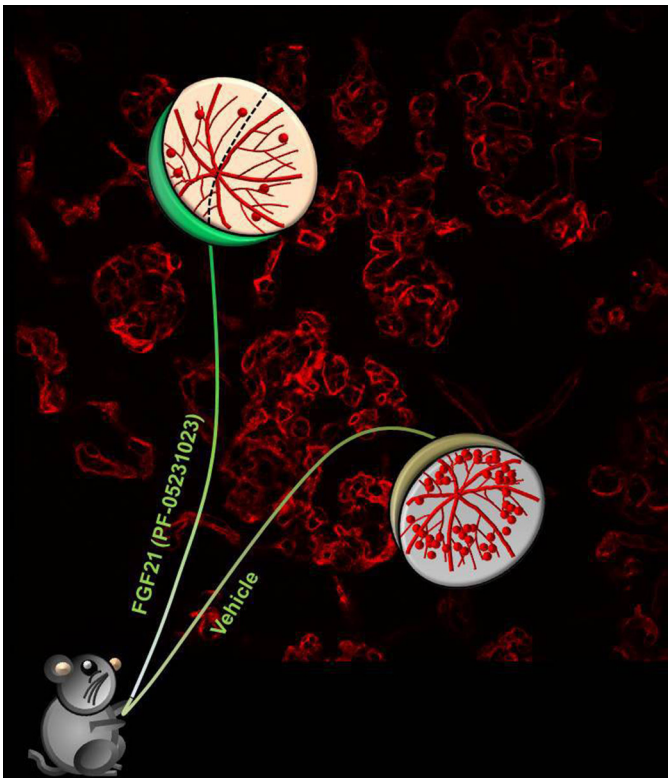
430 Diabetic Retinopathy
Wednesday, May 10, 2017 11:00 AM–12:45 PM
Ballroom 1 Paper Session
Program #/Board # Range: 4245–4251
Organizing Section: Retinal Cell Biology

Program Number: 4245
Presentation Time: 11:00 AM–11:15 AM
Fibroblast growth factor 21 administration suppresses pathologic retinal vessel growth in oxygen-induced retinopathy
Zhongjie Fu[1], Yan Gong[1], Raffael Liegl[1], Zhongxiao Wang[1], Chi-Hsiu Liu[1], Steven Meng[1], Samuel Burnim[1], Ann Hellstrom[3], Saswata Talukdar[2], Lois E. Smith[1]. [1]Ophthalmology, Boston Children's Hospital / Harvard Medical School, Boston, MA; [2]Merck Research Laboratories, Boston, MA; [3]Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden.
Purpose: Fibroblast growth factor 21 (FGF21), mediated by adiponectin (APN), improves the lipid profile in rodents, non-human primates and obese patients with diabetes. However, it is unknown if FGF21 inhibits complications of diabetes, specifically pathologic retinal neovascularization in diabetic retinopathy. We investigated if FGF21 suppressed retinal neovascularization in a mouse model of oxygen-induced retinopathy (OIR), modeling proliferative diabetic retinopathy.
Methods: To induce OIR, C57BL/6J mouse pups were exposed to 75% oxygen from postnatal day P7-12. Native FGF21 or long-acting FGF21, PF-05231023, or vehicle was injected intraperitoneally from P12-16 to littermates and neovascularization evaluated at P17 (n=14-19). Retinal neovascularization was also compared in OIR FGF21 wild-type (*Fgf21*$^{+/+}$) and knockout (*Fgf21*$^{-/-}$) retinas (n=12-13). To examine the direct retinal effect on C57BL/6J OIR mice, P17 neovascularization was compared after P12 intra-vitreal PF-05231023 treatment of one eye and with vehicle treatment of the contralateral eye (n=7). To determine if APN mediates the effects on neovascularization of FGF21 in OIR, PF-05231023 and vehicle was injected i.p. in *Apn*$^{-/-}$ mice (n=11-14). Retinal FGF21 receptors, *Apn* and tumor necrosis factor alpha (*Tnf*α) levels were examined by qPCR. Both male and female mice were used. All data except low quality images that were insufficient for analysis were used. The percentage of retinal neovascular area over the total retinal area was quantified at P17.
Results: FGF21 administration suppressed (vehicle: 10.8±0.6%; native FGF21: 8.8±0.4%; PF-05231023: 5.4±0.7%) and FGF21 deficiency (*Fgf21*$^{+/+}$: 6.5±0.3%; *Fgf21*$^{-/-}$:7.5±0.3%) worsened pathologic retinal neovascularization. Intravitreal delivery of PF-05231023 directly inhibited neovascularization (vehicle: 6.6±0.8%; PF-05231023: 4.5±0.8%). FGF21 receptors were expressed in mouse retinas and PF-05231023 increased retinal levels of *Apn* and decreased *Tnf*α. FGF21 protection against pathologic neovessel growth was completely abolished with APN deficiency (vehicle: 9.2±0.6%; PF-05231023: 8.2±0.6%) and the reduction in retinal *Tnf*α levels.
Conclusions: Our data suggest that FGF21 inhibits retinal neovascularization through APN pathway activation. FGF21 administration may prevent pathological retinal vascular proliferation in patients with neovascular retinopathy.



