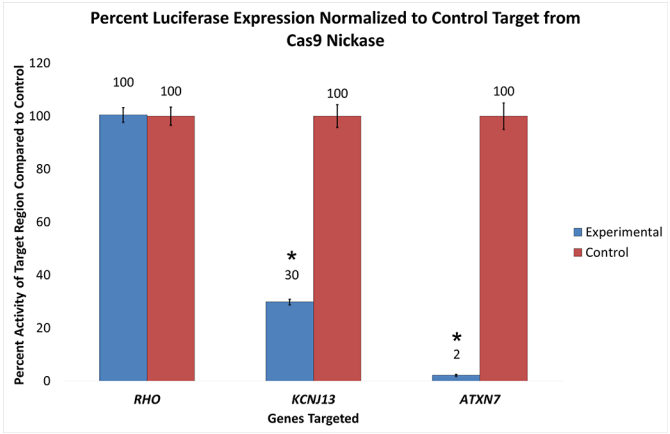
**445 Gene editing and gene therapies**
Wednesday, May 10, 2017 11:00 AM–12:45 PM
Exhibit/Poster Hall Poster Session
**Program #/Board # Range:** 4468–4509/B0123–B0164
**Organizing Section:** Biochemistry/Molecular Biology

**Program Number:** 4468 **Poster Board Number:** B0123
**Presentation Time:** 11:00 AM–12:45 PM
**CRISPR/Cas9-Mediated Genome Editing as a Therapeutic Approach for Leber Congenital Amaurosis 10**
*Guoxiang Ruan, Elizabeth Barry, Dan Yu, Michael Lukason, Seng Cheng, Abraham Scaria*. Gene Therapy, Sanofi Genzyme, Framingham, MA.
**Purpose:** As the most common subtype of Leber congenital amaurosis (LCA), LCA10 is a severe retinal dystrophy caused by mutations in the *CEP290* gene. The most frequent mutation found in LCA10 patients is a deep intronic mutation in *CEP290* that generates a cryptic splice donor site. The large size of the *CEP290* gene prevents its use in AAV-mediated gene augmentation therapy. Here, we test the hypothesis that targeted genomic deletion using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system can be used as a therapeutic approach for the treatment of LCA10 patients bearing the *CEP290* splice mutation.
**Methods:** A cellular model of LCA10 was developed by introducing the *CEP290* splice mutation into human embryonic kidney (HEK) 293FT cells using CRISPR/Cas9-mediated homology-directed repair (HDR). Paired sgRNAs and Cas9 were employed to excise the intronic fragment containing the *CEP290* splice mutation. Dual AAV vectors (AAV5-SpCas9 and AAV5-sgRNA pairs) were co-injected into the subretinal space of C57BL/6J mice to delete an intronic fragment in the *Cep290* gene of mouse photoreceptors. A self-limiting CRISPR/Cas9 system was developed to limit the expression of SpCas9 by incorporating sgRNA recognition sites into the SpCas9 plasmid.
**Results:** The LCA10 cellular model recapitulated the *CEP290* expression pattern observed in LCA10 patient fibroblasts. In this model, guide RNA pairs coupled with SpCas9 were highly efficient at removing the intronic splice mutation and restoring the expression of wild-type *CEP290*. In addition, we demonstrated that the dual AAV system could effectively delete an intronic fragment of the *Cep290* gene in the mouse retina. The self-limiting CRISPR/Cas9 system was effective at minimizing the duration of SpCas9 expression and rescuing the levels of wild-type *CEP290* mRNA.
**Conclusions:** These results support further studies to determine the therapeutic potential of CRISPR/Cas9-based strategies for the treatment of LCA10 patients bearing the *CEP290* splice mutation.
**Commercial Relationships: Guoxiang Ruan**, Sanofi Genzyme (E); **Elizabeth Barry**, Sanofi Genzyme (E); **Dan Yu**, Sanofi Genzyme (E); **Michael Lukason**, Sanofi Genzyme (E); **Seng Cheng**, Sanofi Genzyme (E); **Abraham Scaria**, Sanofi Genzyme (E)

**Program Number:** 4469 **Poster Board Number:** B0124
**Presentation Time:** 11:00 AM–12:45 PM
**Optimized Homology Directed Repair for Treatment of Inherited Retinal Diseases Using the CRISPR/Cas9 System**
*Brian Rossmiller, Takeshi Iwata*. National Institute of Sensory Organs, National Hospital Organization, Tokyo, Japan.
**Purpose:** Nearly 1.5 million people, worldwide, are affected by hereditary vision impairment each year. For many of these, there is little to no effective treatment. Advancements in CRISPR/Cas mediated site specific gene editing provide a new and powerful tool for disease treatment but relies on inefficient homology directed recombination (HDR). The purpose of this project is to treat three models of inherited retinal degeneration using CRISPR/Cas9 mediated HDR. Prior to treatment, we will assess optimal conditions for use of the CRISPR/Cas9 system in the retina. These include age of injection, delivery vehicle (nanoparticle or adeno-associated virus (AAV)), and method of increasing homologous integration of gene replacement (ligase IV inhibitors and neurotrophic factors).
**Methods:** LPD lipid ratios were tested through transfection of 2.5 ug of plasmid containing control cytomegalovirus promoter driven firefly luciferase DNA into HEK293 cells. Luciferase activity was measured 48 hours post-transfection. Assessment of sgRNA cleavage efficiency was performed using a novel dual luciferase approach with a molar ratio of 1:4 target to H1-sgRNA expression vector. Luciferase activity was measured 48 hours post-transfection. Each vector is assessed in three animal models of inherited retinal degeneration. First, a mouse model of retinitis pigmentosa, Rho (I307N), kindly provided by Dr. Nishina of Jackson laboratory. The second model is of normal tension glaucoma, OTPN (E50K) and produced in the Iwata lab. Finally, we created a third model featuring a premature truncation of KCNJ13 (W53X) resulting in Leber's congenital amaurosis.
**Results:** Here, we have confirmed transfection, of CMV-GFP plasmid, by LPDs, into HEK293 cells by GFP expression. These results confirm that a ratio of 1:2:3:15, DNA, nuclear localization signal and trans-acting transcription peptide, protamine sulfate, and lipids, respectively, provided the best DNA transfection rates in tissue culture and will be used moving forward in mouse models. Cleavage of KCNJ13, by CRISPR/Cas9, resulted in a significant reduction in luciferase activity (70%, $p < 0.05$).
**Conclusions:** Our current LPD mixture and sgRNAs targeting KCNJ13 have shown to provide significant transfection and targeted knock-down *in vitro*.




**Commercial Relationships: Brian Rossmiller**, None; **Takeshi Iwata**
**Support:** The Japan Agency for Medical Research and Development

**Program Number:** 4470 **Poster Board Number:** B0125
**Presentation Time:** 11:00 AM–12:45 PM
***Rgr* gene mutation and exon-skipping RGR mRNA in mice**
*Zhaoxia Zhang*[1, 2], *Henry KW Fong*[1, 3]. [1]Department of Ophthalmology, USC Roski Eye Institute, University of Southern California, Los Angeles, CA; [2]Department of Ophthalmology, Shanxi Dayi Hospital, Taiyuan, China; [3]Department of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA.
**Purpose:** Various abnormal RGR opsins lead to different retinal disease phenotypes. A rare human RGR mutation (c.196A>C,

p.S66R) causes autosomal recessive retinitis pigmentosa, and another (c.824dupG, p.I276Nfs*77) leads to progressive peripapillary choroidal atrophy that is dominantly inherited. Our purpose is to study intracellular processing and biological effect of a mouse isoform of the human RGR-d exon-skipping protein in an animal model.
**Methods:** Multiple RGR-d mutant mouse lines (strains: B6D2F1/J and Rosa26-Cas9 knockin) were created by mutation of the splice site at each end of exon VI of the mouse *Rgr* gene. Destruction of the splice sites was performed by multiplex gene editing using CRISPR methods. A pair of single guide RNAs (gRNA), that targets the two splice sites of mouse *Rgr* exon VI, and Cas9 mRNA were microinjected into single-cell wildtype B6D2F1/J embryos. The pair of gRNAs alone was microinjected into embryos from Rosa26-Cas9 knockin mice.
**Results:** We obtained mice with mutation of either the 5' or 3' splice site, both splice sites, or with total deletion of exon VI. We analyzed one litter of 8 viable pups from microinjection of wildtype B6D2F1/J embryos. The founders were designated F81, F82, F83, F89, M91, M94, F95, and F97. We obtained a litter of 10 pups from microinjection of Rosa26-Cas9 knockin embryos. Exon-VI-skipping RGR mRNA, or frameshift RGR transcripts, in positive F1 mice were confirmed by RT-PCR mRNA analysis and direct sequencing of the amplicons. Full-length RGR was completely absent on Western immunoblots of proteins from homozygous mutant (*Rgr-d*) mouse eyes, in which we found that the RGR-d isoform was expressed at very low levels (<1% of RGR in wildtype mice). At two weeks of age, little change was noted by light microscopy between the retinas of wildtype and *Rgr-d* F2 littermates. At five months of age, RPE cells in *Rgr-d* mice showed apical displacement of intracellular pigment and appeared vacuolated compared to RPE in the wildtype littermate. Similar changes were not seen in RGR knockout mice.
**Conclusions:** In contrast to the RGR-d isoform in humans, mouse RGR-d undergoes rapid degradation, and abnormal RGR-d protein does not accumulate in the eyes of otherwise healthy, untreated young mice. These animals provide a model for lifelong expression of an *Rgr-d* gene and potentially chronic endoplasmic-reticulum stress or age-related proteopathy.
**Commercial Relationships: Zhaoxia Zhang**, None; **Henry KW Fong**, None

**Program Number:** 4471 **Poster Board Number:** B0126
**Presentation Time:** 11:00 AM–12:45 PM
**OPTIMIZING THE REPAIR TEMPLATE FOR HOMOLOGY DIRECTED REPAIR OF DOUBLE STRAND BREAKS**
*Knut Stieger, Fei Song, Birgit Lorenz*. Department of Ophthalmology, Justus-Liebig-University Giessen, Giessen, Germany.
**Purpose:** In vivo genome editing takes center stage in the development of therapeutic applications of inherited retinal dystrophies. In such therapeutic applications it is crucial to bias repair outcomes towards high fidelity homology directed repair (HDR) or microhomology mediated end-joining (MMEJ), and to avoid error prone nonhomologous end-joining (NHEJ). In this study, we analyzed the impact of different repair templates on the frequency of HDR and NHEJ in a well-defined cell culture system.
**Methods:** The modified TLR system (TLR3) comprises a bicistronic expression system of a non-functional green fluorescent protein (GFP) gene, followed by a self-cleaving T2A peptide and a second blue fluorescent protein (BFP) gene in a reading frame shifted by 2 bp. A stable HEK293 cell line expressing TLR3 was generated by transfecting a linearized pcDNA3.1(-)-TLR3 plasmid followed by neomycin selection. Donor templates of 1000 bp length containing the corrected GFP sequence were generated as circular plasmid, linearized plasmid with long 3' or 5' backbone overhang, or as PCR product. The sequence to be corrected was either centrally located (RS55), with a shorter 5' homologous region (RS37), or a shorter 3' homologous region (RS73). Eight different guide RNAs were generated and the Cas9 from the streptococcus pyogenes was used. DNA repair activity was measured by FACS.
**Results:** Guide RNAs targeting the active strand (T5, T7) showed NHEJ frequencies of about 20%, while guide RNAs targeting the inactive strand showed NHEJ frequencies of less than 5%. HDR activity was highest (about 2%) when using the linearized plasmid with the short 5' backbone overhang and the RS37 design, followed by the PCR product or the linearized plasmid with the long 5' backbone overhang, both with RS73 design (1-1.4%). Circular plasmid was least efficient in generating HDR events (<1%). The effect of the different repair templates on NHEJ frequencies was marginal.
**Conclusions:** We show that a linearized plasmid with short 5' backbone and short 5' homology arm is the most efficient variant to induce HDR, proving the importance of the repair template design when developing therapeutic applications for genome editing. However, NHEJ activity is not reduced by different repair templates, meaning that this pathway needs to be repressed by other mechanisms.
**Commercial Relationships: Knut Stieger**, None; **Fei Song**, None; **Birgit Lorenz**, Spark Therapeutics (C)
**Support:** ERC starting grant #311244

**Program Number:** 4472 **Poster Board Number:** B0127
**Presentation Time:** 11:00 AM–12:45 PM
***In vivo* gene editing of zebrafish *rho* to model human photoreceptor disease**
*Chris Zelinka, James M. Fadool*. Biological Science, Florida State University, Tallahassee, FL.
**Purpose:** Mutations in *RHO* are among the most prevalent causes of hereditary retinal degeneration. *RHO* alterations lead to the death of rods, and secondary loss of cones. The conservation of photoreceptor structure, gene expression and function among vertebrates has led us to hypothesize that zebrafish *rho* mutations will model human photoreceptor disease. The purpose of this study was to apply *in vivo* genome editing to disrupt zebrafish *rho*.
**Methods:** CRISPR/Cas9 was used to induce mutations within the zebrafish *rho* gene. One- and two-cell staged embryos were injected with a solution containing a single guide (g) RNA targeting *rho* plus *in vitro* transcribed, capped RNA encoding Cas9. Embryos were reared for several days. Genomic DNA was isolated from injected larvae and used as the template to PCR amplify the entire coding sequence for *rho*. Mutations were detected with Sanger sequencing and/or restriction fragment length polymorphism. Retinal cryosections of injected and uninjected siblings were immunostained with the 1D1 monoclonal antibody which recognizes rhodopsin or 4C12, an independent rod-specific marker to verify the loss of rhodopsin expression.
**Results:** *rho* in teleosts is characterized by a single exon with no introns. DNA sequencing of a PCR product spanning the entire *rho* exon showed that the larvae injected with the gRNA/Cas9 mRNA are mosaic for mutations in *rho*. The most common mutations are predicted to disrupt the *rho* coding sequence. Immunolabeling of serial sections of larvae at 6 days post fertilization showed absent or altered expression of rhodopsin in injected-larvae, however immunolabeling with an antibody to a second protein expressed by rods showed that the rod photoreceptors were present. To test for germline transmission, a subset of injected larvae were reared to

adults and outcrossed. Novel *rho* alleles were identified in the F1 generation.
**Conclusions:** Our recovery of novel mutations shows that *rho* can be successfully targeted in zebrafish using the CRISPR/Cas9 system. These offer a proof-of-concept for the successful targeted mutagenesis of *rho* in zebrafish and their potential as models of disease.
**Commercial Relationships: Chris Zelinka**, None; **James M. Fadool**, None
**Support:** Foundation Fighting Blindness

**Program Number:** 4473 **Poster Board Number:** B0128
**Presentation Time:** 11:00 AM–12:45 PM
**Allele-specific gene editing for the treatment of autosomal dominant Retinitis Pigmentosa**
*Gene Liau[1], Kollu N. Rao[2], Victor V. Bartsevich[1], Jeff Smith[1], Derek Jantz[1], Matthew Hirsch[2]*. [1]Precision Biosciences, Durham, NC; [2]Department of Ophthalmology, Gene Therapy Center, University of North Carolina, Chapel Hill, NC.
**Purpose:** Targeted gene knockout is an emerging approach for the treatment of autosomal-dominant disorders such as P23H *RHO* mediated autosomal dominant retinitis pigmentosa (adRP), a common form of inherited blindness. A single nucleotide distinguishes P23H *RHO* from the WT copy which collectively present in a heterozygous manner. Previous works have suggested that down-regulation of the mutant allele in presence of the WT copy prevents retinal degeneration. Therefore, a 930nt *I-CreI* based designer homing endonuclease (HE) to specifically knockout the mutant RHO allele was engineered and validated in a self-complementary AAV vector. The specificity of this HE for P23H RHO was demonstrated *in vitro*. Preclinical evaluations in a human P23H RHO adRP mouse model were undertaken to determine if this approach demonstrates clinical potential for preventing vision loss in adRP patients.
**Methods:** A proprietary ARCUS platform was employed to generate a HE that can specifically target the human *RHO* allele carrying the P23H mutation. The targeted 22 nucleotide sequence differs from the wild type allele only by a single base pair. Specificity of the nuclease was first evaluated in CHO cell lines engineered to contain a single strand annealing GFP reporter that harbors either the WT or P23H *RHO* sequence. Gene editing efficiency was evaluated by flow cytometry and immunoblotting. To evaluate HE activity *in vivo*, a scAAV5 vector harboring P23H RHO HE cDNA under control of a photoreceptor specific promoter was administered to the human P23H *RHO* adRP mouse model via sub-retinal injection. scAAV5-GRK-HE cleavage efficiency of the mutant *RHO* allele was calculated using sequencing and by digital PCR. *In vivo* retinal function was evaluated by electroretinography and the subcellular localization of rhodopsin in photoreceptors by histology.
**Results:** We have generated a HE that is highly specific for the mutant P23H RHO allele. Sub-retinal delivery of scAAV5-GRK-HE decreases the amount of rhodopsin in outer nuclear layer in transduced photoreceptors when compared to non-transduced cells.
**Conclusions:** This is the first study to demonstrate that a single chain HE can efficiently discriminate a one nucleotide difference and specifically target the P23H *RHO* allele. This result coupled with decreased rhodopsin mislocalization in an adRP model, foreshadows possible use of scAAV HE-based gene editing of P23H *RHO* to treat adRP patients.
**Commercial Relationships: Gene Liau**; **Kollu N. Rao**, Precision Biosciences (F); **Victor V. Bartsevich**, Precision Biosciences (E); **Jeff Smith**, Precision Biosciences (E); **Derek Jantz**, Precision Biosciences (E); **Matthew Hirsch**, Precision Biosciences (F)

