]Central Texas VA Health Care System, Temple, TX.
Purpose: To characterize and correlate the patterns of fundus autofluorescence (FAF) and histological features of laser-induced choroidal neovascularization (CNV) in Brown Norway (BN) rats.
Methods: CNV was induced by laser photocoagulation in BN rats. FAF images were obtained and the histological features of CNV lesions were evaluated by light microscopy (LM) at 10 min, 72 hr, 1 wk, 2 wk, and 3 wk after laser injury.
Results: The size of the laser injured area on FAF peaked at 72 hr and gradually decreased after 1 wk. The lesion on FAF exhibited a central punctate dark area surrounded by a discrete bright core with a dark rim 10 min after laser. The lesion boundaries became less distinct after 72 hr. After 1 wk, the lesion exhibited a bright core surrounded by a dark inner ring and a bright outer ring. The highest FAF intensity was observed 10 min after laser and significantly decreased 1 wk after laser. After acute laser injury (72 hr), LM revealed that the central core was composed of loose tissue with chronic inflammatory cells and marked loss of the photoreceptor inner segments (IS), outer segments (OS) and RPE. Pigment migration into the retina and Müller cell activation/proliferation were present. At the margin of the central core, disruption and loss of IS/OS were observed. In the peripheral aspect of the CNV lesion, disorganization of the IS/OS and RPE and rounding-up of pigment were present. In later laser injury (2 wk), LM revealed dense fibrovascular connective tissue with total loss of the IS/OS and RPE in the central core. A glial scar was present with pigment migration into the outer retina. At the margin of the central core, the IS/OS and RPE cell size and number were attenuated. In addition, a thin, loose fibrovascular layer was interposed between the RPE and Bruch membrane with focal RPE hypertrophy in the peripheral aspect of the CNV lesion.
Conclusions: The laser insult elicits a “target-like” configuration of FAF in CNV lesions. The intensity and area of FAF inversely change over time, with a distinct alteration in tissue morphology. FAF appears to correlate with the integrity of the photoreceptors and RPE overlying CNV lesions, and thus may serve as an in vivo, non-invasive assessment for photoreceptor and RPE morphology and function in rodent models of CNV.
Commercial Relationships: Min Zhao, None; **Wankun Xie**, None; **Shu-Huai Tsai**, None; **Brent A. Rocke**, None; **Travis W. Hein**, None; **Lih Kuo**, None; **Robert H. Rosa**, None
Support: Scott & White Kruse Chair Fund, Retina Research Foundation and Scott & White Healthcare Foundation

Program Number: 2311 **Poster Board Number:** B0265
Presentation Time: 3:45 PM–5:30 PM
Inhibition of Micro-Fibrillar Associated Protein 4 as a Potential Therapy Targeting Choroidal Neovascularisation in Age Related Macular Degeneration
Andew Benest[1], Claire Allen[1], Nikita Ved[1], Zoe Blackley[1], Grith Lykke Sørensen[2], David O. Bates[1]. [1]Division of Cancer and Stem Cells, University of Nottingham, Nottingham, United Kingdom; [2]Department of Cancer and Inflammation Research, University of Southern Denmark, Odense, Denmark.
Purpose: Age-Related Macular Degenration (AMD) is the leading cause of blindness in Europe, the USA and Australia. This study tested the hypothesis the hypothesis that targeting MFAP4 (which is upregulated at sites of vascular remodelling) would reduce choroidal vessel growth following laser induced choroidal neovascularisation in murine eyes.
Methods: Female C57/Bl6 mice were anaesthetised and laser coagulation of the Bruch’s membrane was performed (Phoenix Micron IV retinal imaging microscope). Intraocular injection of either 1mg mouse IgG (DAKO) 1 or 5mg aMFAP4 or 1mg aVEGF. Injections were performed using a 36 Gauge Hamilton syringe, in a total of 2ml. Injections were performed at day 0 and day 7. Fundus Fluorescein Angiography (FFA) was performed on day 7 and day 14. Mice were culled and eyes excised, fixed in 4% paraformaldehyde. Choroids were dissected and immunostained for isolectin IB4 and CD45 and imaged by confocal microscopy. Quantification of lesion size, both of FFA and confocal data was performed using ImageJ.
Results: aMFAP4 (1mg n=4), (5mg n=6)) reduced average lesion size and density (measured by FFA) by day 7 dose dependently ($p<0.001$ vs IgG control, n=8) whereas aVEGF (n=12) treatment required 2 doses to significantly reduce lesion size and density (n=4, $p<0.05$). IB4 staining of flatmounted choroids demonstrated reduced burn density following either aVEGF (n=4) or 5mg aMFAP4 (n=6) intraocular injection versus IgG injection (n=8). Inhibition of either VEGF (n=4), or MFAP4 (1mg, n=4 or 5mg n=6) was sufficient to significantly reduce CD45+ myeloid cell infiltration into the choroidal wound area ($p<0.001$).
Conclusions: MFAP4 biology has not yet been studied in the eye, but our results are consistent with MFAP4 upregulation being upregulated by vascular injury (as seen in carotid intimal formation). Collectively this experiments suggest value in MFAP4 inhibition as a potential additional way to managed wet AMD.
Commercial Relationships: Andew Benest; **Claire Allen**, None; **Nikita Ved**, None; **Zoe Blackley**, None; **Grith Lykke Sørensen**, None; **David O. Bates**, Exonate Ltd (P), Exonate Ltd (I)