Commercial Relationships: Zhongjie Fu, None; **Yan Gong**, None; **Raffael Liegl**, None; **Zhongxiao Wang**, None; **Chi-Hsiu Liu**, None; **Steven Meng**, None; **Samuel Burnim**, None; **Ann Hellstrom**, None; **Saswata Talukdar**, Merck (E); **Lois E. Smith**, None
Support: Knights Templar Eye Foundation Competitive Renewal Award

Program Number: 4246
Presentation Time: 11:15 AM–11:30 AM
Long-term Intermittent Fasting (IF) Initiated at Night Prevents Development of Diabetic Retinopathy by Restoration of Bile Acid Metabolism in db/db Mice
Maria B. Grant[2, 1], Leni Moldovan[2, 1], Eleni Beli[2, 1], Yuanqing Yan[3], Ashay D. Bhatwadekar[2, 1], Ruli Gao[4], James Domingquez[2, 1], Todd Lydic[5], Xiaoxin Wang[6], Yuhuan Luo[6], Dong Wang[6], Moshe Levi[6], Julia V. Busik[5], Michael E. Boulton[2, 1]. [1]Eugene and Marilyn Glick Eye Institute/Indiana University, Indianapolis, IN; [2]Ophthalmology, Indiana University, Indianapolis, IN; [3]Phamacology, University of Florida, Gainesville, FL; [4]Computer and Information Sciences, University of Florida, Gainesville, FL; [5]Physiology, Michigan State University, East Lansing, MI; [6]Medicine, University of Colorado Denver, Denver, CO.
Purpose: Diabetes is associated with an altered composition of the bile acid pool and with gut dysbiosis. We investigated whether influencing the commensal microbes by use of intermittent fasting (IF) would alter key microbes that influence bile acid metabolism and impact development of diabetic retinopathy (DR).
Methods: db/db and db/m mice (8 weeks old) were maintained either on *ad libitum* (ad lib) or every other 24-h interval IF regimen (food removed at night time) for 7 months. Fecal samples were collected every 4 h for 48 h and genomic DNA sequencing was used to discover and distinguish various bacterial taxa. The presence/severity of DR was assessed by enumeration of acellular capillaries in the