**Program Number:** 4474 **Poster Board Number:** B0129
**Presentation Time:** 11:00 AM–12:45 PM
***In vivo* allele-specific CRISPR/Cas9 gene editing in the rhodopsin P23H knockin mouse model**
*Pingjuan Li[1], Benjamin Kleinstiver[2], Mihoko Leon[1], Keith Joung[2], Eric A. Pierce[1], Qin Liu[1]*. [1]Department of Ophthalmology, Ocular Genomics Institute, Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Center for Cancer Research, Massachusetts General Hospital, Charlestown, MA.
**Purpose:** P23H mutation in the rhodopsin (*RHO*) gene is the most common cause (15-18%) of autosomal dominant retinitis pigmentosa (adRP) in USA. Strategies for treating RHO-P23H RP have predominantly involved the suppression of the wild-type and mutant alleles, which can therefore require supplementation of a wild-type gene. In this study, we aim to develop an allele-specific CRISPR/Cas9 system to knock out only the P23H mutant allele at its native locus in a well-characterized *Rho-P23H* knock-in mouse model.
**Methods:** Three sgRNAs were designed with PAM sequences for alternative Cas9 variants to target the P23H mutation site. The sgRNAs, together with CAG-Cas9-T2A-EGFP encoding plasmids, were transfected into retinal cells of the wild type or mutant mice at P0-P2 by sub-retinal injection and *in vivo* electroporation. The targeted cleavage efficiency in the EGFP-positive retinal cells was determined 5-7 days post injection by next generation sequencing (NGS). The selected sgRNA with the highest efficiency and specificity, along with its Cas9-EGFP variants, were co-transfected into *Rho-P23H* heterozygous mice. The effect of this CRISPR/Cas9-sgRNA treatment on the retinal structure was evaluated by comparing the outer nuclear layer (ONL) of the EGFP-positive area with the adjacent EGFP-negative region on the frozen sections from the injected retinas.
**Results:** Of the three sgRNAs tested, sgRNA2 with SpCas9 variants VQR or VRQR specifically cleaved the P23H mutant allele, with a cleavage efficiency of 43.20 ± 1.7% (n=3) in *Rho-P23H* homozygous retinas, and 0.94 ± 0.35% (n=3) in the wild type littermates. However, sgRNA1 and sgRNA3 showed no allele specificity between P23H mutant and wild type allele, with cutting efficiencies at 13.66% vs. 38.29% and 33.16% vs. 38.38%, respectively. NGS results showed that 70-90% of the Indels are out-of-frame. Preservation of photoreceptor degeneration in the transfected retina of *Rho-P23H* heterozygous mice was observed with 1-2 more rows of ONL than the adjacent non-transfected area at age of 40 days.
**Conclusions:** Our study showed that CRISPR/Cas9 can selectively disrupt the *Rhodopsin-P23H* allele, which differed by only a single nucleotide from the wild type allele in mouse retina. This demonstrates the feasibility of an allele-specific CRIPSR-Cas9 gene editing approach that could be applied to a wide range of dominant diseases.
**Commercial Relationships: Pingjuan Li**; **Benjamin Kleinstiver**, None; **Mihoko Leon**, None; **Keith Joung**, None; **Eric A. Pierce**, None; **Qin Liu**, None

**Program Number:** 4475 **Poster Board Number:** B0130
**Presentation Time:** 11:00 AM–12:45 PM
**Multiplex CRISPR/Cas9-based Genetic Screen for Retinal Regeneration-deficient Zebrafish Mutants**
*Arife Eroglu, Timothy Mulligan, Sumitra Sengupta, Jeff S. Mumm*. Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.
**Purpose:** Müller glia (MG) cells function as retinal stem cells in zebrafish. Intriguingly, human MG appears to retain regenerative potential; neurons derived from MG in culture can restore visual function when transplanted into retinal degeneration models. Accordingly, the mechanisms regulating the regenerative potential

of MG are of great interest. To gain a comprehensive understanding of the factors and cognate signaling systems controlling retinal regeneration, we apply forward and reverse genetics screening approaches to identify regeneration-deficient mutant zebrafish lines. Here, we discuss the use of a multiplexed CRISPR/Cas9-based targeted mutational strategy to evaluate ~300 transcriptomics-implicated genes as factors necessary for rod photoreceptor regeneration in zebrafish.
**Methods:** We have developed complementary cell-specific ablation and whole-organism high-throughput screening (HTS) methods to 1) facilitate studies of cellular regeneration, 2) model degenerative disease, and 3) discover regeneration-promoting factors in zebrafish. Here, a model of retinitis pigmentosa—where apoptosis can be induced specifically in rod cells and a fluorescent reporter allows quantification of cell loss and replacement kinetics—is being used to identify genes required for rod cell regeneration. To implicate genes in rod cell replacement, we performed a microarray analysis comparing two cell-specific ablation paradigms, rod cells versus retinal bipolar cells. Nearly 300 genes upregulated in the rod cell were selected for multiplexed CRISPR/Cas9-based mutational analysis. Using this method, phenotyping assays can be performed in the F1 generation, vastly increasing screening efficiency, and thereby enabling large-scale reverse genetics.
**Results:** Targeting oligos for 144 candidate genes have been generated. Multiplexing is used to concurrently target 3 gene candidates, resulting in a total of 96 pooled founder groups for 288 gene candidates. Our recent progress in generating mutant lines and identifying regeneration-deficient phenotypes will be discussed.
**Conclusions:** Multiplexed CRISPR/Cas9 methods enable large-scale mutational analyses, providing a means of functionally evaluating candidate genes at a rate of high-throughput bioinformatics-based datasets. The approaches outlined here can therefore provide fundamental insights into the mechanisms regulating regenerative potential in the retina
**Commercial Relationships: Arife Eroglu**, None; **Timothy Mulligan**, None; **Sumitra Sengupta**, None; **Jeff S. Mumm**, None
**Support:** NEI R01EY022810

**Program Number:** 4476 **Poster Board Number:** B0131
**Presentation Time:** 11:00 AM–12:45 PM
**Safety and post hoc analysis of subretinal rAAV.sFLT-1 for wet age-related macular degeneration following a phase 2a randomized clinical trial**
*Elizabeth P. Rakoczy[1, 2], Chooi-May Lai[1, 2], Aaron Magno[2], Martyn French[3], Steve Butler[4], Samuel Barone[4], Steven D. Schwartz[5], Mark Blumenkranz[6], Mariapia Degli-Esposti[1], Ian Constable[1, 2].*
[1]Centre for Ophthalmol & Visual Sciences, University of Western Australia, Perth, WA, Australia; [2]Molecular Ophthalmology, Lions Eye Institute, Perth, WA, Australia; [3]School of Pathology, University of Western Australia, Perth, WA, Australia; [4]Adverum Biotechnologies, San Francisco, CA; [5]University of California, Los Angeles, Los Angeles, CA; [6]Byers Eye Institute, Stanford University, Palo Alto, CA.
**Purpose:** To assess the safety and tolerability of rAAV.sFLT-1 in patients with wet age-related macular degeneration (wAMD).
**Methods:** Thirty-two patients (55 years or older) with wAMD and best corrected visual acuity worse (BCVA) than 20/40 secondary to wAMD due to active subfoveal choroidal neovascularization in their study eye were recruited. Patients were randomized in a 2:1 ratio (gene therapy:control). All patients received intravitreal ranibizumab at baseline (Day 0) and at day 28. Gene therapy patients received rAAV.sFLT-1, $1x10^{11}$ vg subretinally at Day 7. Both arms received intravitreal ranibizumab pro nata after day 28. Patients were assessed every 4 weeks to the 52-week primary endpoint using clinical laboratory testing, physical and ophthalmic examinations. Biodistribution and immune response to the vector was evaluated by a range of laboratory tests.
**Results:** Treatment group patients injected subretinally with rAAV. sFlt-1 tolerated the procedure well. All procedure-related ocular adverse events self-resolved. No systemic safety signals were observed and serious AEs were deemed not associated with rAAV. sFLT-1. There was no change in sFLT-1 levels and AAV2 capsid was not detected in the bodily fluids. rAAV.sFLT-1 DNA was detected transiently in the tears of 13 rAAV.sFLT-1-injected patients. There were no notable changes in T-cell response identified by ELISPOT analysis. The presence of AAV neutralizing antibodies at baseline was not associated with diminished efficacy. In the treatment group patients, best corrected visual acuity improved by a median of 1.0 (IQR: 1.0 to 9.0) Early Treatment Diabetic Retinopathy Study (ETDRS) letter from baseline compared to a median -5.0 (IQR: -17.5 to 1.0) ETDRS letter change in control patients. Twelve rAAV.sFLT-1-injected patients maintained or improved vision compared to 4 control patients. The median number of ranibizumab retreatments in rAAV.sFLT-1-injected and control group patients was 2.0 (IQR: 1.0 to 6.0) and 4.0 (IQR: 3.5 to 4.0), respectively.
**Conclusions:** These results suggest that ocular gene therapy combined with the option for intravitreal anti-VEGF protein rescue treatment appears safe and may be a viable approach to reducing the treatment burden of wAMD.
**Commercial Relationships: Elizabeth P. Rakoczy**; **Chooi-May Lai**, Avalanche Biotechnologies (E), Avalanche Bitechnologies (P); **Aaron Magno**, Avalanche Biotechnologies (E); **Martyn French**, None; **Steve Butler**, Avalanche Biotechnologies (E); **Samuel Barone**, Avalanche Biotechnologies (E); **Steven D. Schwartz**, Avalanche Biotechnologies (I); **Mark Blumenkranz**, Avalanche Biotechnologies (I); **Mariapia Degli-Esposti**, None; **Ian Constable**, Avalanche Biotechnologies (F), Avalanche Biotechnologies (P)
**Clinical Trial:** NCT01494805

**Program Number:** 4477 **Poster Board Number:** B0132
**Presentation Time:** 11:00 AM–12:45 PM
**Co-expression of human L- or M-opsin restores M-Cone Function in the *Opn1mw* Knock-out mouse, a model for blue cone monochromacy (BCM)**
*Wentao Deng[1], Ji-Jing Pang[1], Jie Li[1], Ping Zhu[1], Wolfgang Baehr[2], W. Clay Smith[1], William W. Hauswirth[1].* [1]Ophthalmology, University of Florida, Gainesville, FL; [2]University of Utah, Salt Lake City, UT.
**Purpose:** BCM is an X-linked congenital disorder characterized by loss of both L- and M- cone function. Here we tested gene therapy for BCM using an M-cone knock out mouse model by AAV-mediated expression of either or both human M- and L-opsin.
**Methods:** Since human L- and M-opsin are highly homologous there is no antibody that can distinguish between them. We generated AAV5 vectors expressing either C-terminal myc tagged L-opsin-myc cDNA or C-terminal HA tagged M-opsin-HA cDNA, both driven by the cone-specific PR2.1 promoter. Vectors were delivered either individually or as a mixture containing equal numbers of vector genomes of each. 1 µL of vector(s) was injected subretinally into one eye of one month old M-opsin Knockout (*Opn1mw*$^{-/-}$) mice, while the contralateral eyes served as controls. M-cone function was assessed by photopic ERG under middle wavelength (510nm) flash conditions. In retinal sections human L- and M-opsin expression were analyzed by myc and HA antibodies respectively, and their cellular

localizations analyzed by peanut agglutinin (PNA) and anti-human L/M-opsin and anti-mouse S-opsin.
**Results:** Retinal functional analysis of *Opn1mw*$^{-/-}$ mice treated with either vector individually or as an equal mixture showed significant ERG rescue compared to untreated controls. In eyes treated with individual vectors, expression of myc, HA and M/L-opsin were detected and co-localized correspondingly. In the dorsal retina where no mouse M-opsin is expressed, human M- or/and L-opsin expression co-localized with PNA labeled cone sheaths. In the ventral retina, where mouse S-opsin is present in cone outer segments, they co-localized with endogenous S-opsin. In retinas treated with the vector mixture, a majority of the cones co-express both M- and L-opsin as seen by HA and Myc co-localization. We also note that individual cones can contain different ratios of human M- and L-opsin.
**Conclusions:** We demonstrate that C-terminal tagging human opsins does not interfere either function or cellular localization. Therefore, these tags can be employed to distinguish L- from M-opsin in the same retina. Functional and structural analyses in *Opn1mw*$^{-/-}$ retinas confirm that either human opsin or their mixture can restore both significant M-cone function and cone outer segments. These observations provide preclinical proof-of-principle justifying gene therapy treatment of human BCM.
**Commercial Relationships: Wentao Deng**, None; **Ji-Jing Pang**, None; **Jie Li**, None; **Ping Zhu**, None; **Wolfgang Baehr**, None; **W. Clay Smith**, None; **William W. Hauswirth**, AGTC (F), AGTC (P), AGTC (C)

**Program Number:** 4478 **Poster Board Number:** B0133
**Presentation Time:** 11:00 AM–12:45 PM
**Restoration of M-cone function in an S-cone only mouse model of human blue cone monochromacy by AAV-mediated expression of human L- or M-opsin**
*Ji-Jing Pang*[1]*, Wentao Deng*[1]*, Yuxin Zhang*[1, 2]*, Jie Li*[1]*, Ping Zhu*[1]*, Wolfgang Baehr*[3]*, William W. Hauswirth*[1]. [1]Ophthalmology, University of Florida, Gainesville, FL; [2]Ophthalmology, First Affiliated Hospital, Nanjing Medical University, Nanjing, China; [3]Ophthalmology, University of Utah, Salt Lake City, UT.
**Purpose:** Blue cone monochromacy (BCM) is an X-linked congenital disorder with severe cone dysfunction due to the absence of the long and medium wavelength-sensitive cone function. In this study, we used an M-opsin knockout (*Opn1mw*$^{-/-}$) mice with only S-cone function remaining as a model to test gene therapy for BCM by AAV-mediated delivery of either human M- or L-opsin, homologues of the mouse M-opsin.
**Methods:** We generated AAV5 vectors expressing either human L-opsin or human M-opsin driven by the cone-specific PR2.1 promoter. 1 microliter ($5 \times 10^{12}$ vector genomes/mL) of each vector was injected subretinally into one eye of one month old *Opn1mw*$^{-/-}$ mice, while the contralateral eyes remain untreated and served as controls. M-cone mediated retinal function was analyzed by full-field photopic ERG under middle wavelength flash conditions (510nm). Human L- and M-opsin transgene expression and their cellular localization were examined by immunohistochemistry using anti-L/M-opsin, anti-S-opsin antibodies and peanut agglutinin (PNA). The levels of transgene expression were assessed by Western blot analysis.
**Results:** Untreated *Opn1mw*$^{-/-}$ mice have no M-cone function and only S-cone function primarily in their ventral retina. Treatment with either human M- or L-opsin rescued middle wavelength ERG responses to about 60% of normal b-wave amplitudes. AAV delivered human M-opsin or L-opsin was detected panretinally. In dorsal retinas, M-opsin or L-opsin was localized within PNA-labeled cone sheaths in cone outer segments. In ventral retinas where S-cones are present, M-opsin or L-opsin was co-localized with endogenous mouse S-opsin in cone outer segments. Western blot analysis showed that AAV-delivered M- or L-opsin was about 65% of the wildtype level which is consistent with the level of ERG rescue.
**Conclusions:** We demonstrate that AAV-mediated expression of either human M-opsin or L-opsin can restore ERG responses to middle wavelength light in *Opn1mw*$^{-/-}$ mice. Vectored human opsins were expressed in cone outer segments in both dorsal and ventral retinas. These results also demonstrate that cones with one type of opsin, endogenous S-opsin in this case, can co-exist with a second type of vector delivered opsin, human L-opsin or M-opsin without physiological effects. These observations provide proof-of-concept for eventually treating BCM patients by gene therapy.
**Commercial Relationships: Ji-Jing Pang**; **Wentao Deng**, None; **Yuxin Zhang**, None; **Jie Li**, None; **Ping Zhu**, None; **Wolfgang Baehr**, None; **William W. Hauswirth**, AGTC (F), AGTC (P), AGTC (C)
**Support:** NIH grant EY021721, EY023543, BCM Families Foundation, AGTC, MVRF, and RPB, Inc.