Program Number: 2312 **Poster Board Number:** B0266
Presentation Time: 3:45 PM–5:30 PM
A2E-blue light induced endothelial invasion by using 3-deimensional choroidal neovascularization model
Kyoung-pil Lee, Man-Il Huh, Jeongho Kim, Byeong-Ung Park, Soo-Jin Yi, Myung-Jun Kim, Hong Kyun Kim. Kyungpook National University, School of medicine, Daegu, Korea (the Republic of).
Purpose: Accumulation of A2E and Exposure of blue light are a major factor of marcular degeneration. These are related with angiogenesis between retinal pigment epithelial (RPE) cells and choroid. There were no 3-deimensional (D) choroidal neovascularization (CNV) model to describe both accumulation of A2E, optical stimulus and movement of endothelial cells. We developed 3D retinal-vascular mimetic system reflecting angiogenesis induced by A2E-blue light damage.
Methods: Human RPE cells and peripheral micro-vascular endothelial (PMVE) cells were co-cultured in a microfluidic channel.

hRPEs were pretreated with 10 μM A2E, and then the cells were exposed with/without blue light (the channel of RPE cells were exposed to 400 ± 20 nm for 30 seconds). A hypoxic condition for 24hrs was used as positive control of CNV. We examined the chemical and optical stimulus on 3D retinal-vascular mimetics system, and then evaluated cell invasion, Vascular endothelial growth factor (VEGF) secretion and activation of the signal mediators.
Results: In the 3D retinal-vascular mimetic system between RPE and PMVE cells, PMVE cell were invaded into RPE cells in hypoxia (21.0 ± 3.93 cell/unit area), A2E (22.3 ± 4.26 cell/unit area), and A2E with blue light (36.3 ± 5.02 cell/unit area) conditions for 24hrs. In the addition, Secretion of VEGF by RPE in the 3D retinal-vascular mimetics system increased A2E with/without blue light and hypoxia conditions by ELISA assay for 24 hrs.
Conclusions: In this study, data indicated A2E-blue light induced the secretion of VEGF and the invasion of PMVE cells in 3D co-culture model. We established new 3D retinal-vascular mimetic model, an angiogenesis model related with CNV, would be able to detect invasion of endothelial cells under a disease condition. This model would be to employ for a candidate selection before a pre-clinical trial.
Commercial Relationships: Kyoung-pil Lee; **Man-Il Huh**, None; **Jeongho Kim**, None; **Byeong-Ung Park**, None; **Soo-Jin Yi**, None; **Myung-Jun Kim**, None; **Hong Kyun Kim**, None
Support: This study was supported by Korean National Research Fund (NRF-2012R1A1A1010163) and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI15C0001)

