IV. Online Supplement (Proof of concept)

A. *AIPL1*-Leber congenital amaurosis (LCA4). Accounting for approximately 5% of LCA cases, mutations in aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) are associated with the most severe form of LCA(1,2). AIPL1 is expressed predominantly in rods of human and mouse retina, but is also expressed at lower levels in adult cones(3-6). AIPL1-LCA patients exhibit very low visual acuity in infancy, little or no measurable visual fields, hyperopia, nondetectable ERG, pigmentary retinopathy and prominent maculopathy. A recent study revealed with cross sectional OCT imaging that foveal inner/outer segment laminae were absent and foveal ONL was either barely discernible or not detectable in a group of six AIPL1-LCA patients (ages 16-40). ONL is absent from a wide expanse of the central retina (7) . Studies in mice lacking AIPL1 (Aipl1-/-) revealed that both rods and cones lack function and rapidly degenerate, making this a reliable model of the human phenotype(8,9). This mouse model was first used to show that AIPL1 acts as a specialized chaperone by enhancing the farnesylation of the alpha subunit of rod PDE6β (cyclic GMP phosphodiesterase) promoting its folding to assemble the PDE complex(9,10). At that point, it was unclear whether cone degeneration in Aipl1-/- mice was the indirect result of rod cell death (bystander effect) or if AIPL1 also played a direct role in cones. In an effort to understand more about the potential function of this protein in cones, a mouse was developed which transgenically expressed human AIPL1 exclusively in rod photoreceptors in the Aipl1-/- mouse (hAipl1;Aipl1-/-) (6). Investigators found that cones lacking AIPL1 in the presence of viable rod photoreceptors failed to produce an ERG response and exhibited a reduced rate of degeneration indicating that AIPL1 is indeed necessary for the function and survival of cones. The study also showed that destabilization of cone PDE6 subunits occurs in the absence of AIPL1 prompting investigators to theorize that this protein also acts as a chaperone, assisting in the folding and/or assembly of the catalytic subunit of cone PDE6β (α')(6). Loss of PDE6β activity in rods and cones is thought to result in increased cGMP levels which, in turn, alters calcium levels inside photoreceptors leading to their demise. Degeneration in transgenic hAipl1;Aipl1-/- mice

reveal that cone cell death is inherently slow in the presence of 'healthy' rods while in Aipl1-/- mice cone loss is accelerated by rod cell death(6). These results suggest that cones of the transgenic mouse might be analogous to those found in the fovea of Aipl1-LCA patients, an area less susceptible to the bystander effect of rod cell death. Investigators thus hypothesized that Aipl1 gene replacement therapy could be successful if foveal cone cell bodies of these patients survived long enough.

In addition to the Aipl1-/- and hAipl1;Aipl1-/- mice described above, a hypomorphic model that exhibits reduced levels of Aipl1 mRNA and protein (the Aipl1 h/h mouse) was created (11). Photoreceptor loss is considerably slower in the Aipl1 h/h hypomorph relative to Aipl1-/- mice, with the outer nuclear layer reduced by 50% by 6-8 months of age. It was recently shown that AAV2, AAV5 and AAV8 containing either the ubiquitous CMV or photoreceptor-specific hGRK1 promoter and the human or murine Aipl1 cDNA conferred therapy to mouse models carrying either null (Aipl1-/-) or hypomorphic (Aipl1h/h) alleles(12,13) . AAV-mediated AIPL1 expression was found exclusively in photoreceptors of treated mice and led to a corresponding increase in PDE synthesis. Treatment resulted in improved photoreceptor function and survival in both mouse models. Most recently, the Ramamurthy lab elegantly demonstrated that early (P2-P10) treatment of Aipl1-/- mice with self-complimentary AAV8(Y733F) vector containing the hGRK1 promoter driving Aipl1 resulted in restoration of retinal function (out to at least P60), visually-guided behavior (out to P35) and a slowing of photoreceptor degeneration(14). These improvements are likely due to the increased kinetics of transgene expression achieved with both the self-complimentary genome and AAV8(Y733F) serotype used in this study.

A recent report evaluating the severity of human disease in AIPL1-LCA suggests that the encouraging proof-of-concept studies performed in mice may not translate to successful gene-replacement therapy in most AIPL1-LCA patients. The outer nuclear layer is absent throughout the central retinas (including the cone-rich fovea) of these patients and the shape of their foveal pits suggest that AIPL1 deficiency

impacts cones early in development (7). The absence of 'treat-able' photoreceptors in AIPL1 LCA patients may preclude them from being good candidates for gene replacement. It should be noted, however that patients with the more slowly progressing AIPL1-RD (modeled by the Aipl1h/h hypomorph) may respond to treatment.

***B. RPGRIP1*-Leber congenital amaurosis (LCA6).** Mutations in the human RPGRIP1 gene which encodes RP GTPase Regulator Interacting Protein 1 (RPGRIP1) are associated with ~5% of LCA cases(15-18). RPGRIP1 is expressed predominantly in photoreceptors and is also found in other ocular tissues albeit at greatly reduced levels(19-22). Multiple isoforms exist with species-specific subcellular localization patterns including the PR connecting cilium, PR inner and outer segments and basal bodies of cells with primary cilia(19,21,23-25). RPGRIP1 was originally identified through its capacity to bind RP GTPase regulator (RPGR), a protein which also localizes to the connecting cilia and, when mutated, is linked to the majority of cases of X-linked retinitis pigmentosa(20,22,23,26). Generation of mutant mice lacking RPGRIP1 revealed that one of its main roles is to anchor RPGR at the connecting cilium, but not vice versa(27) . The absence of RPGRIP1 in RPGRIP1$^{-/-}$ mice results in mislocalization of RPGR. However, LCA patients with mutations in RPGRIP1 have a much more severe phenotype than patients with RPGR-RP suggesting that RPGRIP1 performs additional functions in photoreceptors other than anchoring RPGR. Studies in mice lacking RPGRIP1 reveal that this protein is required for disc morphogenesis and also for the formation of rod outer segments(27,28). RPGRIP1$^{-/-}$ mice display profound disorganization of outer segment structure, mislocalization of rod and cone opsins and loss of all photoreceptors by 5 months of age. The progression of disease in the RPGRIP1$^{-/-}$ mouse is akin to that seen in RPGRIP1 LCA patients. A more recent study demonstrates that RPGRIP1 likely functions as a scaffold, providing a platform upon which cilium-specific kinases, regulators and substrates can be assembled(29). For instance, it was shown that Nek4, an unexplored kinase which is involved in cilium assembly, interacts directly with RPGRIP1 at the PR cilium suggesting that Nek4 may also be a ciliopathy candidate gene(29).

In the first photoreceptor-targeted gene replacement study in an animal model of RPGRIP LCA, investigators showed that subretinal delivery of a serotype 2 AAV vector containing a proximal domain of the mouse rhodopsin promoter was capable of driving murine RPGRIP1 expression in the connecting cilia of photoreceptors in the RPGRIP1$^{-/-}$ mouse(30). Treated mice exhibited significant preservation of photoreceptors, normal localization of rhodopsin and restoration of rod function for at least 5 months postinjection(30) . Improving upon this study which provided therapy only to rods, treatment with an AAV8 vector containing the hGRK1 promoter driving human *RPGRIP* cDNA resulted in photoreceptor-specific RPGRIP1 expression, significant preservation of photoreceptors and restoration of rod and cone function in RPGRIP$^{-/-}$ mice(31,32). However, retinal function declined over time in treated eyes. Investigators hypothesize this resulted from the relatively low levels of human RPGRIP1 expression in the mouse retina and/or the divergence between human and mouse RPGRIP1 sequences. Whether these exciting, but incomplete, proof-of-concept results in the RPGRIP1$^{-/-}$ mice will translate to a more favorable outcome in the human eye remains to be seen. Gene replacement studies in a large animal model of RPGRIP1 deficiency such as the miniature longhaired dachshund (MLHD) may represent a valuable tool for assessing the *in vivo* efficacy of gene therapy vectors prior to clinical application(33-35). However, recent evidence that the hGRK1 promoter is not active in canine cones highlights the need for alternative photoreceptor-specific promoters in such a study(36).

C. Autosomal recessive RP- ***PDE6β.*** Mutations in the gene *PDE6β* which encodes the β-subunit (βPDE) of the rod cGMP-phosphodiesterase (PDE6), are associated with an aggressive and early onset form arRP(37,38) (39). PDE6 is a heterotetramer composed of the catalytic α and β subunits, and two inhibitory γ subunits. During the phototransduction cascade, PDE6 catalyzes the hydrolysis of cGMP to 5'-GMP thereby lowering the concentration of cGMP which eventually results in hyperpolarization of the cell and neural signaling. In the absence of βPDE, high levels of intracellular cGMP accumulate upon light stimulation leading to photoreceptor apoptosis. There exist two mouse models of RP that are

associated with mutations in *Pde6β*, the *rd1* and *rd10* mouse. The *rd*1 (formerly known as simply retinal degeneration, *rd* mouse) was first described Keeler in 1924(40) and represents the first discovered model with an inherited structural abnormality of the nervous system(41). The *rd1* mouse contains two mutations in *Pde6b*; the integration of the xenotropic murine leukemia virus in the first intron and a nonsense mutation in exon 7(42,43). By comparison the *rd10* mouse was discovered relatively recently at the Jackson Laboratory and contains a missense mutation in exon 13(44). *rd1* mice exhibit very rapid retinal degeneration, with photoreceptor cell death initiating prior to terminal differentiation in the first week of life. There have been many attempts at gene replacement in *rd1* mice using adenoviral, lentiviral and AAV based vectors, all of which resulted in at best only transient slowing of photoreceptor degeneration (45-47). In the case of adenoviral vectors it is unclear whether photoreceptor preservation was primarily associated with restoration of PDE6B or a consequence of the immune response to the carrier adenoviral vector(48). In contrast, the *rd10* mouse exhibits rapid but somewhat slower photoreceptor degeneration than rd1, starting at P18 and peaking at P25(49,50). Degeneration in the rd10 mouse can be further slowed by dark rearing(49). In terms of evaluating strategies for gene replacement, the *rd10* mouse is a more suitable model of human arRP-PDE6B than *rd1*. Attempts at gene replacement therapy in the *rd10* mouse have utilized AAV5, AAV8 and AAV8 capsid mutant vectors(51-53). AAV5 and AAV8 vectors mediate only transient therapy either when injected alone or when combined with nilvadipine, a calcium ion (Ca^{2+}) blocker(51,53). However when P14 *rd10* mice were treated with an AAV8(Y733F) vector containing the same promoter (smCBA) and gene combination used in one of the earlier studies(51), significant preservation of photoreceptor function and structure was observed out to at least 6 months postinjection(52) . Most recently, a large animal model of arRP-PDE6β, the *rcd1* dog, was treated subretinally with either AAV5 or AAV8 vectors containing the hGRK1 promoter driving canine pde6β(54,55). Treatment resulted in in significant preservation of retinal structure, restoration of rod function and rod-mediated visual behavior. What remains to be determined

is whether sufficient photoreceptors remain in patients affected by *PDE6β*-RP to warrant a gene replacement clinical trial.

D. X-linked retinitis pigmentosa-*RPGR*. Approximately 70% of cases of X-Linked Retinitis Pigementosa (XLRP) are associated with mutations in the retinitis pimentosa GTPase regulator (*RPGR*) gene(56-58). Like most forms of RP, RPGR-XLRP patients first present with night blindness that progresses to a loss of visual field and legal blindness by the fourth decade (59,60). RPGR has a complex splicing pattern in the 3' portion which is conserved among taxa(61). The highly repetitive, purine rich exon ORF15 is a mutational hotspot(56). Two naturally occurring canine models with micro deletions in ORF15 (XLRPA1 and XLRPA2, respectively) exist (62). These models bracket the spectrum of human XLRP; with XLRPA1 being a slow progressing form and XLRPA2 being early onset and rapidly progressive(62,63). Utilizing subretinally delivered AAV5 vectors carrying either hIRBP or hGRK1 promoters driving full length *hRPGR,* Beltran and colleagues observed marked improvement in retinal structure and function in treated areas of retina in both models, with more effective treatment seen with the hIRBP promoter(64). Multiple mouse models of XLRP-RPGR exist and have been compared for their fidelity to the progression of human disease(65). Gene replacement studies in several XLRP-RPGR murine models are ongoing with the vectors shown to be therapeutic in the aforementioned canine study.

E. Bardet-Biedl syndrome (BBS). BBS is a predominantly autosomal recessive condition characterized by a wide spectrum of clinical features including severe rod-cone dystrophy, polydactyly, obesity, mental retardation, male hypogonadism and renal dysfunction(66,67). Strabismus and myopia are common with attenuation in ERG by 10 years of age and progression to legal blindness by the age of 16(66). BBS is genetically heterogeneous; there are currently 15 genetic loci known to be associated the disease, the most consistent feature of which is retinal degeneration. It was determined that seven evolutionarily conserved BBS proteins form a network called the "BBSome" which plays a role in ciliary function(68) via

recruitment of cargo to the ciliary basal body and intraflagellar transport (IFT)(69-72). IFT is responsible for the transport of proteins such as rhodopsin to and from the photoreceptor outer segment and thus is crucial for maintenance of photoreceptor function.

Multiple mouse models carrying mutations in different BBS genes have been generated, all of which exhibit retinal degeneration(73-78). Bbs4- deficient mice exhibit gross mislocalization of rhodopsin and cone opsins, followed by deterioration of ERG and apoptosis of PRs(76). Gene expression analsysis revealed that photoreceptor cell loss is associated with increased expression of stress-response genes(79). BBS4 is required for normal, light-dependent transport of transducin and arrestin between photoreceptor inner and outer segments and is also required for normal synaptic transmission from photoreceptors to second order neurons(80). Despite protein mislocalization and functional deficits, the connecting cilia and basal bodies of Bbs4-/- photoreceptors appeared structurally intact at early ages(80) (~1 month). The temporal dissociation between functional and structural impairment is similar to a clinical finding in which a BBS patient presented with severely attenuated ERG but a normal fundus appearance(81) and suggests that BBS patients may be viable candidates for gene-replacement therapy. A recent study showed that subretinal injection of a serotype 5, self-complimentary AAV vector containing the rod-specific rhodopsin (MOPS500) promoter drove BBS4 expression exclusively in rod photoreceptors of Bbs4-/- mice. Investigators focused their efforts on rod photoreceptor therapy because of the slower course of degeneration relative to cones(82). Cone opsin mislocalization (obvious by 2 weeks of age) and loss of cone function (~10% of normal by 1 month of age) precede rhodopsin mislocalization or loss of rod ERG in this model(80). AAV-mediated BBS4 expression reduced opsin mislocalization in transduced rods, preserved rod structure and improved function (ERG) of transduced rods in Bbs4-/- mice(82). Preliminary evidence that AAV-BBS4 treatment can restore rod-mediated behavior to Bbs4-/- mice is currently being evaluated. Taken together, these results suggest that BBS-associated retinal degeneration is treatable.

F. Color blindness. The loss of either the X-linked L- or M-opsin gene leads to red-green color blindness, the most common single gene disorder of humans. The discovery of a tribe of trichromatic squirrel monkeys (*Saimiri sciureus*) that were missing the L opsin gene identified male primates exhibiting protanopic color blindness from birth(83), thus raised the possibility of testing a gene therapy correction for color blindness(84). This idea was tested using an AAV5 vector with the human L-opsin cDNA regulated by a human L/M opsin enhancer and promoter(85). Before vector injection, affected monkeys were behaviorally trained to perform a computer-based color vision test(86) modified for analysis of nonhuman primate color vision(87). The question was whether adding a new cone opsin to existing cones in an adult long wavelength color-deficient primate would be sufficient to produce trichromatic color vision behavior. Prior to treatment, affected and normal monkeys exhibited normal (low) thresholds for blue and yellow wavelengths, but failed to discriminate the diagnostic blue and green wavelengths, with thresholds for detection orders of magnitude higher than normal. Two affected animals were then treated subretinally with the human L-opsin AAV5 vector(88). The treated area encompassed most of the foveal cones, the region in humans in which red-green color discrimination is derived. Gene therapy changed the spectral sensitivity of a subset of the cones within the vector treated retinal area by enhancing their response to red wavelengths as determined by chromatic multifocal ERG. More dramatically, both treated monkeys exhibited an increased behavioral sensitivity to long-wavelength light, with thresholds for detection now being nearly normal. Restored color vision in the treated animals remained stable for over 2 years. Since the subject animals were adults when treated, a novel developmental process is unlikely, and it appears that simple addition of L-opsin protein to an existing cone that is missing this opsin class is sufficient to restore red sensitivity. Although contrary to classical visual deprivation experiments in which neural connections established during early development would not appropriately process an input unless present from birth(89,90), this simple gene therapy-mediated addition of a novel opsin in an adult is an apparent exception to this concept

and opens a range of possibilities for treating foveal cone diseases that have affected patients from birth. A particularly interesting target is Blue Cone Monochromacy (BCM), in which both L- and M-opsin genes are not functional(91) because the vector used in the color blind monkeys could in theory be used unmodified to treat BCM foveal cones to restore red sensitivity.