**Program Number:** 4479 **Poster Board Number:** B0134
**Presentation Time:** 11:00 AM–12:45 PM
**The effect of timing on visual restoration of *Gnat1/Pde6c* double-knockout mouse through AAV-mediated gene supplementation**
*Koji M. Nishiguchi*[1]*, Kosuke Fujita*[3]*, Yukihiro Shiga*[2]*, Toru Nakazawa*[1, 2]. [1]Advanced Ophthamic Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Japan; [3]Retinal Disease Control, Tohoku University Graduate School of Medicine, Sendai, Japan.
**Purpose:** To determine the differential effect of treatment timing on visual restoration using mice defective of both rod and cone function.
**Methods:** Mice severely defective of both rod and cone function from birth was generated through cross-breeding (*Gnat1/Pde6c* double-knockout mice). The mice were supplemented with *Gnat1* using AAV2/8 at 3W, 3M, and 9M. Histology was examined with immunohistochemistry and visual function was assessed by measuring visually evoked potentials (VEPs) and opto-kinetic responses (visual acuity and contrast sensitivity) 2 weeks after the treatment. The data was compared between the treatment groups.
**Results:** The double KO mice were confirmed to be blind as measured by opto-kinetic responses, while the mice retained weak light perception as measured by VEP. Progressive decline in PNA-positive cones were noted with increasing age while the number of cells in the outer nuclear layer, mostly representing rods, remained essentially unchanged. Visual function as probed by VEPs and visual acuity did not differ between the treatment groups treated at 3W, 3M, and 9 M. Meanwhile, a modest decline in contrast sensitivity was detected only in mice treated at 9M ($58.8 \pm 2.5\%$) compared to 1M ($50.6 \pm 4.9\%$) and 3M ($45.8 \pm 2.9\%$).
**Conclusions:** The timing of visual restoration had only a modest impact on the visual function in blind mice. The visual cortex have the capacity to accomodate and process novel visual inputs mediated by the gain of retinal function that takes place well beyond adulthood in mice.
**Commercial Relationships: Koji M. Nishiguchi**; **Kosuke Fujita**, None; **Yukihiro Shiga**, None; **Toru Nakazawa**, None
**Support:** JSPS KAKENHI Grants-in-Aid for Scientific Research C (16K11315)

**Program Number:** 4480 **Poster Board Number:** B0135
**Presentation Time:** 11:00 AM–12:45 PM
**AAV Gene Therapy in a Canine Model of MPS1 Prevents and Reverses Corneal Blindness**
*Matthew Hirsch[1, 2], Brian C. Gilger[4], Telmo Llanga[1], Richard Davis[1], Joanne Kurtzberg[5], Richard J. Samulski[2], Keiko Miyadera[3].*
[1]Ophthalmology, University of North Carolina, Chapel Hill, NC; [2]Gene Therapy Center, University of North Carolina, Chapel Hill, NC; [3]University of Pennsylvania, Philadelphia, PA; [4]North Carolina State University, Raleigh, NC; [5]Duke University, Durham, NC.
**Purpose:** Mucopolysacharidosis type 1 (MPS1) is a devastating lysosomal storage disease that can be lethal in the first decade of life. Bone marrow transplantation has proven effective at prolonging life by decades, however, the patient's quality of life is compromised due to non-lethal MPS1 manifestations including blindness. In particular, approximately 90% of MPS1 children present corneal opacity with about 50% of cases causing complete vision loss. There is no effective treatment for MPS1 corneal blindness, which is the deficit we seek to address in this work using a viral based gene addition strategy. This technology is envisioned as complementary therapeutic to approaches such as cell therapy that address systemic MPS1 disease symptoms.
**Methods:** A codon optimized IDUA cassette was packaged in a chimeric AAV capsid identified efficient for cornea transduction. Previous evaluations in human corneas ex vivo demonstrated supraphysiological IDUA activity and safety following intrastromal injection of AAV8G9-optIDUA. These optimistic data warranted an efficacy study in naturally occurring MPS1 canines that also suffer from corneal clouding. Three canines with advanced (13 m.o.) and one with early (9 m.o.) corneal diseases were administered AAV8G9-GFP (negative control) in one eye and AAV8G9-optIDUA in the contralateral eye via cornea stromal injections. These canines have been monitored by slit lamp biomicroscopy and corneal imaging currently out 14 weeks post-injections.
**Results:** Corneal clearing, encompassing both the actual cloudy storage disease and regression of associated vascularization was apparent as early as 1 week and near complete disease reversal is now evident 13 weeks in all corneas injected with AAV-8G9-optIDUA in the symptomatic cohort. In the younger animal with the early disease, corneal disease progression continued in the control eye while the AAV8G9-optIDUA injected cornea demonstrated clearance of microscopic storage materials and did not develop vascularization. No adverse effect was observed specific to the injection or either of the transgenes, except for transient reversible corneal edemas that developed in each eye of one animal at 6 and 10 weeks post injection.
**Conclusions:** The collective data of a corneal gene addition strategy using AAV vectors for MPS1 blindness demonstrate safety and efficacy and lend support for the evaluation of this technology in MPS1 children.

**Commercial Relationships: Matthew Hirsch**; **Brian C. Gilger**, None; **Telmo Llanga**, None; **Richard Davis**, None; **Joanne Kurtzberg**, None; **Richard J. Samulski**, University of North Carolina (P); **Keiko Miyadera**, None
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**Program Number:** 4481 **Poster Board Number:** B0136
**Presentation Time:** 11:00 AM–12:45 PM
**Towards Corneal Gene Therapy in the Aniridia Mouse Model *Pax6*$^{Sey/+}$**
*Elizabeth M. Simpson[1, 2], Siu Ling Lam[1], Andrea Korecki[1], Beatrice M. Tam[3], Orson L. Moritz[3], Jack Hickmott[1, 2].* [1]Centre for Molecular Medicine and Therapeutics (CMMT), University of British Columbia, Vancouver, BC, Canada; [2]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; [3]Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada.
**Purpose:** Aniridia is a rare autosomal dominant disorder caused by mutations in *PAX6* (Paired Box 6). Although named for lack of iris, aniridia is a panocular disorder, with vision loss attributable to three main causes: 1) hypomorphic fovea, 2) progressive corneal clouding, and 3) progressive glaucoma. Current treatments may delay vision loss, but do not prevent the blindness experienced by most people with aniridia. Gene therapy is a promising treatment strategy that may delay, or even prevent, vision loss. Here we focus on gene therapy for the corneal pathology.
**Methods:** EmGFP (emerald GFP), PAX6, 3xFLAG/PAX6, and PAX6/3xFLAG mRNAs were injected into *Xenopus laevis* embryos, which were fixed and examined 14 days later. Additionally, EmGFP and 3xFLAG/PAX6 constructs, driven by smCBA (ubiquitous) or a human MiniPromoter (targeted to the cornea) were cloned into a custom rAAV genome, and packaged into rAAV9 at the University of Pennsylvania Vector Core. Viruses were introduced into wild-type and *Pax6*$^{Sey/+}$ mice using several different injections routes. Mouse eyes were longitudinally scored for corneal opacification and then harvested by enucleation, and fixed in 4% paraformaldehyde.

Cryosections were stained with antibodies against EmGFP, PAX6, and FLAG tag for immunofluorescent analysis.
**Results:** Since human and mouse PAX6 are indistinguishable by immune detection, we required a tagged version of PAX6 that did not jeopardize PAX6 *in vivo* function. Thus, we tested PAX6 fused to either amino- or carboxyl-terminal 3xFLAG for the ability to generate ectopic eye structures in *X. laevis* tadpoles. PAX6 and 3xFLAG/PAX6 mRNA drove similar ectopic eye formation, suggesting that this amino-terminal 3xFLAG tag did not disrupt PAX6 function. Delivery of rAAV to the cornea was then explored using topical, intravitreal, intravenous, and subconjunctival routes with ssAAV9 smCBA-EmGFP-WPRE and ssAAV9 Ple253-EmGFP-WPRE. The MiniPromoter Ple253 was derived from the human *PITX3* gene and had previously shown, using a different methodology, to express in the corneal stroma (de Leeuw et al., 2016). In this study, stromal expression was achieved with both intravenous and subconjunctival routes.
**Conclusions:** These results have laid the foundation for rAAV9 delivery of *in vivo* functional 3xFLAG/PAX6, with either a ubiquitous or cell-type restricted promoter, by either intravenous or subconjunctival routes, to treat corneal pathology in *Pax6*$^{Sey/+}$ mice.
**Commercial Relationships: Elizabeth M. Simpson**; **Siu Ling Lam**, None; **Andrea Korecki**, None; **Beatrice M. Tam**, None; **Orson L. Moritz**, None; **Jack Hickmott**, None
**Support:** Brain Canada, Quebec Consortium for Drug Discovery (CQDM), Canadian Institutes of Health Research, Sharon Stewart Aniridia Research Trust, Foundation Fighting Blindness – Canada, National Science and Engineering Research Council, Studentships from the University of British Columbia

**Program Number:** 4482 **Poster Board Number:** B0137
**Presentation Time:** 11:00 AM–12:45 PM
**Improved intravitreal AAV-mediated inner retinal gene transduction by surgical internal limiting membrane (ILM) peeling in cynomolgus monkeys**
*Kazuhisa Takahashi[1, 2], Tsutomu Igarashi[1, 2], Koichi Miyake[2], Maika Kobayashi[1], Chiemi Yaguchi[1], Osamu Iijima[2], Yoshiyuki Yamazaki[2], Noriko Miyake[1], Shuhei Kameya[3], Takashi Shimada[2], Hiroshi Takahashi[1], Takashi Okada[2].*
[1]Ophthalmology, Nippon Medical School, Tokyo, Japan; [2]Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan; [3]Ophthalmology, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan.
**Purpose:** The adeno-associated virus (AAV) vector is an ideal tool for retinal gene therapy, and there are two administration routes, subretinal injection (SR) and intravitreal injection (IV). SR is used in many studies because of its efficiency of gene transduction of outer retina. However, SR induces an iatrogenic retinal detachment, which causes several adverse effects. On the other hand, IV is considered to be safer than SR, although the transduction efficiency in large animals was insufficient, as the vitreous and internal limiting membrane (ILM) acted as barriers to transduction. To overcome this issue, we performed vitrectomy (VIT) and ILM peeling before AAV vector injection.
**Methods:** Among the six eyes in the three female cynomolgus monkeys (aged 10-13 years old), two eyes received standard 3-port VIT (VIT group), two eyes received ILM peeling in addition to the standard 3-port VIT (VIT+ILM group), and the remaining two received no pretreatment (IV group) at 4 weeks before AAV injection. All eyes were penetrated by 30-G needle into the vitreous at the pars plana, and 50 µl of triple-mutated self-complementary AAV serotype 2 vector ($1.9 \times 10^{13}$ v.g./ml) encoding enhanced green fluorescent protein (GFP) was administered. Nineteen weeks after intravitreal injection of AAV vector, transduction efficiency was analyzed. To detect potential adverse effects, the retinas were monitored using color fundus photography, optical coherence tomography (OCT) and electroretinography (ERG).
**Results:** Little expression of GFP was detected in the IV and VIT groups. On the other hand, strong GFP expression was detected within the peeled ILM area in the VIT+ILM group. Intraocular inflammation was observed in 4 of 6 eyes, however the inflammation was transient and no morphological changes were detected on color fundus images or OCT. Transient reduction of ERG amplitude was detected during the period of intraocular inflammation.
**Conclusions:** These results indicate that surgical ILM peeling before intravitreal AAV vector administration would be safe and useful for efficient transduction of the nonhuman primate retina and provide therapeutic benefits for the treatment of glaucoma and retinal diseases.
**Commercial Relationships: Kazuhisa Takahashi**, None; **Tsutomu Igarashi**, None; **Koichi Miyake**, None; **Maika Kobayashi**, None; **Chiemi Yaguchi**, None; **Osamu Iijima**, None; **Yoshiyuki Yamazaki**, None; **Noriko Miyake**, None; **Shuhei Kameya**, None; **Takashi Shimada**, None; **Hiroshi Takahashi**, None; **Takashi Okada**, None

**Program Number:** 4483 **Poster Board Number:** B0138
**Presentation Time:** 11:00 AM–12:45 PM
**AAV-mediated Knockdown and Replacement of Rhodopsin Protects Rods in a Canine Model of *RHO*-ADRP**
*William A. Beltran[1], Artur V. Cideciyan[2], Raghavi Sudharsan[1], Michael Massengill[3], Brian Rossmiller[3], Valerie L. Dufour[1], felipe Pompeo Marinho[2], Tatyana Appelbaum[1], Malgorzata Swider[2], Mychajlo S. Kosyk[2], Simone Iwabe[1], William W. Hauswirth[4], Samuel G. Jacobson[2], Alfred S. Lewin[3], Gustavo D. Aguirre[1].* [1]Clinical Studies, Univ of Pennsylvania Sch Vet Med, Philadelphia, PA; [2]Ophthalmology, University of Pennsylvania, USA, PA; [3]Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; [4]Ophthalmology, University of Florida, Gainesville, FL.
**Purpose:** To develop an allele-independent corrective gene therapy approach for *RHO*-ADRP that combines the knockdown (KD) of both the mutant and WT *RHO* alleles and their replacement with a "resistant" codon-modified human *RHO* cDNA, and to test its efficiency in preventing rod loss in the light-sensitive canine T4R RHO mutant dog.
**Methods:** An shRNA ($shRNA_{820}$) designed to target a homologous region of both human and dog *RHO*, and shown to efficiently KD *RHO* in 293T cells expressing GFP-tagged human *RHO* was packaged in an AAV2/5 vector under the H1 promoter (cloned as a self-complementary cassette). Four adult WT ($RHO^{+/+}$) and 4 mutant ($RHO^{T4R/+}$) dogs were each injected subretinally (volume: ~ 150 ml) with one of four titers ranging from $1 \times 10^{11}$ to $1 \times 10^{12}$ vg/ml. Seven to eight weeks post-injection, retinal integrity was assessed by cSLO/OCT imaging, and by histology /IHC on cryosections. Efficiency of RHO KD was measured both at the RNA and protein levels from retinal tissue punches collected both in the treated (vector bleb) and untreated areas. Following identification of the optimal therapeutic titer, an AAV2/5 construct containing both $shRNA_{820}$ (under H1 promoter control), and a resistant codon-modified human RHO cDNA ($RHO_{820}$) under control of a human opsin (HOP) promoter was tested in $RHO^{T4R/+}$ dogs. Testing employed an acute light damage paradigm that rapidly assesses the effect of therapeutic intervention in preventing light-induced loss of rods in the T4R RHO mutant dog.
**Results:** scAAV2/5-H1-$shRNA_{820}$ achieved a KD efficiency that ranged between 5% and 100 % at the protein level and between 26% and 100 % at the RNA level with titers ranging between $1x10^{11}$

