ARVO 2016 Annual Meeting Abstracts

204 Diabetic Retinopathy
Monday, May 02, 2016 8:30 AM–10:15 AM
6C Paper Session
Program #/Board # Range: 1350–1356
Organizing Section: Retina

Program Number: 1350
Presentation Time: 8:30 AM–8:45 AM

Ranibizumab induces regression of diabetic retinopathy (DR) in more than three-quarters of patients with high-risk nonproliferative diabetic retinopathy (NPDR) independent of retinal nonperfusion

Charles C. Wykoff1, 2, Shamika Gune3, Lauren Hill3, Miranda Hemphill3, Zdenka Haskova3. 1Vision Care, Retina Consultants of Houston, Houston, TX; 2Blanton Eye Institute and Houston Methodist Hospital, Houston, TX; 3Genentech, Inc., South San Francisco, CA.

Purpose: DR is a progressive disease, and patients with moderately severe or severe NPDR (levels 47 and 53, respectively, on the Early Treatment Diabetic Retinopathy Study [ETDRS] DR Severity Scale [DRSS]) are at high risk of worsening to proliferative DR (PDR) (ETDRS Report 12, Ophthalmology, 1991). The effect of ranibizumab (RBZ) therapy on DR in patients with diabetic macular edema (DME) at high risk of worsening to PDR was evaluated in this post hoc analysis of RIDE/RISE.

Methods: In the randomized phase 3 RIDE/RISE studies, patients with DME (N=759) received monthly RBZ (0.3 mg or 0.5 mg) or sham injections for 24 months. DR severity was graded on the ETDRS-DRSS by masked evaluators using 7-field fundus photographs. Retinal nonperfusion was evaluated by fluorescein angiography. Outcomes by baseline DR severity were retrospectively analyzed.

Results: At baseline, 33% of patients in RIDE/RISE had moderately severe or severe NPDR (ETDRS-DRSS level 47/53) and these patients were evenly distributed among the treatment groups (88, 74, and 86 patients for 0.3 mg RBZ, 0.5 mg RBZ, and sham, respectively). Among these patients, rates of ≥2-step DR improvement were significantly greater for RBZ-treated patients vs sham at months 12 (76.1%, 75.7%, and 2.3% for 0.3 mg RBZ, 0.5 mg RBZ, and sham, respectively) and 24 (78.4%, 81.1%, and 11.6%, respectively) (all RBZ vs sham comparisons, P<0.0001); these improvements were independent of the presence or absence of retinal nonperfusion at baseline. Rates of ≥3-step DR improvement at month 24 were 22.7%, 28.4%, and 1.2% (each RBZ arm vs sham, P<0.0001). In addition, fewer RBZ-treated patients progressed to PDR (ETDRS-DRSS ≥60) compared with sham-treated patients at months 12 (0%, 0%, and 10.5% for 0.3 mg RBZ, 0.5 mg RBZ, and sham, respectively) and 24 (0%, 2.7%, and 18.6%, respectively).

Conclusions: Ranibizumab treatment resulted in statistically significant and clinically meaningful regression of DR to milder severity in the large majority of patients at high risk of progression to PDR, independent of retinal nonperfusion. More than 75% of ranibizumab-treated patients with moderately severe or severe NPDR (ETDRS-DRSS 47/53) experienced ≥2-step DR improvements at 12 and 24 months, and almost none worsened to PDR.

Commercial Relationships: Charles C. Wykoff, Iconic Therapeutics (F), Apellis Pharmaceuticals (F), Alcon/Novartis (F), Alimera Sciences (C), Allergan (F), Alcon (C), Allergan (R), Regeneron (C), DRCR.net (F), Regeneron (R), Roche (C), Regeneron/Bayer (F), Bayer (C), Genentech, Inc. (C), Allegro Ophthalmic (F), Thrombogenics (C), Genentech/Roche (F), Allergan (C), Valeant (C); Shamika Gune, Genentech, Inc.; Lauren Hill, Genentech, Inc.; Miranda Hemphill, Genentech, Inc.; Zdenka Haskova, Genentech, Inc.