G. NEUROTROPHIC/NEUROPROTECTIVE STRATEGIES

1. Ciliary neurotrophic factor (CNTF) was discovered in 1979 as a survival factor for ciliary ganglion neurons in developing chicken embyos(92). CNTF is a member of the interleukin 6 (IL-6) family of cytokines and is actively expressed in response to neuronal damage(93). The first test of CNTF as a neuroprotective agent for the retina was carried out in a light damage model of retinal degeneration where it was found to slow the progression of photoreceptor loss when delivered to the vitreous as a protein(94). Subsequently, CNTF has been tested in at least 18 different animal models of retinal degeneration, 14 of which showed clear evidence of neuroprotection(95). In over half of the studies, either adenovirus or AAV was used to deliver the CNTF gene(95). Notably, ocular side effects have been seen after CNTF administration. The most common adverse effect has been an acute decrease in ERG amplitudes upon adminitration(96-99). It has been postulated that these ERG changes are primarily caused by downregulation of the phototransduction machinery, however morphological changes to photoreceptors and peripheral retina may also contribute(100). CNTF treatment has been shown to result in changes in photoreceptor morphology (shortening of rod outer segments and regeneration of cone outer segments) and remodeling of the peripheral retina suggesting that it may also be a mitogenic and/or de-differentiating agent(97,101,102). Indeed, intravitreal injection of CNTF protein leads to deconstruction and regeneration of photoreceptor outer segments in normal dogs and has been found to improve the success rate of AAV-mediated gene replacement in a dog model of *CNGB3*-achromatopsia(103).

The most completely tested form of CNTF delivery has been the use of the encapsulated cell technology (ECT)(104-106). The CNTF eluting ECT device (CNTF-ECT) is composed of a polymer scaffold containing human RPE cells stably transfected with plasmid DNA containing the human CNTF gene. The cell scaffold is contained within a hollow fiber membrane with a pore size that allows free diffusion of CNTF out while preventing large molecules such as antibodies from entering the device. The implant is secured in the vitreous cavity outside the visual axis, and allows for *in situ* CNTF protein production. Pre-clinical efficacy and safety studies of CNTF-ECT were carried out in the rcd1 dog, a model of arRP(105). These studies showed that the device mediated a sustained release of CNTF which resulted in dose dependent increase in retinal survival(105). CNTF-ECT has been evaluated in a phase I clinical trial for the treatment of retinal degeneration. In this study patients with advanced RP were enrolled and treated with either a low dose or high dose CNTF-ECT, with the device remaining in place for a period of 6 months(107). The results of the trial indicated that the CNTF-ECT devices released CNTF for the duration of the implantation period and there were no adverse outcomes, suggesting that CNTF-ECT is safe for the treatment of degenerative retina. Additionally there were indications that the CNTF treatment may have improved visual acuity as 3 of the 7 study eyes showed improvements of 2-3 lines of vision (10-15 letters). However the small sample size and non-placebo controlled nature of the study prevent a definitive conclusion from being made. Subsequently phase II clinical trials for RP and dry age-related macular degeneration (AMD)/geographic atrophy (GA) were initiated. Published results for the dry AMD/GA indicate that best corrected visual acuity (BCVA) was consistently stabilized in the high dose group relative to the low dose and sham groups, and that this was more pronounced when evaluating a subgroup of patients with starting BCVA at 20/63 or better(108). The same company that manufactures the CNTF-ECT plans to test an ECT that delivers a vascular endothelial growth factor (VEGF) antagonist in phase I trial for wet AMD (http://www.neurotechusa.com/ect/nt-503.asp).

2. Glial cell line-derived neurotrophic factor (GDNF), initially found in dopaminergic neurons(109), is part of the transforming growth factor β (TGFβ) superfamily. As a therapeutic, it was initially shown to promote survival of central or motor neurons(110-113) Interestingly, GDNF is also found in the retina(114). Frasson et al.(115)initially studied GDNF protein as a retina survival enhancing agent in the *rd1* mouse and found that treatment led to structural and functional (ERG) improvements.

McGee Sanftner et al. initially studied AAV-mediated expression of GDNF in the rodent retina(116). When regulated by the CBA promoter and delivered by subretinal AAV serotype 2, GDNF could delay photoreceptor degeneration in a transgenic S334ter-4 rhodopsin rat model of retinitis pigmentosa. Immunohistochemical studies showed that vectored GDNF localized to photoreceptor inner and outer segments and the RPE. Quantitative morphometric studies showed that treatment led to enhanced rod photoreceptor survival. Functionally, there was a significant improvement in rod-mediated ERG amplitudes. Subsequently it was found that a similar therapy also attenuated retinal ischemia in Sprague-Dawley rats and ameliorated photoreceptor injury due to retinal detachment(117). Thus, GDNF gene therapy protected the rat retina from multiple genetic, physiological and physical insults, likely by preventing retinal cell apoptosis.

An evaluation of the long term safety of intravitreally delivered AAV2-GDNF in rat retina showed that one year after vitreal vector, there was no abnormality in retinal thickness, no decreases in ganglion cell counts or evidence of immune cell infiltration in treated eyes relative to untreated controls(117). There were also no statistical differences in rod mediated a- and b-wave ERG amplitudes or latency between treated and control eyes. Thus, long-term expression of vectored GDNF within the eye can not only be achieved by intravitreal AAV vector, but appears to be safe immunologically, structurally and functionally for photoreceptors and RGCs for a significant fraction of a rat's life.

In addition to promoting photoreceptor structure and function, GDNF has also been shown to enhance more direct gene replacement therapy in retinal disease. Buch et al. (2006) reported that AAV-mediated GDNF expression improves gene replacement therapy in several rodent models of retinal degeneration(118). Additionally, it was shown that GDNF did not lead to the transient ERG suppression seen with CNTF gene therapy(97). Thus, GDNF gene therapy not only slows retinal degeneration but combination GDNF/gene replacement therapy may prove to be more therapeutic than simple single gene therapies. Issues regarding whether this sort of dual gene therapy would raise regulatory issues in a clinical setting remain to be resolved.

One disappointing feature of GDNF gene therapy, and neurotrophic factors in general, is that retinal degeneration is not permanently halted and eventually the degenerative process leads to loss of retinal cells and hence loss of GDNF, most likely because the underlying degenerative mechanism has not been dealt with, except in the case dual gene therapy discussed above. An alternative that may promote longer lasting GDNF therapy is to express it from cells not directly involved in the degenerative process. Vitreal delivery of vector to transduce RGCs is one possibility, but transduction of Muller glial cells whose cell bodies span the entire retinal thickness, naturally secrete neurotrophins and come into close proximity to photoreceptors is a more attractive possibility(119). Accordingly, Klimczak et al. identified an AAV variant (ShH10) that was able to selectively transduce Muller glial when delivered intravitreally in the rodent(120). When tested in transgenic rats with a well characterized retinal degeneration due to a S334ter mutation in rhodopsin, ShH10-mediated GDNF secretion from glia led to high intra-retinal GDNF levels, to preservation of photoreceptors and improved rod ERG amplitudes for at least 5 months relative to untreated eyes(121). Longer term follow up will be necessary to fully assess whether this approach is superior to transduction of photoreceptors or RGCs, however the ability to enhance GDNF levels from a natural retinal cell reservoir that is not involved in most degenerative genetic retinal diseases suggest it is a promising therapeutic approach for a wide variety of retinal degenerations.

3. X-linked inhibitor of apoptosis (XIAP) is a key member of the gene family of apotosis inhibitors(122). XIAP exerts its effect by binding to and suppressing caspases 3, 7, and 9(123)and is a promising reagent for treating degenerative diseases involving apoptotic cell death(124). AAV- delivered XIAP has been studied in the context of retinal cell loss resulting from both physical and chemical insult as well as for loss due to genetic origin. The benefit of XIAP gene therapy has been reported for retinal ischemia, retinal hypertension, chemotoxic retinal insult, oxidative retinal stress, and models of RP. Additionally it has been tested as an adjuvant for retinal cell transplantation therapy and for gene replacement therapy in genetic models of retinal disease.

Retinal ischemia, either acute or prolonged is at the heart of the pathology leading to vision loss in a variety of conditions that include acute glaucoma, diabetic retinopathy, hypertensive retinopathy and retinal vascular occlusion. As XIAP-mediated gene therapy protects neurons from apoptosis, Renwick et al. tested whether AAV- delivered XIAP would preserve retinal cells following ischemia-induced cell death in normal rats(125). AAV2-CBA-XIAP was delivered vitreally and 6 weeks later XIAP-treated and control GFP vector-treated eyes were made ischemic by raising intraocular pressure. ERG studies showed that XIAP-treated eyes maintained significantly larger rod mediated b-wave amplitudes than GFP vector-treated eyes for up to 4 weeks post-ischemia. Both the inner and outer nuclear layers were significantly better preserved in XIAP vs. control vector-treated eyes. Optic nerve axon counts, a measure of inner retinal RGC survival, was near normal in XIAP-treated eyes and significantly reduced in controls. This was confirmed by fewer TUNEL-positive inner retinal cells seen in XIAP-treated retinas. Transient ischemic retinal cell damage can therefore be ameliorated by XIAP gene delivery and leads to both functional and structural benefit, at least in the short term(125).

Sustained intraocular pressure (IOP) is a primary cause of glaucoma, a prevalent and blinding retinal disease involving the loss of RGCs in response to abnormal pressure regulation. This condition can be

modeled in animal models by blocking the normal ocular aqueous outflow apparatus of the trablecular meshwork. AAV2-CBA-XIAP, when delivered to the vitreous of normal rats, transduces cells of the TM and cilliary body(126). Subsequent to XIAP vector delivery, eyes were exposed to chronic intraocular pressure elevation by blocking TM-mediated ocular fluid outflow. At six months, optic nerve axon counts were performed to determine the neuroprotective effects of vectored XIAP in comparison with normal pressure eyes and control vector treated eyes. XIAP treatment was found to reduce RGC loss by 50%. Although XIAP mediated protection of RGCs from apoptotic cell death clearly played a role in this therapy, vector pretreatment also unexpectedly lowered the level of elevated IOP. This suggests that in addition to directly protecting RGCs, XIAP may also play a positive role in protecting TM cells from induced apoptosis. Thus XIAP mediated protection of RGCs in the inner retina, perhaps involving simultaneous protection of cells controlling aqueous outflow, shows promise as an approach for pressure related glaucoma. Additional studies in other glaucoma models seem warranted.

XIAP has also been evaluated for its ability to protect the retina against chemotoxic insult. Petrin et al. evaluated the neuroprotective effects of AAV-delivered XIAP against N-methyl-N-nitrosourea (MNU)-induced retinal toxicity in Sprague-Dawley rats that were injected subretinally with either AAV2-CBA-XIAP or the corresponding control vector with a GFP transgene(127). Six weeks post-treatment, rats received an intraperitoneal injection of MNU at a retinotoxic dose of 60 mg/kg. Real-time PCR and Western analysis confirmed XIAP over-expression in vector treated eyes. ERG analysis demonstrated *in vivo* protection of retinal function from MNU toxicity. At 24 hours post-MNU treatment, TUNEL analysis showed significantly fewer photoreceptors experiencing apoptosis in XIAP-treated vs. control eyes. Histological analysis of control vector treated photoreceptors showed their near complete loss 72 hours after MNU. In contrast, XIAP treated eyes showed clear photoreceptor cell preservation throughout the full 7 day sampling period. These results suggested that XIAP is protective against this potent

chemotoxic agent that may be modeling the slower apoptotic processes taking place in genetically determined retinal disease.

Retinal detachment, physical separation of the retina from the underlying RPE, is a common form of retinal trauma and is a significant cause of visual loss(128,129), particularly if it encompasses macula. Photoreceptors are the principle cell type sustaining injury upon retinal detachment and undergo rapid apoptotic cell death(128,130).In animal model studies, detachment initiated apoptosis begins within one day and is maximal at 3 days(129,131). Hence, XIAP, as a potent inhibitor of the key caspases that initiate and maintain apoptosis, is a natural therapeutic candidate. Zadro-Lamoureux et al (2009) documented the protective effects of AAV2-CBA-XIAP when delivered subretinially two weeks before experimental retinal detachment in normal rats(132). One day after detachment in control AAV-GFP treated eyes, caspase 3 and 9 activities increased measurably in the detached areas. In contrast, AAV-XIAP-treated detached retinas had caspase levels near that seen in undetached controls. At 3 days post-detachment, TUNEL assays in XIAP-treated detachments showed a reduction in apoptotic photoreceptors relative to control treatment. Two months post-detachment, immunohistochemistry demonstrated that, within regions of vector-mediated XIAP expression, rod cell bodies were preserved whereas significant ONL cell loss had occurred in control, detached retinas. The challenge will be to develop a system for expressing XIAP sufficiently rapidly after a retinal detachment to be therapeutic in the face of rapid photoreceptor apoptosis in the clinic. This will be a challenge for any vectored gene expression system and suggests that protein therapy may be a better option currently.

Intracellular damage due to oxidative stress in the retinal pigment epithelial (RPE) is thought to play a critical role in the pathogenesis of AMD and other degenerative retinal diseases(133,134). Shan et al. assessed whether human XIAP delivered by an AAV2 vector could prolong survival of cultured human RPE cells after oxidative insult due to H_2O_2 exposure(135). Upon H_2O_2 exposure, ARPE-19 cells exhibited

an increase in levels of reactive oxygen species relative to control conditions. AAV-XIAP treated cells had XIAP levels 11 times higher than controls and significantly fewer apoptotic nuclei by TUNEL staining. Mitochondrial dysfunction, a central initiator of the apoptotic pathway, was only slightly reduced in treated cells, consistent with XIAP's role primarily in the downstream caspase- mediated steps in apoptosis. It remains to be seen how XIAP will affect more chronic types of oxidative stress such as smoking, chronic low level inflammation and light exposure that are risk factors in a variety of retinal diseases.

XIAP has also been evaluated for its ability to augment retinal cell transplantation. Neonatal cells from the mouse retina can be transplanted into adult mouse outer retina(136) with a small fraction able to survive, express rod and/or cone specific proteins, elaborate outer segments, make synaptic connections with the inner retina, mediate ERG responses to light stimuli and rescue a degenerating retina to at least a limited extent(137-139). A major issue with this strategy for restoring retinal function is that the transplanted cells frequently lose viability within a few weeks or months. As a potential means of enhancing cell transplant survival in the retina, vector-mediated XIAP pretreatment of donor cells was tested by Yao et al. in a model for X-linked RP, the *rd9* mouse(140). Retinal cell suspensions from normal postnatal day 4 GFP transgenic mice were subretinally transplanted into 2-, 5-, and 8-month-old *rd9* mice. GFP-positive transplanted cells integrated into the ONL of recipient *rd9* retinas, developed inner and outer segments and elaborated synaptic projections to bipolar cells. The efficiency of donor cell integration increased with host age at the time of transplantation with the integrated cell count peaking at one month post-transplantation and decreasing thereafter. When donor cells were treated with AAV-XIAP prior to transplantation, donor cell survival was significantly enhanced. This was not due to avoidance of any immune-mediated processes since no donor cell-specific immune responses were noted. Hence, retinal cell transplant efficiency can be increased through XIAP-mediated anti-

apoptotic therapy, presumably by preventing programmed cell death, thus suggesting that *ex vivo* XIAP gene therapy may be an important adjunct to therapeutic retinal cell transplantation in the future.

Clearly, XIAP gene therapy has potential for prolonging photoreceptor survival in the face of apoptotic cell death in degenerative retinal diseases of the outer retina, RP being the prime example(141). Leonard et al. asked whether XIAP could slow photoreceptor degeneration in several rodent models of RP(142). HA- tagged XIAP in an AAV2-CBA-XIAP vector was delivered subretinally on postnatal days 14-17 to either the P23H or S334ter rhodopsin rat models of autosomal dominant RP. In both, western blots and immunostaining of retinal sections confirmed XIAP overexpression in photoreceptors. Analysis of outer nuclear layer thickness, a measure of photoreceptor cell bodies, was also significantly enhanced by XIAP treatment in both models. However, ERG analysis showed significant preservation of the rod-mediated responses out to 7 months post-treatment only in the P23H line but not in the S334ter line. This may suggest limitations to XIAP gene therapy depending on the mechanism leading to cell death since P23H rhodopsin is thought to lead to rod cell death via protein misfolding(143) whereas S334ter rhodopsin accumulates in the plasma membrane due to incorrect trafficking(144,145). Although data show that vectored XIAP expression is able to protect photoreceptors at some level in two models of autosomal dominant RP, it will be important to more completely confirm the potential of XIAP gene therapy in a broader range of genetically determined retinal disease models.

Since XIAP gene therapy shows protective effects in a broad range of genetic and toxic contexts within the retina, particularly for photoreceptors, it is logical to ask whether XIAP treatment can prolong the window of treatability for gene replacement therapy for models in which simple supplementation with a normal gene is already known to be therapeutic. *Rd10* mice, an autosomal-recessive model of RP caused by a point mutation of the *Pde6b* gene(49), undergoes rapid photoreceptor degeneration in normal ambient light conditions(51) but is responsive to gene replacement therapy if treated early with the

mice maintained under low light conditions(51,52). Accordingly, Yao et al. analyzed *rd10* eyes after pretreatment with AAV5-CBA-XIAP(140). Subretinal AAV5-XIAP at postnatal days 4 or 21 significantly delayed light-induced retinal degeneration, as measured by outer nuclear thickness and cell counts. In contrast, under normal vivarium light, AAV5-XIAP alone did not improve outer segment structure or correct the rhodopsin mislocalization. In contrast, co-injection of AAV5-XIAP and AAV5-PDEβ increased levels of rod rescue and decreased rates of retinal degeneration compared to treatment with AAV5-PDEβ alone. Mice treated with AAV5-XIAP at P4, but not P21, remained responsive to rescue by AAV5-PDEβ when injected two weeks after moving them to a normal intensity cycling light environment. Thus, anti-apoptotic XIAP gene therapy confers an adjunctive therapeutic effect to gene replacement therapy in the *rd10* mouse. Critically, when given early, AAV5-XIAP prolongs the age window within which gene-replacement therapy remains effective. If this approach can be shown to apply to a range of genetic retinal diseases, it could be a novel approach for clinical disease, particularly in those conditions for which there is no current gene replacement therapy or the causative gene is presently unknown.