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IF and ad lib cohorts and in mice treated with the dual FXR/TGR5 agonist INT-767 (30 mg/kg bw/day).
Results: IF showed a beneficial effect on survival, lipid metabolism, liver function and the microbiota of db/db mice. Fecal genomic DNA showed that IF corrected dysbiosis and restored toward nondiabetic levels key microbial species that are known to influence bile acid metabolism. Specifically, the family of Clostridiaceae are increased in db/db and restored to nondiabetic levels with IF. In addition, Bacillus firmus, Lactobacillus reuteri, Clostridium perfringens, Methanobrevibacter sp., Lactobacillaceae, Ruminonococcaceae and Bifidobacterium, were beneficially altered by IF in db/db mice. A long-term IF regimen resulted in reduced development of DR. db/db mice of eleven months of age showed the expected increased numbers of acellular capillaries ($20\pm4mm^2$ $p<0.05$) compared to age-matched control mice ($5\pm1mm^2$). db/db mice under the IF regimen did not show this increase in acellular capillaries ($9\pm 2/mm^2$). We next examined whether INT-767 would similarly prevent development of DR as did IF. Treatment of diabetic mice with INT-767, resulted in reduced numbers of acellular capillaries compared to vehicle treated mice ($p<0.05$).
Conclusions: The protective effects of IF on DR occur by restoring key species of the gut microbiota that influence bile acid metabolism and can be duplicated by use of a bile acid agonist.
Commercial Relationships: Maria B. Grant, None; **Leni Moldovan**; **Eleni Beli**, None; **Yuanqing Yan**, None; **Ashay D. Bhatwadekar**, None; **Ruli Gao**, None; **James Domingquez**, None; **Todd Lydic**, None; **Xiaoxin Wang**, None; **Yuhuan Luo**, None; **Dong Wang**, None; **Moshe Levi**, None; **Julia V. Busik**, None; **Michael E. Boulton**, None
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Program Number: 4247
Presentation Time: 11:30 AM–11:45 AM
Neutralization of placental growth factor as a novel treatment option in diabetic retinopathy
Tine Van Bergen[1], Tjing-Tjing Hu[1], Isabelle Etienne[1], Geert Reyns[1], Lieve K. Moons[2], Jean Feyen[1]. [1]ThromboGenics, Leuven, Belgium; [2]Biology, KU Leuven, Leuven, Belgium.
Purpose: Anti-vascular endothelial growth factor (VEGF) therapy has shown a significant improvement in visual acuity in patients with diabetic retinopathy (DR), however treatment response can be variable and might be associated with potential side effects. In this study, inhibition of placental growth factor (PlGF) was explored as a possible alternative therapy for DR by investigating its effect on different hallmarks of DR in various experimental murine models.
Methods: The *in vivo* efficacy of the anti-PlGF antibody (5D11D4; 0.77 – 5.4 μg/eye) was tested in diabetic streptozotocin (STZ; n=15-30/group) and Akimba mouse models (n=20-27/group) and in the laser-induced mouse model of choroidal neovascularization (CNV; n=10-20/group). Intravitreal (IVT) administration of the anti-PlGF antibody was compared to anti-VEGFR-2 antibody (DC101; 3.1 - 6.2μg/eye), VEGF-Trap (aflibercept; 2.4 -20 μg/eye), triamcinolone acetonide (TAAC; 40 μg/eye) and PBS as negative control. Vascular leakage was investigated by FITC-labelled bovine serum albumin perfusion or by fluorescein angiography. Immunohistological stainings were performed to check for neurodegeneration (Brn3a), inflammation (CD45, F4/80) and fibrosis (collagen type 1a and Sirius Red).
Results: In the diabetic STZ and Akimba models, repeated IVT administration of 5D11D4 reduced vascular leakage with 34±14% and 22±13% ($P<0.05$), respectively. This effect was equally efficacious as DC101 treatment in STZ mice ($P=0.43$). 5D11D4 treatment did not alter retinal ganglion cell (RGC) density, whereas DC101 significantly reduced RGC density with 20±6% ($P=0.04$). In the CNV model, 5D11D4 injection dose-dependently reduced inflammation and fibrosis, as compared to PBS treatment ($P<0.05$). Equimolar administration of 5D11D4, aflibercept and TAAC decreased leukocyte and macrophage infiltration with 45±5% ($P<0.001$), whereas DC101 had no effect on the inflammatory response ($P=0.96$). Administration of 5D11D4 and TAAC similarly reduced collagen I and total collagen deposition with 40±5% and 35±8% ($P<0.001$), while no effect was observed after equimolar DC101 ($P=0.82$) nor aflibercept administration ($P=0.66$).
Conclusions: The neutralization of PlGF showed equal efficacy compared to VEGF inhibition on the process of vascular leakage, but differentiates itself by also reducing inflammation and fibrosis, without triggering a neurodegenerative response.
Financial disclosure: ThromboGenics NV.
Commercial Relationships: Tine Van Bergen, ThromboGenics NV (E); **Tjing-Tjing Hu**, ThromboGenics NV (E); **Isabelle Etienne**, ThromboGenics NV (E); **Geert Reyns**, ThromboGenics NV (E); **Lieve K. Moons**, ThromboGenics NV (C); **Jean Feyen**, ThromboGenics NV (C)