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Clinical Trial: NCT00473382, NCT00473330

Program Number: 1351
Presentation Time: 8:45 AM–9:00 AM

Intravitreal Bevacizumab for Proliferative Diabetic Retinopathy: Results from the Pan-American Collaborative Retina Study Group (PACORES) at 24 Months of Follow-up

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Purpose: To evaluate the effects of intravitreal bevacizumab (IVB) on retinal neovascularization (RN) in patients with proliferative diabetic retinopathy (PDR).

Methods: Retrospective multicenter interventional case series. Clinical records of 81 consecutive patients (97 eyes) with RN due to PDR who received at least one intravitreal injection of bevacizumab (IVB) were included. Patients examination included measurement of best-corrected Snellen visual acuity (BCVA), ophthalmoscopy, fluorescein angiography (FA), and optical coherence tomography (OCT) at baseline and follow-up visits.

Results: The mean age of patients was 55.6±11.6 years. The mean number of IVB injections per eye was 4.2±2.5 (range, 1 to 7 injections). The mean interval between IVB applications was 3.5±3.3 months. Sixty two (63.9%) eyes showed total regression of RN on fundus examination with absence of fluorescein leakage, 26 (26.8%) eyes demonstrated partial regression of RN and 9 (9.3%) eyes had no regression. The mean duration of follow-up was 29.6±2 months (range, 24 to 30 months). BCVA and OCT improved statistically significantly (P<0.0001, both comparisons). Three eyes without previous PRP (‘naive’ eyes) and with vitreous hemorrhage did not require vitrectomy. Five (5.2%) eyes with PDR progressed to tractional retinal detachment requiring vitrectomy, and 1 (1%) eye had vitreous hemorrhage with increased intraocular pressure (ghost cell glaucoma). No systemic adverse events were noted.

Conclusions: IVB resulted in marked regression of RN in patients with PDR and previous PRP. In a subgroup of eyes with no previous laser, only 42.1% achieved control or regression of PDR with IVB injections during 24 months of follow up without necessity of adjunctive laser or vitrectomy. There were no safety concerns over 2 years follow up of IVB for PDR.

Commercial Relationships: J Fernando Arevalo, None; Andres Lasave, None; Lihteh Wu, None; Mauricio Maia, None; Manuel Diaz-Llopis, None; Arturo Alezzandrini, None; Miguel Brito, None

Program Number: 1352
Presentation Time: 9:00 AM–9:15 AM

Galectin-1 is an Angiogenic Factor Associated with Proliferative Diabetic Retinopathy: Novel Target for Afibeccept

Presentation Time: 9:00 AM–9:15 AM

Galectin-1 is an Angiogenic Factor Associated with Proliferative Diabetic Retinopathy: Novel Target for Afibeccept
**Purpose:** Aflibercept is an anti-vascular endothelial growth factor (VEGF) agent pharmacologically engineered as a chimeric receptor-based decoy protein fused to the immunoglobulin Fc fragment (i.e., VEGFR1/VEGFR2-Fc). We revealed a novel anti-angiogenic function for aflibercept beyond its antagonism against VEGF family members, and characterized the newly identified aflibercept-binding protein in vitro and in vivo using patient samples as well.

**Methods:** Immunoprecipitation and mass spectrometry analyses were carried out to identify aflibercept-interacting proteins. Protein binding affinities were quantified by biolayer interferometry. Peptide-N-glycosidase F was used to cleave N-linked glycans of aflibercept. Real-time polymerase chain reaction was performed to measure mRNA expression levels in hypoxic cell culture and disease mouse models including streptozotocin-induced diabetes and laser-induced choroidal neovascularization. Human surgical samples were examined by immunofluorescence and enzyme-linked immunosorbent assay.