and $1x10^{12}$ vg/ml. Efficient (85-100 %) KD of RHO protein was achieved at $5 \times 10^{11}$ vg/ml. Light-induced retinal degeneration in $RHO^{T4R/+}$ dogs was prevented, yet was associated with a loss of rod OS and reduced RHO immunolabeling. However, treatment with the combination virus (scAAV2/5-HOP-$RHO_{820}$-H1-$shRNA_{820}$) at $5x10^{11}$ vg/ml preserved ONL thickness and rod OS integrity.
**Conclusions:** We have identified a very efficient KD reagent ($shRNA_{820}$) able to suppress canine RHO expression when delivered with viral titers that appear to be safe. Its combination with the replacement (resistant human RHO cDNA) component results in protection from light-induced retinal degeneration and paves the way for a novel allele-independent gene therapy for *RHO*-ADRP.
**Commercial Relationships: William A. Beltran**; **Artur V. Cideciyan**, None; **Raghavi Sudharsan**, None; **Michael Massengill**, None; **Brian Rossmiller**, None; **Valerie L. Dufour**, None; **felipe Pompeo Marinho**, None; **Tatyana Appelbaum**, None; **Malgorzata Swider**, None; **Mychajlo S. Kosyk**, None; **Simone Iwabe**, None; **William W. Hauswirth**, AGTC (F), AGTC (P), AGTC (C); **Samuel G. Jacobson**, None; **Alfred S. Lewin**, None; **Gustavo D. Aguirre**, None
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**Program Number:** 4484 **Poster Board Number:** B0139
**Presentation Time:** 11:00 AM–12:45 PM
**LHON Mouse model carrying human *ND1G3460A***
*Hong Yu, John Guy*. Ophthalmology, Bascom Palmer Eye Inst, Univ of Miami, Miami, FL.
**Purpose:** To generate an LHON mouse model with the same genotype and phenotype as human G3460A mutation in *ND1*
**Methods:** The *hND1G3460* gene fused with *MYC* epitope tag was cloned into a self-complementary AAV backbone, under the control of the mitochondrial heavy strand promoter including 3 upstream conserved sequence blocks of the D-loop responsible for replication. Mitochondrial encoded Cherry(*mCherry*) was put downstream of the mutant gene to visualize transgene expression in live mice. After packaging with mito-targeted AAV. rAAV was injected into the adult mouse vitreous or was microinjected into fertilized oocytes to generate transgenic mitomice. Expression and function of the mutant gene were assessed by laser capture microdissection, qPCR, immunostaining, pattern electroretinography(PERG), and spectral domain optical coherence tomography(SD-OCT)
**Results:** Mutant *hND1* DNA was 25 times that of endogenous mouse *ND1*(*mND1*) in the RGC layer, 3 times in the INC layer and 2 times in the ONC layer of injected mice. Longitudinal sections of the retina reacted with antibodies against MYC and mCherry showed the expression of hND1 and mCherry in RGC layer. SD-OCT showed thinning of RGC layer and inner plexiform layer in *hND1G3460A* injected mice. Compared to control mice injected with *mCherry*, the PERG amplitude dropped by 50% in mice injected with *hND1G3460A*(p=8.6E-5) by 12 months after injection, and the rate of ATP synthesis was reduced by 28% in the optic nerve(p=0.02). Intravitreal injection of wildtype human *ND1* rescued visual function caused by *hND1G3460A* and significantly preserve PERG amplitude compared to unrescued mice(p=0.02). In addition, we generated 109 mitomice carrying *hND1G3460A* using microinjections. qPCR showed that mutant *hND1* was 10 times that of *mND1* in the RGC layer, 3 times in the INC layer and 0.19 times in the ONC layer of the mitomice. Immunostaining demonstrated expression hND1 and mCherry in the RGC layer of mitomouse retina. PERG showed a progressive decrease of the amplitude in mitomice as the mice aged and the reduction became significant when the mice are 12 months old(p=0.003). SD-OCT revealed optic atrophy in the mitomice at 10 months of age. Complex I activity decreased by 80% in the brain of mitomice relative to wild type controls(p=0.04).
**Conclusions:** Mice carrying *hND1G3460A* developed visual loss, optic atrophy and decreasing respiratory chain function. This mouse model may be used to test potential treatments for human LHON
**Commercial Relationships: Hong Yu**, None; **John Guy**, None
**Support:** NIH Grants R01 EY017141, EY012355, and P30-EY014801

**Program Number:** 4485 **Poster Board Number:** B0140
**Presentation Time:** 11:00 AM–12:45 PM
**AAV2-*hCHM* Subretinal Delivery to the Macula in Choroideremia: Preliminary Six Month Safety Results of an Ongoing Phase I/II Gene Therapy Trial**
*Tomas S. Aleman[1, 2], Leona Serrano[1], Grace K. Han[1], Denise J. Pearson[1], Sarah McCague[2, 3], Kathleen A. Marshall[2, 3], Daniel C. Chung[4], Emily Liu[4], Jessica I. Morgan[1], Jean Bennett[1, 2], Albert M. Maguire[1, 2]*. [1]Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA; [2]Center for Advanced Retinal and Ophthalmic Therapeutics, Department of Ophthalmoilogy, University of Pennsylvania, Philadelphia, PA; [3]The Children's Hospital of Philadelphia, Philadelphia, PA; [4]Spark Therapeutics, Philadelphia, PA.
**Purpose:** To assess preliminary safety data of the investigational subretinal delivery of a recombinant adeno-associated virus serotype 2 (AAV2) vector carrying a human REP-1-encoding cDNA in choroideremia (CHM).
**Methods:** Nine subjects with CHM (ages 26-57 years), received uniocular subfoveal injections of low dose (up to $5x10^{10}$ vector genome (vg) per eye, n=5) or high dose (up to $1x10^{11}$ vg per eye, n=4) AAV2-hCHM. Patients were evaluated pre- and post-operatively for 6 months with follow-up ongoing. Ocular safety was assessed by ophthalmic examination, perimetry, spectral domain optical coherence tomography (SD-OCT) and short-wavelength autofluorescence (SW-FAF).
**Results:** There were no surgery-related complications or unexpected adverse events. Visual acuity (VA) returned to baseline in all but one patient who slowly recovered to within 20 letters of baseline by 6 months. This subject had foveal thinning (to ~80% of baseline) and foveal sensitivity loss. All other patients showed no obvious changes in foveal structure post-injection. Mean sensitivity by light-adapted perimetry (10-2 protocol) was unchanged in treated and control eyes. Fundus perimetry showed non-significant (<3SD of the intervisit variability) increases in light sensitivity in some locations and subjects in the operated eye. Cone-mediated sensitivities by two-color dark-adapted perimetry showed non-significant increases in sensitivity above mean baseline for some locations in the operated eye of three subjects. Residual islands of retinal pigment epithelium preservation by SW-FAF showed trace reduction in area that was not significantly different in the injected eye compared to the control. There were no obvious dose-dependent relationships.
**Conclusions:** VA returned to baseline in most subjects after subfoveal injections of AAV2-hCHM. Foveal thinning and slow recovery of VA with unchanged perifoveal function in one patient raises the possibility of non-vector related individual vulnerability to the subfoveal injection. Not unexpectedly, short-term increases in sensitivity in the operated eyes of a subset of patients did not exceed the intervisit or inter-ocular variability estimates. Longer observation intervals are required to better evaluate the significance of the preliminary changes.

**Commercial Relationships: Tomas S. Aleman**, None; **Leona Serrano**, None; **Grace K. Han**, None; **Denise J. Pearson**, None; **Sarah McCague**, None; **Kathleen A. Marshall**, None; **Daniel C. Chung**, Spark Therapeutics (E), Spark Therapeutics (I); **Emily Liu**, Spark Therapeutics (E), Spark Therapeutics (I); **Jessica I. Morgan**, AGTC (F), US Patent 8226236 (P), Canon (F); **Jean Bennett**, Biogen (F), Spark Therapeutics (S), Sanofi (C), Limelight (F), Gensight Biologics (S), Spark Therapeutics (P); **Albert M. Maguire**, Spark Therapeutics (F)
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**Clinical Trial:** NCT-02341807

**Program Number:** 4486 **Poster Board Number:** B0141
**Presentation Time:** 11:00 AM–12:45 PM
**Evaluation of AAV2tYF-GRK1-RPGR vectors in a canine model of *RPGR*-XLRP**
*Guo-jie Ye[1], William A. Beltran[2], Valerie L. Dufour[2], Felipe Pompeo Marinho[2], Inna Martynyuk[2], Chuanjian Song[1], David Knop[1], Gustavo D. Aguirre[2], Jeffrey D. Chulay[1], Mark S. Shearman[1].*
[1]Applied Genetic Technologies Corp, Alachua, FL; [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.
**Purpose:** In previous studies, an AAV2tYF capsid and GRK1 promoter were more efficient than an AAV5 and IRBP promoter for transducing primate photoreceptors, and two stable human RPGR cDNAs (the full length codon-optimized RPGRco or stablized RPGRstb) were both effective at early-stage disease in the XLPRA2 dog model of XLRP due to a mutation in the *RPGR* gene. We now compared the efficacy of 3 dose levels of RPGRco and RPGRstb vectors with a AAV2tYF capsid and GRK1 promoter in XLPRA2 dogs at mid-stage disease (~12 wks of age).
**Methods:** Two dogs per group received a 0.15 mL subretinal injection of AAV2tYF-RPGRco in one eye and AAV2tYF-RPGRstb in the other eye at each of 3 doses ($1.2 \times 10^{11}$, $6 \times 10^{11}$ or $3 \times 10^{12}$ vg/ml). One dog received the mid-dose of AAV5-RPGRco and 1 dog received vehicle, in both eyes. Rescue of photoreceptor structure was assessed by clinical examination, and histology/IHC on retinal cryosections at termination 8 wks post-injection.
**Results:** No abnormal ophthalmic findings were noted in any eyes at the mid- or low-dose levels, but there was retinal inflammation and retinal detachment in the 4 eyes treated with the high-dose of either vector. Retinal perivascular infiltration consisting primarily in T cytotoxic (CD8) and B (CD20) lymphocytes, with some T helper (CD4) cells were found in the bleb area of all eyes treated with the high doses and one eye with AAV2tYF-RPGRco at mid-dose. Both RPGRco and RPGRstb vectors achieved dose-dependent RPGR transgene expression in photoreceptors, with greater RPGR expression in eyes injected with RPGRco vector than in contralateral eyes injected with RPGRstb vector at the same dose levels. Correction of rod opsin and middle/long wavelength (M/L) cone opsin mislocalization was demonstrated in all AAV-RPGR treated eyes. Müller cell activation was decreased in eyes injected with the low and mid-doses but not in eyes injected with the high dose, likely due to the associated inflammation and retinal detachment.
**Conclusions:** With the AAV2tYF capsid and GRK1 promoter, greater RPGR expression was achieved with the vector expressing the RPGRco cDNA than with the vector expressing the RPGRstb cDNA. Optimal correction at mid-stage disease with limited inflammation, including improved structure of cones, correction of rod and M/L cone opsin mislocalization and reduced reactivity of Müller cells, was achieved in this model with the mid-doses of both vectors (0.15 mL at $6 \times 10^{11}$ vg/ml).
**Commercial Relationships: Guo-jie Ye**, AGTC (E), AGTC (I); **William A. Beltran**, Biogen (P), AGTC (P); **Valerie L. Dufour**, None; **Felipe Pompeo Marinho**, None; **Inna Martynyuk**, None; **Chuanjian Song**, AGTC (E), AGTC (I); **David Knop**, AGTC (E), AGTC (I); **Gustavo D. Aguirre**, Biogen (P), AGTC (P); **Jeffrey D. Chulay**, AGTC (E), AGTC (I); **Mark S. Shearman**, AGTC (E), AGTC (I)

**Program Number:** 4487 **Poster Board Number:** B0142
**Presentation Time:** 11:00 AM–12:45 PM
**Fundus Autofluorescence Analysis of the Transition Zone in Choroideremia: Outcomes Following Gene Therapy**
*Ioannis S. Dimopoulos[1], Jake Knowles[1], Tanvir Sajed[2], Ian M. MacDonald[1].* [1]Ophthalmology and Visual Sciences, University of Alberta, Edmonton, AB, Canada; [2]Computing Science, University of Alberta, Edmonton, AB, Canada.
**Purpose:** To evaluate rescue of the transition zone (TZ) autofluorescence in six subjects undergoing unilateral subretinal AAV2.REP1 gene replacement therapy (NCT02077361).
**Methods:** Bilateral serial 30-degree fundus autofluorescence (FAF) images (Spectralis BluePeak, Heidelberg Engineering) were acquired at baseline and at 6-month intervals post-intervention. Longitudinal images were aligned using the i2k Align Retina platform (Dual-Align LCC, Clifton Park, NY) to compensate for image distortion, changes in resolution, pixel density and focus. Semi-automated serial segmentation of stacked images was performed using custom code written in Matlab (MathWorks Inc.). Expert graders also manually segmented the images using the polygon selection tool in ImageJ (NIH) to determine the ground-truth. Change from baseline in FAF area was quantified at 6-month intervals and compared between treated and untreated eyes.
**Results:** Baseline residual FAF areas ranged from 8.63 to 72.64 $mm^2$ (median: 12.26 $mm^2$), with high symmetry between the two eyes (r=0.83). The semi-automated algorithm showed on average 88.95% similarity (Dice similarity coefficient) with the manually segmented ground truth, with a test-retest repeatability of 3%. After 6 months, an nonsignificant increase in FAF area loss was noted in the treated eyes (8.1% vs. 4.5% in the untreated; p=0.30). FAF signal loss occurred exclusively at the TZ between healthy and degenerated retina. At the 12-month timepoint, change from baseline in FAF area was similar between the treated (11.86%) and untreated (9.86%) eyes.
**Conclusions:** FAF allows accurate and reliable monitoring of the TZ activity in CHM. A semi-automated segmentation algorithm reduced significantly the measurement bias and error in FAF area calculation. In this limited cohort of CHM subjects, TZ appears not to be rescued by gene therapy. Longer follow-up is required to determine whether gene therapy shows any benefit in modifying the natural history of RPE loss in CHM.
**Commercial Relationships: Ioannis S. Dimopoulos**, None; **Jake Knowles**, None; **Tanvir Sajed**, None; **Ian M. MacDonald**, None
**Support:** Foundation Fighting Blindness Canada/Canadian Institutes of Health Research/Choroideremia Research Foundation Canada Team Grant, Alberta Innovates Health Solutions CRIO Grant 201201139
**Clinical Trial:** NCT02077361

**Program Number:** 4488 **Poster Board Number:** B0143
**Presentation Time:** 11:00 AM–12:45 PM
**Safety and Efficacy of AAV2tYF-PR1.7-CNGA3 in CNGA3-deficient Sheep**
*mark S. shearman[1], Elisha Gootwine[2], Eyal Banin[3], Ron Ofri[4], Raaya Ezra-Elia[4], Guo-jie Ye[1], Paulette Robinson[1], David Knop[1], Jeffrey D. Chulay[1]*. [1]AGTC, Alachua, FL; [2]Institute of Animal Science, Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel; [3]Hadassah-Hebrew University Medical Center, Kiryat Hadassah, Israel; [4]Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel.
**Purpose:** Achromatopsia is an inherited retinal disorder characterized by markedly reduced visual acuity, extreme light sensitivity and absence of color discrimination. AGTC is developing AAV2tYF-PR1.7-CNGA3, a recombinant adeno-associated virus (rAAV) vector expressing *CNGA3*, for treatment of CNGA3-related achromatopsia. Here we report results of a toxicology and efficacy study of this vector administered by subretinal injection in CNGA3-deficient sheep.
**Methods:** Groups of 4 or 5 animals received a 0.5 mL subretinal injection in the right eye of AAV2tYF-PR1.7-CNGA3 at one of two dose levels or AAV5-PR2.1-hCNGA3, a vector previously shown to rescue cone photoreceptor responses. The left eye received a 0.5 mL subretinal injection of vehicle (4 animals) or was untreated (9 animals). Toxicity assessment was based on mortality, clinical observations, ophthalmic examinations, ERG, and clinical and anatomic pathology. CNGA3 expression was assessed by immunohistochemistry. Efficacy was assessed by cone ERG responses and maze navigation testing performed before, then 6 and 12 weeks after treatment.
**Results:** Treatment was not associated with systemic toxicity. Most animals had mild to moderate conjunctival hyperemia, chemosis and subconjunctival hemorrhage immediately after surgery that generally resolved by post-operative Day 7. No clear and consistent test article-related effects were noted in any group during the 12 week study. Two animals treated with the higher dose of AAV2tYF-PR1.7-CNGA3 had microscopic findings of outer retinal atrophy, with or without inflammatory cells in the retina and choroid, that were procedural- and/or test article-related. All vector-treated eyes developed cone-mediated ERG responses with no change in rod-mediated ERG responses. Behavioral maze testing showed significantly improved navigation times and reduced numbers of obstacle collisions in all vector-treated eyes compared to their control eye or pre-dose results in the treated eye.
**Conclusions:** Subretinal injection of AAV2tYF-PR1.7-CNGA3 in CNGA3-deficient sheep was well tolerated with no clinically important toxicology findings. Rescue of cone-mediated ERG responses was recorded in all vector-treated eyes. These results support the use of AAV2tYF-PR1.7-CNGA3 in clinical studies in patients with achromatopsia caused by CNGA3 mutations. A Phase 1/2 clinical trial evaluating AAV2tYF-PR1.7-hCNGA3 is scheduled to begin in 2017.
**Commercial Relationships: mark S. shearman**, AGTC (E), AGTC (I); **Elisha Gootwine**, None; **Eyal Banin**, None; **Ron Ofri**, None; **Raaya Ezra-Elia**, None; **Guo-jie Ye**, AGTC (E), AGTC (I); **Paulette Robinson**, AGTC (E), AGTC (I); **David Knop**, AGTC (E), AGTC (I); **Jeffrey D. Chulay**, AGTC (E), AGTC (I)