Program Number: 4248
Presentation Time: 11:45 AM–12:00 PM
Downregulation of the SIRT1 and Liver X Receptor α/β signaling axis promotes diabetic retinopathy pathogenesis
Sandra Hammer[1], Todd Lydic[1], Qi Wang[1], Sugata Hazra[2], Goldis Malek[3], Michael E. Boulton[4], Maria B. Grant[4], Julia V. Busik[1]. [1]Physiology, Michigan State University, East Lansing, MI; [2]The University of Utah, Salt Lake City, UT; [3]Duke Univeristy, Durham, NC; [4]Indiana University, Indianapolis, IN.
Purpose: Clinical trials have demonstrated a strong association between dyslipidemia and the progression of diabetic retinopathy (DR). Activation of LXRα/β prevents diabetes-induced retinal vascular damage. Moreover, SIRT1-mediated LXR activation has been shown to be beneficial in non-retinal systems, but the involvement of this signaling axis in DR remains unknown. The goal of this project was to investigate the role that the SIRT1-LXR signaling cascade plays in the progression of DR.
Methods: Bovine retinal endothelial cells (BRECs) were isolated and validated according to a previously published protocol. Human retinal endothelial cells (HRECs) were isolated from control or diabetic patients. BRECs and HRECs were treated with TNFα (10ng) for 24hrs. DMHCA (1ug) was used as a LXR activator. LXRα/β, SIRT1, CYP46A1, -27A1, -11A1, ABCA1 and -G1 were analyzed by qRT-PCR. Oxysterols and cholesterol esters were measured by high resolution/accurate mass LC-MS and direct infusion MS, respectively, on an Orbitrap Velos mass spectrometer. db/db diabetic mice were used to model diabetic retinopathy *in vivo*.
Results: HRECs isolated from diabetic donors had significantly lower expression levels of LXRα/β and SIRT1 when compared to non-diabetic donors ($n=6$; $p<0.01$). Additionally, LXRα was significantly decreased in BRECs and control HRECs treated with TNFα (10ng/ml) ($n=9$, $n=3$; $p<0.01$, respectively). Cholesterol metabolizing enzymes, CYP46A1 and -11A1 were significantly increased after TNFα treatment ($n=9$; $p<0.05$) in BREC while CYP46A1, -27A1 and -11A1 were downregulated in control HRECs after TNFα treatment ($n=3$; $p<0.01$). LXR activation (DMHCA) prevented TNFα-induced ABCA1 and -G1 downregulation ($n=9$; $p<0.05$). Oxysterol levels were decreased ($n=9$; $p<0.05$)

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and total retinal cholesterol ester abundance was increased in diabetic animals compared to control animals. Lastly, retinal levels of SIRT1 and LXRα/β were downregulated in diabetic mice after 8 weeks of diabetes and LXR activation prevented development of acellular capillaries in db/db mice (n=6; p<0.05).
Conclusions: This study demonstrates the detrimental effect diabetes has on LXR and SIRT1 retinal levels. Activation of LXR prevents DR-induced pathology in diabetic animals. Hence, these studies suggest that retinal-specific activation of LXR has the potential to be a novel therapeutic target in the treatment of DR.
Commercial Relationships: Sandra Hammer, None; **Todd Lydic**, None; **Qi Wang**, None; **Sugata Hazra**, None; **Goldis Malek**, None; **Michael E. Boulton**, None; **Maria B. Grant**, None; **Julia V. Busik**, None
Support: NH Grant EY025383