**Results:** Aflibercept exhibited firm binding to galectin-1 with higher affinity than VEGFR1-Fc and VEGFR2-Fc, which was abolished by deglycosylation of aflibercept. Retinal LGALS1/Galectin-1 mRNA expression was enhanced in vitro by hypoxic stimulation and in vivo by induction of various retinal diseases. Galectin-1 immunoreactivity co-localized with VEGFR2 in neovascular tissues surgically excised from human eyes with PDR (proliferative diabetic retinopathy). Compared with non-diabetic controls, intravitreal galectin-1 protein levels were elevated in PDR eyes, showing no correlation with increased VEGF-A levels. Preoperative injection of bevacizumab, a monoclonal antibody to VEGF-A, reduced the VEGF-A, but not galectin-1, levels. Galectin-1 as well as VEGF-A application to human retinal microvascular endothelial cells up-regulated VEGFR2 phosphorylation, both of which were eliminated by aflibercept.

**Conclusions:** Our present findings demonstrated the neutralizing efficacy of aflibercept against galectin-1, an angiogenic factor associated with PDR independently of VEGF-A.

**Commercial Relationships:** Atsuhiro Kanda, None; Kousuke Noda, None; Wataru Saito, None; Susumu Ishida, None

**Program Number:** 1353

**Presentation Time:** 9:15 AM–9:30 AM

**Changes in Disorganization of Retinal Inner Layers (DRIL) Predict Retinal Sensitivity in Eyes with Diabetic Macular Edema**

**Timothy Peiris**, 1 Oliver Comyn, 2 Luke Nicholson, 2 Amanda Delacruz, 3 Migil Ledesma, 4 Phillip Huykin, 5 Novartis (C), Bayer (C), Bayer (F), Novartis (F), Allergan (F), Sobha Sivaprasad, 5 Novartis (C), Bayer (C), Bayer (F), Allergan (C), Allergan (F), Novartis (F), Paolo S. Silva, 5 None; 6 Lloyd Paul Aiello, 5 None; Jennifer K. Sun, 5 None, 7 Boston Micromachine (F), Optovue (F), Genentech (F)  

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**Program Number:** 1354

**Presentation Time:** 9:30 AM–9:45 AM

**Title:** Complement component 3 (C3) is increased in vitreous in diabetic macular edema and is required for bradykinin (BK)-induced retinal edema

**Nivetha Murugesan**, 1 Allen C. Clermont, 2 Takeshi Kita, 3 Edward P. Feener, 1 Vascular Cell Biology, Joslin Diabetes Center, Harvard Medical School, Boston, MA; 4Kyushu University, Fukuoka, Japan.

**Purpose:** The plasma kallikrein kinin system has been implicated in diabetic macular edema (DME), however its downstream mediators are not fully understood. To identify factors that may contribute to BK’s effects in DME, we compared the proteome from rat retina with BK-induced edema with the proteome from DME patients. This analysis revealed an association of increased C3 with retinal thickness, which was investigated in C3 deficient (C3−/−) mice subjected to BK-induced retinal edema.

**Methods:** LC-MS/MS-based proteomics was performed on retina from Sprague Dawley rats with BK (2μM)-induced retinal edema compared to PBS-injected controls. Protein changes were correlated with retinal thickening measured by optical coherence tomography (OCT) and compared with human vitreous (DME, n=10 and macular hole (MH), n=5) proteomes. Western blotting of C3 in vitreous was performed. C3−/− and wildtype (WT) mice received intravitreal (IVT) injections with BK, C3, or PBS. Retinal thickness was quantified by OCT at baseline and 24 hrs.