Program Number: 2313 **Poster Board Number:** B0267
Presentation Time: 3:45 PM–5:30 PM
Cytochrome P450 lipid metabolites alter leukocyte kinetics in a mouse model of choroidal inflammation
Clifford B. Kim, Eiichi Hasegawa, Saori Inafuku, Deeba Husain, Joan W. Miller, Kip M. Connor. Ophthalmology, Harvard Medical School/Massachusetts Eye and Ear Infirmary, Boston, MA.
Purpose: Systemic leukocyte recruitment from blood vessels into tissues is a key process in many inflammatory ocular conditions, such as the development of choroidal neovascularization (CNV) in advanced age-related macular degeneration. Systemic leukocytes attach and roll along endothelial cells via adhesion molecules, such as intercellular cell-adhesion molecules (ICAM) or selectins. We previously found that omega fatty acid metabolites from the cytochrome P450 (CYP) pathway are integral to attenuating CNV severity. To explore mechanisms for this finding, this study examines how CYP-derived lipid metabolites affect leukocyte dynamics under inflammatory ocular conditions.
Methods: A 532 nm laser was used to induce CNV in C57BL/6 mice. Directly after laser treatment, mice were given daily intraperitoneal injections, for 3 to 7 days, of CYP-derived omega fatty acid metabolites: epoxydocosapentaenoic acid (EDP), epoxyeicosaquatraenoic acid (EEQ), or epoxyeicosatrienoic acid (EET). 3 days post-laser, an autoperfused microflow chamber assay was used to measure leukocyte rolling velocity in the blood, and fluorescence-activated cell sorting (FACS) of blood leukocytes was used to determine the expression of CD11b and CD18 (cell adhesion molecules). 7 days post-laser, real-time PCR of CNV lesions isolated via laser-capture dissection was conducted to determine expression of E-selectin and ICAM-1.
Results: Mice administered EEQ or EDP had higher leukocyte rolling velocities, while mice administered EET had lower rolling velocities, compared to mice administered PBS ($P<0.05$, n=3 mice/group). From FACS analysis, CD11b and CD18 expression decreased in mice administered EDP and EEQ, respectively, while CD18 expression increased in mice administered EET, compared to mice administered PBS ($P<0.05$, n=10 mice/group). Finally, PCR analysis of isolated CNV lesions showed decreased expression of E-selectin and ICAM-1 in mice administered EEQ ($P<0.05$, n=6 mice/group), while mice administered EET showed increased ICAM-1 ($P<0.001$, n=6 mice/group), compared to mice administered PBS.
Conclusions: CYP-derived lipid metabolites EEQ and EDP decrease leukocyte adhesion ability, while EET increases leukocyte adhesion. The ability of these lipid metabolites to modulate adhesion molecule expression and leukocyte rolling velocity, and thus leukocyte recruitment, may influence disease severity in ocular inflammatory diseases.
Commercial Relationships: Clifford B. Kim, None; **Eiichi Hasegawa**, None; **Saori Inafuku**, None; **Deeba Husain**, None; **Joan W. Miller**, Valeant Pharmaceuticals (P), Maculogix, Inc. (C), Amgen, Inc. (C), KalVista Pharmaceuticals Ltd. (C), ONL Therapeutics, LLC (P), Alcon (C); **Kip M. Connor**, Achillion (F), OMEICOS (F), OMEICOS (C), MEEI (P)
Support: Research to Prevent Blindness Medical Student Fellowship

Program Number: 2314 **Poster Board Number:** B0268
Presentation Time: 3:45 PM–5:30 PM
Regression of choroidal neovascularization in exudative age-related macular degeneration following postinjection endophthalmitis
Peter Mortensen, Denise Gallagher, Joseph N. Martel. Ophthalmology, University of Pittsburgh Medical Center, Pittsburgh, PA.
Purpose: We report three cases in which patients receiving chronic intravitreal anti-VEGF therapy for exudative age-related macular degeneration (ARMD) exhibited inactivation of CNV following development of endophthalmitis, negating the need for anti-VEGF therapy following endophthalmitis resolution.
Methods: We retrospectively reviewed the medical records of endophthalmitis patients with a history of exudative ARMD and identified three female patients with mean age 87 (range: 79-93) receiving anti-VEGF therapy for exudative ARMD who developed postinjection endophthalmitis. Examination for post endophthalmitis exudative maculopathy was performed by clinical examination and OCT.
Results: Following resolution of the endophthalmitis, there was notable inactivation of their exudative maculopathy in all patients as observed on OCT. Prior to endophthalmitis, all patients received anti-VEGF therapy (bevacizumab n=2 both every 5 -6 weeks; ranibizumab n=1 every 8 weeks). All three patients received intravitreal vancomycin and ceftazidime. Two patients were additionally treated with pars plana vitrectomy surgery.
In all three cases, inactivation of the exudative maculopathy following endophthalmitis resolution was observed and no subsequent anti-VEGF injections were needed in the eye affected by endophthalmitis (mean follow-up time following endophthalmitis diagnosis: 218.7 days, range 148-263). All three patients also had exudative ARMD in their contralateral eye, with one of three patients no longer requiring anti-VEGF injections in their contralateral eye.
Conclusions: Development of anti-VEGF post-injection endophthalmitis was associated with inactivation of the exudative maculopathy in patients with exudative ARMD. Further study is warranted to elucidate the etiology of presumed CNV inactivation after resolution of endophthalmitis.