**Program Number:** 4489 **Poster Board Number:** B0144
**Presentation Time:** 11:00 AM–12:45 PM
**Genetic engineering of freshly isolated primary human pigment epithelial cells by non-viral gene delivery for the treatment of neovascular AMD**
*Sandra Johnen[1], Sabine Diarra[1], Nina Harmening[2], Corinne Marie[3], Daniel Scherman[3], Zsuzsanna Izsvák[4], Peter Walter[1], Gabriele Thumann[2, 5]*. [1]Department of Ophthalmology, University Hospital RWTH Aachen, Aachen, Germany; [2]Laboratory of Ophthalmology, University of Geneva, Geneva, Switzerland; [3]Unité de Technologies Chimiques et Biologiques pour la Santé UMR 8258, CNRS, Paris, France; [4]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany; [5]Department of Ophthalmology, University Hospitals of Geneva, Geneva, Switzerland.
**Purpose:** Gene therapy is a promising treatment for many diseases, and viral vectors are already used in clinical applications. However, their drawbacks have encouraged the improvement of non-viral strategies. We have developed an approach for the treatment of neovascular age-related macular degeneration (nAMD). nAMD is characterized by choroidal blood vessels growing into the subretinal space, which results from an overexpression of the vascular endothelial growth factor and a decreased expression of the pigment epithelium-derived factor (PEDF). Our therapeutic approach involves the transplantation of pigment epithelial (PE) cells that have been engineered to stably overexpress PEDF. The *PEDF* transgene is incorporated into the cell's genome by the *Sleeping Beauty* (*SB100X*) transposon system delivered by electroporation of non-viral miniplasmids free of antibiotic resistance markers (pFAR).
**Methods:** Immediately after isolation, PE cells from human donor eyes were co-transfected with two pFAR4 derivatives encoding *PEDF* and the *SB100X* transposase using two different electroporation systems. PEDF secretion was evaluated by immunoblotting and quantified by ELISA. *PEDF* gene expression was analyzed via quantitative real-time PCR (qRT-PCR).
**Results:** Effective transfection was demonstrated in various independently performed experiments using as few as $2x10^4$ freshly isolated cells. The amount of secreted PEDF was 0.6 ng/h/$1x10^4$ cells, and thus 6-fold higher compared to the PEDF secretion rate of non-transfected cells. qRT-PCR analysis revealed a 3.2-fold increase in the median of total *PEDF* gene expression compared to the endogenous level. However, extended postmortem times until cell isolation resulted in reduced PEDF ratios.
**Conclusions:** *SB100X*-mediated transfection of freshly isolated PE cells resulted in an increase in both PEDF secretion and *PEDF* gene expression. The pFAR technology further improves the biosafety of non-viral gene transfer, as it allows for plasmid propagation in the absence of antibiotics, which is a crucial safety issue for human trials. The transfection of freshly isolated PE cells is an important step towards the ultimate goal of the TargetAMD project, which is the efficient and safe delivery of the *PEDF* transgene to autologous cells *ex vivo*, followed by the transplantation to the subretinal space of nAMD patients.
**Commercial Relationships: Sandra Johnen**, None; **Sabine Diarra**, None; **Nina Harmening**, None; **Corinne Marie**, None; **Daniel Scherman**, None; **Zsuzsanna Izsvák**, None; **Peter Walter**, None; **Gabriele Thumann**, None
**Support:** European Union's Seventh Framework Programme for Research, Technological Development and Demonstration, grant No. 305134

**Program Number:** 4490 **Poster Board Number:** B0145
**Presentation Time:** 11:00 AM–12:45 PM
**Intravitreally administered AAV-mediated short-hairpin RNA against mTOR efficiently reduces neovascularization in the murine model of laser-induced choroidal neovascularization**
*Tae Kwann Park[1], Jun-Sub Choi[2], Hee Jong Kim[2], Hayan Park[4], Seung Kwan Nah[1], Eung Suk Lee[1], Young-Kook Choi[2], Heuiran Lee[3], Young-Hoon Ohn[1], Keerang Park[2]*. [1]Ophthalmology, Soonchunhyang Univ Hospital, Bucheon-si, Korea (the Republic of); [2]Cdmogen Co. ltd, Cheongju-si, Korea (the Republic of); [3]Microbiology, Asan Medical center, University of Ulsan, Seoul, Korea (the Republic of); [4]Institute for laboratory medicine, Soonchunhyang Hospital Bucheon, Bucheon, Korea (the Republic of).
**Purpose:** we evaluated the therapeutic effect of a self complement adeno-associated virus (scAAV)-delivered mammalian target of rapamycin (mTOR) short hairpin RNA in the laser-induced choroidal neovascularization of the mouse eye.
**Methods:** Self complementray adeno-associated virus (scAAV) based expression vector including GFP and mTOR shRNA (scAAV-GFP-mTOR shRNA) originally designed for the cancer treatment was used in this study.
Choroidal neovascularization was induced via laser photocoagulation (LP) in 8 week-old male C57/B6 mice (n=45). Intravitreal injection of AAV-GFP-mTOR shRNA (1μl of 5.0E+10 vg/ml) or AAV-GFP-scrambled shRNA (1 μl of 5.0E+10 vg/ml) was performed to the right eye of each animal (15 mice per each group) at 5 days after LP. Fifteen laser-treated mice with the same volume of 0.1% PBS injection were used as negative control. Transduction patterns and therapeutic effects of AAV-GFP-mTOR shRNA were evaluated at 14 days after LP. Anti-GFP antibody was used for the evaluation of the transduction efficiency into the CNV-induced retinal tissue. The expression pattern of mTOR through the chorioretinal tissue was detected with anti-mTOR antibody. Anti-LC3B and ATG7 antibodies were used to detect the autophagy. Furthermore, anti-F4/80, CD31, and Brn3 antibodies were also used. Neuroretinal injury was assessed with TUNEL staining.
**Results:** Expression of GFP was observed through the inner retina and cellular components in the CNV. These pattern of GFP expression were also well collocalized with CD31 (+) and F4/80 (+) cells located in the CNV tissue. In scAAV-GFP-mTOR shRNA-treated group, CNV volume was significantly reduced ($p<0.05$). Furthermore, CD31 and F4/80 expression were decreased. In addition, the expression of LC3B abd ATG7 were dramatically increased in the CNV sites of the scAAV-GFP-mTOR shRNA-treated eye. However, expression of these autophagic markers in the neuroretinal tissue was not significantly changed. Interestingly, the number of TUNEL (+) cells detected in the outer nuclear layer around the CNV tissue was dramatically decreased in the scAAV-GFP-mTOR shRNA-treated eye.
**Conclusions:** scAAV espressing mTOR shRNA along with GFP as a reporter gene did efficiently suppress laser-induced CNV in the mouse eye. These data confirm the induction of autophagy in the CNV can be used for the treatment of nAMD.
**Commercial Relationships: Tae Kwann Park**; **Jun-Sub Choi**, None; **Hee Jong Kim**, None; **Hayan Park**, None; **Seung Kwan Nah**, None; **Eung Suk Lee**, None; **Young-Kook Choi**, None; **Heuiran Lee**, None; **Young-Hoon Ohn**, None; **Keerang Park**, None

**Program Number:** 4491 **Poster Board Number:** B0146
**Presentation Time:** 11:00 AM–12:45 PM
**Investigation of intracellular innate immune responses to AAV gene therapy in the retina**
*Laurel C. Chandler[1, 2], Howell Fu[1], Sarah Caddy[3], Alun R. Barnard[1, 2], Maria I. Patrício[1, 2], Leo James[3], Cristina Rada[3], Robert E. MacLaren[1, 2], Kanmin Xue[1, 2]*. [1]Nuffield Laboratory of Ophthalmology, Department of Clinical Neuroscience, University of Oxford, Oxford, United Kingdom; [2]Oxford Eye Hospital, Oxford University Hospitals, NHS Foundation Trust, Oxford, United Kingdom; [3]MRC Laboratory of Molecular Medicine, Cambridge, United Kingdom.
**Purpose:** Gene therapy using recombinant adeno-associated viral (AAV) vectors holds great promise for treating inherited retinal dystrophies. Recent research has established intracellular innate immune mechanisms for the detection of viral DNA and restriction of several pathogenic viruses, including HIV, hepatitis B and human papilloma virus. However, little is known about this response in relation to AAV, which is generally thought to have low immunogenicity. We investigated intracellular innate immunity to AAV transduction in the retina, with the aim of uncovering its impact on the efficacy of retinal gene therapy.
**Methods:** An AAV2 vector encoding green fluorescent protein (GFP) was injected subretinally into 4 week old female C57BL/6 mice, with sham injections of phosphate-buffered saline undertaken in the contralateral eye (n=3 per time point). RNA was extracted from the mouse retina at baseline and on day 3, 7 and 15 post-injection. The expression of a panel of genes implicated in intracellular innate immunity was quantified using reverse transcription qPCR.
**Results:** GFP expression was observed in the AAV injected eyes at all time points analysed. A range of cytosolic viral nucleic acid sensors (*cGas*, *Trim21*, *Rig-I* and *Sting*) and anti-viral cytokines (*Tnf*-α, *Cxcl10*, *Isg15* and *Ifn*-γ) were upregulated 7 days post-injection in the AAV injected eyes. Furthermore, by day 7, the expression of *Apobec3* and *Apobec1* were upregulated 16 and 10 fold respectively compared to sham. The induction of *Apobec3* and *Apobec1*, which both encode enzymes implicated in the deamination and degradation of viral DNA in the nucleus, was statistically significant both relative to sham and baseline expression (ANOVA followed by Tukey HSD or paired 2-tailed Student's t-test respectively; $p<0.05$). There was no detectable change in other *Apobec* family members (*Apobec2* and *Aicda*).
**Conclusions:** Intracellular innate immune responses involving anti-viral sensors (*Apobec3*, *Apobec1*, *cGAS* and *Rig-I*), mediators (*Sting*) and effectors (*Trim21* and pro-inflammatory cytokines) are activated in response to AAV gene therapy in the retina, independent of surgical trauma. Further work is needed to elucidate the effects of these responses on the efficacy and longevity of gene therapy.
**Commercial Relationships: Laurel C. Chandler**, None; **Howell Fu**, None; **Sarah Caddy**, None; **Alun R. Barnard**, None; **Maria I. Patrício**, None; **Leo James**, None; **Cristina Rada**, None; **Robert E. MacLaren**, None; **Kanmin Xue**, None
**Support:** University of Oxford and National Institute for Health Research, UK

**Program Number:** 4492 **Poster Board Number:** B0147
**Presentation Time:** 11:00 AM–12:45 PM
**Gene Therapy for LHON Suppresses Neurodegeneration**
*John Guy, Rajeshwari D. Koilkonda, William J. Feuer, Janet L. Davis, Vittorio Porciatti, Phillip Gonzalez, Huijan Yuan, Byron L. Lam*. Bascom Palmer Eye Institute, MIAMI, FL.
**Purpose:** Contradicting our initial preclinical studies, Oca-Cossio and coworkers showed that allotopic expression by delivering a

mitochondrial targeting sequence appended to a nuclear-encoded *ND4* gene killed cells suggesting it was harmful. Here we show that the retinal nerve fiber layer (RNFL) of patients with LHON caused by the G11778A is not damaged by allotopic ND4 gene therapy.
**Methods:** 14 patients with mutated G11778A mtDNA were injected with gene therapy vector AAV-P1ND4v2. As of 11/2016, they returned for 5 or more post-injection visits (3 to 18 months). Eyes with the worse visual acuity were treated except two with unilateral visual loss injected into the good eye before losing vision. Testing included ETDRS acuity, visual fields, OCT, PERG and neuro-ophthalmic examinations. Protocol post-injection OCTs were not collected between 2 and 12 months follow up.
**Results:** Nine treated patients had at least 12 months of follow-up. In eyes selected for treatment average temporal RNFL thickness at the baseline2 was 54 μm and at month12, it was 55 μm and average overall RNFL thickness was 71 μm at baseline2 and 58.8 μm at month12. Month 12 average acuity improvements with treatment relative to baseline2 were 0.24 logMAR. Fellow eyes had a 0.1 logMAR improvement with the temporal RNFL being 55.8 μm at baseline2 and dropping to 50.1 at month12. Average temporal RNFL was thicker in treated than in fellow eyes at month12, p = 0.013. Average overall RNFL thickness in fellow eyes was 82.2 μm at baseline2 and 59.6 μm at month12. Although average RNFL thickness of injected eyes at baseline2 was thinner than fellow eyes (p=0.043), no significant differences of average overall RNFL thickness were detected at month12. Eight patients were treated before 24 months of visual loss. Four patients improved by ~0.3 logMAR or more and four did not. The average temporal RNFL at the first baseline examination was 65 μm in four patients whose vision improved, but lower in the 4 patients who did not improve with an average of 51.75 μm (not statistically significant). On the day prior to injection, measurements were 55.2 μm in the eyes selected for injection vs 60 μm in fellow eyes. Twelve months after treatment, average temporal RNFL thickness was 56 μm in treated eyes relative to 48 μm in the fellow eyes, p = 0.026.
**Conclusions:** OCT suggests that allotopic ND4 does not damage the temporal RNFL subserving macular fibers targeted by an intravitreal AAV2 injection.
**Commercial Relationships: John Guy**, U.S. Patent No. 7,405, 284 (P), U.S. Patent No.: 8,278,428 (P); **Rajeshwari D. Koilkonda**, None; **William J. Feuer**, None; **Janet L. Davis**, None; **Vittorio Porciatti**, None; **Phillip Gonzalez**, None; **Huijan Yuan**, None; **Byron L. Lam**, None
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**Clinical Trial:** NCT0216380