4. Erythropoietin (EPO) is a cytokine produced in the adult kidney that is classically known for stimulating erythropoiesis (proliferation and differentiation of erythroid progenitor cells), a process stimulated by hypoxia. However, EPO has several other functions including positive roles in neuroprotection, anti-inflammation and regeneration(146) as well as protection of neurons following acute or chronic injury(147,148). While erythropoiesis is promoted by interaction of EPO with the EPO receptor ($EPOR_2$), non-erythropoietic functions are mediated via interactions with other poorly characterized EPOR complexes(149-154). The beta common receptor (βCR) has been shown to interact with EPOR and play a role in EPO-mediated neuroprotection(155-159). Both EPOR and βCR are expressed in the retina, primarily in ganglion cells, inner retinal neurons and some photoreceptors(160). Harnessing EPO's ability to cross the blood retina barrier, studies have shown that systemically-delivered EPO protects against light damage, certain inherited retinal degenerations, ischemic injury and

glaucoma(161-165). More direct, intraocular delivery of EPO protects RGCs from axotomy- induced degeneration and experimental glaucoma and prevents apoptosis in models of light-induced and inherited retinal degeneration and diabetes(166-169). While the majority of these studies involved repeated injections of recombinant EPO protein, four utilized AAV to deliver EPO resulting in sustained expression of the therapeutic(160,162,170,171).

Despite its protective effects, systemic delivery of EPO is associated with significant hematocrit increase(147,162). Non-erythropoietic EPO derivatives (EPO-D) have been engineered which are neuroprotective and do not cause significant hematocrit changes. AAV-mediated delivery of such EPO-D constructs prevents neuronal loss following either systemic or intraocular delivery to various models of induced or inherited retinal degeneration(160,170,171). These studies suggest that the level of protection conferred is dependent on the specific EPO or EPO-D construct delivered, the route of injection and the etiology of degeneration in the treated animal. Taken together, data suggests that AAV-mediated delivery of EPO-D has the potential to safely treat of a wide variety of retinal degenerations.

H. OPTOGENETIC STRATEGIES

The *Chlamydomonas reinhardtii* algal protein channel rhodopsin-2 (ChR2) is an ion channel that opens in response to specific wavelengths of light(172-175). If expressed in mammalian neurons, light-activated ChR2 allows cation influx, membrane depolarization and neuronal signaling. ChR2 could in theory therefore, through targeted gene delivery, allow externally regulated control of one class of neural signaling(176). This concept has led to an active field of research aimed at restoring useful light sensitivity to degenerated retinas in animal models that have lost photoreceptor mediated light responses.

AAV-mediated expression of ChR2 or other light sensitive channels has been shown to restore function to rodents that have lost light responses due to either genetic or physical causes of photoreceptor cell loss. Bi et al. showed that expression of ChR2 in the murine retina via intravitreal AAV2 vector led to long-term transgene expression primarily in RGCs(177) of the *rd1* mouse that lacks photoreceptors within a month after birth. Vector treatment restored the *rd1* retina's response to light and its ability to encode and transmit light-induced signals to the visual cortex by showing improved visually evoked cortical potentials (VEPs). Tomita et al. employed a similar AAV2 approach, this time expressing a Ch2R-Venus reporter gene fusion, to treat late-stage RCS rats homozygous for a mutation in the *Mertk* gene that leads to RPE dysfunction and photoreceptor cell death(178). Venus fluorescence was seen predominantly in RGCs upon vitreal vector delivery, but also in some presumptive amacrine cells. VEPs elicited by chromatic light flashes and recorded from the visual cortex at various periods of time after vector injection showed that a stable, but only partial cortical response was restored. Subsequently, it was also shown that behavioral responses to a moving image stimulus, the optokinetic response, in these treated rats also improved significantly(179). Additionally, when the ChR2, under regulation of the Thy1.2 promoter, was targeted exclusively to RGCs in transgenic rats and photoreceptors ablated by continuous high intensity light exposure, the optomoter contrast sensitivity response was similar to that in non-transgenic rats with a normal functioning retina(180).

Recently, vectored retinal expression of Ch2R studies have been extended to the nonhuman primate. Ivanova et al. examined AAV2-mediated expression of a fusion construct in inner retinal neurons in the common marmoset *Callithrix jacchus* following intravitreal injection*(181).* Expression of ChR2-GFP and functional properties of transduced ChR2 were examined 3 months after treatment. The retinal expression pattern ChR2-GFP was topographical and age-dependent with the highest fraction of positive RGCs seen in the far-peripheral retina and in the foveal and para-foveal region. In young animals

receiving high vector doses, all major classes of RGCs were transduced when the CBA promoter was used. ChR2-mediated light responses by multielectrode recordings of *ex vivo* transduced retinas were consistent with spiking being initiated by light activated Ch2R(181). Thus, the primate retina also seems amenable to optogenetic manipulation.

The safety of expressing ChR2 in retinal neurons has been indirectly confirmed in the above studies in mice(176,177,182,183), since there were are no changes in resting membrane and activation potentials in RGCs when transduced with ChR2 for at least two years. Ivanova and Pan(184) and Sugano et al.(185) then specifically studied the safety of vitreal AAV2- ChR2 in normal mice at different delivered doses and in the RCS rat. Transgene expression was stable for up to 18 months and seen mainly in RGCs and retinal amacrine cells with ChR2-GFP under control of the promiscuous CBA promoter. Neither delivered vector dose, illumination conditions, or fraction of inner retina cells positive for ChR2-GFP had any effect on the density or survival of the RGC or amacrine cell populations. Doroudchi et al. also studied nonocular toxicity measures in *rd1* mice after targeting ChR2 to bipolar cells and found its expression to be nontoxic, with no measurable immune or inflammatory response for at least 10 months(186).

This body of work establishes that ChR2 expression primarily in RGCs when excited by the proper wavelengths of visible light, is safe and can restore some physiological and visual responses in blind rodents. An alternative to targeting RGCs with light activatable channel proteins is to target cone photoreceptors because they are typically the last photoreceptor cell type to be lost in many forms of inherited blindness and cones mediate high acuity central vision originating in the primate fovea. The issue in this context is that the introduced light sensitive channel must close rather than open like ChR2 in response to light in order to mimic the hyperpolarization experienced by cones upon photon absorption. Archaebacterial halorhodopsin possesses these properties(187,188). Zhang et al. and

Busskamp et al. tested its effect when delivered by a cone-targeted AAV vector in two mouse lines modeling relative cone preservation in the context of rod loss: the Cnga3$^{-/-}$; Rho$^{-/-}$ double-knockout mouse with rod degeneration followed by slow cone degeneration(189), and the and *rd1* mouse, also used above, with rapid rod degeneration and relative early cone sparing(190). Using cone specific promoters(85,191,192), expression was targeted to residual cone inner segments even when their light sensing outer segments had been lost. Through transmission of transduced cone signals to the inner retina, treatment elicited appropriate spiking activities in a variety of RGC subtypes and improved visually guided behavior and optokinetic performance relative to untreated controls. Thus, restoring a light response to cones in a degenerating retina is clearly feasible. However, its clinical applicability is likely to be very context dependent since the rate of loss and level of residual cone function is quite variable among different retinal genetic diseases and even among patients with the same genetic disease. Additionally, this outer retinal optogenetic gene therapy approach would in theory not slow the loss of cones in a degenerating retina and therefore may be of shorter term benefit to the patient than inner retinal cell optogenetic therapy.

A second alternative to transducing ganglion cells is to target bipolar cells, an upstream component of the neural pathway between photoreceptors and the primary visual cortex. This approach has the potential advantage of allowing the remaining interneurons in a degenerated retina to process bipolar cell light-elicited spike trains in a more normal manner than is possible from ChR2-transduced RGCs. Doroudchi et al.(186) report targeting mouse bipolar cells with ChR2 using a mGRM6 ON bipolar cell promoter(193) within a capsid mutated AAV2 vector that is known to penetrate multiple retinal layers(194). Targeted bipolar cell expression led to ChR2-mediated, postsynaptic RGC ON electrophysiological responses in three mouse models of photoreceptor cell loss and genetic blindness. Positive effects persisted for at least 10 months. This response was accompanied by a significant

improvement in water maze performance, a visually-guided behavioral test, relative to untreated, affected mice. However, the light intensities required to elicit improved visually guided behavior were at the very high end of the normal range response and may themselves be ultimately toxic. Thus new generations of optogenetic reagents are being developed that respond at lower light intensities (see below). A remaining issue is whether the progressive changes seen in bipolar cell-inner retinal neuron connectivity as retinal degeneration advances(195)will compromise the fidelity of this bipolar cell approach to optogenetic therapy.

Although ChR2 has been largely successful in restoring light sensitivity to degenerated retinas, several suboptimal physical properties including its requirement for very high light intensity to elicit efficient activation and its slow return to the inactive state have prompted the development of alternative light sensitive channel proteins. These and other unresolved issues related to optimizing the optogenetic approach to restoring light sensitivity to a late stage degenerated retina have been recently reviewed by Busskamp et al.(196). One option under current study is an iGluR6 receptor protein that has been altered *in vitro* at residue 439 substituting a cysteine for a leucine. This allows covalent attachment of a photoswitch which reversibly activates the receptor(197,198). This novel light-activated channel, termed LiGluR, contains a maleimide linked to a glutamate through a photoisomerizable azobenzene linker, together termed "MAG". At one wavelength, light absorption orients the glutamate into the receptor binding pocket leading to ion channel opening, and at a different wavelength, the glutamate is moved out of the pocket thus closing the channel. This enables the wavelength of incident light to modulate LiGluR between open and closed configurations. Employing a vitreal AAV2-LiGluR vector to transduce RGCs of adult in photoreceptorless *rd1* mice, Caporale et al. restored light sensitivity to RGCs *in vitro* in whole mount retinas(199). *In vivo*, the primary visual cortex response to light on the retina and the pupillary constriction in response to bright light were also restored. These results suggest that

engineered light-activated channels may have a future in restoring visual function in patients who have retinal lost light sensing function.

I. ANTI ROS/OXIDATIVE STRESS THERAPIES

Damage from reactive oxygen species (ROS) has been implicated in diseases of the retina ranging from glaucoma to macular degeneration. The high oxygen tension of the retina places photoreceptors at elevated risk of oxidative damage especially as endogenous levels of antioxidant protection diminish with aging. Cone photoreceptors are particularly sensitive oxidative stress(200,201). In diabetic retinopathy, reactive oxygen species are thought to be the link between elevated glucose and diabetic complications(202,203). Oxidative damage to the RPE may also be an initiating factor in age related macular degeneration(133,204-206).

The importance of enzymes such as superoxide dismutase (SOD) and catalase (CAT) in protecting the retina from oxidative damage has been established using knockout and transgenic mice to either reduce or increase the levels of specific enzymes. Campochiarro's group has demonstrated that cones can be protected from degeneration by over-expression of *SOD1* (which encodes the cytoplasmic Cu/Zn SOD) provided that glutathione peroxidase 4 (Gpx4) is also elevated, to deal with the excess production of hydrogen peroxide(207). Elevation of CuZnSOD alone increased oxidative damage and accelerated the loss of cone function in the *rd10* model of retinal degeneration. Similarly co-expression of *SOD2* (mitochondrial manganese SOD) and *CAT* protected *rd10* cones, where over expression of each gene by itself did not(201).

Gene transfer methods have also documented the importance of such enzymes in animal models of ocular disease. Experimental autoimmune encephalomyelitis (EAE) is often used as an animal model for studying the pathogenesis of multiple sclerosis (MS). In both MS and EAE, demyelination and neuronal

loss play key roles in irreversible loss of function and disability. In a mouse EAE model, Qi et al. found that intravitreal injection of AAV2 expressing SOD2 in retinal ganglion cells increased SOD2 levels two-fold in the optic nerve, reduced the loss of myelin fibers by 51% and the loss of retinal ganglion cells (RGC) by 400%. Overall, vector treatment limited ganglion cell axonal loss to just 7% at 1 year(208). The same group also tested a combination of SOD2 and CAT delivered by separate AAV2 vectors and observed long term suppression of neuronal and axonal loss in EAE.

In diseases such as closed-angle glaucoma(209) and diabetic retinopathy(210)retinal ischemia leads to visual impairment. Using a mouse model of retinal ischemia via acute elevation of intraocular pressure followed by reperfusion (I/R), Chen et al.(211) found that liposome-based delivery of *SOD2* or *CAT* significantly reduced levels of superoxide ion, hydrogen peroxide, and 4-hydorxynonenal, a marker of lipid peroxidation. Delivery of *SOD2* or *CAT* inhibited the I/R-induced apoptosis of retinal vascular cell and retinal capillary degeneration. While this was a short-term experiment, it shows promise for treating the long term retinal complications of diabetes provided an efficient method delivering genes to the retinal vasculature can be found. Using a similar model for glaucoma, Chen and Tang recently reported that AAV delivery of *CAT* also protected retinal ganglion cells from I/R injury(212).

Abnormal neovascularization is also associated with localized neuronal death in conditions such as retinal angiomatous proliferation and macular telangiectasia. Mice lacking functional receptors for Very Low Density Lipoprotein (VLDL) display abnormal intra- and sub-retinal neovascularization associated with photoreceptor cell death(213). These mice exhibit increased oxidative stress as demonstrated by acrolein accumulation in the photoreceptor layer. Dorrell *et al*. used AAV2-mediated gene transfer of Neurotrophin-4 driven by the GFAP promoter to transduce retinal Muller glial cells. This neurotrophic factor protected photoreceptors from degeneration in this model as evidenced by elevated levels of rhodopsin and an improved ERG response in treated eyes.

Choroidal neovascularization (CNV), the abnormal growth of blood vessels from behind the retina, is the central pathologic feature of "wet" or neovascular age related macular degeneration (AMD). While oxidative stress has long been implicated as an initiating event in AMD, Li et al. (214) tested the hypothesis that reactive oxygen species are involved in the late, neovascular stage of this disease. NAPDH oxidase (NOX) is the major non-mitochondrial source of superoxide radicals. These authors first demonstrated that NOX is expressed in the retinal pigment epithelium (RPE), and, then, they used subretinal injection of AAV2 expressing a small hairpin RNA targeting the p22phox subunit common to all NOX isoforms. 80% reduction in the level of this subunit in the RPE led to a 3-fold reduction in laser induced CNV. Haruta et al.(215) have used a Cre-lox system to specifically delete the Rac-1 subunit of NADPH oxidase in photoreceptors, and they report that these photoreceptors are normal with respect to the ERG response and therefore protected against light induced injury.

J. CURRENT ISSUES/FUTURE DIRECTIONS

For its success in the clinic and a multitude of proof-of-concept studies in animal models, AAV has emerged as the optimal vector for the treatment of retinal disease. Despite its remarkable success, AAV-mediated ocular gene therapy is not without its hurdles. The three main obstacles currently faced by ocular gene therapists are the fidelity of results across species, the ability to safely transduce outer retina (i.e. avoid potential surgical trauma of subretinal injection) and the relatively small packaging capacity (~5 kb) of the vector system.

1. Fidelity across species. The most common animal model used in proof-of-concept ocular gene therapy studies is the mouse. It is within this species that an innumerable amount of therapeutic cDNAs, promoters and AAV vector serotypes have been tested. However, many differences exist between man and other mammals, including but not limited to overall eye size, the existence of a fovea, and the thickness of nerve fiber layer and inner limiting membranes(216-219). In addition, the biochemical

makeup of retinal cells differs across species. These differences make viral vector transduction studies in mouse a sporadic predictor of transduction in humans. Thus far, only a handful of studies have been conducted which address AAV tropism or promoter activity in higher order species. A summary of the transduction profiles of these vectors across species can be found in Supplemental Table 1.

With a focus on outer retina, AAV2, AAV5, AAV7, AAV8 and AAV9, rh.8R and rh64R1 have been tested for their ability to transduce photoreceptors following subretinal injection in dogs and/or NHP. Collectively, results show that all serotypes effectively transduce both rods/cones and RPE in these species, a pattern identical to that found in rodent. Of these serotypes, the two which transduced NHP foveal cones most effectively were AAV9 and rh.64R1. However, these serotypes failed to efficiently transduce cones in the parafovea. (220) (221). In contrast (despite their lack of fovea), no differences in efficiency of cone transduction following subretinal injection of these serotypes has been observed in rodent retina. On the other hand, AAV5 transduction across species appears to be more similar. AAV5 is known to transduce both foveal and parafoveal cones following subretinal injection in the non-human promate(84,222,223) and AAV5 has been used to deliver long wavelength opsin to cones thereby conferring trichromacy to a previously dichromatic male squirrel monkey (see above)(222). AAV5 has also been used successfully in proof of concept studies in a canine model of CNGB3-achromatopsia(224). More obvious discrepancies have been uncovered following AAV delivery to the inner retina. A recent study showed that AAV2 is capable of transducing ganglion and Muller cells within, but not outside the fovea following intravitreal injection into the macaque eye(225). This result differs from the widespread transduction of inner retina found in intravitreally injected rodents(226,227) and is thought to be attributed to the physical barriers such as the relatively thick inner limiting membrane seen in the primate retina.