Commercial Relationships: Peter Mortensen, None; **Denise Gallagher**, None; **Joseph N. Martel**, None

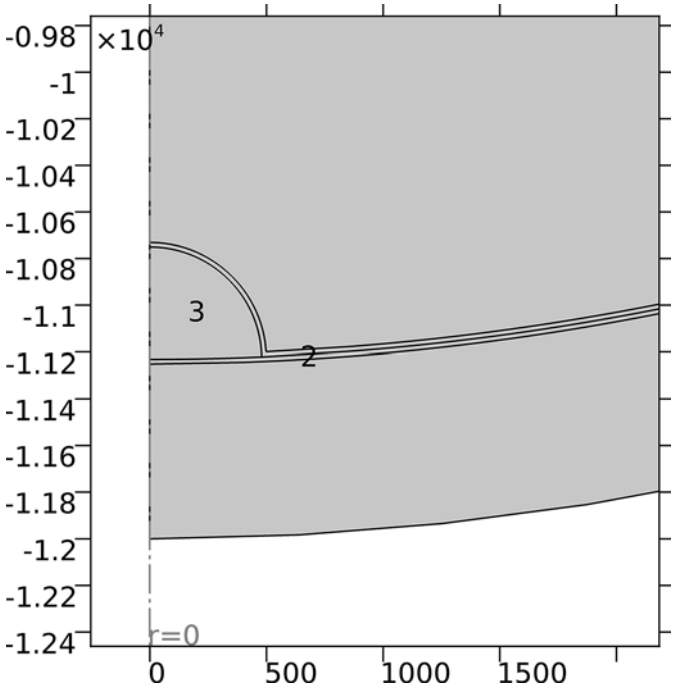
Program Number: 2315 **Poster Board Number:** B0269
Presentation Time: 3:45 PM–5:30 PM
Clinical characteristics and treatment outcomes in Central Serous Chorioretinopathy with secondary choroidal neovascularisation
Danilo Iannetta[1, 2], Savitha Madhusudhan[1], Nicholas V. Beare[1], Ian A. Pearce[1]. [1]St. Paul's Eye unit, Royal Liverpool Hospital, Liverpool, United Kingdom; [2]UOSD Glaucoma, Tor Vergata University, Rome, Italy.
Purpose:
Development of choroidal neovascularization (CNV) secondary to Central Serous Chorioretinopathy (CSCR) is a rare entity with an incidence from 2% to 9%, with no established standard treatment. We present our data on the clinical characteristics and treatment outcomes in this condition.
Methods:
This is a retrospective interventional case series of consecutive patients with an angiographic diagnosis of CNV secondary to CSCR, who were treated at a single tertiary medical retina unit between 2011 and 2016. Collected data includes demographic details, clinical characteristics, best-corrected visual acuity (BCVA), and OCT features at presentation and final follow-up, and mode of treatment. Main outcome measure was the proportion of eyes that had improved, stable, or worse vision at the final follow-up.
Results: Twenty-one eyes of 21 patients, of whom 12 were male, were included. Mean age at diagnosis of CSCR was 52.57 years (range 24-77 years). Six were right eyes. Nine eyes had received half-dose photodynamic therapy (PDT) for CSCR and the rest were untreated. Mean interval between diagnosis of CSCR and diagnosis of CNV was 34.85 months. Sub-type of CNV was occult in 14 and classic in 7 eyes respectively. 14 eyes received anti-vascular endothelial growth factor intravitreal (anti-VEGF) therapy only, 4 had PDT only and 3 had a combination of anti-VEGF and PDT for the CNV. Mean follow-up duration (range) was 18.95 (1-65) months. Mean number of injections during the follow-up was 9 in the group with anti-VEGF monotherapy and 6.42 in the group with combination therapy.
The mean gain in BCVA was 5.23 letters (±7.02 letters). Mean gain in BCVA in eyes with previous PDT for CSCR was 4.85 letters. Visual acuity improved in 14 (66.66%), remained stable in 2 (9.52%) and worsened in 5 (23.80%) eyes overall. Mean gain in BCVA was 5.43, 4.25 and 5.43 letters in patients receiving anti-VEGF monotherapy, PDT only and combination treatment respectively. Mean central subfield thickness (SD) on OCT was 354.67(± 64.12) at baseline and reduced to 252.21 (±34.43) microns at final follow-up. Subretinal fluid was present in all cases at baseline and only in 6 eyes at final follow-up on OCT.There were no adverse events noted.
Conclusions:
Anti-VEGF therapy either as monotherapy or in combination with PDT is effective for CNV secondary to CSCR including in eyes previously treated with PDT for CSCR.
Commercial Relationships: Danilo Iannetta; **Savitha Madhusudhan**, None; **Nicholas V. Beare**, None; **Ian A. Pearce**, None