**Program Number:** 4493 **Poster Board Number:** B0148
**Presentation Time:** 11:00 AM–12:45 PM
**Structural Modelling Predicts Variability in Efficacy of Lead Candidate Hammerhead Ribozyme Between Different Species Rhodopsin mRNA**
*Alexandria J. Trujillo[1, 2], Beau R. Froebel[3], Jack M. Sullivan[1, 4].* [1]Research Service, VA Western NY Healthcare System, Buffalo, NY; [2]Pharmacology and Toxicology, University at Buffalo, Buffalo, NY; [3]Ophthalmology (Ross Eye Institute), University at Buffalo, Buffalo, NY; [4]Ophthalmology (Ross Eye Institute), Pharmacology & Toxicology, Physiology & Biophysics, Neuroscience Program, University at Buffalo, Buffalo, NY.
**Purpose:** Hammerhead ribozymes (hhRz) cleave target mRNAs at 'NUH' (N= any nucleotide H= U, A, C) sites. When developed for therapeutic purposes the target site must be in a region of the mRNA that is accessible in a stable structure *in vivo*. Our lab has designed a hhRz that cleaves the 725nt region of human Rhodopsin mRNA (h*RHO*), as a therapeutic for Autosomal Dominant Retinitis Pigmentosa (adRP.) Conventional methods to bring a therapeutic to clinical trial generally require efficacy and toxicity studies conducted in non-human animals. Rat, mouse, pig, and dog are commonly used as adRP model organisms in therapeutic screening. This study was conducted to determine the potential impact that use of non-human animals would have on evaluating a ribozyme that targets h*RHO* mRNA, and to further characterize and optimize our 725 GUC↓ hhRz.
**Methods:** *RHO* mRNA sequences for Human and 15 non-human animals (relevant to adRP/evolutionary relations) were folded at the secondary level using SFold, MFold, and RNAStructure (OligoWalk) algorithms. These algorithms predict structure based on single stranded accessibility by Boltzmann weighting, minimal folding energy, and local free energy, respectively. The product of these output vectors determine the Multiparameter Prediction of RNA accessibility (MPPRNA) and were graphed and area under the curve (AUC) was calculated.
**Results:** For all animals (n=15) MPP values and AUC varied from human regardless of sequence homology in both regions. One-way ANOVA demonstrated MPP values were significantly different ($p<0.05$) between all species, and 2-way independent t-tests showed AUC values were significantly different ($p<0.05$) from human for the majority of animal *RHO* mRNAs tested (n=9, 60%).
**Conclusions:** Primary mRNA sequence or protein homology is insufficient information to predict mRNA accessibility and efficacy of post-transcriptional gene silencing agents such as hhRzs. Algorithms that predict mRNA folding provide an expedited analysis for structural similarity to the human mRNA target as a proxy for cleavage efficacy. Because of the predicted structural variations between even evolutionarily close organisms, in a single gene that encodes highly homologous proteins, development studies for PTGS agents should be conducted with human samples or mRNAs derived from human genes in transgenic animals.
**Commercial Relationships: Alexandria J. Trujillo**, None; **Beau R. Froebel**, None; **Jack M. Sullivan**, None
**Support:** NIH/NEI R01EY013433 (JMS), Veterans Administration Merit Award 1I01 BX000669 (JMS), SUNY Health Now Award (JMS), Research to Prevent Blindness Unrestricted Award (Univ. Buffalo)

**Program Number:** 4494 **Poster Board Number:** B0149
**Presentation Time:** 11:00 AM–12:45 PM
**Cleavage of Human Rhodopsin mRNA and Protein Knockdown using Lead HHRz-725, a Candidate Therapeutic for Autosomal Dominant Retinitis Pigmentosa**
*Zahra Fayazi[1, 2], Mark C. Butler[1, 2], Jack M. Sullivan[1, 3].* [1]Research Service, VA Western NY Healthcare System, Buffalo, NY; [2]Ophthalmology (Ross Eye Institute), University at Buffalo-SUNY, Buffalo, NY; [3]Ophthalmology (Ross Eye Institute), Pharmacology/Toxicology, Physiology/Biophysics and Program in Neuroscience, University at Buffalo-SUNY, Buffalo, NY.
**Purpose:** Lead hammerhead ribozyme (hhRz)-725 embedded in an engineered anticodon domain of the small human tRNA-Lys3 scaffold has potential as a therapeutic agent for autosomal dominant retinitis pigmentosa (adRP). Following demonstrated improved catalytic activity of hhRz-725 *in vitro*, we tested activity in cell culture. Advancement in characterization places us one step closer to testing this post transcriptional gene silencing (PTGS) agent in an adRP animal model.

**Methods:** tRNA-Ribozymes were cloned into a plasmid with T7 promoter to drive *in vitro* transcription. Full length human RHO (hRHO) including 5′ UTR and part of 3′UTR was cloned into plasmids driven by T7 promoter for *in vitro* and CMV for expression into HEK-293S cells. *In vitro* cleavage reactions used T7 RNA polymerase for co-transcription and cleavage of hhRz and hRHO RNAs with analysis by PAGE. Real time PCR for hRHO was performed using primers spanning exon1 and 2 and an internal probe containing a fluorescent dye (FAM) at the 5′ and a quenching dye (BHQ1) at the 3′ end and b-actin as endogenous control and analyzed by comparative CT method (ΔΔCT method).
**Results:** We studied cleavage activity of hhRz-725 *in vitro* and cell culture on short and full length versions of hRHO mRNA. Since we found hhRz-725 has superior cleavage activity in the context of a smaller tRNA scaffold than prior adenoviral VAI scaffold, we tested it with no scaffold and four different constructs containing a smaller stem-II in hhRz-725 (Mini-725) but showed no enzymatic improvement. Looking at the combination of hhRz-725 and hhRz-266 and hhRz-1364 with hRHO the catalytic function showed independent cleavage activity for each hhRz. Quantification of hRHO mRNA by real time RT/PCR and protein using 1D4 antibody to hRHO in cells transfected with VAI-hhRz-725 showed ~40% knockdown both in mRNA (p=1.58865E-4) and protein level (p=1.08485E-6). Also tRNA-hhRz-725 showed ~40 % hRHO mRNA (p=0.01688) knockdown. While less specific PTGS agent shRNA at the (725 GUC↓) showed more than 90% (p=2.92915E-7) RHO mRNA knockdown by real time RT/PCR.
**Conclusions:** We are optimizing our lead hhRz-725 in the human tRNA scaffold and showed hRHO mRNA cleavage *in vitro* and also knockdown of hRHO mRNA and protein in cell culture. We are now in the process of advancing the ribozyme for testing in a humanized mouse model of adRP.
**Commercial Relationships: Zahra Fayazi**; **Mark C. Butler**, None; **Jack M. Sullivan**, Research Foundation of SUNY (P)
**Support:** NIH/NEI R01EY013433 (JMS); Veterans Administration Merit Award 1I01 BX000669 (JMS); SUNY Health Now Award (JMS); Research to Prevent Blindness Unrestricted Award (Univ. Buffalo)

**Program Number:** 4495 **Poster Board Number:** B0150
**Presentation Time:** 11:00 AM–12:45 PM
**RNA Mango - Finding a Needle in a Haystack: Tracking Rhodopsin mRNA using RNA Aptamers**
*Jennifer B. Breen[2], Alexandria J. Trujillo[2, 3], Mark C. Butler[1, 2], Sriganesh Ramachandra Rao[2], Jack M. Sullivan[1, 4].* [1]Research Service, VA Western NY Healthcare System, Buffalo, NY; [2]Ophthalmology (Ross Eye Institute), University at Buffalo-SUNY, Buffalo, NY; [3]Pharmacology/Toxicology, University at Buffalo-SUNY, Buffalo, NY; [4]Ophthalmology (Ross Eye Institute), Pharmacology/Toxicology, Physiology/Biophysics and Program in Neuroscience, University at Buffalo-SUNY, Buffalo, NY.
**Purpose:** Specific 3′UTR mRNA sequences (e.g. zip codes) traffic RNA out of the nucleus and into the cytoplasm for protein production, and this knowledge is critical for success of post-transcriptional gene silencing agents (e.g. ribozymes) as co-localization is essential to enzymatic efficacy. We hypothesize a newly developed RNA Aptamer "MANGO", conjugated with a small molecule fluorescent dye "TO1-Biotin", can label a target mRNA, track the path/distribution, and aid in biotechnological applications (e.g. SELEX) to develop more efficient hammerhead ribozymes.
**Methods:** Two MANGO aptamers (M and M-C4) were inserted in the 1410 nt stem/loop of a 1532 nt human rhodopsin (h*RHO*) cDNA and *in vitro* transcribed. The 1410 nt region was chosen based on RNA structure algorithm predictions of accessibility and maintenance of hRho overall structure. Equal molar concentrations of TO1-Biotin were added, and fluorescence observed on a gel-imaging box. Controls include WT hRho, buffer, and dye alone. Cells transfected with hRho M and M-C4 cDNA constructs were incubated with TO1-Biotin, Alexa647-1D4 antibody and DAPI and visualized on a confocal microscope (EX/EM 488/535nm). Fluorescence intensity was quantified using ImageJ, and analyzed by two-tailed Student's t-test. HEK293S cells alone, cells with WT hRho serve as controls.
**Results:** With an intrinsic 260nm excitation, visualization of TO1-Biotin binding to *in vitro* transcribed hRho M, hRho M-C4 and WT hRho RNA at 100nM is seen with RFUs at 66.2, 50.2, 24.5 respectively. Using ImageJ, WT hRho mRNA (n=12) Corrected Total Cell Fluorescence (CTCF) for TO1-Biotin binding is 4605.96 (p=0.94); while hRho M-C4 (n=11) had a CTCF of 43158.36 (p=1.91E-3), which indicates specificity to the MANGO aptamer in mammalian cells. Images show DAPI (blue) stained cells containing Alexa647 (red) bound to hRho proteins, overlaid with clusters of TO1-Biotin (green), which indicates that hRho MANGO mRNA is present during and does not disrupt protein production.
**Conclusions:** Preliminary data suggests that MANGO aptamer may be used to track hRho mRNA produced and shuttled within the cell and cytoplasm. High-resolution information for mRNA localization requires further optimization. Spatial assessment of mRNA hRho trafficking may allow optimal hammerhead ribozyme delivery to co-localize within photoreceptors of the eye.
**Commercial Relationships: Jennifer B. Breen**, None; **Alexandria J. Trujillo**, None; **Mark C. Butler**, None; **Sriganesh Ramachandra Rao**, None; **Jack M. Sullivan**
**Support:** NIH/NEI R01EY013433 (JMS) Veterans Administration Merit Award 1I01 BX000669 (JMS) SUNY Health Now Award (JMS) Research to Prevent Blindness Unrestricted Award (Univ. Buffalo)

**Program Number:** 4496 **Poster Board Number:** B0151
**Presentation Time:** 11:00 AM–12:45 PM
**Hammerhead ribozyme in a tRNA scaffold: Effects of RNA processing and anti-sense flank length on cleavage in-vivo**
*Jason Myers[1, 2], Zahra Fayazi[2], Mark C. Butler[1, 2], Jack M. Sullivan[1, 3].* [1]Research Service, VA Western NY Healthcare System, Buffalo, NY; [2]Ophthalmology (Ross Eye Institute), University at Buffalo-SUNY, Buffalo, NY; [3]Ophthalmology (Ross Eye Institute), Pharmacology/Toxicology, Physiology/Biophysics and Program in Neuroscience, University at Buffalo-SUNY, Buffalo, NY.
**Purpose:** Embed a therapeutic hammerhead ribozyme (hhRz) within a human tRNA-Lys3 scaffold and screen modifications to achieve and optimize a gene therapy agent for Stargardt/dry AMD/adRP by suppression of human rod rhodopsin (hRHO).
**Methods:** tRNA hhRz processing and mRNA were assayed by endpoint RT/PCR and real time RT/PCR. hRHO knockdown by various 725 hhRzs and varying anti-sense (AS) flank lengths was assayed by RT/PCR, Alexa-647-1D4 IHC, and a reporter from a bicistronic construct (phRHO-IRES-SuperNova). HhRz kinetic model of Stage-Zimmermann and Uhlenbeck (1998) and RNAstructure were used for modelling.
**Results:** tRNAs are transcribed by RNA Pol III, but addition of an upstream H1 promoter/enhancer increases tRNA levels substantially. Fractionation and RNA isolation from H1 tRNA hhRz transfected cells with a 3' CCA lead to loss of hhRz during processing, while lack of 3' CCA allows nuclear export where the tRNA hhRz reaches a cytoplasmic steady-state (SS) concentration which modulates collision frequency with hRHO mRNA. When SS is reached cleavage efficiency depends on the hhRz enzyme kinetics which are influenced

by the length of the hhRz AS flanks. Kinetic modelling of our initial hhRz with 11 nt 5' and 7 nt 3' AS flanks predicted reaction kinetics with a lack of binding specificity ($K_{binding}$ = 1.74E-17 M) and product inhibition (product leaving rates of 6.67E-4 $min^{-1}$ and 2.1 $min^{-1}$). In transfected cells this tRNA hhRz led to an increase in hRHO levels (mRNA, protein) compared to tRNA scaffold alone (p=0.01009). Decreasing 725 AS flanks to 7/7, 6/6 and 5/5 dropped RHO levels significantly (>50%) compared to Scaffold (7/7 p=0.02072, 6/6 p=1.42E-5, 5/5 p=1.16E-4). Our new lead candidate H1 725 6/6 -CCA had a binding affinity of 0.49 nM and leaving rates of 5 $min^{-1}$ and 17.8 $min^{-1}$ which provides specificity without product inhibition. Using Supernova as a reporter for hhRz cleavage, H1 tRNA 725 6/6 –CCA suppressed fluorescence levels by 72% compared to scaffold (p=3.25E-52).

**Conclusions:** A therapeutic 725 hhRz driven by the H1 promoter/enhancer with 6nt/6nt AS flanks suppresses levels of membrane bound hRHO protein by 50% in transient transfection experiments. This tRNA hhRz delivered by AAV to photoreceptors has potential to rescue light-induced damage of the ABCA4/RDH8 double knockout mouse (Stargardt, dAMD model) and intrinsic retinal degeneration in our humanized adRP hRHO mouse.

**Commercial Relationships: Jason Myers**; **Zahra Fayazi**, None; **Mark C. Butler**, None; **Jack M. Sullivan**, Research Foundation of SUNY (P)

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**Program Number:** 4497 **Poster Board Number:** B0152
**Presentation Time:** 11:00 AM–12:45 PM

**Adeno-associated virus dual vector-mediated gene therapy for ABCA4 Stargardt Disease**

*Frank M. Dyka, Vince A. Chiodo, William W. Hauswirth*. Ophthalmology, University of Florida, Gainesville, FL.

**Purpose:** ATP binding cassette transporter A4 (ABCA4) is a flippase localized in the rim region of photoreceptor outer segments. It is involved in the clearance of all-trans-retinal from the disks. Loss of this function leads to the accumulation of N-retinylidene-N-retinylethanolamine (A2E) in the RPE, a major component of Lipofuscin and ultimately the Stargardt Disease phenotype. Mutations in the ABCA4 gene are associated with the most common form of recessive Stargardt Disease. As a monogenic disease it is a viable target for gene replacement therapy. Lentiviral vectors are suboptimal for the transduction of differentiated cells like photoreceptors. In contrast, Adeno-associated virus vectors have shown great potential for gene transfer due to their low immunogenicity and their ability to infect dividing and post-mitotic cells equally efficiently. However, the payload capacity of AAV is limited to 4.8 kb. Hence the large size of the ABCA4 coding sequence of 6.8 kilobases, make it impossible to deliver the transgene in a single capsid. To overcome this drawback dual vectors were developed where the coding sequence is split between two vectors and packaged in separate capsids. After co-infection the two halves come together through homologous recombination or nonhomologous end joining to reconstitute the full length coding sequence. Here we present data on AAV dual vector mediated gene expression of ABCA4 and its effect on A2E accumulation *in vivo* in an ABCA4 knock out mouse.

**Methods:** The human *ABCA4* coding sequence was cloned into AAV dual vector pairs, where one vector contained the promoter and the 5' portion of the cDNA sequence and the second vector contained the 3' portion and a polyA signal. ABCA4 knock out mice were injected with AAV dual vector pairs and retinal function was measured by electroretinography followed by lipofuscin/A2E autofluorescence employing a confocal scanning laser ophthalmoscope.

**Results:** Expression of full length ABCA4 from AAV dual vectors was detected *in vitro* and *in vivo*. *In vivo* measurements revealed a marked reduction in A2E accumulation by fundus autofluorescence in injected versus uninjected eyes of ABCA4 knock out mice.

**Conclusions:** cDNAs exceeding the payload capacity of AAV can be expressed with high efficiency and specificity using dual AAV vectors. Our results show that AAV dual vector delivered ABCA4 can have a significant effect on A2E accumulation.

**Commercial Relationships: Frank M. Dyka**, None; **Vince A. Chiodo**, None; **William W. Hauswirth**, AGTC (F), AGTC (P), AGTC (C)

**Support:** grant from AGTC, NIH grant EY021721, the Macular Vision Research Foundation, and Research to Prevent Blindness, Inc.