**Results:** LC-MS/MS-based proteomics revealed that C3 was increased 4.7 fold in DME compared with MH vitreous (441 vs 94 spectral/peptide counts, p<0.001) and this increase was

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confirmed by western blotting (8.2 fold). C3 levels were elevated 7.8 fold (23.8 ± 7.2 vs 3.3 ± 2.8 spectral/peptide counts, p=0.027) in BK-stimulated rat retina 24hrs post IVT injection compared to control retina and C3 peptide counts correlated (R²= 0.90) with retinal thickening measured by OCT. BK-induced retinal edema measured 24hrs post IVT was reduced in C3⁻/⁻ mice by 67.3% in males (thickness change:23.5±2.7μm in WT vs 7.7±3.6μm in C3⁻/⁻, p<0.01) and 78.1% in females (21.5±2.5μm WT vs 4.7±1.6μm C3⁻/⁻, p<0.0015). VEGF (10ng/eye) induced retinal thickening 24hrs post IVT was reduced in C3⁻/⁻ mice (22.5±7.4μm in WT vs 9.0 ±3.8μm in C3⁻/⁻). IVT injection of C3 (1μg/eye) increased retinal thickness by 10.6±3.0μm compared to 6.2 ±1.1μm with vehicle alone (p=0.132).

**Conclusions:** C3 levels are elevated in DME patients vitreous and increased C3 levels correlated with retinal thickness in rat retina with BK-induced edema. Mice with C3 deficiency are protected against both BK- and VEGF-induced retinal edema, however intravitreal injection of C3 alone did not significantly affect retinal thickness. These results identify C3 as a potential therapeutic target for DME.

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**Program Number:** 1355  
**Presentation Time:** 9:45 AM–10:00 AM

**Non-targeted plasma metabolomics of diabetic retinopathy demonstrates increased arginine and related metabolites**  
Milam A. Brantley¹, David J. Herren¹, Karan Uppal², L. G. Burgess³, Isaac Chocron³, M. W. Calcutt¹, David C. Samuels¹, Dean P. Jones³. ¹Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN; ²Department of Medicine, Emory University, Atlanta, GA; ³Department of Biochemistry and Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN; ⁴Vanderbilt Genetics Institute, Vanderbilt University, Nashville, TN.

**Purpose:** To determine plasma metabolite differences between Type 2 diabetics with retinopathy (T2DR) and Type 2 diabetics without retinopathy (T2DM).

**Methods:** We performed untargeted metabolomic analysis using ultra-high resolution mass spectrometry with C18 liquid chromatography on frozen plasma samples from 83 T2DR patients and 90 T2DM controls. Metabolic features were extracted using aplCMS with xMSanalyzer. LIMMA was used to isolate differentially expressed metabolites (DEM) after correcting for multiple testing. DEM were further analyzed using MetaboAnalyst to identify metabolic pathways altered in diabetic retinopathy (DR). Four specific metabolites were measured individually in targeted fashion using liquid chromatography-mass spectrometry (LC-MS). For these targeted measurements, t-tests and multivariate linear regressions were used to analyze differences between the groups.

**Results:** Of 11,763 mass/charge (m/z) features recovered, 78 differed significantly between T2DR cases and T2DM controls. Twenty-eight features possessed putative matches to the Metlin metabolomics database. Three of these matched to arginine-related metabolites, including arginine, asymmetric/symmetric dimethyl arginine (ADMA/SDMA), and citrulline. Pathway analysis also indicated arginine metabolism was altered in DR. Targeted LC-MS measurements of each arginine metabolite confirmed a significant difference between T2DM and T2DR patients (arginine, p=1.3x10⁻¹⁰; ADMA, p=1.8x10⁻⁹; SDMA, p=0.0008; citrulline, p=0.0084). After adjusting for age, sex, race, duration of diabetes, and hemoglobin A1c, each of the four metabolites remained significantly different between T2DM and T2DR (arginine, p=1.3x10⁻⁸; ADMA, p=7.0x10⁻⁶; SDMA, p=0.0042; citrulline, p=0.0040). None of the four metabolites differed between nonproliferative DR (n=49) and proliferative DR (n=24) patients (arginine, p=0.96; ADMA, p=0.89; SDMA, p=0.43; citrulline, p=0.25).