Program Number: 2316 **Poster Board Number:** B0270
Presentation Time: 3:45 PM–5:30 PM
Establishment of a novel tissue engineered product consisting of RPE derived from human embryonic stem cells cultured on human amniotic membrane for clinical applications
Walter Habeler[1, 2], Karim Ben M'Barek[1], Alexandra Plancheron[1, 2], Mohamed Jarraya[3], Marc Peschanski[1, 2], Christelle Monville[1]. [1]I-Stem, INSERM UEVE UMR861, AFM, Corbeil-Essonnes, France; [2]CECS/I-Stem, AFM, Corbeil-Essonnes, France; [3]Banque de tissus humaines, Hôpital Saint Louis, Paris, France.
Purpose: Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are the main causes of blindness in the developed world. Stem cell-based therapy represents an alternative approach in the treatment of retinal diseases. The Retinal Pigment Epithelium (RPE) is a continuous monolayer of cuboidal epithelial cells, localized between the photoreceptors and fenestrated choroid capillaries. The RPE interacts with the photoreceptors for the maintenance of visual function. RP and AMD could be caused by degeneration or malfunction of the RPE cell layer. Two important features of human embryonic stem cells (hESC), self-renew and the ability to generate an unlimited number of RPE cells, make them very attractive tools in the cell therapy approach for retinal diseases. Maintaining the epithelial morphology of RPE cells is a crucial parameter to consider in order restoring some visual function by cell therapy.
Methods: To achieve this goal, we have developed a tissue engineered product (TEP) that consists of RPE derived from hESC cultured on denuded human amniotic membrane (hAM). In our study, we have optimized a differentiation protocol allowing us to generate RPE from hESC in a large amount using xeno-free culture media.
Results: Our results show that more than 98% of differentiated cells expressed RPE typical markers. In parallel, we have established high quality standard to definite their characteristics and impurities. This cells could be banked and maintain their phenotype when cultured on hAM. In fact, when cultured for 1 month on denuded hAM, the hESC-derived RPE cells formed a typical hexagonal pigmented epithelium. RPE cells secrete VEGF in polarized manner (basal >apical).
Conclusions: Finally, our TEP will be used for phase I/II clinical trials for the treatment of RP caused by a RPE defect.
Commercial Relationships: Walter Habeler, None; **Karim Ben M'Barek**, None; **Alexandra Plancheron**, None; **Mohamed Jarraya**, None; **Marc Peschanski**, None; **Christelle Monville**, None

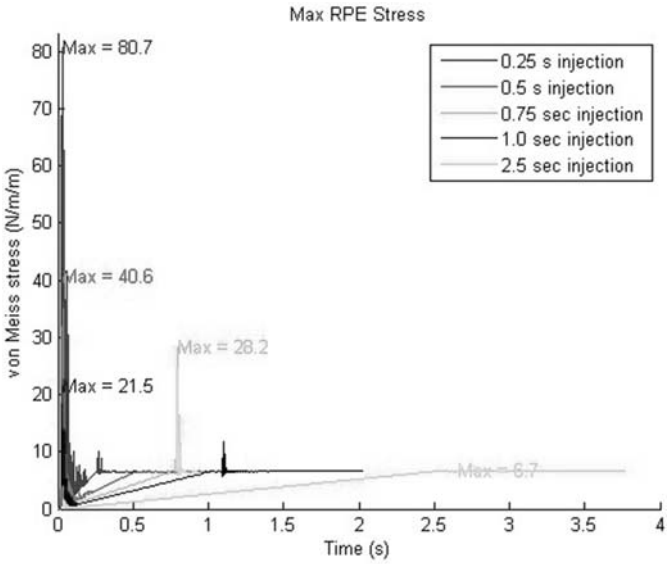
Program Number: 2317 **Poster Board Number:** B0271
Presentation Time: 3:45 PM–5:30 PM
In vitro testing of fibrin as a temporary support for RPE transplantation
Jarel Gandhi, Zahid Manzar, Hannah Schmidt, Benjamin Gilles, Travis Knudsen, Matthew Hill, Lori Bachman, Raymond Iezzi, Jose Pulido, Alan D. Marmorstein. Ophthalmology, Mayo Clinic, Rochester, MN.
Purpose: Retinal pigment epithelium (RPE) transplantation holds promise as a therapy for macular degeneration. While RPE transplanted without support appear safe, they form clumps in vivo. Using synthetic polymers to preserve the RPE monolayer phenotype necessitates an extended half-life for the support, which in turn can cause inflammation and fibrosis in vivo. Our goal in this study is to produce a natural support that is retained during RPE culture while being rapidly (hours to days) degraded following transplantation.
Methods: Hydrogels of various thickness and fibrinogen concentration were produced and mechanical rigidity tested qualitatively. The degradation kinetics of the hydrogel were studied