Differences in promoter activity have also been documented across species. For instance, the human rhodopsin kinase promoter has exclusive activity in both rods and cones of the rodent retina(31) but drives transgene expression exclusively in rod photoreceptors of the canine retina(36). This difference is likely due to a species-specific difference in expression of G-protein coupled receptor kinases in rods and cones. GRK1 is expressed in rods and cones of mice and rats, but is only found in rods of the canine retina(228). Desensitization of cones in these species is modulated by GRK7, a cone-specific isoform(228). In monkey and human retinas, GRK1 is localized to rods but is also co-expressed with GRK7 in cones(228). Preliminary data indicates that this promoter is active in both rod and cones of NHP(223). Species differences have also been found following delivery of AAV vectors containing the human L/M opsin (PR2.1) promoter. This promoter is active in all cones (and some rods) of the murine retina but its activity is restricted to L/M cones of both canine and NHP retinas. The ubiquitous "small CBA" (smCBA) promoter is a truncated form of the "CBA" (chicken beta actin promoter/cytomegalovirus enhancer) promoter. Both smCBA and CBA have identical activity in retinal cells of the rodent retina. As such, the smaller smCBA promoter is attractive for use in conjunction with large transgenes and has been used in multiple proof of concept studies(229,230). Interestingly, in the context of intravitreally-delivered AAV2, a recent study showed that CBA, but not smCBA, drove transgene expression in inner retinal cells of the monkey(225). Reasons for this remain to be elucidated.

2. Transducing outer retina via the vitreous. The majority of retinal degenerations are known to be associated with mutations in genes expressed in photoreceptors and/or RPE. The ability to target these cell types with AAV vectors via the vitreous, thereby avoiding the potential trauma associated with subretinal injection, would represent a significant advancement in the field (see Supplemental Figure 1 for schematic of administration routes). By mutating tyrosine residues on AAV vector capsids that improve the transduction efficiency, increase the kinetics and enhance the penetration ability of this vector system, Srivastava and colleagues have brought us closer to realizing this goal(223,231-233).

Recent studies show that single or multiple mutations on the capsid surfaces of AAV2, AAV8 and AAV9 result in altered transduction characteristics in murine retina relative to standard AAV serotypes, with some exhibiting the ability to express transgenes in the outer retina following intravitreal delivery(194,223,233,234). Alternatively, directed evolution using large mutant capsid libraries to select for AAV variants with enhanced properties has also been used to isolate vectors with enhanced retinal penetration(235). Despite these encouraging results, the structural differences between mouse and primate retina must be considered. Characterization of these 'enhanced' serotypes in the non-human primate retina should precede clinical application.

3. Packaging large genes. Recently, multiple groups responded to the assertion that AAV vectors were capable of packaging ~9 kb of genetic information(236). They determined that the packaging of large DNAs initiates primarily from the 3' end of DNA single strands and proceeds until the AAV capsid reaches capacity (~5 kb). Resultant vector DNAs are therefore heterogeneous in length and truncated at their 5' ends(237-239). Full-length large gene reconstruction occurs from these incomplete, opposite polarity cDNAs post-infection. To avoid this heterogeneity, dual AAV vector systems with definable genetic content have been developed that have potential for clinical application (Supplemental Figure 2).

In the "dual overlap" platform, two independent AAV particles are produced, each carrying a portion of an intact expression cassette. A promoter and N' terminal portion of the transgene are packaged into a front-half vector and the C' terminal portion of the transgene and polyA addition sequence are packaged into the back-half vector. Each vector contains both 3' and 5' ITRs and a central region of shared DNA sequence. Homology in this shared DNA sequence promotes homologous recombination post-infection and reconstitution of full-length transgene(240,241). Concomitant with the first reports of "dual overlap" vectors, "trans-splicing" AAV technology surfaced(242,243). "Trans-splicing" vectors are

designed such that the front-half vector contains a promoter, the N' terminal portion of the transgene, a splicing donor and both ITRs. The back-half vector contains a splicing acceptor, the C' terminal portion of the transgene, the polyA addition sequence and both ITRs. Upon co-infection, ITR-mediated recombination and subsequent RNA splicing of the recombinant transcript occurs leading to production of full length protein. To improve upon "dual overlap" and "trans-splicing" vectors whose efficacy depends on the recombinogenic nature of the transgene and/or the efficiency of its endogenous splicing signals, a generic vector system has been developed which is independent of the target gene(244) . Such "hybrid vectors" containing a recombinogenic portion of the alkaline phosphatase (AP) gene and splicing signals (splice donor and splice acceptor) promote AP sequence-mediated homologous recombination and reconstitution of full-length gene product. A small fraction of these vectors also undergo traditional head-to-tail vector ITR-mediated recombination, after which the splicing signals remove the ITR junction and reconstitute the full length transgene(244). "Hybrid vectors" have demonstrated therapeutic relevance in a mouse disease model(244). Most recently, "oligo-assisted vectors" were developed. Investigators suggested that this technology could be adjunctive to the aforementioned dual vector platforms. "Oligo-assisted" vectors bias concatemerization of partial cDNAs and eliminate the inhibitory ITR junction(245). Using a single-strand oligonucleotide complementary to distinct regions of the cDNA fragments of interest, the orientation of AAV concatemerization can be directed resulting in expression levels approaching that of a conventional sized single vector system(245). All dual vector platforms described herein have proven valuable for full-length, large-gene reconstruction via concatemerization of distinct genomes delivered by separate capsids and have promoted a paradigm shift in AAV-mediated gene therapy by presenting the opportunity to treat retinal diseases associated with mutations in genes with coding regions larger than 5kb.

In summary, taken together, the large body of preclinical efficacy and safety data now available in a wide variety of animal models of retinal diseases coupled with the emergence of multiple early stage

gene therapy clinical trials for several genetic forms of retinal degeneration and recent advancements in our understanding of how to more efficiently and safely target gene delivery to specific subsets of retinal cells bodes extremely well for the continued successful application of AAV vectors in the pursuit of therapies for genetic forms of human blindness.

Reference List

1. Dharmaraj,S., Leroy,B.P., Sohocki,M.M., Koenekoop,R.K., Perrault,I., Anwar,K., Khaliq,S., Devi,R.S., Birch,D.G., De,P.E*., et al.* (2004) The phenotype of Leber congenital amaurosis in patients with AIPL1 mutations. *Arch. Ophthalmol.*, **122**, 1029-1037.

2. Sohocki,M.M., Bowne,S.J., Sullivan,L.S., Blackshaw,S., Cepko,C.L., Payne,A.M., Bhattacharya,S.S., Khaliq,S., Qasim,M.S., Birch,D.G*., et al.* (2000) Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. *Nat. Genet.*, **24**, 79-83.

3. van der Spuy,J., Kim,J.H., Yu,Y.S., Szel,A., Luthert,P.J., Clark,B.J., Cheetham,M.E. (2003) The expression of the Leber congenital amaurosis protein AIPL1 coincides with rod and cone photoreceptor development. *Invest Ophthalmol. Vis. Sci.*, **44**, 5396-5403.

4. Hendrickson,A., Bumsted-O'Brien,K., Natoli,R., Ramamurthy,V., Possin,D., Provis,J. (2008) Rod photoreceptor differentiation in fetal and infant human retina. *Exp. Eye Res.*, **87**, 415-426.

5. van der,S.J., Munro,P.M., Luthert,P.J., Preising,M.N., Bek,T., Heegaard,S., Cheetham,M.E. (2005) Predominant rod photoreceptor degeneration in Leber congenital amaurosis. *Mol. Vis.*, **11**, 542-553.

6. Kirschman,L.T., Kolandaivelu,S., Frederick,J.M., Dang,L., Goldberg,A.F., Baehr,W., Ramamurthy,V. (2010) The Leber congenital amaurosis protein, AIPL1, is needed for the viability and functioning of cone photoreceptor cells. *Hum. Mol. Genet.*, **19**, 1076-1087.

7. Jacobson,S.G., Cideciyan,A.V., Aleman,T.S., Sumaroka,A., Roman,A.J., Swider,M., Schwartz,S.B., Banin,E., Stone,E.M. (2011) Human retinal disease from AIPL1 gene mutations: foveal cone loss with minimal macular photoreceptors and rod function remaining. *Invest Ophthalmol. Vis. Sci.*, **52**, 70-79.

8. Dyer,M.A., Donovan,S.L., Zhang,J., Gray,J., Ortiz,A., Tenney,R., Kong,J., Allikmets,R., Sohocki,M.M. (2004) Retinal degeneration in Aipl1-deficient mice: a new genetic model of Leber congenital amaurosis. *Brain Res. Mol. Brain Res.*, **132**, 208-220.

9. Ramamurthy,V., Niemi,G.A., Reh,T.A., Hurley,J.B. (2004) Leber congenital amaurosis linked to AIPL1: a mouse model reveals destabilization of cGMP phosphodiesterase. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 13897-13902.

10. Ramamurthy,V., Roberts,M., van den Akker,F., Niemi,G., Reh,T.A., Hurley,J.B. (2003) AIPL1, a protein implicated in Leber's congenital amaurosis, interacts with and aids in processing of farnesylated proteins. *Proc. Natl. Acad. Sci. U. S. A*, **100**, 12630-12635.

11. Liu,X., Bulgakov,O.V., Wen,X.H., Woodruff,M.L., Pawlyk,B., Yang,J., Fain,G.L., Sandberg,M.A., Makino,C.L., Li,T. (2004) AIPL1, the protein that is defective in Leber congenital amaurosis, is essential for the biosynthesis of retinal rod cGMP phosphodiesterase. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 13903-13908.

12. Tan,M.H., Smith,A.J., Pawlyk,B., Xu,X., Liu,X., Bainbridge,J.B., Basche,M., McIntosh,J., Tran,H.V., Nathwani,A.*, et al.* (2009) Gene therapy for retinitis pigmentosa and Leber congenital amaurosis caused by defects in AIPL1: effective rescue of mouse models of partial and complete Aipl1 deficiency using AAV2/2 and AAV2/8 vectors. *Hum. Mol. Genet.*, **18**, 2099-2114.

13. Sun,X., Pawlyk,B., Xu,X., Liu,X., Bulgakov,O.V., Adamian,M., Sandberg,M.A., Khani,S.C., Tan,M.H., Smith,A.J.*, et al.* (2010) Gene therapy with a promoter targeting both rods and cones rescues retinal degeneration caused by AIPL1 mutations. *Gene Ther.*, **17**, 117-131.

14. Ku,C.A., Chiodo,V.A., Boye,S.L., Goldberg,A.F., Li,T., Hauswirth,W.W., Ramamurthy,V. (2011) Gene therapy using self-complementary Y733F capsid mutant AAV2/8 restores vision in a model of early onset Leber congenital amaurosis. *Hum. Mol. Genet.*, **20**, 4569-4581.

15. Dryja,T.P., Adams,S.M., Grimsby,J.L., McGee,T.L., Hong,D.H., Li,T., Andreasson,S., Berson,E.L. (2001) Null RPGRIP1 alleles in patients with Leber congenital amaurosis. *Am. J. Hum. Genet.*, **68**, 1295-1298.

16. Gerber,S., Perrault,I., Hanein,S., Barbet,F., Ducroq,D., Ghazi,I., Martin-Coignard,D., Leowski,C., Homfray,T., Dufier,J.L.*, et al.* (2001) Complete exon-intron structure of the RPGR-interacting protein (RPGRIP1) gene allows the identification of mutations underlying Leber congenital amaurosis. *Eur. J. Hum. Genet.*, **9**, 561-571.

17. Hanein,S., Perrault,I., Gerber,S., Tanguy,G., Barbet,F., Ducroq,D., Calvas,P., Dollfus,H., Hamel,C., Lopponen,T.*, et al.* (2004) Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. *Hum. Mutat.*, **23**, 306-317.

18. den Hollander,A.I., Roepman,R., Koenekoop,R.K., Cremers,F.P. (2008) Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog. Retin. Eye Res.*, **27**, 391-419.

19. Castagnet,P., Mavlyutov,T., Cai,Y., Zhong,F., Ferreira,P. (2003) RPGRIP1s with distinct neuronal localization and biochemical properties associate selectively with RanBP2 in amacrine neurons. *Hum. Mol. Genet.*, **12**, 1847-1863.

20. Roepman,R., Bernoud-Hubac,N., Schick,D.E., Maugeri,A., Berger,W., Ropers,H.H., Cremers,F.P., Ferreira,P.A. (2000) The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. *Hum. Mol. Genet.*, **9**, 2095-2105.

21. Mavlyutov,T.A., Zhao,H., Ferreira,P.A. (2002) Species-specific subcellular localization of RPGR and RPGRIP isoforms: implications for the phenotypic variability of congenital retinopathies among species. *Hum. Mol. Genet.*, **11**, 1899-1907.

22. Boylan,J.P., Wright,A.F. (2000) Identification of a novel protein interacting with RPGR. *Hum. Mol. Genet.*, **9**, 2085-2093.

23. Hong,D.H., Yue,G., Adamian,M., Li,T. (2001) Retinitis pigmentosa GTPase regulator (RPGRr)-interacting protein is stably associated with the photoreceptor ciliary axoneme and anchors RPGR to the connecting cilium. *J. Biol. Chem.*, **276**, 12091-12099.

24. Lu,X., Ferreira,P.A. (2005) Identification of novel murine- and human-specific RPGRIP1 splice variants with distinct expression profiles and subcellular localization. *Invest Ophthalmol. Vis. Sci.*, **46**, 1882-1890.

25. Shu,X., Fry,A.M., Tulloch,B., Manson,F.D., Crabb,J.W., Khanna,H., Faragher,A.J., Lennon,A., He,S., Trojan,P.*, et al.* (2005) RPGR ORF15 isoform co-localizes with RPGRIP1 at centrioles and basal bodies and interacts with nucleophosmin. *Hum. Mol. Genet.*, **14**, 1183-1197.

26. Vervoort,R., Lennon,A., Bird,A.C., Tulloch,B., Axton,R., Miano,M.G., Meindl,A., Meitinger,T., Ciccodicola,A., Wright,A.F. (2000) Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. *Nat. Genet.*, **25**, 462-466.

27. Zhao,Y., Hong,D.H., Pawlyk,B., Yue,G., Adamian,M., Grynberg,M., Godzik,A., Li,T. (2003) The retinitis pigmentosa GTPase regulator (RPGR)- interacting protein: subserving RPGR function and participating in disk morphogenesis. *Proc. Natl. Acad. Sci. U. S. A*, **100**, 3965-3970.

28. Won,J., Gifford,E., Smith,R.S., Yi,H., Ferreira,P.A., Hicks,W.L., Li,T., Naggert,J.K., Nishina,P.M. (2009) RPGRIP1 is essential for normal rod photoreceptor outer segment elaboration and morphogenesis. *Hum. Mol. Genet.*, **18**, 4329-4339.

29. Coene,K.L., Mans,D.A., Boldt,K., Gloeckner,C.J., van,R.J., Bolat,E., Roosing,S., Letteboer,S.J., Peters,T.A., Cremers,F.P.*, et al.* (2011) The ciliopathy-associated protein homologs RPGRIP1 and RPGRIP1L are linked to cilium integrity through interaction with Nek4 serine/threonine kinase. *Hum. Mol. Genet.*, **20**, 3592-3605.

30. Pawlyk,B.S., Smith,A.J., Buch,P.K., Adamian,M., Hong,D.H., Sandberg,M.A., Ali,R.R., Li,T. (2005) Gene replacement therapy rescues photoreceptor degeneration in a murine model of Leber congenital amaurosis lacking RPGRIP. *Invest Ophthalmol. Vis. Sci.*, **46**, 3039-3045.

31. Khani,S.C., Pawlyk,B.S., Bulgakov,O.V., Kasperek,E., Young,J.E., Adamian,M., Sun,X., Smith,A.J., Ali,R.R., Li,T. (2007) AAV-mediated expression targeting of rod and cone photoreceptors with a human rhodopsin kinase promoter. *Invest Ophthalmol. Vis. Sci.*, **48**, 3954-3961.

32. Pawlyk,B.S., Bulgakov,O.V., Liu,X., Xu,X., Adamian,M., Sun,X., Khani,S.C., Berson,E.L., Sandberg,M.A., Li,T. (2010) Replacement gene therapy with a human RPGRIP1 sequence slows photoreceptor degeneration in a murine model of Leber congenital amaurosis. *Hum. Gene Ther.*, **21**, 993-1004.

33. Curtis,R., Barnett,K.C. (1993) Progressive retinal atrophy in miniature longhaired dachshund dogs. *Br. Vet. J.*, **149**, 71-85.

34. Mellersh,C.S., Boursnell,M.E., Pettitt,L., Ryder,E.J., Holmes,N.G., Grafham,D., Forman,O.P., Sampson,J., Barnett,K.C., Blanton,S.*, et al.* (2006) Canine RPGRIP1 mutation establishes cone-rod dystrophy in miniature longhaired dachshunds as a homologue of human Leber congenital amaurosis. *Genomics*, **88**, 293-301.

35. Turney,C., Chong,N.H., Alexander,R.A., Hogg,C.R., Fleming,L., Flack,D., Barnett,K.C., Bird,A.C., Holder,G.E., Luthert,P.J. (2007) Pathological and electrophysiological features of a canine cone-

rod dystrophy in the miniature longhaired dachshund. *Invest Ophthalmol. Vis. Sci.*, **48**, 4240-4249.