**Conclusions:** Using non-targeted high-resolution metabolomics, we determined that plasma arginine metabolites were altered in our cohort of Type 2 diabetics with retinopathy. Targeted LC-MS measurements confirmed that four specific arginine-related metabolites were elevated in plasma in T2DR patients compared to T2DM controls. This suggests that arginine metabolites might be effective biomarkers for DR and that the arginine pathway might be a target for future DR therapy.

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**Program Number:** 1356  
**Presentation Time:** 10:00 AM–10:15 AM

**Myeloid-derived VEGF and HIF are dispensable for ocular neovascularisation**  
Sidath Liyanage¹, Alessandro Fantin¹, Pilar Villacampa¹, Clemens Lange², ³, Laura Dentii, Enrico Cristante, ³, Alexander J. Smith¹, Robin R. Ali¹, Ulrich F. Luhmann¹,¹, James W. Bainbridge¹, Christiana Ruhrberg¹, ¹Division of Genetics, UCL Institute of Ophthalmology, London, United Kingdom; ²Division of Cell Biology, UCL Institute of Ophthalmology, London, United Kingdom; ³University Eye Hospital Freiburg, Freiburg, Germany; ⁴F. Hoffmann-La Roche Ltd, Basel, Switzerland.

**Purpose:** Ocular neovascularisation (ONV) is a pathological feature of diseases such as diabetic retinopathy and age-related macular degeneration. Myeloid cells are considered an important source of the vascular endothelial growth factor A (VEGF) in mouse models of ONV. By inducing neovascularisation in transgenic mouse models using oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV), we investigated the hypothesis that myeloid-derived VEGF and its upstream regulators hypoxia-inducible factor (HIF)-1α and HIF2α are key drivers of ONV.

**Methods:** The Vegfa−/− descendant reporter and in situ hybridisation (ISH) were used to analyse Vegfa expression. Cre-lox systems used Lysm−/− or Tie2-Cre to target myeloid cells for reporter expression with Rosa26Rº and Rosa26RºCre or deletion of Hif1aºº, Hif2aºº or Vegfaºº. Established OIR and CNV protocols were performed with littermate controls. Anti-isoelectin B4 staining on P17 retinal flatmounts was used to identify avascular (AV) areas and retinal neovascularisation (RNV) in OIR. The area of resultant CNV post-laser rupture of Bruch’s membrane was analysed using fluorescence angiography at day D7 and D14. Vegfa gene locus recombination, VEGF protein levels and mRNA levels of Vegfa, Hif1a and Hif2a were analysed using genomic PCR, ELISA and quantitative RT-PCR respectively. Flow cytometry was used to isolate and analyse myeloid subsets. The t test and ANOVA were used for statistical analysis.

**Results:** Immunofluorescence and flow cytometry on reporter lines confirmed that Cre-targeted myeloid cells accumulate at sites of RNV and CNV. The Vegfa−/− reporter and ISH showed that Vegfa is highly expressed by several cell types, but not by myeloid cells. The Lysm−/− allele did not affect the extent of AV, RNV or CNV. Conditional deletion of Vegfa, Hif1a and Hif2a was confirmed at genomic and transcript level (P=0.05). Myeloid-specific VEGF ablation did not reduce total ocular VEGF during CNV and RNV.

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In agreement, the deletion of Vegfa, Hif1a or Hif2a in recruited and resident myeloid cells that accumulated at sites of neovascularisation did not significantly reduce CNV or RNV (P>0.05).

**Conclusions:** These findings show that myeloid-derived HIF1α, HIF2α and VEGF are not significant in these mouse models of ONV, suggesting that myeloid function and VEGF production in neovascular eye disease differs from wound healing and other neovascular pathologies.

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