Program Number: 4249
Presentation Time: 12:00 PM–12:15 PM
Elevated expression of RUNX1 by vascular endothelial cells in proliferative diabetic retinopathy
Jonathan D. Lam[1, 2], Daniel Oh[1, 2], Patricia A. D'Amore[1, 2], Leo A. Kim[1, 2], Joseph Arboleda-Velasquez[1, 2]. [1]Schepens Eye Research Institute, Boston, MA; [2]Ophthalmology, Massachusetts Eye and Ear, Boston, MA.
Purpose: A major sight threatening complication of proliferative diabetic retinopathy (PDR) is the formation of fibrovascular membranes (FVMs). The pathophysiology of this process is poorly understood. Our whole transcriptomic analysis of CD31+ endothelial cells isolated from patient derived FVMs previously identified elevated transcripts of Runt-related transcription factor 1 (RUNX1), implicating it in PDR. RUNX1 has been studied extensively in hematopoietic development and various cancers but its role in endothelial cells is not fully elucidated. We test the hypothesis that RUNX1 plays an active role in endothelial cell glucose response and angiogenesis using *in vitro* tools.
Methods: Human microvascular retinal endothelial cells (HMREC) and human umbilical vein endothelial cells (HUVEC) were cultured in endothelial growth media supplemented with D-glucose or L-glucose as a control for osmolarity at concentrations of 5 mM (normal baseline) to 30 mM (high glucose) for 72 hours before analysis. qRT-PCR and Western blot with antibodies against RUNX1 and β-actin were performed. RUNX1 gene knockdown was achieved using small interfering RNA (siRNA) transfection using Dharmafect1 and migration and cell proliferation were studied using the scratch-wound assay and immunostaining for Ki67 respectively. Student's t-test was used for statistical analysis.
Results: RNA expression of RUNX1 was elevated 2.8 ± 0.2 fold in HMREC cultured in high D-glucose (30 mM) compared to baseline controls (p<0.01, n=3). No difference in RNA expression of RUNX1 was seen in osmotic controls (L-glucose) (p>0.05). Western blotting analysis showed increased RUNX1 protein in both HMREC (1.28 ± 0.03, p<0.05, n=3) and HUVEC (1.3 ± 0.1, p<0.05, n=3) exposed to high glucose. Wound-closure in HMREC was significantly reduced by RUNX1 knockdown (0.052 ± 0.003 mm^2/hr) compared to transfection control (0.131 ± 0.003 mm^2/hr; p<0.001, n=5). RUNX1 knockdown in HMREC was also associated with decreased Ki67 staining (65% ± 4 of nuclei positively stained compared to 93% ± 2 in control; p<0.001, n=6).
Conclusions: These findings are consistent with the hypothesis that RUNX1 is inducible by hyperglycemia and contributes to a pro-angiogenic phenotype. Furthermore, these findings support the observation of elevated RUNX1 in endothelial cells from fibrovascular membranes of patients with PDR, suggesting a role for RUNX1 in pathologic retinal angiogenesis.
Commercial Relationships: Jonathan D. Lam, None; **Daniel Oh**, None; **Patricia A. D'Amore**, 62/422,523 (P); **Leo A. Kim**, 62/422,523 (P); **Joseph Arboleda-Velasquez**, 62/422,523 (P)
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Program Number: 4250
Presentation Time: 12:15 PM–12:30 PM
Characterization of early retinal vascular changes in murine models of diabetic retinopathy: Comparison of phase-variance OCT angiography and fluorescein angiography
Susanna S. Park[1], Suman Manna[2], Pengfei Zhang[2], Jonathan Lu[1], Parisa Emami-naeini[1], Robert J. Zawadzki[1, 2]. [1]UC Davis Eye Center/ University of California, Sacramento, CA; [2]Cell Biology and Human Anatomy, UC Davis EyePod Laboratory, Davis, CA.
Purpose: To characterize the development of retinal vascular changes associated with diabetic retinopathy in murine models of type 1 and 2 diabetes mellitus using a multi-modal in vivo imaging system built for mouse retinal imaging.
Methods: Murine models of type 1 diabetes mellitus (i.e. streptozotocin-induced) and type 2 diabetes mellitus (i.e. C57BLKS-*Leprdb* homozygotes mice, db/db) were imaged serially using a multi-modal in vivo retinal imaging system built for mouse retinal imaging. This system combines a SLO fundus camera and a spectral-domain OCT instrument such that simultaneous fluorescein angiography and phase-variance OCT angiography can be performed (pv-OCTA) to evaluate retinal perfusion.
Results: Early retinal vascular changes consistent with diabetic retinopathy were noted using both fluorescein angiography and pv-OCTA in type 1 and 2 diabetic mice. Fluorescein angiography detected microaneurysmal changes in the retinal vasculature and associated vascular leakage in type 1 diabetic mice consistent with diabetic retinopathy. Although simultaneous pv-OCTA did not detect retinal vascular leakage, some of the retinal vascular microaneurysmal changes noted on fluorescein angiography also could be detected using pv-OCTA. Among db/db type 2 diabetic mice, subtle areas of probable retinal capillary non-perfusion detected on fluorescein angiography were visualized more clearly using pv-OCTA. Phase variance-OCTA allowed 3-dimensional vascular imaging, localizing the retinal vascular changes seen on fluorescein angiography images more precisely to a specific retinal layer, feature not possible using fluorescein angiography alone.
Conclusions: Simultaneous multi-modal in vivo retinal vascular imaging using fluorescein angiography and pv-OCTA allows improved detection and characterization of early retinal vascular changes associated with diabetic retinopathy in murine models of type 1 and 2 diabetes mellitus.
Commercial Relationships: Susanna S. Park, None; **Suman Manna**, None; **Pengfei Zhang**, None; **Jonathan Lu**, None; **Parisa Emami-naeini**, None; **Robert J. Zawadzki**, None
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Program Number: 4251
Presentation Time: 12:30 PM–12:45 PM