by varying concentrations of fibrinogen, plasminogen and tissue plasminogen activator (tPA). The degraded fibrin was quantified by spectrophotometry over time, and a first order reaction was assumed to find the rate constant.
Fibrin was polymerized and seeded with RPE to form a monolayer. RPE viability was studied before and after fibrin degradation using a live/dead assay. Immunofluorescence was used to confirm monolayer phenotype by staining for RPE markers.
Results: Hydrogels, thinner than 500μm, required a high fibrinogen concentration to support their own weight. Qualitatively, these gels rebounded back to their original, flat shape after manipulation and bending. Implantation of the fibrin sheets into the subretinal space of an ex vivo eyecup was successful.
Degradation of the hydrogel was only observed after the addition of plasminogen and tPA, with all tested conditions degrading within 48 hours. The degradation rate constant was independent of fibrinogen concentration ($p=0.35$) but dependent on both plasminogen ($p=0.005$) and tPA concentrations ($p<0.001$).
On the hydrogel, the RPE reformed as a flat monolayer, with confirmation of ZO-1, a cell-cell junction marker, throughout the monolayer. RPE viability remained the same before and after complete degradation of the hydrogel ($p=0.88$). With the degradation of fibrin, the RPE monolayer folded onto itself. ZO-1 was similarly present after fibrin degradation.
Conclusions: Fibrin hydrogels were formed to mechanically support a flat RPE monolayer and were rapidly degraded, without harm to the RPE. Fibrin provides a great alternative for RPE transplantation, as this strategy maintains a flat, viable RPE monolayer without a persisting material between the choroid and RPE.
Commercial Relationships: Jarel Gandhi, None; **Zahid Manzar**, None; **Hannah Schmidt**, None; **Benjamin Gilles**, None; **Travis Knudsen**, None; **Matthew Hill**, None; **Lori Bachman**, None; **Raymond Iezzi**, Alcon Inc (C); **Jose Pulido**, None; **Alan D. Marmorstein**, LAgen Laboratories LLC (I)
Support: Gordon and Llura Gund Fund for Career Development in Retinal Degenerative Disease Research, VitreoRetinal Surgery Foundation Research Fellowship

Program Number: 2318 **Poster Board Number:** B0272
Presentation Time: 3:45 PM–5:30 PM
Simulated Stress on a Pigment Epithelial Detachment During an Intravitreal Injection
Steven N. Luminais[1], Shlomit Schaal[3], Amir Hadayer[4], William J. Foster[2, 5]. [1]Lewis Katz School of Medicine at Temple University, Philadelphia, PA; [2]Department of Ophthalmology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA; [3]Department of Ophthalmology & Visual Sciences, University of Massachusetts Medical School, Worcester, MA; [4]Department of Ophthalmology & Visual Sciences, University of Louisville, Louisville, KY; [5]Department of Bioengineering, College of Engineering, Temple University, Philadelphia, PA.
Purpose: Retinal pigment epithelial (RPE) tears are known clinically to be associated with intravitreal injections in eyes with pigment epithelial detachments (PEDs). We hypothesize that the expansion of the eye at the time of the injection may be the reason for this serious condition. To date, the origin and magnitude of the stress imposed on the retinal tissue by the injection itself has not been well described. This study aims to characterize the stresses the injection causes in the RPE and retina and to assess what impact, if any, changing the duration of the injection has on these forces. Further understanding of these stresses may allow the development of techniques and/or devices to reduce the risk of retinal tears during treatment of PEDs.
Methods: A serous PED between Bruch's membrane and the retina was modeled using COMSOL Multiphysics v5.2a finite element analysis system (Fig 1). Intravitreal injections were modeled by moving the sclera radially outward at a uniform velocity to match the volume increase in a 0.05mL injection. The PED fluid was modeled as water while the vitreous was modeled as a visco-elastic material, as described in the literature.
Results: There are transient periods of oscillating stresses on the retina at the start and end of the simulated injection with a linear increase in stress between the oscillations. The maximum stress was found at the junction between the PED and the normal retina tissue for all injection durations. Maximal retina stress was found at the start of the injection for injection durations 0.25, 0.5 and 1.0 s (80.7, 40.6 and 21.5 N/m^2 respectively) while the post injection oscillation produced the largest stress for injection durations 0.75 and 2.5 s (28.2 and 6.7 N/m^2 respectively). Injection durations longer than 2.5 seconds did not differ significantly from the results at 2.5s.
Conclusions: Modeling of intravitreal injections demonstrates that the retina is subject to transient stresses at the beginning and end of the injection, with the junction between the PED and the normal retinal tissue being subjected to the largest stress. Different injection lengths modulated the timing of and the maximum stress on the PED.