36. Beltran,W.A., Boye,S.L., Boye,S.E., Chiodo,V.A., Lewin,A.S., Hauswirth,W.W., Aguirre,G.D. (2010) rAAV2/5 gene-targeting to rods:dose-dependent efficiency and complications associated with different promoters. *Gene Ther.*, **17**, 1162-1174.

37. McLaughlin,M.E., Sandberg,M.A., Berson,E.L., Dryja,T.P. (1993) Recessive mutations in the gene encoding the beta-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. *Nat. Genet.*, **4**, 130-134.

38. McLaughlin,M.E., Ehrhart,T.L., Berson,E.L., Dryja,T.P. (1995) Mutation spectrum of the gene encoding the beta subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa. *Proc. Natl. Acad. Sci. U. S. A*, **92**, 3249-3253.

39. Danciger,M., Blaney,J., Gao,Y.Q., Zhao,D.Y., Heckenlively,J.R., Jacobson,S.G., Farber,D.B. (1995) Mutations in the PDE6B gene in autosomal recessive retinitis pigmentosa. *Genomics*, **30**, 1-7.

40. Keeler,C.E. (1924) The Inheritance of a Retinal Abnormality in White Mice. *Proc. Natl. Acad. Sci. U. S. A*, **10**, 329-333.

41. Pittler,S.J., Keeler,C.E., Sidman,R.L., Baehr,W. (1993) PCR analysis of DNA from 70-year-old sections of rodless retina demonstrates identity with the mouse rd defect. *Proc. Natl. Acad. Sci. U. S. A*, **90**, 9616-9619.

42. Pittler,S.J., Baehr,W. (1991) Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. *Proc. Natl. Acad. Sci. U. S. A*, **88**, 8322-8326.

43. Bowes,C., Li,T., Frankel,W.N., Danciger,M., Coffin,J.M., Applebury,M.L., Farber,D.B. (1993) Localization of a retroviral element within the rd gene coding for the beta subunit of cGMP phosphodiesterase. *Proc. Natl. Acad. Sci. U. S. A*, **90**, 2955-2959.

44. Chang,B., Hawes,N.L., Hurd,R.E., Davisson,M.T., Nusinowitz,S., Heckenlively,J.R. (2002) Retinal degeneration mutants in the mouse. *Vision Res.*, **42**, 517-525.

45. Bennett,J., Tanabe,T., Sun,D., Zeng,Y., Kjeldbye,H., Gouras,P., Maguire,A.M. (1996) Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. *Nat. Med.*, **2**, 649-654.

46. Jomary,C., Vincent,K.A., Grist,J., Neal,M.J., Jones,S.E. (1997) Rescue of photoreceptor function by AAV-mediated gene transfer in a mouse model of inherited retinal degeneration. *Gene Ther.*, **4**, 683-690.

47. Takahashi,M., Miyoshi,H., Verma,I.M., Gage,F.H. (1999) Rescue from photoreceptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer. *J. Virol.*, **73**, 7812-7816.

48. Reichel,M.B., Bainbridge,J., Baker,D., Thrasher,A.J., Bhattacharya,S.S., Ali,R.R. (2001) An immune response after intraocular administration of an adenoviral vector containing a beta galactosidase reporter gene slows retinal degeneration in the rd mouse. *Br. J. Ophthalmol.*, **85**, 341-344.

49. Chang,B., Hawes,N.L., Pardue,M.T., German,A.M., Hurd,R.E., Davisson,M.T., Nusinowitz,S., Rengarajan,K., Boyd,A.P., Sidney,S.S*., et al.* (2007) Two mouse retinal degenerations caused by missense mutations in the beta-subunit of rod cGMP phosphodiesterase gene. *Vision Res.*, **47**, 624-633.

50. Gargini,C., Terzibasi,E., Mazzoni,F., Strettoi,E. (2007) Retinal organization in the retinal degeneration 10 (rd10) mutant mouse: a morphological and ERG study. *J. Comp Neurol.*, **500**, 222-238.

51. Pang,J.J., Boye,S.L., Kumar,A., Dinculescu,A., Deng,W., Li,J., Li,Q., Rani,A., Foster,T.C., Chang,B*., et al.* (2008) AAV-mediated gene therapy for retinal degeneration in the rd10 mouse containing a recessive PDEbeta mutation. *Invest Ophthalmol. Vis. Sci.*, **49**, 4278-4283.

52. Pang,J.J., Dai,X., Boye,S.E., Barone,I., Boye,S.L., Mao,S., Everhart,D., Dinculescu,A., Liu,L., Umino,Y*., et al.* (2011) Long-term retinal function and structure rescue using capsid mutant AAV8 vector in the rd10 mouse, a model of recessive retinitis pigmentosa. *Mol. Ther.*, **19**, 234-242.

53. Allocca,M., Manfredi,A., Iodice,C., Di,V.U., Auricchio,A. (2011) AAV-mediated gene replacement, either alone or in combination with physical and pharmacological agents, results in partial and transient protection from photoreceptor degeneration associated with betaPDE deficiency. *Invest Ophthalmol. Vis. Sci.*, **52**, 5713-5719.

54. Petit,L., Lheriteau,E., Weber,M., Le,M.G., Deschamps,J.Y., Provost,N., Mendes-Madeira,A., Libeau,L., Guihal,C., Colle,M.A*., et al.* (2012) Restoration of Vision in the pde6beta-deficient Dog, a Large Animal Model of Rod-cone Dystrophy. *Mol. Ther.*, **20**, 2019-2030.

55. Suber,M.L., Pittler,S.J., Qin,N., Wright,G.C., Holcombe,V., Lee,R.H., Craft,C.M., Lolley,R.N., Baehr,W., Hurwitz,R.L. (1993) Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. *Proc. Natl. Acad. Sci. U. S. A*, **90**, 3968-3972.

56. Vervoort,R., Lennon,A., Bird,A.C., Tulloch,B., Axton,R., Miano,M.G., Meindl,A., Meitinger,T., Ciccodicola,A., Wright,A.F. (2000) Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. *Nat. Genet.*, **25**, 462-466.

57. Bader,I., Brandau,O., Achatz,H., Apfelstedt-Sylla,E., Hergersberg,M., Lorenz,B., Wissinger,B., Wittwer,B., Rudolph,G., Meindl,A., Meitinger,T. (2003) X-linked retinitis pigmentosa: RPGR mutations in most families with definite X linkage and clustering of mutations in a short sequence stretch of exon ORF15. *Invest Ophthalmol. Vis. Sci.*, **44**, 1458-1463.

58. Sharon,D., Sandberg,M.A., Rabe,V.W., Stillberger,M., Dryja,T.P., Berson,E.L. (2003) RP2 and RPGR mutations and clinical correlations in patients with X-linked retinitis pigmentosa. *Am. J. Hum. Genet.*, **73**, 1131-1146.

59. Jacobson,S.G., Buraczynska,M., Milam,A.H., Chen,C., Jarvalainen,M., Fujita,R., Wu,W., Huang,Y., Cideciyan,A.V., Swaroop,A. (1997) Disease expression in X-linked retinitis pigmentosa caused by a putative null mutation in the RPGR gene. *Invest Ophthalmol. Vis. Sci.*, **38**, 1983-1997.

60. Sandberg,M.A., Rosner,B., Weigel-DiFranco,C., Dryja,T.P., Berson,E.L. (2007) Disease course of patients with X-linked retinitis pigmentosa due to RPGR gene mutations. *Invest Ophthalmol. Vis. Sci.*, **48**, 1298-1304.

61. Zeiss,C.J., Ray,K., Acland,G.M., Aguirre,G.D. (2000) Mapping of X-linked progressive retinal atrophy (XLPRA), the canine homolog of retinitis pigmentosa 3 (RP3). *Hum. Mol. Genet.*, **9**, 531-537.

62. Zhang,Q., Acland,G.M., Wu,W.X., Johnson,J.L., Pearce-Kelling,S., Tulloch,B., Vervoort,R., Wright,A.F., Aguirre,G.D. (2002) Different RPGR exon ORF15 mutations in Canids provide insights into photoreceptor cell degeneration. *Hum. Mol. Genet.*, **11**, 993-1003.

63. Beltran,W.A., Hammond,P., Acland,G.M., Aguirre,G.D. (2006) A frameshift mutation in RPGR exon ORF15 causes photoreceptor degeneration and inner retina remodeling in a model of X-linked retinitis pigmentosa. *Invest Ophthalmol. Vis. Sci.*, **47**, 1669-1681.

64. Beltran,W.A., Cideciyan,A.V., Lewin,A.S., Iwabe,S., Khanna,H., Sumaroka,A., Chiodo,V.A., Fajardo,D.S., Roman,A.J., Deng,W.T.*, et al.* (2012) Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proc. Natl. Acad. Sci. U. S. A*, **109**, 2132-2137.

65. Huang,W.C., Wright,A.F., Roman,A.J., Cideciyan,A.V., Manson,F.D., Gewaily,D.Y., Schwartz,S.B., Sadigh,S., Limberis,M.P., Bell,P.*, et al.* (2012) RPGR-associated retinal degeneration in human X-linked RP and a murine model. *Invest Ophthalmol. Vis. Sci.*, **53**, 5594-5608.

66. Beales,P.L., Elcioglu,N., Woolf,A.S., Parker,D., Flinter,F.A. (1999) New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. *J. Med. Genet.*, **36**, 437-446.

67. Katsanis,N., Ansley,S.J., Badano,J.L., Eichers,E.R., Lewis,R.A., Hoskins,B.E., Scambler,P.J., Davidson,W.S., Beales,P.L., Lupski,J.R. (2001) Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science*, **293**, 2256-2259.

68. Ansley,S.J., Badano,J.L., Blacque,O.E., Hill,J., Hoskins,B.E., Leitch,C.C., Kim,J.C., Ross,A.J., Eichers,E.R., Teslovich,T.M.*, et al.* (2003) Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature*, **425**, 628-633.

69. Kim,J.C., Badano,J.L., Sibold,S., Esmail,M.A., Hill,J., Hoskins,B.E., Leitch,C.C., Venner,K., Ansley,S.J., Ross,A.J.*, et al.* (2004) The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat. Genet.*, **36**, 462-470.

70. Nachury,M.V., Loktev,A.V., Zhang,Q., Westlake,C.J., Peranen,J., Merdes,A., Slusarski,D.C., Scheller,R.H., Bazan,J.F., Sheffield,V.C., Jackson,P.K. (2007) A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell*, **129**, 1201-1213.

71. Blacque,O.E., Reardon,M.J., Li,C., McCarthy,J., Mahjoub,M.R., Ansley,S.J., Badano,J.L., Mah,A.K., Beales,P.L., Davidson,W.S*., et al.* (2004) Loss of C. elegans BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev.*, **18**, 1630-1642.

72. Lechtreck,K.F., Johnson,E.C., Sakai,T., Cochran,D., Ballif,B.A., Rush,J., Pazour,G.J., Ikebe,M., Witman,G.B. (2009) The Chlamydomonas reinhardtii BBSome is an IFT cargo required for export of specific signaling proteins from flagella. *J. Cell Biol.*, **187**, 1117-1132.

73. Kulaga,H.M., Leitch,C.C., Eichers,E.R., Badano,J.L., Lesemann,A., Hoskins,B.E., Lupski,J.R., Beales,P.L., Reed,R.R., Katsanis,N. (2004) Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat. Genet.*, **36**, 994-998.

74. Nishimura,D.Y., Fath,M., Mullins,R.F., Searby,C., Andrews,M., Davis,R., Andorf,J.L., Mykytyn,K., Swiderski,R.E., Yang,B*., et al.* (2004) Bbs2-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 16588-16593.

75. Mykytyn,K., Mullins,R.F., Andrews,M., Chiang,A.P., Swiderski,R.E., Yang,B., Braun,T., Casavant,T., Stone,E.M., Sheffield,V.C. (2004) Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 8664-8669.

76. Eichers,E.R., Abd-El-Barr,M.M., Paylor,R., Lewis,R.A., Bi,W., Lin,X., Meehan,T.P., Stockton,D.W., Wu,S.M., Lindsay,E*., et al.* (2006) Phenotypic characterization of Bbs4 null mice reveals age-dependent penetrance and variable expressivity. *Hum. Genet.*, **120**, 211-226.

77. Fath,M.A., Mullins,R.F., Searby,C., Nishimura,D.Y., Wei,J., Rahmouni,K., Davis,R.E., Tayeh,M.K., Andrews,M., Yang,B*., et al.* (2005) Mkks-null mice have a phenotype resembling Bardet-Biedl syndrome. *Hum. Mol. Genet.*, **14**, 1109-1118.

78. Davis,R.E., Swiderski,R.E., Rahmouni,K., Nishimura,D.Y., Mullins,R.F., Agassandian,K., Philp,A.R., Searby,C.C., Andrews,M.P., Thompson,S*., et al.* (2007) A knockin mouse model of the Bardet-Biedl syndrome 1 M390R mutation has cilia defects, ventriculomegaly, retinopathy, and obesity. *Proc. Natl. Acad. Sci. U. S. A*, **104**, 19422-19427.

79. Swiderski,R.E., Nishimura,D.Y., Mullins,R.F., Olvera,M.A., Ross,J.L., Huang,J., Stone,E.M., Sheffield,V.C. (2007) Gene expression analysis of photoreceptor cell loss in bbs4-knockout mice reveals an early stress gene response and photoreceptor cell damage. *Invest Ophthalmol. Vis. Sci.*, **48**, 3329-3340.

80. Abd-El-Barr,M.M., Sykoudis,K., Andrabi,S., Eichers,E.R., Pennesi,M.E., Tan,P.L., Wilson,J.H., Katsanis,N., Lupski,J.R., Wu,S.M. (2007) Impaired photoreceptor protein transport and synaptic transmission in a mouse model of Bardet-Biedl syndrome. *Vision Res.*, **47**, 3394-3407.

81. Spaggiari,E., Salati,R., Nicolini,P., Borgatti,R., Pozzoli,U., Polenghi,F. (1999) Evolution of ocular clinical and electrophysiological findings in pediatric Bardet-Biedl syndrome. *Int. Ophthalmol.*, **23**, 61-67.

82. Simons,D.L., Boye,S.L., Hauswirth,W.W., Wu,S.M. (2011) Gene therapy prevents photoreceptor death and preserves retinal function in a Bardet-Biedl syndrome mouse model. *Proc. Natl. Acad. Sci. U. S. A*, **108**, 6276-6281.

83. Mollon,J.D., Bowmaker,J.K., Jacobs,G.H. (1984) Variations of colour vision in a New World primate can be explained by polymorphism of retinal photopigments. *Proc. R. Soc. Lond B Biol. Sci.*, **222**, 373-399.

84. Mancuso,K., Hendrickson,A.E., Connor,T.B., Jr., Mauck,M.C., Kinsella,J.J., Hauswirth,W.W., Neitz,J., Neitz,M. (2007) Recombinant adeno-associated virus targets passenger gene expression to cones in primate retina. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.*, **24**, 1411-1416.

85. Wang,Y., Macke,J.P., Merbs,S.L., Zack,D.J., Klaunberg,B., Bennett,J., Gearhart,J., Nathans,J. (1992) A locus control region adjacent to the human red and green visual pigment genes. *Neuron*, **9**, 429-440.

86. Regan,B.C., Reffin,J.P., Mollon,J.D. (1994) Luminance noise and the rapid determination of discrimination ellipses in colour deficiency. *Vision Res.*, **34**, 1279-1299.

87. Mancuso,K., Neitz,M., Neitz,J. (2006) An adaptation of the Cambridge Colour Test for use with animals. *Vis. Neurosci.*, **23**, 695-701.

88. Mancuso,K., Hauswirth,W.W., Li,Q., Connor,T.B., Kuchenbecker,J.A., Mauck,M.C., Neitz,J., Neitz,M. (2009) Gene therapy for red-green colour blindness in adult primates. *Nature*, **461**, 784-787.

89. WIESEL,T.N., HUBEL,D.H. (1963) EFFECTS OF VISUAL DEPRIVATION ON MORPHOLOGY AND PHYSIOLOGY OF CELLS IN THE CATS LATERAL GENICULATE BODY. *J. Neurophysiol.*, **26**, 978-993.

90. WIESEL,T.N., HUBEL,D.H. (1963) SINGLE-CELL RESPONSES IN STRIATE CORTEX OF KITTENS DEPRIVED OF VISION IN ONE EYE. *J. Neurophysiol.*, **26**, 1003-1017.

91. Ayyagari,R., Kakuk,L.E., Bingham,E.L., Szczesny,J.J., Kemp,J., Toda,Y., Felius,J., Sieving,P.A. (2000) Spectrum of color gene deletions and phenotype in patients with blue cone monochromacy. *Hum. Genet.*, **107**, 75-82.

92. Adler,R., Landa,K.B., Manthorpe,M., Varon,S. (1979) Cholinergic neuronotrophic factors: intraocular distribution of trophic activity for ciliary neurons. *Science*, **204**, 1434-1436.

93. Sendtner,M., Stockli,K.A., Thoenen,H. (1992) Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during regeneration. *J. Cell Biol.*, **118**, 139-148.

94. LaVail,M.M., Unoki,K., Yasumura,D., Matthes,M.T., Yancopoulos,G.D., Steinberg,R.H. (1992) Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proc. Natl. Acad. Sci. U. S. A*, **89**, 11249-11253.