Inhibition of Nox1, 4 and 5 attenuates vasculopathy and inflammation in rats with hypertensive diabetic retinopathy

Devy Deliyanti, Saeed Alrashdi, David R. Berka, Karin A. Jandeleit-Dahm, Mark E. Cooper, Jennifer L. Wilkinson-Berka. Diabetes, Monash University, Melbourne, VIC, Australia.

Purpose: Hypertension is a causal factor in the vasculopathy and inflammation that occurs in diabetic retinopathy, and there is evidence that reactive oxygen species (ROS) has an important role. We hypothesized that inhibition of ROS derived from NADPH oxidase (Nox) isoforms 1, 4 and 5 would attenuate vasculopathy and related macroglial Muller cell dysfunction as well as inflammation in the retina of hypertensive rats with type-1 diabetes.

Methods: Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were made diabetic with streptozotocin and studied for 8 weeks. GKT139601 (GKT) inhibits Nox1 and 4 and also the human/bovine Nox5 isoform and was administered from the onset of diabetes (60mg/kg/day, gavage) (N=21-26/group). Because Nox5 is absent in rodents, transgenic mice expressing human Nox5 in endothelial cells ($Nox5^{VE\text{-}Cad+}$) were generated and studied for 10 weeks. In retina, immunohistochemistry, qPCR or ELISA were used to measure oxidative stress, vasculopathy, Muller cell injury and inflammation. Studies were performed in primary cultures of rat Muller cells and bovine retinal endothelial cells (BREC) exposed to hyperglycaemia (HG, 25mM D-glucose) or HG+GKT (5μM) for 72 hours. Data were analysed by a one-way ANOVA and Mann Whitney U test or a Student's t-test.

Results: SBP was increased in diabetic SHR compared to diabetic WKY (143.7±2.6 vs 103.2±2.8) and GKT had no effect. All parameters were elevated in diabetic WKY compared to non-diabetic controls. Further increases occurred in diabetic SHR with respect to oxidative stress (8OHdG, $p<0.01$), vasculopathy (leakage, tight junction proteins, $p<0.05$; VEGF, angiopoietin2, $p<0.01$), Muller cell gliosis ($p<0.01$) and inflammation (microglial density, TNFα, ICAM-1, $p<0.05$). Treatment with GKT improved all parameters ($p<0.01$). $Nox5^{VE\text{-}Cad+}$ mice exhibited increased retinal gliosis and vascular leakage ($p<0.001$). In cultured Muller cells, HG increased Nox1 and 4, VEGF and MCP-1 ($p<0.001$), which were reduced with GKT ($p<0.01$). In BREC, HG increased Nox1, 4 and 5, VEGF and ICAM-1, which were reduced with GKT ($p<0.01$). Differential expression of tight junction proteins occurred. HG reduced occludin and ZO-1 levels in BREC and ZO-3 levels in Muller cells, which were restored by GKT ($p<0.05$).

Conclusions: These data indicate the potential of Nox1, 4 and 5 inhibition to reduce vision-threatening hypertensive diabetic retinopathy.

Commercial Relationships: Devy Deliyanti, None; **Saeed Alrashdi**, None; **David R. Berka**, None; **Karin A. Jandeleit-Dahm**, None; **Mark E. Cooper**, None; **Jennifer L. Wilkinson-Berka**, Genkyotex (S)

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