The axisymmetric PED model showing layers for the retina, Bruch's membrane and the sclera.



Maximal von Meiss stress on the retina during a simulated 0.05mL intravitreal injection for different injection durations.

Commercial Relationships: Steven N. Luminais, None; **Shlomit Schaal**, None; **Amir Hadayer**, None; **William J. Foster**, None

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Program Number: 2319 **Poster Board Number:** B0273
Presentation Time: 3:45 PM–5:30 PM

IND-enabling In vitro and In vivo Functional Authentication of AMD-patient Derived Clinical-Grade iPSC-RPE Tissue

Kapil Bharti, Vladimir Khristov, Ruchi Sharma, Balendu Jha, Aaron Rising, Yichao Li, Congxiao Zhang, Nathan Hotaling, Arvydas Maminishkis, Juan Amaral, Sheldon S. Miller. National Eye Institute, Bethesda, MD.

Purpose: Age-related macular degeneration (AMD) affects more than 30 million individuals worldwide. Literature evidence suggests that in the advanced AMD stage called Geographic Atrophy (GA) RPE cell death precedes photoreceptor death that leads to vision loss. Therefore, RPE replacement has been suggested as an option to treat GA. Here we develop an autologous replacement therapy for AMD using an RPE-tissue developed from patient-specific induced pluripotent stem cells (iPSCs).

Methods: Clinical-grade iPSC-RPE tissues were manufactured from eight independent iPSC clones derived from three AMD patients. In vitro purity, epithelial character, and functionality of all eight iPSC-RPE tissues was authenticated using flow cytometry, tissue transepithelial resistance (TER), ability to phagocytose photoreceptor outer segments, polarized secretion of VEGF cytokine, ultrastructural and gene expression analysis. In vivo functionality of iPSC-RPE from all three AMD patients was confirmed using a swine model of RPE injury that mimics RPE loss seen in GA patients.

Results: Clinicial-grade iPSC-RPE tissue from all three AMD patients have pure RPE cells (without any iPSC contamination) and have ultra-structural features of epithelial cells. RPE tissues derived from all eight iPSC clones show comparable TER, levels of VEGF on apical/basal sides, ability of phagocytose photoreceptor outer segments, and RPE-specific gene expression that suggests adult-RPE properties. All functional features of clinical-grade iPSC-RPE are also comparable to native mammalian RPE. Clinical-grade RPE tissue is able to survive and integrate in the swine model of RPE injury and able to protect overlying photoreceptors from dying as confirmed by imaging and electrophysiological analysis of the retina.

Conclusions: AMD patient's iPSCs produce functionally authentic and mature RPE tissue in clinically compatible conditions. AMD patient derived clinicial-grade RPE tissue is efficacious in a swine model of RPE injury. This work supports a phase I IND application to test this potential autologous cell therapy in AMD patients.

Commercial Relationships: Kapil Bharti, None; **Vladimir Khristov**, None; **Ruchi Sharma**, None; **Balendu Jha**, None; **Aaron Rising**, None; **Yichao Li**, None; **Congxiao Zhang**, None; **Nathan Hotaling**, None; **Arvydas Maminishkis**, None; **Juan Amaral**, None; **Sheldon S. Miller**, None

Support: NEI Intramural grant and NIH Common Fund grant

Program Number: 2320 **Poster Board Number:** B0274
Presentation Time: 3:45 PM–5:30 PM

Phase I/IIa clinical trial of human embryonic stem cell (hESC)-derived retinal pigmented epithelium (RPE, OpRegen®) transplantation in advanced dry form age-related macular degeneration (AMD): interim results

Eyal Banin[1], Yitzchak Hemo[1], Tareq Jaouni[1], Devora Marks-Ohana[1], Shelly Stika[2], Svetlana Zheleznykov[1], Alexey Obolensky[1], Maria Gurevich[2], Charles S. Irving[2], Benjamin Reubinoff[3]. [1]Center for Retinal and Macular Degenerations, Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [2]Cell Cure Neurosciences Ltd, Jerusalem, Israel; [3]Center for Embryonic Stem Cells and the Department of Gynecology and Obstetrics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Purpose: Transplantation studies using autologous RPE cells in AMD patients suggest that introducing healthy RPE cells may be of benefit. Over the last decade we developed the technology to derive RPE cells in-vitro from hESCs using a directed differentiation, xeno-free protocol. Safety and tolerability of this cell product, OpRegen, is now being evaluated in a dose-escalating Phase I/IIa clinical study in patients with advanced dry AMD accompanied by geographic atrophy (NCT02286089). Here we report accumulated safety and imaging data from the 1st and 2nd cohorts of patients, who received a subretinal transplant of 50k or 200k OpRegen cells in suspension, with up to 1 year follow up.