95. Beltran,W.A. (2008) On the role of CNTF as a potential therapy for retinal degeneration: Dr. Jekyll or Mr. Hyde? *Adv. Exp. Med. Biol.*, **613**, 45-51.

96. Liang,F.Q., Aleman,T.S., Dejneka,N.S., Dudus,L., Fisher,K.J., Maguire,A.M., Jacobson,S.G., Bennett,J. (2001) Long-term protection of retinal structure but not function using RAAV.CNTF in animal models of retinitis pigmentosa. *Mol. Ther.*, **4**, 461-472.

97. Bok,D., Yasumura,D., Matthes,M.T., Ruiz,A., Duncan,J.L., Chappelow,A.V., Zolutukhin,S., Hauswirth,W., LaVail,M.M. (2002) Effects of adeno-associated virus-vectored ciliary neurotrophic factor on retinal structure and function in mice with a P216L rds/peripherin mutation. *Exp. Eye Res.*, **74**, 719-735.

98. Schlichtenbrede,F.C., MacNeil,A., Bainbridge,J.W., Tschernutter,M., Thrasher,A.J., Smith,A.J., Ali,R.R. (2003) Intraocular gene delivery of ciliary neurotrophic factor results in significant loss of retinal function in normal mice and in the Prph2Rd2/Rd2 model of retinal degeneration. *Gene Ther.*, **10**, 523-527.

99. Bush,R.A., Lei,B., Tao,W., Raz,D., Chan,C.C., Cox,T.A., Santos-Muffley,M., Sieving,P.A. (2004) Encapsulated cell-based intraocular delivery of ciliary neurotrophic factor in normal rabbit: dose-dependent effects on ERG and retinal histology. *Invest Ophthalmol. Vis. Sci.*, **45**, 2420-2430.

100. Wen,R., Song,Y., Kjellstrom,S., Tanikawa,A., Liu,Y., Li,Y., Zhao,L., Bush,R.A., Laties,A.M., Sieving,P.A. (2006) Regulation of rod phototransduction machinery by ciliary neurotrophic factor. *J. Neurosci.*, **26**, 13523-13530.

101. Beltran,W.A., Wen,R., Acland,G.M., Aguirre,G.D. (2007) Intravitreal injection of ciliary neurotrophic factor (CNTF) causes peripheral remodeling and does not prevent photoreceptor loss in canine RPGR mutant retina. *Exp. Eye Res.*, **84**, 753-771.

102. Li,Y., Tao,W., Luo,L., Huang,D., Kauper,K., Stabila,P., LaVail,M.M., Laties,A.M., Wen,R. (2010) CNTF induces regeneration of cone outer segments in a rat model of retinal degeneration. *PLoS. One.*, **5**, e9495.

103. Komaromy,A., Iwabe,S., Rowlan,J.S., Duncan,A.D., Rao,S., Oh,A., Wen,R., Aguirre,G.D. (2012) *ARVO*. Vol. Ft. Lauderdale, Fl.

104. Thanos,C.G., Bell,W.J., O'Rourke,P., Kauper,K., Sherman,S., Stabila,P., Tao,W. (2004) Sustained secretion of ciliary neurotrophic factor to the vitreous, using the encapsulated cell therapy-based NT-501 intraocular device. *Tissue Eng*, **10**, 1617-1622.

105. Tao,W., Wen,R., Goddard,M.B., Sherman,S.D., O'Rourke,P.J., Stabila,P.F., Bell,W.J., Dean,B.J., Kauper,K.A., Budz,V.A*., et al.* (2002) Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. *Invest Ophthalmol. Vis. Sci.*, **43**, 3292-3298.

106. Tao,W. (2006) Application of encapsulated cell technology for retinal degenerative diseases. *Expert. Opin. Biol. Ther.*, **6**, 717-726.

107. Sieving,P.A., Caruso,R.C., Tao,W., Coleman,H.R., Thompson,D.J., Fullmer,K.R., Bush,R.A. (2006) Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF

delivered by encapsulated cell intraocular implants. *Proc. Natl. Acad. Sci. U. S. A*, **103**, 3896-3901.

108. Zhang,K., Hopkins,J.J., Heier,J.S., Birch,D.G., Halperin,L.S., Albini,T.A., Brown,D.M., Jaffe,G.J., Tao,W., Williams,G.A. (2011) Ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for treatment of geographic atrophy in age-related macular degeneration. *Proc. Natl. Acad. Sci. U. S. A*, **108**, 6241-6245.

109. Lin,L.F., Doherty,D.H., Lile,J.D., Bektesh,S., Collins,F. (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*, **260**, 1130-1132.

110. Kaddis,F.G., Zawada,W.M., Schaack,J., Freed,C.R. (1996) Conditioned medium from aged monkey fibroblasts stably expressing GDNF and BDNF improves survival of embryonic dopamine neurons in vitro. *Cell Tissue Res.*, **286**, 241-247.

111. Gash,D.M., Zhang,Z., Ovadia,A., Cass,W.A., Yi,A., Simmerman,L., Russell,D., Martin,D., Lapchak,P.A., Collins,F.*, et al.* (1996) Functional recovery in parkinsonian monkeys treated with GDNF. *Nature*, **380**, 252-255.

112. Choi-Lundberg,D.L., Lin,Q., Chang,Y.N., Chiang,Y.L., Hay,C.M., Mohajeri,H., Davidson,B.L., Bohn,M.C. (1997) Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science*, **275**, 838-841.

113. Oppenheim,R.W., Houenou,L.J., Johnson,J.E., Lin,L.F., Li,L., Lo,A.C., Newsome,A.L., Prevette,D.M., Wang,S. (1995) Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. *Nature*, **373**, 344-346.

114. Nosrat,C.A., Tomac,A., Lindqvist,E., Lindskog,S., Humpel,C., Stromberg,I., Ebendal,T., Hoffer,B.J., Olson,L. (1996) Cellular expression of GDNF mRNA suggests multiple functions inside and outside the nervous system. *Cell Tissue Res.*, **286**, 191-207.

115. Frasson,M., Picaud,S., Leveillard,T., Simonutti,M., Mohand-Said,S., Dreyfus,H., Hicks,D., Sabel,J. (1999) Glial cell line-derived neurotrophic factor induces histologic and functional protection of rod photoreceptors in the rd/rd mouse. *Invest Ophthalmol. Vis. Sci.*, **40**, 2724-2734.

116. McGee Sanftner,L.H., Abel,H., Hauswirth,W.W., Flannery,J.G. (2001) Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. *Mol. Ther.*, **4**, 622-629.

117. Wu,W.C., Lai,C.C., Chen,S.L., Sun,M.H., Xiao,X., Chen,T.L., Tsai,R.J., Kuo,S.W., Tsao,Y.P. (2004) GDNF gene therapy attenuates retinal ischemic injuries in rats. *Mol. Vis.*, **10**, 93-102.

118. Buch,P.K., Maclaren,R.E., Duran,Y., Balaggan,K.S., Macneil,A., Schlichtenbrede,F.C., Smith,A.J., Ali,R.R. (2006) In Contrast to AAV-Mediated Cntf Expression, AAV-Mediated Gdnf Expression Enhances Gene Replacement Therapy in Rodent Models of Retinal Degeneration. *Mol. Ther.*.

119. Bringmann,A., Pannicke,T., Grosche,J., Francke,M., Wiedemann,P., Skatchkov,S.N., Osborne,N.N., Reichenbach,A. (2006) Muller cells in the healthy and diseased retina. *Prog. Retin. Eye Res.*, **25**, 397-424.

120. Klimczak,R.R., Koerber,J.T., Dalkara,D., Flannery,J.G., Schaffer,D.V. (2009) A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Muller cells. *PLoS. One.*, **4**, e7467.

121. Dalkara,D., Kolstad,K.D., Guerin,K.I., Hoffmann,N.V., Visel,M., Klimczak,R.R., Schaffer,D.V., Flannery,J.G. (2011) AAV mediated GDNF secretion from retinal glia slows down retinal degeneration in a rat model of retinitis pigmentosa. *Mol. Ther.*, **19**, 1602-1608.

122. Deveraux,Q.L., Takahashi,R., Salvesen,G.S., Reed,J.C. (1997) X-linked IAP is a direct inhibitor of cell-death proteases. *Nature*, **388**, 300-304.

123. Rehm,M., Huber,H.J., Dussmann,H., Prehn,J.H. (2006) Systems analysis of effector caspase activation and its control by X-linked inhibitor of apoptosis protein. *EMBO J.*, **25**, 4338-4349.

124. Holcik,M., Gibson,H., Korneluk,R.G. (2001) XIAP: apoptotic brake and promising therapeutic target. *Apoptosis.*, **6**, 253-261.

125. Renwick,J., Narang,M.A., Coupland,S.G., Xuan,J.Y., Baker,A.N., Brousseau,J., Petrin,D., Munger,R., Leonard,B.C., Hauswirth,W.W*., et al.* (2006) XIAP-mediated neuroprotection in retinal ischemia. *Gene Ther.*, **13**, 339-347.

126. McKinnon,S.J., Lehman,D.M., Tahzib,N.G., Ransom,N.L., Reitsamer,H.A., Liston,P., LaCasse,E., Li,Q., Korneluk,R.G., Hauswirth,W.W. (2002) Baculoviral IAP repeat-containing-4 protects optic nerve axons in a rat glaucoma model. *Mol. Ther.*, **5**, 780-787.

127. Petrin,D., Baker,A., Brousseau,J., Coupland,S., Liston,P., Hauswirth,W.W., Komeluk,R.G., Tsilfidis,C. (2003) XIAP protects photoreceptors from n-methyl-n-nitrosourea-induced retinal degeneration. *Adv. Exp. Med. Biol.*, **533**, 385-393.

128. Cook,B., Lewis,G.P., Fisher,S.K., Adler,R. (1995) Apoptotic photoreceptor degeneration in experimental retinal detachment. *Invest Ophthalmol. Vis. Sci.*, **36**, 990-996.

129. Zacks,D.N., Hanninen,V., Pantcheva,M., Ezra,E., Grosskreutz,C., Miller,J.W. (2003) Caspase activation in an experimental model of retinal detachment. *Invest Ophthalmol. Vis. Sci.*, **44**, 1262-1267.

130. Chang,C.J., Lai,W.W., Edward,D.P., Tso,M.O. (1995) Apoptotic photoreceptor cell death after traumatic retinal detachment in humans. *Arch. Ophthalmol.*, **113**, 880-886.

131. Lewis,G.P., Charteris,D.G., Sethi,C.S., Leitner,W.P., Linberg,K.A., Fisher,S.K. (2002) The ability of rapid retinal reattachment to stop or reverse the cellular and molecular events initiated by detachment. *Invest Ophthalmol. Vis. Sci.*, **43**, 2412-2420.

132. Zadro-Lamoureux,L.A., Zacks,D.N., Baker,A.N., Zheng,Q.D., Hauswirth,W.W., Tsilfidis,C. (2009) XIAP effects on retinal detachment-induced photoreceptor apoptosis [corrected]. *Invest Ophthalmol. Vis. Sci.*, **50**, 1448-1453.

133. Beatty,S., Koh,H., Phil,M., Henson,D., Boulton,M. (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv. Ophthalmol.*, **45**, 115-134.

134. Zarbin,M.A. (2004) Current concepts in the pathogenesis of age-related macular degeneration. *Arch. Ophthalmol.*, **122**, 598-614.

135. Shan,H., Ji,D., Barnard,A.R., Lipinski,D.M., You,Q., Lee,E.J., Kamalden,T.A., Sun,X., MacLaren,R.E. (2011) AAV-mediated gene transfer of human X-linked inhibitor of apoptosis protects against oxidative cell death in human RPE cells. *Invest Ophthalmol. Vis. Sci.*, **52**, 9591-9597.

136. Lamba,D., Karl,M., Reh,T. (2008) Neural regeneration and cell replacement: a view from the eye. *Cell Stem Cell*, **2**, 538-549.

137. MacLaren,R.E., Pearson,R.A., MacNeil,A., Douglas,R.H., Salt,T.E., Akimoto,M., Swaroop,A., Sowden,J.C., Ali,R.R. (2006) Retinal repair by transplantation of photoreceptor precursors. *Nature*, **444**, 203-207.

138. Kwan,A.S., Wang,S., Lund,R.D. (1999) Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Exp. Neurol.*, **159**, 21-33.

139. Lamba,D.A., Gust,J., Reh,T.A. (2009) Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. *Cell Stem Cell*, **4**, 73-79.

140. Yao,J., Feathers,K.L., Khanna,H., Thompson,D., Tsilfidis,C., Hauswirth,W.W., Heckenlively,J.R., Swaroop,A., Zacks,D.N. (2011) XIAP therapy increases survival of transplanted rod precursors in a degenerating host retina. *Invest Ophthalmol. Vis. Sci.*, **52**, 1567-1572.

141. Marigo,V. (2007) Programmed cell death in retinal degeneration: targeting apoptosis in photoreceptors as potential therapy for retinal degeneration. *Cell Cycle*, **6**, 652-655.

142. Leonard,K.C., Petrin,D., Coupland,S.G., Baker,A.N., Leonard,B.C., LaCasse,E.C., Hauswirth,W.W., Korneluk,R.G., Tsilfidis,C. (2007) XIAP protection of photoreceptors in animal models of retinitis pigmentosa. *PLoS. One.*, **2**, e314.

143. Mendes,H.F., van der Spuy,J., Chapple,J.P., Cheetham,M.E. (2005) Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. *Trends Mol. Med.*, **11**, 177-185.

144. Sung,C.H., Makino,C., Baylor,D., Nathans,J. (1994) A rhodopsin gene mutation responsible for autosomal dominant retinitis pigmentosa results in a protein that is defective in localization to the photoreceptor outer segment. *J. Neurosci.*, **14**, 5818-5833.

145. Green,E.S., Menz,M.D., LaVail,M.M., Flannery,J.G. (2000) Characterization of rhodopsin mis-sorting and constitutive activation in a transgenic rat model of retinitis pigmentosa. *Invest Ophthalmol. Vis. Sci.*, **41**, 1546-1553.

146. Maiese,K., Chong,Z.Z., Li,F., Shang,Y.C. (2008) Erythropoietin: elucidating new cellular targets that broaden therapeutic strategies. *Prog. Neurobiol.*, **85**, 194-213.

147. Brines,M., Cerami,A. (2005) Emerging biological roles for erythropoietin in the nervous system. *Nat. Rev. Neurosci.*, **6**, 484-494.

148. Hasselblatt,M., Ehrenreich,H., Siren,A.L. (2006) The brain erythropoietin system and its potential for therapeutic exploitation in brain disease. *J. Neurosurg. Anesthesiol.*, **18**, 132-138.

149. Um,M., Gross,A.W., Lodish,H.F. (2007) A "classical" homodimeric erythropoietin receptor is essential for the antiapoptotic effects of erythropoietin on differentiated neuroblastoma SH-SY5Y and pheochromocytoma PC-12 cells. *Cell Signal.*, **19**, 634-645.

150. Masuda,S., Nagao,M., Takahata,K., Konishi,Y., Gallyas,F., Jr., Tabira,T., Sasaki,R. (1993) Functional erythropoietin receptor of the cells with neural characteristics. Comparison with receptor properties of erythroid cells. *J. Biol. Chem.*, **268**, 11208-11216.

151. Sakanaka,M., Wen,T.C., Matsuda,S., Masuda,S., Morishita,E., Nagao,M., Sasaki,R. (1998) In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc. Natl. Acad. Sci. U. S. A*, **95**, 4635-4640.

152. Siren,A.L., Knerlich,F., Poser,W., Gleiter,C.H., Bruck,W., Ehrenreich,H. (2001) Erythropoietin and erythropoietin receptor in human ischemic/hypoxic brain. *Acta Neuropathol.*, **101**, 271-276.

153. Chen,Z.Y., Asavaritikrai,P., Prchal,J.T., Noguchi,C.T. (2007) Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. *J. Biol. Chem.*, **282**, 25875-25883.

154. Sanchez,P.E., Fares,R.P., Risso,J.J., Bonnet,C., Bouvard,S., Le-Cavorsin,M., Georges,B., Moulin,C., Belmeguenai,A., Bodennec,J.*, et al.* (2009) Optimal neuroprotection by erythropoietin requires elevated expression of its receptor in neurons. *Proc. Natl. Acad. Sci. U. S. A*, **106**, 9848-9853.

155. Hanazono,Y., Sasaki,K., Nitta,H., Yazaki,Y., Hirai,H. (1995) Erythropoietin induces tyrosine phosphorylation of the beta chain of the GM-CSF receptor. *Biochem. Biophys. Res. Commun.*, **208**, 1060-1066.

156. Jubinsky,P.T., Krijanovski,O.I., Nathan,D.G., Tavernier,J., Sieff,C.A. (1997) The beta chain of the interleukin-3 receptor functionally associates with the erythropoietin receptor. *Blood*, **90**, 1867-1873.

157. Blake,T.J., Jenkins,B.J., D'Andrea,R.J., Gonda,T.J. (2002) Functional cross-talk between cytokine receptors revealed by activating mutations in the extracellular domain of the beta-subunit of the GM-CSF receptor. *J. Leukoc. Biol.*, **72**, 1246-1255.

158. Su,K.H., Shyue,S.K., Kou,Y.R., Ching,L.C., Chiang,A.N., Yu,Y.B., Chen,C.Y., Pan,C.C., Lee,T.S. (2011) beta Common receptor integrates the erythropoietin signaling in activation of endothelial nitric oxide synthase. *J. Cell Physiol*, **226**, 3330-3339.