**Program Number:** 4498 **Poster Board Number:** B0153
**Presentation Time:** 11:00 AM–12:45 PM

**Gene therapy with constitutively active focal adhesion kinase (FAK) suppresses irreversible visual loss in experimental optic neuritis mice**

*Venu Talla, M Livia Bajenaru, Vittorio Porciatti, John Guy*. Ophthalmology, Bascom Palmer Eye Institute, Miami, FL.

**Purpose:** Vision loss in optic neuritis and other optic neuropathies is attributed to the loss of retinal ganglion cells (RGCs) and its axons. Knockout of phosphatase tensin homologue (PTEN) in RGCs is well known for its effect on RGC survival and axonal outgrowth in optic neuropathies. FAK is under the control of PTEN and in the absence of PTEN, FAK activates the cell survival signaling pathways involved in RGC survival and axon outgrowth. The purpose of the current study is to evaluate the effect of constitutively active FAK gene therapy on visual loss and optic neuropathy in the experimental autoimmune encephalomyelitis (EAE) mouse model

**Methods:** Constitutively active FAK with an HA epitope tag (Y397E FAK-HA) was cloned in pAAV vector and packaged into AAV2 capsids (AAV2-FAK-E). Expression of FAK-E in the retina was confirmed two weeks after gene transfer by HA tag immunofluorescence (IF). EAE was induced in female DBA/1J (n=20) mice by subdermal injection of 0.1 ml homologous spinal cord emulsion in CFA. Ten EAE sensitized mice received intravitreal injection of AAV2-FAK-E in both eyes. EAE mice (n=10) and CFA sensitized mice (n=10) injected with scAAV-GFP served as controls. Visual function was assessed by pattern electroretinograms (PERG) at 3 and 6 months post injection (MPI). All mice were euthanized 9MPI; retinas and ONs were dissected for histological evaluation. RGC survival was assessed by immunofluorescence in retinal flat-mounts.

**Results:** Immunofluorescence with HA tag antibody revealed expression of FAK-E in 75% of RGCs in the retina of AAV2-FAK-E injected mice. FAK gene therapy significantly rescued the PERG amplitudes in EAE mice compared to GFP ($p<0.05$). PERG amplitudes in EAE-GFP mice were decreased by 22% compared to CFA controls (p=024; p=0.037; at 3 and 6 MPI). Whereas, amplitude in EAE FAK-E group was comparable to CFA control. FAK-E gene transfer into EAE mice retina significantly increased RGC (RBPMS positive cells) survival compared to EAE-GFP group (p=0.023). RGC numbers were 33% less in EAE GFP group compared to EAE-FAK-E injected group.

**Conclusions:** FAK-E gene therapy preserves vision by limiting the RGC loss in experimental optic neuritis. Use of FAK-E gene therapy might be an option to treat the optic neuritis and multiple sclerosis patients.

**Commercial Relationships: Venu Talla**, None; **M Livia Bajenaru**, None; **Vittorio Porciatti**, None; **John Guy**, None
**Support:** R01EY07892 (JG), EY017141 (JG), EY012355 (JG), P30-EY014801 (VP)

**Program Number:** 4499 **Poster Board Number:** B0154
**Presentation Time:** 11:00 AM–12:45 PM
**Preclinical evaluation of rAAV8.CNGA3 in the *Cnga3* knockout mouse model of ACHM2**
*Stylianos Michalakis[1], Christian Schön[1], Regine Mühlfriedel[2], Vithiyanjali Sothilingam[2], Bernd Wissinger[2], Mathias W. Seeliger[2], Martin Biel[1]*. [1]Center for Integrated Protein Science Munich CiPSM at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany; [2]Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany.
**Purpose:** Mutations in the cyclic nucleotide-gated channel alpha 3 subunit (*CNGA3*) gene are known to cause achromatopsia type 2 (ACHM2) characterized by poor visual acuity, photophobia and lack of color discrimination. Here, a novel recombinant adeno-associated virus (AAV) vector for gene supplementation therapy of *CNGA3*-linked ACHM2 was developed and tested for efficacy in the preclinical *Cnga3* knockout (KO) mouse model of ACHM2.
**Methods:** An AAV-based gene supplementation vector coding for full length human *CNGA3* under control of the human, cone-specific cone arrestin promoter was generated and used for packaging of AAV8-pseudotyped vectors. The resulting vector (rAAV8.CNGA3) was tested for efficacy by subretinal injections in two-week-old *Cnga3* KO mice with short-term and long-term analysis at 8 weeks and 12 months post-injection, respectively. AAV vector-mediated expression of human CNGA3 protein was assessed by immunohistochemistry using a CNGA3-specific antibody combined with confocal microscopy. Efficacy was assessed in vivo by electroretinography (ERG) with protocols designed to isolate cone-specific light responses.
**Results:** We designed and generated rAAV8.CNGA3, a novel AAV vector optimized for efficient CNGA3 gene expression in human cone photoreceptors. Transgene expression assay of rAAV8.CNGA3 in *Cnga3* KO mice confirmed efficient and specific human CNGA3 protein expression that localized to cone photoreceptor outer segments. A biological activity assay for rAAV8.CNGA3 based on ERG analysis confirmed a beneficial effect of the treatment at both observation time points. Additionally, we found prolonged survival of cone photoreceptors up to 12 months after treatment.
**Conclusions:** The novel rAAV8.CNGA3 vector supports efficient and specific transgene expression and biological activity in the preclinical *Cnga3* KO mouse model of ACHM2.
**Commercial Relationships: Stylianos Michalakis**; **Christian Schön**, None; **Regine Mühlfriedel**, None; **Vithiyanjali Sothilingam**, None; **Bernd Wissinger**, None; **Mathias W. Seeliger**, EYESERV (P); **Martin Biel**, EYESERV (P)
**Support:** Tistou and Charlotte Kerstan Foundation

**Program Number:** 4500 **Poster Board Number:** B0155
**Presentation Time:** 11:00 AM–12:45 PM
**Preclinical evaluation of rAAV8.PDE6A in the *Pde6a*$^{D670G}$ mutant mouse model of RP43**
*Christian Schön[1], Regine Mühlfriedel[2], Vithiyanjali Sothilingam[2], Francois Paquet-Durand[2], Bernd Wissinger[2], Mathias W. Seeliger[2], Martin Biel[1], Stylianos Michalakis[1]*. [1]Center for Integrated Protein Science Munich CiPSM at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany; [2]Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany.
**Purpose:** Mutations in the phosphodiesterase 6 alpha (*PDE6A*) gene are known to cause retinitis pigmentosa type 43 (RP43) – A currently cureless blinding disease. Here, a novel recombinant adeno-associated virus (AAV) vector for gene supplementation therapy of *PDE6A*-linked RP was developed and tested for efficacy in the preclinical *Pde6a*$^{D670G}$ mouse model of RP43.
**Methods:** An AAV-based gene supplementation vector coding for full length human *PDE6A* under control of the human, rod-specific rhodopsin promoter was generated and used for packaging of AAV8-pseudotyped vectors. The resulting vector (rAAV8.PDE6A) was tested for efficacy by subretinal injections in two-week old *Pde6a*$^{D670G}$ mutant mice with short-term and long-term analysis at 4 weeks and 6 months post-injection, respectively. AAV vector-mediated expression of human PDE6A protein was assessed by immunohistochemistry using a PDE6A-specific antibody combined with confocal microscopy. Efficacy was assessed by measuring morphological preservation *in vivo* using optical coherence tomography (OCT) and *ex vivo* by analyzing histological cross-section of treated and untreated retinas.
**Results:** We designed and generated rAAV8.PDE6A, a novel AAV vector optimized for efficient PDE6A gene expression in human rod photoreceptors. Transgene expression assay of rAAV8.PDE6A confirmed efficient and specific human PDE6A protein expression that localized to rod photoreceptor outer segments. A biological activity assay for rAAV8.PDE6A based on analysis of morphological preservation confirmed a beneficial effect of the treatment at both observation time points. Additionally, we found a preservation of rod outer segment structure and prolonged survival of cone photoreceptors up to 6 months after treatment.
**Conclusions:** The novel rAAV8.PDE6A vector supports efficient and specific transgene expression and biological activity in the preclinical *Pde6a*$^{D670G}$ mouse model of RP43.
**Commercial Relationships: Christian Schön**, EyeServ (P); **Regine Mühlfriedel**, None; **Vithiyanjali Sothilingam**, None; **Francois Paquet-Durand**, None; **Bernd Wissinger**, None; **Mathias W. Seeliger**, EyeServ (P); **Martin Biel**, EyeServ (P); **Stylianos Michalakis**, EyeServ (P)
**Support:** Tistou and Charlotte Kerstan Foundation

**Program Number:** 4501 **Poster Board Number:** B0156
**Presentation Time:** 11:00 AM–12:45 PM
**In-frame exon skipping fails to rescue retinal or hair cell defects in zebrafish models of *PCDH15* (USH1F)**
*Jennifer B. Phillips, Jeremy Wegner, Monte Westerfield*. Inst of Neuroscience, University of Oregon, Eugene, OR.
**Purpose:** The R245X mutation in *PCDH15* R245X mutation causes a severe form of Usher Syndrome. The affected codon lies in an in-frame exon that encodes part of two consecutive E-cadherin domains in the extracellular region of PCDH15. This region is proximal to a protein-protein interaction domain required in stereocilia, but of unknown function in retinal tissue. To evaluate in-frame exon-skipping as a therapy for *PCHD15(R245X)* patients, we deleted

Exon 8 from zebrafish *pcdh15* genes and assayed visual and auditory function.
**Methods:** CRISPRs with zebrafish-optimized Cas9 were injected into zygotes to generate germline mutations. Sequence analysis of gDNA and cDNA confirmed the deletions and revealed production of transcripts lacking exon 8. Behavioral and histological analyses were performed to assay hearing, balance, cellular morphology and cell death. Images were captured with an iOS camera or Zeiss LSM5 Confocal microscope.
**Results:** We analyzed behavior, cell morphology and photoreceptor cell death in zebrafish *pcdh15aE8*Δand *pcdh15bE8*Δ lines, each containing in-frame deletions of Exon 8. We also examined truncating mutations in exon 8 of both zebrafish *PCDH15* orthologs (*pcdh15aE8X* and *pcdh15bE8X*, respectively) showing partially overlapping functions with a greater requirement for *pcdh15a* in inner ear and for *pcdh15b* in retina. We used these lines as positive controls for experiments with the in-frame exon deletions. *pcdh15aE8*Δ and *pcdh15bE8*Δ animals recapitulated the swimming and balance phenotypes seen in the E8 truncating mutants for each gene, with no improvements or differences noted in mechanosensory hair cell structure or function. Mild photoreceptor cell morphology defects and low levels of photoreceptor death that was not exacerbated by exposure to increased illumination observed in *pcdh15aE8X* larvae were recapitulated in *pcdh15aE8*Δ larvae. Severe PRC morphology defects and marked PRC death that increased with elevated light intensity was the same in *pcdh15bE8X* and *pcdh15bE8*Δ larvae.
**Conclusions:** We conclude that disruption of EC1 and EC2 domains is incompatible with normal PCDH15 protein function in auditory and visual sensory cells, even when more C-terminal portions of the protein remain intact. Our rapid and inexpensive evaluation of this therapeutic approach in zebrafish disease models informs future approaches for alternative allele-specific treatments of the R245X mutation
**Commercial Relationships: Jennifer B. Phillips**; **Jeremy Wegner**, None; **Monte Westerfield**, None
**Support:** NIH P01HD22486, Vision for a Cure and the Usher 1F Collaborative

**Program Number:** 4502 **Poster Board Number:** B0157
**Presentation Time:** 11:00 AM–12:45 PM
**Intracellular expression of mini-chaperone confers resistance to apoptosis from oxidative and thermal stress in cos7 cells**
*Puttur Santhoshkumar, K Krishna Sharma*. Ophthalmology, University of Missouri-Columbia, Columbia, MO.
**Purpose:** Therapeutic potentials of peptide chaperone derived from αA-crystallin is well established. The objective of this study is to prepare a mini-gene construct capable of expressing functional peptide chaperone(s) in mammalian cells.
**Methods:** A stable cell line expressing a mini-chaperone (MGKFVIFLDVKHFSPEDLTVK) was prepared by transfecting Cos7 cells with linearized pcDNA3.1(+) vector containing the engineered mini-gene inserted between EcoRI and XbaI sites and grown on Geneticin selective medium including 5% serum and maintained at 37$^{o}$C and 5% $CO_2$. Cos7 cells transfected with linearized empty vector served as control. The anti-apoptotic activity of the intracellularly expressed mini-chaperone was tested by treating the cells grown on a 96-well plate with 75, 150 and 300 μM $H_2O_2$ for 16 h followed by TUNEL assay. The anti-apoptotic activity of the mini-chaperone against hyperthermia was investigated by incubating the culture plates at 55$^{o}$C for 1h and the live/dead cell in the two groups were visualized by staining the cells with HCS NuclearMask™ Red stain and Image-IT® DEAD Green™ Viability Stain at 37$^{o}$C for 30 min. The plates were imaged using the SpectraMax Minimax 300 Imaging Cytometer (Molecular Devices) and the live/dead cells were quantified using Softmax Pro software.
**Results:** Cell cultures transfected with mini-gene showed 55 – 80% less TUNEL positive cells when compared to control groups. Cells transfected with empty vector (positive control) failed to provide any protection against $H_2O_2$ and was comparable to untransfected cells (negative control). In the Live/Dead assay, cells transfected with empty vector showed increased number of dead cells (8.6 + 2.3%) compared to cells transfected with mini-gene (2.2 + 1.2 %). Our data suggests that the intracellular expression of mini-chaperone protects Cos7 cells from oxidative and thermal stress.
**Conclusions:** Functional mini-chaperones can be expressed in cells. Mini-gene therapy can be a possible strategy for treating diseases involving oxidative stress, protein aggregation, and apoptosis.
**Commercial Relationships: Puttur Santhoshkumar**, None; **K Krishna Sharma**, None
**Support:** NIH Grant EY 023219

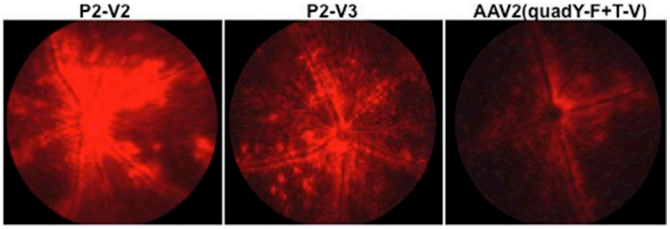
**Program Number:** 4503 **Poster Board Number:** B0158
**Presentation Time:** 11:00 AM–12:45 PM
**Overexpression of human Nrf2 rescues cone photoreceptors and retinal pigment epithelial cells from degeneration in the rd1 mouse**
*David M. Wu[1, 2], Xuke J. Ji[1, 2], Brian P. Hafler[2], Yunlu Xue[2], Sophia Zhao[2], Parimal Rana[2], Wenjun Xiong[3], Connie Cepko[2].*
[1]Retina Service, Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Genetics, Harvard Medical School, Boston, MA; [3]Biomedical Sciences, City University of Hong Kong, Kowloon, Hong Kong.
**Purpose:** Overexpression of mouse Nrf2 in the rd1 mouse, a model of retinitis pigmentosa, results in rescue of cones and retinal pigment epithelial cells. The next step towards evaluating the potential for Nrf2 for human disesae therapy is to assess its ability to rescue retinal degeneration in larger animals. In planning such studies, it would be useful to know whether the product of the human Nrf2 gene is able to rescue across species, since this is the gene product of most interest in the development of human therapies.
**Methods:** An AAV 2/8 was constructed using a CMV promoter and human-beta globin intron to drive expression of the human Nrf2 gene (AAV 2/8 CMV human Nrf2). A construct with the same promoter complex encoding GFP strongly drives GFP expression in cone photoreceptors and retinal pigment epithelial cells in mouse retina (AAV 2/8 CMV GFP). The AAV 2/8 driving the human Nrf2 gene was delivered to the subretinal space of rd1 mouse eyes at p0. For controls, some eyes were left uninjected and other eyes were injected with AAV 2/8 with the same promoter complex driving GFP or mouse Nrf2 gene (AAV 2/8 CMV mouse Nrf2). The mice were sacrificed at P40 or P70. Cones were identified by immunostaining for cone arrestin and retinal pigment epithelial cells were identified by phalloidin.
**Results:** All rd1 eyes at P40 and P70 showed evidence of retinal degeneration, with cone and retinal pigment epithelial cell loss higher in the central eye compared to the peripheral eye. However, there was significantly less loss in eyes infected with AAV 2/8 CMV human Nrf2 or AAV 2/8 mouse Nrf2 than in those that had been uninjected, or injected with AAV 2/8 CMV GFP. There was not a marked difference in eyes receiving AAV 2/8 CMV human Nrf2 compared to AAV 2/8 CMV mouse Nrf2.
**Conclusions:** Overexpression of human Nrf2 in the degenerating eyes of rd1 mice rescues cone photoreceptors and retinal pigment epithelial cells. There is significant homology between the coding sequence and protein sequence for human and mouse Nrf2 (83 and 82% respectively). Given that sequence homology is similar or greater in Nrf2 genes from large animals (89% in dogs and 99% in

rhesus monkeys) that may be utilized in studies to evaluate Nrf2 as a potential therapeutic gene for human gene therapy trials, these results suggest that it may be feasible to assess rescue by the human Nrf2 gene across species.
**Commercial Relationships: David M. Wu**, None; **Xuke J. Ji**, None; **Brian P. Hafler**, None; **Yunlu Xue**, None; **Sophia Zhao**, None; **Parimal Rana**, None; **Wenjun Xiong**, None; **Connie Cepko**, Astellas (F)
**Support:** NIH NEI EY023993-03, Massachusetts Lion Eye Research Fund, Inc., HHMI