Methods: Transplantation was performed by subretinal injection following conventional 23G vitrectomy under local anesthesia. Systemic immunosuppression is administered from 1 week prior to transplantation until 1 year after. Systemic and ocular safety is closely monitored. Retinal function and structure are assessed using various techniques including BCVA, and color, OCT and fundus autofluorescence (FAF) imaging.

Results: At date of writing, dosing of cohort 1 of 3 patients (ages 74-80 years) who received 50k cells has been completed with a follow-up of 1 year, 9 and 6 months, and 2 out of 3 patients from cohort 2 (ages 65 and 82) were dosed with 200k cells. Surgery was uneventful, with subretinal fluid absorbing within <48 hours. OCT imaging showed healing of the retinal penetration site by 2 weeks post-op. Treatment has been well tolerated systemically and with regard to ocular findings. Imaging changes associated with OpRegen include subretinal pigmentation in area of transplant in 4 out of 5 patients, often accompanied by hypo- and hyper-fluorescent spots on FAF imaging and irregular reflectance above areas of atrophy and host RPE on OCT scans. These changes develop over the first 2-3 months and persist through the latest time point examined. Of note, epiretinal membranes that do not require surgical intervention were observed.

Conclusions: Subretinal transplantation of OpRegen in patients with advanced dry AMD appears well tolerated to date. Findings on imaging suggest presence of cells in the subretinal space. These results provide a framework for functional assessments in cohorts at higher doses.
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3-Year Interim Safety Profile of Adeno-Associated Virus Serotype 2-soluble Variant of the Vascular Endothelial Growth Factor Receptor Type 1 (AAV2-sFLT01) Administered by Intravitreal Injection in Patients with Neovascular Age-Related Macular Degeneration
Rafael Varona[3], Annaig Le-Halpere[3], Peter A. Campochiaro[2], Jeffrey Heier[4], Pravin U. Dugel[6], Mark Barsamian[1], Annie Purvis[5], Mario Cabrera-Salazar[5], Cheryl Delacono[5], Najwa Mostafa[5], Abraham Scaria[5], Ronald BUGGAGE[3]. [1]UMass Memorial Medical Center, Hyannis, MA; [2]Johns Hopkins Wilmer Eye Institute, Baltimore, MD; [3]Sanofi R&D, Chilly Mazarin, France; [4]Ophthalmic Consultants of Boston, Boston, MA; [5]Sanofi R&D (US), Cambridge, MA; [6]Retinal Consultants of Arizona, Phoenix, AZ.
Purpose: To characterize the 3-year preliminary safety profile of AAV2-sFLT01 administered by intravitreal injection (IVT) in patients with neovascular age-related macular degeneration participating in a Phase I trial
Methods: All reported treatment emergent adverse events (TEAEs) including descriptions, severity, relatedness to procedure or the investigational medicinal product (IMP) and event coding using MedDRA dictionary were collected and analyzed from an 52-week trial and through 2 years of a 4-year extended follow-up (EFU) program
Results: All patients treated with a single uniocular IVT completed the 52 weeks of the core trial. 17 of them elected to the EFU program and 12 completed the first 2 years of the extension. After 3 years, 18 (95%) patients experienced 136 TEAE, the most common being conjunctival hemorrhage, retinal hemorrhage, vitreous floaters and eye pain. The majority of TEAE were mild 85 (63%), occurred during the first year of FU and were related neither to the IVT procedure or IMP. 2 patients treated at highest dose experienced 6 TEAE related to IMP (vitritis, vitreous floaters, iritis and corneal deposits in one patient and pyrexia in the other). 9 patients experienced 13 TEAE related to the study procedure (conjunctival hemorrhage, edema, hyperemia, eyelid pruritus and eye pain). 7 patients reported 14 SAE, all non-ocular except for 3 retinal tears in the same patient. A death in a 91 year old patient could not be assessed by the investigator and thus was reported as a SAE attributed to the IMP and study procedure by the Sponsor. No AE that could be related to systemic exposure of the IMP or its transgene (sFLT01) were observed. A sustained reduction in retinal fluid was observed in 4 patients
Conclusions: A IVT administered gene providing sustained anti-VEGF expression may offer a convenient alternative to anti-VEGF treatments for wet AMD requiring repetitive injections and other gene therapies in development requiring delivery by subretinal injection following vitrectomy. After three years of follow up, AAV2-sFLT01 appears to be generally safe, well tolerated and does not appear to raise any new safety concerns relative to the established long-term safety profile of other intravitreally administered anti-VEGF therapies
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