159. Brines,M., Grasso,G., Fiordaliso,F., Sfacteria,A., Ghezzi,P., Fratelli,M., Latini,R., Xie,Q.W., Smart,J., Su-Rick,C.J.*, et al.* (2004) Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 14907-14912.

160. Colella,P., Iodice,C., Di,V.U., Annunziata,I., Surace,E.M., Auricchio,A. (2011) Non-erythropoietic erythropoietin derivatives protect from light-induced and genetic photoreceptor degeneration. *Hum. Mol. Genet.*, **20**, 2251-2262.

161. Grimm,C., Wenzel,A., Groszer,M., Mayser,H., Seeliger,M., Samardzija,M., Bauer,C., Gassmann,M., Reme,C.E. (2002) HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. *Nat. Med.*, **8**, 718-724.

162. Rex,T.S., Allocca,M., Domenici,L., Surace,E.M., Maguire,A.M., Lyubarsky,A., Cellerino,A., Bennett,J., Auricchio,A. (2004) Systemic but not intraocular Epo gene transfer protects the retina from light-and genetic-induced degeneration. *Mol. Ther.*, **10**, 855-861.

163. Junk,A.K., Mammis,A., Savitz,S.I., Singh,M., Roth,S., Malhotra,S., Rosenbaum,P.S., Cerami,A., Brines,M., Rosenbaum,D.M. (2002) Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. *Proc. Natl. Acad. Sci. U. S. A*, **99**, 10659-10664.

164. Grimm,C., Wenzel,A., Acar,N., Keller,S., Seeliger,M., Gassmann,M. (2006) Hypoxic preconditioning and erythropoietin protect retinal neurons from degeneration. *Adv. Exp. Med. Biol.*, **588**, 119-131.

165. Zhong,L., Bradley,J., Schubert,W., Ahmed,E., Adamis,A.P., Shima,D.T., Robinson,G.S., Ng,Y.S. (2007) Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Invest Ophthalmol. Vis. Sci.*, **48**, 1212-1218.

166. King,C.E., Rodger,J., Bartlett,C., Esmaili,T., Dunlop,S.A., Beazley,L.D. (2007) Erythropoietin is both neuroprotective and neuroregenerative following optic nerve transection. *Exp. Neurol.*, **205**, 48-55.

167. Tsai,J.C., Wu,L., Worgul,B., Forbes,M., Cao,J. (2005) Intravitreal administration of erythropoietin and preservation of retinal ganglion cells in an experimental rat model of glaucoma. *Curr. Eye Res.*, **30**, 1025-1031.

168. Rex,T.S., Wong,Y., Kodali,K., Merry,S. (2009) Neuroprotection of photoreceptors by direct delivery of erythropoietin to the retina of the retinal degeneration slow mouse. *Exp. Eye Res.*, **89**, 735-740.

169. Zhang,J., Wu,Y., Jin,Y., Ji,F., Sinclair,S.H., Luo,Y., Xu,G., Lu,L., Dai,W., Yanoff,M*., et al.* (2008) Intravitreal injection of erythropoietin protects both retinal vascular and neuronal cells in early diabetes. *Invest Ophthalmol. Vis. Sci.*, **49**, 732-742.

170. Sullivan,T., Kodali,K., Rex,T.S. (2011) Systemic gene delivery protects the photoreceptors in the retinal degeneration slow mouse. *Neurochem. Res.*, **36**, 613-618.

171. Sullivan,T.A., Geisert,E.E., Hines-Beard,J., Rex,T.S. (2011) Systemic adeno-associated virus-mediated gene therapy preserves retinal ganglion cells and visual function in DBA/2J glaucomatous mice. *Hum. Gene Ther.*, **22**, 1191-1200.

172. Nagel,G., Szellas,T., Huhn,W., Kateriya,S., Adeishvili,N., Berthold,P., Ollig,D., Hegemann,P., Bamberg,E. (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc. Natl. Acad. Sci. U. S. A*, **100**, 13940-13945.

173. Nagel,G., Brauner,M., Liewald,J.F., Adeishvili,N., Bamberg,E., Gottschalk,A. (2005) Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. *Curr. Biol.*, **15**, 2279-2284.

174. Nagel,G., Szellas,T., Kateriya,S., Adeishvili,N., Hegemann,P., Bamberg,E. (2005) Channelrhodopsins: directly light-gated cation channels. *Biochem. Soc. Trans.*, **33**, 863-866.

175. Sineshchekov,O.A., Jung,K.H., Spudich,J.L. (2002) Two rhodopsins mediate phototaxis to low- and high-intensity light in Chlamydomonas reinhardtii. *Proc. Natl. Acad. Sci. U. S. A*, **99**, 8689-8694.

176. Boyden,E.S., Zhang,F., Bamberg,E., Nagel,G., Deisseroth,K. (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.*, **8**, 1263-1268.

177. Bi,A., Cui,J., Ma,Y.P., Olshevskaya,E., Pu,M., Dizhoor,A.M., Pan,Z.H. (2006) Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron*, **50**, 23-33.

178. Tomita,H., Sugano,E., Yawo,H., Ishizuka,T., Isago,H., Narikawa,S., Kugler,S., Tamai,M. (2007) Restoration of visual response in aged dystrophic RCS rats using AAV-mediated channelopsin-2 gene transfer. *Invest Ophthalmol. Vis. Sci.*, **48**, 3821-3826.

179. Tomita,H., Sugano,E., Isago,H., Hiroi,T., Wang,Z., Ohta,E., Tamai,M. (2010) Channelrhodopsin-2 gene transduced into retinal ganglion cells restores functional vision in genetically blind rats. *Exp. Eye Res.*, **90**, 429-436.

180. Tomita,H., Sugano,E., Fukazawa,Y., Isago,H., Sugiyama,Y., Hiroi,T., Ishizuka,T., Mushiake,H., Kato,M., Hirabayashi,M.*, et al.* (2009) Visual properties of transgenic rats harboring the channelrhodopsin-2 gene regulated by the thy-1.2 promoter. *PLoS. One.*, **4**, e7679.

181. Ivanova,E., Hwang,G.S., Pan,Z.H., Troilo,D. (2010) Evaluation of AAV-mediated expression of Chop2-GFP in the marmoset retina. *Invest Ophthalmol. Vis. Sci.*, **51**, 5288-5296.

182. Han,X., Boyden,E.S. (2007) Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS. One.*, **2**, e299.

183. Zhang,F., Wang,L.P., Brauner,M., Liewald,J.F., Kay,K., Watzke,N., Wood,P.G., Bamberg,E., Nagel,G., Gottschalk,A., Deisseroth,K. (2007) Multimodal fast optical interrogation of neural circuitry. *Nature*, **446**, 633-639.

184. Ivanova,E., Pan,Z.H. (2009) Evaluation of the adeno-associated virus mediated long-term expression of channelrhodopsin-2 in the mouse retina. *Mol. Vis.*, **15**, 1680-1689.

185. Sugano,E., Isago,H., Wang,Z., Murayama,N., Tamai,M., Tomita,H. (2011) Immune responses to adeno-associated virus type 2 encoding channelrhodopsin-2 in a genetically blind rat model for gene therapy. *Gene Ther.*, **18**, 266-274.

186. Doroudchi,M.M., Greenberg,K.P., Liu,J., Silka,K.A., Boyden,E.S., Lockridge,J.A., Arman,A.C., Janani,R., Boye,S.E., Boye,S.L.*, et al.* (2011) Virally delivered channelrhodopsin-2 safely and

effectively restores visual function in multiple mouse models of blindness. *Mol. Ther.*, **19**, 1220-1229.

187. Zhang,F., Wang,L.P., Brauner,M., Liewald,J.F., Kay,K., Watzke,N., Wood,P.G., Bamberg,E., Nagel,G., Gottschalk,A., Deisseroth,K. (2007) Multimodal fast optical interrogation of neural circuitry. *Nature*, **446**, 633-639.

188. Busskamp,V., Duebel,J., Balya,D., Fradot,M., Viney,T.J., Siegert,S., Groner,A.C., Cabuy,E., Forster,V., Seeliger,M.*, et al.* (2010) Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science*, **329**, 413-417.

189. Claes,E., Seeliger,M., Michalakis,S., Biel,M., Humphries,P., Haverkamp,S. (2004) Morphological characterization of the retina of the CNGA3(-/-)Rho(-/-) mutant mouse lacking functional cones and rods. *Invest Ophthalmol. Vis. Sci.*, **45**, 2039-2048.

190. Farber,D.B., Heckenlively,J.R., Sparkes,R.S., Bateman,J.B. (1991) Molecular genetics of retinitis pigmentosa. *West J. Med.*, **155**, 388-399.

191. Pickrell,S.W., Zhu,X., Wang,X., Craft,C.M. (2004) Deciphering the contribution of known cis-elements in the mouse cone arrestin gene to its cone-specific expression. *Invest Ophthalmol. Vis. Sci.*, **45**, 3877-3884.

192. Allocca,M., Mussolino,C., Garcia-Hoyos,M., Sanges,D., Iodice,C., Petrillo,M., Vandenberghe,L.H., Wilson,J.M., Marigo,V., Surace,E.M., Auricchio,A. (2007) Novel adeno-associated virus serotypes efficiently transduce murine photoreceptors. *J. Virol.*, **81**, 11372-11380.

193. Lagali,P.S., Balya,D., Awatramani,G.B., Munch,T.A., Kim,D.S., Busskamp,V., Cepko,C.L., Roska,B. (2008) Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat. Neurosci.*, **11**, 667-675.

194. Petrs-Silva,H., Dinculescu,A., Li,Q., Deng,W.T., Pang,J.J., Min,S.H., Chiodo,V., Neeley,A.W., Govindasamy,L., Bennett,A.*, et al.* (2011) Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. *Mol. Ther.*, **19**, 293-301.

195. Marc,R.E., Jones,B.W., Anderson,J.R., Kinard,K., Marshak,D.W., Wilson,J.H., Wensel,T., Lucas,R.J. (2007) Neural reprogramming in retinal degeneration. *Invest Ophthalmol. Vis. Sci.*, **48**, 3364-3371.

196. Busskamp,V., Roska,B. (2011) Optogenetic approaches to restoring visual function in retinitis pigmentosa. *Curr. Opin. Neurobiol.*, **21**, 942-946.

197. Volgraf,M., Gorostiza,P., Numano,R., Kramer,R.H., Isacoff,E.Y., Trauner,D. (2006) Allosteric control of an ionotropic glutamate receptor with an optical switch. *Nat. Chem. Biol.*, **2**, 47-52.

198. Gorostiza,P., Volgraf,M., Numano,R., Szobota,S., Trauner,D., Isacoff,E.Y. (2007) Mechanisms of photoswitch conjugation and light activation of an ionotropic glutamate receptor. *Proc. Natl. Acad. Sci. U. S. A*, **104**, 10865-10870.

199. Caporale,N., Kolstad,K.D., Lee,T., Tochitsky,I., Dalkara,D., Trauner,D., Kramer,R., Dan,Y., Isacoff,E.Y., Flannery,J.G. (2011) LiGluR restores visual responses in rodent models of inherited blindness. *Mol. Ther.*, **19**, 1212-1219.

200. Shen,J., Yang,X., Dong,A., Petters,R.M., Peng,Y.W., Wong,F., Campochiaro,P.A. (2005) Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J. Cell Physiol*, **203**, 457-464.

201. Usui,S., Komeima,K., Lee,S.Y., Jo,Y.J., Ueno,S., Rogers,B.S., Wu,Z., Shen,J., Lu,L., Oveson,B.C*., et al.* (2009) Increased expression of catalase and superoxide dismutase 2 reduces cone cell death in retinitis pigmentosa. *Mol Ther*, **17**, 778-786.

202. Kowluru,R.A., Kowluru,V., Xiong,Y., Ho,Y.S. (2006) Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. *Free Radic. Biol Med*, **41**, 1191-1196.

203. Kowluru,R.A., Chan,P.S. (2007) Oxidative stress and diabetic retinopathy. *Exp. Diabetes Res.*, **2007**, 43603.

204. Cai,J., Nelson,K.C., Wu,M., Sternberg,P., Jr., Jones,D.P. (2000) Oxidative damage and protection of the RPE. *Prog. Retin. Eye Res.*, **19**, 205-221.

205. Liang,F.Q., Godley,B.F. (2003) Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. *Exp. Eye Res.*, **76**, 397-403.

206. Wu,Z., Lauer,T.W., Sick,A., Hackett,S.F., Campochiaro,P.A. (2007) Oxidative stress modulates complement factor H expression in retinal pigmented epithelial cells by acetylation of FOXO3. *J Biol. Chem.*, **282**, 22414-22425.

207. Usui,S., Oveson,B.C., Iwase,T., Lu,L., Lee,S.Y., Jo,Y.J., Wu,Z., Choi,E.Y., Samulski,R.J., Campochiaro,P.A. (2011) Overexpression of SOD in retina: Need for increase in H(2)O(2)-detoxifying enzyme in same cellular compartment. *Free Radic. Biol Med*.

208. Qi,X., Lewin,A.S., Sun,L., Hauswirth,W.W., Guy,J. (2007) Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis. *Invest Ophthalmol. Vis. Sci.*, **48**, 681-691.

209. Osborne,N.N., Ugarte,M., Chao,M., Chidlow,G., Bae,J.H., Wood,J.P., Nash,M.S. (1999) Neuroprotection in relation to retinal ischemia and relevance to glaucoma. *Surv. Ophthalmol*, **43 Suppl 1**, S102-S128.

210. Zheng,L., Gong,B., Hatala,D.A., Kern,T.S. (2007) Retinal ischemia and reperfusion causes capillary degeneration: similarities to diabetes. *Invest Ophthalmol. Vis. Sci.*, **48**, 361-367.

211. Chen,B., Caballero,S., Seo,S., Grant,M.B., Lewin,A.S. (2009) Delivery of antioxidant enzyme genes to protect against ischemia/reperfusion-induced injury to retinal microvasculature. *Invest Ophthalmol Vis Sci*, **50**, 5587-5595.

212. Chen,B., Tang,L. (2011) Protective effects of catalase on retinal ischemia/reperfusion injury in rats. *Exp Eye Res.*.

213. Dorrell,M.I., Aguilar,E., Jacobson,R., Yanes,O., Gariano,R., Heckenlively,J., Banin,E., Ramirez,G.A., Gasmi,M., Bird,A.*, et al.* (2009) Antioxidant or neurotrophic factor treatment preserves function in a mouse model of neovascularization-associated oxidative stress. *J Clin Invest*, **119**, 611-623.

214. Li,Q., Dinculescu,A., Shan,Z., Miller,R., Pang,J., Lewin,A.S., Raizada,M.K., Hauswirth,W.W. (2008) Downregulation of p22phox in retinal pigment epithelial cells inhibits choroidal neovascularization in mice. *Mol Ther*, **16**, 1688-1694.

215. Haruta,M., Bush,R.A., Kjellstrom,S., Vijayasarathy,C., Zeng,Y., Le,Y.Z., Sieving,P.A. (2009) Depleting Rac1 in mouse rod photoreceptors protects them from photo-oxidative stress without affecting their structure or function. *Proc Natl Acad Sci U S A*, **106**, 9397-9402.

216. Stieger,K., Lheriteau,E., Moullier,P., Rolling,F. (2009) AAV-mediated gene therapy for retinal disorders in large animal models. *ILAR. J.*, **50**, 206-224.

217. Curcio,C.A., Sloan,K.R., Kalina,R.E., Hendrickson,A.E. (1990) Human photoreceptor topography. *J. Comp Neurol.*, **292**, 497-523.

218. Frenkel,S., Morgan,J.E., Blumenthal,E.Z. (2005) Histological measurement of retinal nerve fibre layer thickness. *Eye (Lond)*, **19**, 491-498.

219. Matsumoto,B., Blanks,J.C., Ryan,S.J. (1984) Topographic variations in the rabbit and primate internal limiting membrane. *Invest Ophthalmol. Vis. Sci.*, **25**, 71-82.

220. Vandenberghe,L.H., Bell,P., Maguire,A.M., Cearley,C.N., Xiao,R., Calcedo,R., Wang,L., Castle,M.J., Maguire,A.C., Grant,R.*, et al.* (2011) Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. *Sci. Transl. Med.*, **3**, 88ra54.

221. Vandenberghe,L.H., Bell,P., Maguire,A.M., Xiao,R., McMenamin,D., Munden,R., Grant,R., Bennett,J., Wilson,J.M. (2011) *ARVO*. Vol. Ft. Lauderdale, Fl.

222. Mancuso,K., Hauswirth,W.W., Li,Q., Connor,T.B., Kuchenbecker,J.A., Mauck,M.C., Neitz,J., Neitz,M. (2009) Gene therapy for red-green colour blindness in adult primates. *Nature*, **461**, 784-787.

223. Boye,S.E. (2012) *ARVO*. Vol. Ft. Lauderdale, Fl.

224. Komaromy,A.M., Alexander,J.J., Rowlan,J.S., Garcia,M.M., Chiodo,V.A., Kaya,A., Tanaka,J.C., Acland,G.M., Hauswirth,W.W., Aguirre,G.D. (2010) Gene therapy rescues cone function in congenital achromatopsia. *Hum. Mol. Genet.*, **19**, 2581-2593.

225. Yin,L., Greenberg,K., Hunter,J.J., Dalkara,D., Kolstad,K.D., Masella,B.D., Wolfe,R., Visel,M., Stone,D., Libby,R.T.*, et al.* (2011) Intravitreal injection of AAV2 transduces macaque inner retina. *Invest Ophthalmol. Vis. Sci.*, **52**, 2775-2783.