**Program Number:** 4504 **Poster Board Number:** B0159
**Presentation Time:** 11:00 AM–12:45 PM
**Characterization of hypoxia–regulated, glial cell specific AAV vector for targeting retinal neovascularization**
*Janet C. Blanks, Howard Prentice, James Sullivan*. Ctr for Complex Systems & Brain Sci, Florida Atlantic University, Boca Raton, FL.
**Purpose:** The Muller cell is known to secrete angiogenic factors under conditions of hypoxia exposure similar to the mouse oxygen induced retinopathy (OIR) model or in diabetic retinopathy. Previously, we constructed adeno-associated virus vector (AAV) gene therapy vectors whose transgene expression is driven by a hypoxia responsive domain combined with the glial fibrillary associated protein (GFAP) promoter. Here we developed and tested a hypoxia regulated and glial cell specific vector encoding human decorin which functions as an antiangiogenic protein.
**Methods:** Plasmids for AAV generation were constructed with the human decorin cDNA together with the CMV promoter enabling generation of the virus AAV-CMV-Decorin or with the GFAP promoter giving virus AAV-GFAP-Decorin or with the GFAP promoter PLUS a hypoxia responsive domain (HRE) giving AAV-HRE-GFAP-Decorin. AAVs were then packaged using these plasmids for subsequent infection of primary rat brain astrocytes, PC12 neuronal cells or retinal epithelial ARPE-19 cells. After infection cells were subjected to 72 h of hypoxia. Levels of mRNA for human decorin were measured by quantitative real time RT-PCR.
**Results:** Following infection of primary astrocytes with AAV-HRE-GFAP and hypoxia treatment there was a significant induction in decorin mRNA of greater than 5 fold relative to normoxic cells. Infection with AAV-GFAP-Decorin resulted in high level expression of decorin mRNA either in the absence or presence of hypoxia. Neither AAV-GFAP-Decorin nor regulated AAV-HRE-GFAP-Decorin infected into ARPE-19 cells or PC12 cells resulted in significant decorin mRNA expression with or without hypoxia. By contrast AAV-CMV-Decorin infection resulted in high level expression of decorin mRNA in ARPE19 cells and in PC12 cells with or without hypoxia.
**Conclusions:** Our data indicates that AAV-HRE-GFAP-Decorin drives hypoxia inducible, as well as glial cell specific expression, of decorin mRNA. AAV-GFAP-Decorin showed glial cell specific expression that was not further modified by hypoxic exposure. However, AAV-CMV-Decorin was ubiquitously expressing decorin mRNA in all cell types tested in both conditions of hypoxia and normoxia. These studies contribute to comparing the relative efficacy of hypoxia responsive and glial cell specific AAV-HRE-GFAP-decorin to glial cell specific vector AAV-GFAP-decorin for antiangiogenic treatment in diabetic retinopathy.
**Commercial Relationships: Janet C. Blanks**, None; **Howard Prentice**, None; **James Sullivan**, None

**Program Number:** 4506 **Poster Board Number:** B0161
**Presentation Time:** 11:00 AM–12:45 PM
**AAV-mediated delivery of ABCA4 to the photoreceptors of *Abca4*$^{-/-}$ mice using an overlapping dual vector strategy**
*Michelle E. McClements[1], Alun R. Barnard[1], Mandeep S. Singh[2], Zhichun Jiang[3], Peter Charbel Issa[4], Roxana A. Radu[3], Robert E. MacLaren[1, 4]*. [1]Nuffield Department of Clinical Neurosciences (Ophthalmology), University of Oxford, Oxford, United Kingdom; [2]John Hopkins University, Baltimore, MD; [3]Ophthalmology, Stein Eye Institute, David Geffen School of Medicine, California, CA; [4]Oxford Eye Hospital, Oxford, United Kingdom.
**Purpose:** Gene therapy development for disorders caused by mutations in large genes is an advancing field. A dual vector approach for delivering the large gene split in two parts and carried in two separate adeno-associated viral (AAV) vectors is feasible, but given the likely reduction in transduction efficiency associated with this strategy, achieving therapeutic levels of protein in target cells has to date remained challenging. Here we reveal that a customized overlapping dual vector strategy can successfully deliver robust levels of ABCA4 protein to the photoreceptor outer segments of the *Abca4*$^{-/-}$ mouse as assessed by immunohistochemistry.
**Methods:** Overlapping transgenes were packaged in AAV8 Y733F capsids. The upstream transgene contained the human rhodopsin kinase (*GRK1*) promoter and an upstream portion of the *ABCA4* CDS. The downstream transgene contained a downstream portion of the *ABCA4* CDS, Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) and polyA signal (pA). Both the upstream and downstream transgenes carried a region of *ABCA4* CDS overlap. *Abca4*$^{-/-}$ mice received a 2 µl sub retinal injection at 4-5 weeks of age containing a 1:1 mix of upstream and downstream vectors (1 x 10e13 gc/ml). Eyes were harvested 6 weeks post-injection for immunohistochemical (IHC) assessments. Eyes were fixed, frozen and sectioned prior to IHC staining. Staining for Hcn1 was used as a photoreceptor inner segment marker.
**Results:** Uninjected *Abca4*$^{-/-}$ and injected controls did not stain for Abca4/ABCA4 in the photoreceptor outer segments. However, dual vector treated eyes did show robust ABCA4 staining in the photoreceptor outer segments. No off-target ABCA4 expression was observed in other cell types.
**Conclusions:** An overlapping dual vector approach using the AAV8 Y733F serotype generates readily detectable levels of therapeutic protein in the photoreceptor outer segment structures.
**Commercial Relationships: Michelle E. McClements**, University of Oxford (P); **Alun R. Barnard**, None; **Mandeep S. Singh**, None; **Zhichun Jiang**, None; **Peter Charbel Issa**, None; **Roxana A. Radu**, None; **Robert E. MacLaren**, University of Oxford (P)
**Support:** MRC DPFS/DCS grant MR/K007629/1, Fight for Sight grant 1882

**Program Number:** 4507 **Poster Board Number:** B0162
**Presentation Time:** 11:00 AM–12:45 PM
**Novel AAV variants isolated by directed evolution in primate display enhanced retinal transduction following intravitreal injection**
*Shreyasi Choudhury[1], Damien Marsic[2], James Peterson[1], Diego Fajardo[1], Antonette Bennett[3], Paul D. Gamlin[4], Mavis Agbandje-McKenna[3], Sergei Zolotukhin[2], Sanford L. Boye[1], Shannon E. Boye[1]*. [1]Department of Ophthalmology, University of Florida, Gainesville, FL; [2]Deparment of Pediatrics, University of Florida, Gainesville, FL; [3]Deparment of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL; [4]Department of Ophthalmology, University of Alabama at Birmingham, Birmingham, AL.
**Purpose:** Directed evolution of AAV capsid libraries in mice has identified variants with enhanced transduction of mouse retina following intravitreal (Ivt) injection. In primate retina, improvements were less substantial. We developed a method to generate sortable retinal cells in primate which enabled screening of a highly complex AAV2-based capsid library to identify AAV variants capable of efficient retinal transduction following Ivt injection. In parallel, the same library was screened in mice. After 3 rounds of selection in mice and 2 in macaque, a subset of the most prevalent variants was characterized. The purpose of this study was to evaluate their transduction profiles following Ivt injection in mice and to measure physicochemical parameters previously shown to correlate with increased transduction efficiency by this injection route.
**Methods:** Capsid variants contained a self-complementary AAV genome carrying the truncated CBA promoter driving mCherry (sc-smCBA-mCherry). Transduction was quantified *in vitro* using ocular cell lines. Vectors were Ivt injected into Nrl-GFP mice and transduction was evaluated 4 weeks later by fundoscopy and FACS. AAV2wt and AAV2(quadY-F+T-V) were included for comparison. Measurements of capsid *stability,* as determined by *differential scanning fluorimetry* (DSF), and affinity for heparan sulfate by heparin column chromatography are underway.
**Results:** After 3 rounds of screening in mice, two heavily enriched variants, M3-A (32% relative frequency) and M3-B (21%) were chosen for analysis. After two rounds of selection in macaque, the 4 most prevalent variants P2-V1, P2-V2, P2-V3 and P2-V4 were analyzed. Interestingly, P2-V1 is identical to M3-B and P2-V4 is identical to M3-A. All variants displayed substantially improved transduction *in vitro* compared to AAV2wt. When compared to our most efficient rationally designed capsid, AAV2(quadY-F+T-V), P2-V1, P2-V2 and P2-V3 are all improved, with P2-V3 displaying a >2-fold increase in the number of retinal cells transduced following Ivt injection.
**Conclusions:** AAV capsids identified by screening in primate and mouse retina show enhanced transduction efficiencies both *in vitro* and following Ivt injection. An additional round of screening in both primate and mouse has been completed and results are pending.




Ivt-mediated transduction of enhanced AAVs.
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**Program Number:** 4508 **Poster Board Number:** B0163
**Presentation Time:** 11:00 AM–12:45 PM
**Gene augmentation therapy improves inner and outer segment morphology and preserves retinal structure in a large animal model of *CNGB1*-retinitis pigmentosa**
*Laurence M. Occelli[1], Paige A. Winkler[1], Vince A. Chiodo[2], Sanford L. Boye[2], William W. Hauswirth[2], Simon M. Petersen-Jones[1]*. [1]Small Animal Clinical Sciences, Michigan State University, East Lansing, MI; [2]University of Florida, Gainesville, FL.
**Purpose:** Mutations in cyclic nucleotide gated channel beta 1 (*CNGB1*) cause autosomal recessive RP type 45 (RP45). *Cngb1-/-* dogs have marked loss of rod function and a slowly progressive photoreceptor degeneration recapitulating RP45. We showed at ARVO 2016 that gene augmentation therapy in *Cngb1-/-* dogs rescues rod function. Treated dogs had a dramatic restoration of rod electroretinogram (ERG) and improved vision in dim light. The purpose of this study was to investigate long-term effects of gene therapy in *Cngb1-/-* dogs.
**Methods:** Seven eyes of 5 *Cngb1*$^{-/-}$ dogs received a subretinal injection of 1x10$^{12}$ vg of *AAV2/5*-hGRK1-*cCngb1*. Dark- and light-adapted electroretinograms (ERGs), vision testing, fundus imaging and structural preservation assessment by spectral-domain optical coherence tomography (SD-OCT) imaging was performed regularly post-injection. Immunohistochemistry (IHC) was performed on eyes collected 3, 6, 9 and 23 months post-injection.
**Results:** Gene augmentation rescued rod function (as measured by ERG and vision testing) up to 18 months post-injection (latest time point assessed). SD-OCT showed that there was improved definition of the zones representing the external limiting membrane to the interdigitation zone which was maintained over time. Following an initial decline in Receptor plus (REC+) thickness after subretinal injection further decline halted and thickness was preserved in the longer term (up to 23 months in two eyes). IHC confirmed photoreceptor preservation but only in the treated regions.
**Conclusions:** Rescue of rod function by gene augmentation therapy in *Cngb1-/-* dogs was maintained long term. There was also structural preservation with improved morphology of photoreceptor inner/outer segments. The initial continued decline in REC+ thickness following treatment may represent loss of rods prior to full expression of the transgene coupled with loss of any rods that had inadequate transgene expression or were already irreversibly committed to cell death. This study shows successful long-term gene therapy in a large animal model of RP45 making this an attractive disease for translational therapy.
**Commercial Relationships: Laurence M. Occelli**; **Paige A. Winkler**, None; **Vince A. Chiodo**, None; **Sanford L. Boye**, None; **William W. Hauswirth**, None; **Simon M. Petersen-Jones**, None
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**Program Number:** 4509 **Poster Board Number:** B0164
**Presentation Time:** 11:00 AM–12:45 PM

**Subretinal delivery of RGX-314 AAV8-anti-VEGF Fab gene therapy in NHP**

*Anna Tretiakova[1], Tomas S. Aleman[3], Arkady Lyubarsky[3], Elaine J. Zhou[4], Erik Wielechowski[1], Gui-Shuang Ying[2], Erin Bote[1], Leah Makaron[1], Stephen Yoo[5], Jean Bennett[3, 6], Albert M. Maguire[3, 6], James Wilson[1]*. [1]Gene Therapy Program, Department of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Center for Preventative Ophthalmology and Biostatistics, University of Pennsylvania, Philadelphia, PA; [3]Center for Advanced Retinal and Ocular Therapeutics, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA; [4]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [5]REGENXBIO, Rockville, MD; [6]Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia, Philadelphia, PA.

**Purpose:** To evaluate safety and define an upper dose limit of an anti-VEGF Fab-containing AAV8 gene therapy vector for wet age-related macular degeneration in non-human primates (NHP) following subretinal delivery.

**Methods:** 20 NHPs were used in the study. Animals received a single subretinal injection of 1e10 (low), 1e12 (high) AAV8 GC/eye or a control. Production of anti-VEGF Fab was assessed by VEGF ELISA of anterior chamber (AC) fluid. A separate NHP study assessed a 1e11 (middle) GC/eye dose. Retinal structure was evaluated with spectral domain optical coherence tomography (SD-OCT) imaging at 3 months. Retinal function was evaluated using full-field electroretinography (ERG). Retinal distribution of anti-VEGF Fab mRNA and protein was evaluated.

**Results:** At 30 days post injection, expression in control NHPs (n=4) was not detectable. Median expression in low dose NHPs (n=6) was 889 ng/ml (ranging 679 - 2116 ng/ml) and in high dose NHPs (n=6) 2452 ng/ml (ranging 966 - 3500 ng/ml). The middle 1e11 dose (n=8, separate study) showed median expression of 1505 ng/ml (ranging 431 - 2346 ng/ml). Anti-VEGF Fab mRNA and protein were distributed widely throughout the retina well beyond the injection site. Retinal demelanization was present in all injection sites, with no signs of intraocular inflammation. Low dose NHPs showed no significant difference in retinal thickness relative to vehicle controls. There was 10% decrease in total retinal thickness at injection site in high dose NHPs. 1e11 dosed NHP's SD-OCTs are under evaluation. At 10 months, 4 eyes from 2 NHPs in 1e11 group were normal histologically; minimal perivascular infiltrates were present in one optic nerve. Histopathologic changes at 3 months in the low dose eyes included minimal inflammation in the choroid and in the high dose eyes included inflammation in the retina, choroid, and sclera. No significant changes in thicknesses were observed in the control animals. Control or low dose NHPs did not show measurable changes in retinal function. High dose resulted in a statistically significant ($p<0.05$) decline in the amplitude of ERG as compared to baseline, formulation control, and low dose.

**Conclusions:** The study demonstrated presence of anti-VEGF Fab in AC at 30 days in all RGX-314 doses and the feasibility of using OCT and ERG as structural and functional measures and to define an upper dose limit in NHP dose escalation studies.

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