226. Hellstrom,M., Ruitenberg,M.J., Pollett,M.A., Ehlert,E.M., Twisk,J., Verhaagen,J., Harvey,A.R. (2009) Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after intravitreal injection. *Gene Ther.*, **16**, 521-532.

227. Ali,R.R., Reichel,M.B., De,A.M., Kanuga,N., Kinnon,C., Levinsky,R.J., Hunt,D.M., Bhattacharya,S.S., Thrasher,A.J. (1998) Adeno-associated virus gene transfer to mouse retina. *Hum. Gene Ther.*, **9**, 81-86.

228. Weiss,E.R., Ducceschi,M.H., Horner,T.J., Li,A., Craft,C.M., Osawa,S. (2001) Species-specific differences in expression of G-protein-coupled receptor kinase (GRK) 7 and GRK1 in mammalian cone photoreceptor cells: implications for cone cell phototransduction. *J. Neurosci.*, **21**, 9175-9184.

229. Boye,S.E., Boye,S.L., Pang,J., Ryals,R., Everhart,D., Umino,Y., Neeley,A.W., Besharse,J., Barlow,R., Hauswirth,W.W. (2010) Functional and behavioral restoration of vision by gene therapy in the guanylate cyclase-1 (GC1) knockout mouse. *PLoS. One.*, **5**, e11306.

230. Boye,S.L., Conlon,T., Erger,K., Ryals,R., Neeley,A., Cossette,T., Pang,J., Dyka,F.M., Hauswirth,W.W., Boye,S.E. (2011) Long-term preservation of cone photoreceptors and restoration of cone function by gene therapy in the guanylate cyclase-1 knockout (GC1KO) mouse. *Invest Ophthalmol. Vis. Sci.*, **52**, 7098-7108.

231. Zhong,L., Li,B., Mah,C.S., Govindasamy,L., Agbandje-McKenna,M., Cooper,M., Herzog,R.W., Zolotukhin,I., Warrington,K.H., Jr., Weigel-Van Aken,K.A*., et al.* (2008) Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. *Proc. Natl. Acad. Sci. U. S. A*, **105**, 7827-7832.

232. Zhong,L., Li,B., Jayandharan,G., Mah,C.S., Govindasamy,L., Agbandje-McKenna,M., Herzog,R.W., Weigel-Van Aken,K.A., Hobbs,J.A., Zolotukhin,S*., et al.* (2008) Tyrosine-phosphorylation of AAV2 vectors and its consequences on viral intracellular trafficking and transgene expression. *Virology*, **381**, 194-202.

233. Kay,C.N., Boye,S.L., Ryals,R.C., Sun,J., Neeley,A.W., Hauswirth,W.W., Boye,S.E. (2012) *ARVO*. Vol. Ft. Lauderdale, Fl.

234. Petrs-Silva,H., Dinculescu,A., Li,Q., Min,S.H., Chiodo,V., Pang,J.J., Zhong,L., Zolotukhin,S., Srivastava,A., Lewin,A.S., Hauswirth,W.W. (2009) High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol. Ther.*, **17**, 463-471.

235. Dalkara,D., Klimczak R.R., Visel,M., Hoffmann,N., Schaffer,D., Flannery,J. (2011) *ARVO*. Vol. Ft. Lauderdale.

236. Allocca,M., Doria,M., Petrillo,M., Colella,P., Garcia-Hoyos,M., Gibbs,D., Kim,S.R., Maguire,A., Rex,T.S., Di,V.U*., et al.* (2008) Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice. *J. Clin. Invest*, **118**, 1955-1964.

237. Wu,Z., Yang,H., Colosi,P. (2010) Effect of genome size on AAV vector packaging. *Mol. Ther.*, **18**, 80-86.

238. Dong,B., Nakai,H., Xiao,W. (2010) Characterization of genome integrity for oversized recombinant AAV vector. *Mol. Ther.*, **18**, 87-92.

239. Lai,Y., Yue,Y., Duan,D. (2010) Evidence for the failure of adeno-associated virus serotype 5 to package a viral genome > or = 8.2 kb. *Mol. Ther.*, **18**, 75-79.

240. Duan,D., Yue,Y., Yan,Z., Engelhardt,J.F. (2000) A new dual-vector approach to enhance recombinant adeno-associated virus-mediated gene expression through intermolecular cis activation. *Nat. Med.*, **6**, 595-598.

241. Sun,L., Li,J., Xiao,X. (2000) Overcoming adeno-associated virus vector size limitation through viral DNA heterodimerization. *Nat. Med.*, **6**, 599-602.

242. Nakai,H., Storm,T.A., Kay,M.A. (2000) Increasing the size of rAAV-mediated expression cassettes in vivo by intermolecular joining of two complementary vectors. *Nat. Biotechnol.*, **18**, 527-532.

243. Duan,D., Yue,Y., Engelhardt,J.F. (2001) Expanding AAV packaging capacity with trans-splicing or overlapping vectors: a quantitative comparison. *Mol. Ther.*, **4**, 383-391.

244. Ghosh,A., Yue,Y., Lai,Y., Duan,D. (2008) A hybrid vector system expands adeno-associated viral vector packaging capacity in a transgene-independent manner. *Mol. Ther.*, **16**, 124-130.

245. Hirsch,M.L., Storici,F., Li,C., Choi,V.W., Samulski,R.J. (2009) AAV recombineering with single strand oligonucleotides. *PLoS. One.*, **4**, e7705.

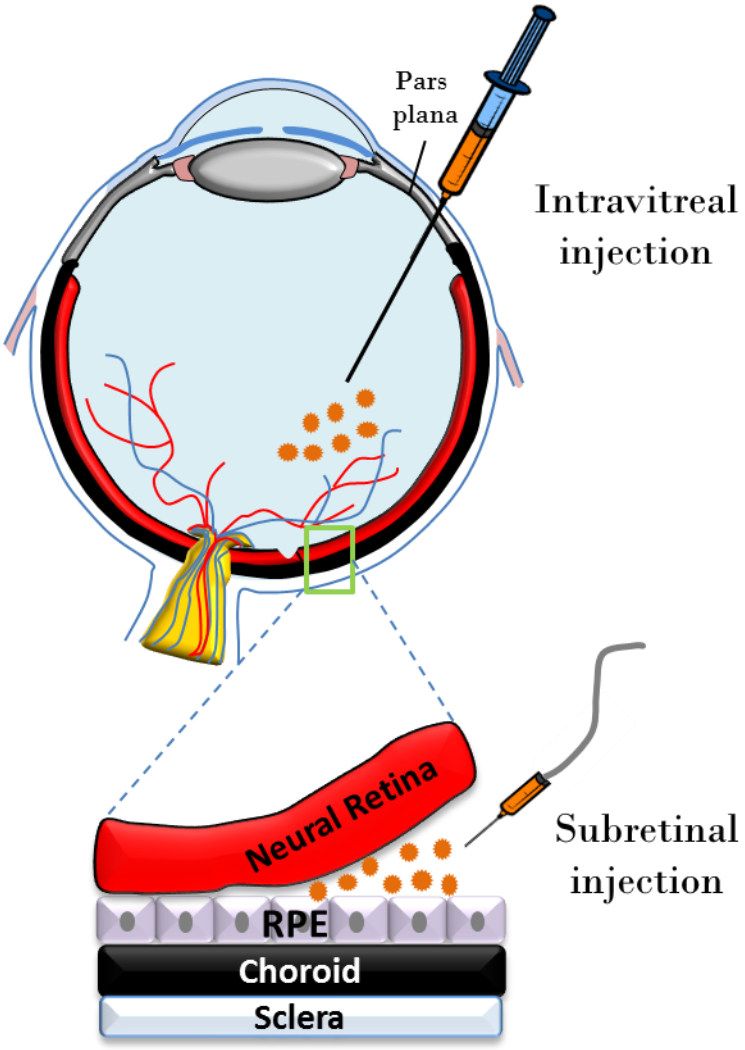
Table 1. Transduction pattern of AAV serotypes in the retina of multiple species					
AAV serotype	**Injection Route**	**Mouse/rat**	**Dog**	**Pig**	**NHP**
AAV2/1	Subretinal	Primarily RPE[2]	RPE[9]		
AAV2/1	Intravitreal	None[2]			
AAV2/2	Subretinal	Photoreceptors/RPE[1,2,8,5]	Photoreceptors/RPE[4,2,5]		Photoreceptors/RPE[5,13,16]
AAV2/2	Intravitreal	Ganglion cells, few Muller cells[2,12,17]			Ganglion cells, Muller cells[19]
AAV2/3	Subretinal	None[8]			
AAV2/4	Subretinal	Solely RPE[5]	Solely RPE[5]		Solely RPE[5]
AAV2/5	Subretinal	Photoreceptors/RPE[2,8,5,15]	Photoreceptors/RPE, few horizontal and Mueller cells[3,5]	Photoreceptors/RPE, few Muller cells[18]	Photoreceptors/RPE[5,10,21]
AAV2/5	Intravitreal	None[2]			
AAV2/6	Subretinal	Primarily RPE[8]			
AAV2/6	Intravitreal	Ganglion cells, inner nuclear layer, Muller cells[17]			
AAV2/7	Subretinal	Photoreceptors/RPE[15]			Photoreceptors/RPE[14]
AAV2/8	Subretinal	Photoreceptors/RPE, inner nuclear layer, ganglion cells[11,15]	Photoreceptors/RPE, inner nuclear layer, ganglion cells[11]	Photoreceptors/RPE, few Muller cells[18]	Photoreceptors/RPE[13,14]
AAV2/8	Intravitreal	Ganglion cells, few Muller cells			
AAV2/9	Subretinal	Photoreceptors/RPE, Muller cells[15]			Photoreceptors/RPE[14]
AAV2/9	Intravitreal	Ganglion cells[20]			
AAV5/5	Subretinal	Photoreceptors/RPE[10]			Photreceptors/RPE[10]

Supplemental Table 1. Transduction profiles of AAV serotypes across species

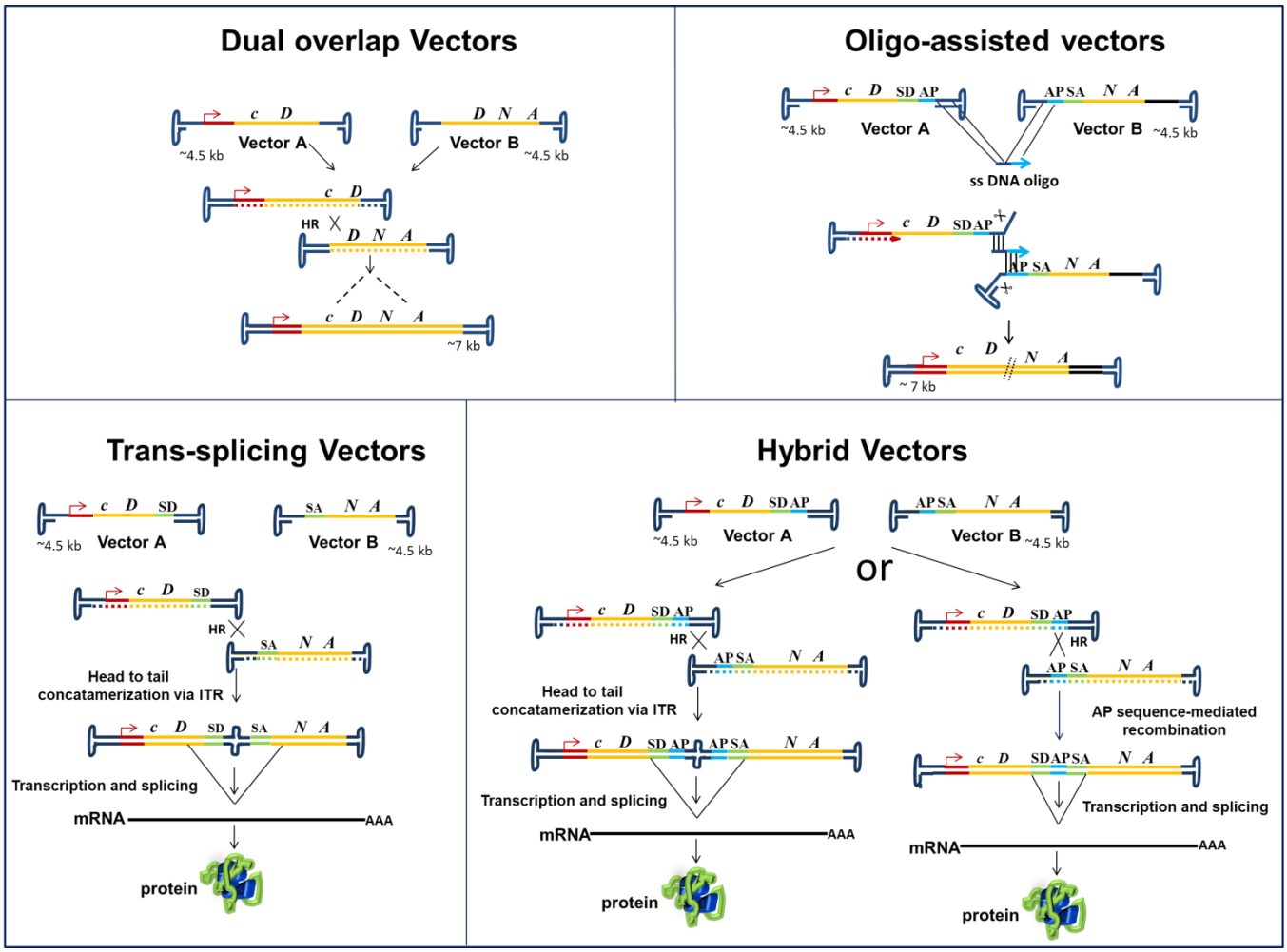
References for Supplemental Table 1:

1. Ali, Reichel, Thrasher et al. Gene transfer into mouse retina mediated by an adeno-associated viral vector. Hum Mol Genet. 1996 5: 591-4
2. Auricchio, Kobinger, Anand et al. Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model. Hum Mol Genet. 2001 10:3075-81.
3. Petersen-Jones SM, Bartoe JT, Fischer AJ et al., AAV retinal transduction in a large animal model species: comparison of a self-complementary AAV2/5 with a single-stranded AAV2/5 vector. Mol Vis. 15:1835-42.
4. Acland GM, Aguuirre GD et al. Gene therapy restores vision in a canine model of childhood blindness. Nat Genet. 2001. 28: 92-5.
5. Weber M, Rabiniowitz J, et al. Recombinant adeno-associated virus serotype 4 mediates unique and exclusive long-term transduction of retinal pigmented epithelium in rat, dog and nonhuman primate after subretinal delivery. Mol Ther. 2003 5:774-81.
6. Le Meur, Stieger et al., Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. Gene Ther. 2007 14:292-303.
7. Ali RR, Reichel MB et al., Adeno-associated virus gene transfer to mouse retina.
8. Yang GS, Schmidt M, Yan Z et al., Virus-mediated transduction of murine retina with adeno-associated virus: effects of viral capsid and genome size. J Virol. 2002, 76:7651-60
9. Acland GM, Aguirre et al. Long term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Mol Ther. 2005, 12: 1072-1082.
10. Lotery AJ, Yang GS et al. Adeno-associated virus type 5: transduction efficiency and cell-type specificity in the primate retina. Human Gene Therapy. 2003. 14:1663-71.
11. Stieger K, Colle MA et al. Subretinal delivery of recombinant AAV serotype 8 in dogs results in gene transfer to neurons in the brain. Mol Ther. 16: 916-23.
12. Dudus, L, Anand, V, Acland, GM, Chen, SJ, Wilson, JM, Fisher, KJ et al. (1999). Persistent transgene product in retina, optic nerve and brain after intraocular injection of rAAV. Vision Res 39: 2545–2553.
13. Vandenberghe LH, Bell P et al. Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. Sci Transl Med. 2011 3:88ra54.
14. Vandenberghe LH, Bell P, Maguire A et al. Cone and rod transduction with alternative AAV serotypes in the macula of non-human primates. ARVO abstract 2011.
15. Allocca M, Mussolino C et al. Novel adeno-associated virus serotypes efficienty transduce murine photoreceptors. Journal of Virology. 2007. 81: 11372-11380.
16. Bennett J *et al.* Stable transgene expression in rod photoreceptors after recombinant adeno-associated virus-mediated gene transfer to monkey retina. *Proc Natl Acad Sci USA* 1999; **96**: 9920–9925.

17. Hellstrom M, Ruitenberg MJ et al. Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after intravitreal injection. Gene Therapy. 2009. 16: 521-532.
18. Mussolino C, della Corte M et al. AAV-mediated photoreceptor transduction of the pig cone-enriched retina. Gene Therapy 2011. 18:637-645.
19. Yin L, Greenberg K et al. Intravitreal injection of AAV2 transduces macaque inner retina. Invest Ophthalmol Vis Scie. 2011. 52: 2775-83.
20. Petrs Silva et al. High-efficiency transduction of the mouse retina by tyrosine mutant AAV serotype vectors. Mol Ther. 2009 17:463-471.
21. Boye SE, Alexander JJ et al. The human rhodopsin kinase promoter in an AAV5 vector confers rod-and cone-specific expression in the primate retina. Human Gene Therapy. 2012. 23:1101-15.



Supplemental Figure 1. Schematic of subretinal vs. intravitreal injection routes



Supplemental Figure 2. Dual AAV vector platforms