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(54) Title: COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING RETINAL DEGENERATION

(57) Abstract: The invention provides ocular compositions containing valproic acid and valproic acid analogs and methods of using such compositions to reduce ocular cell death (e.g., retinal pigment epithelium cell death), particularly cell death associated with age-related macular degeneration.

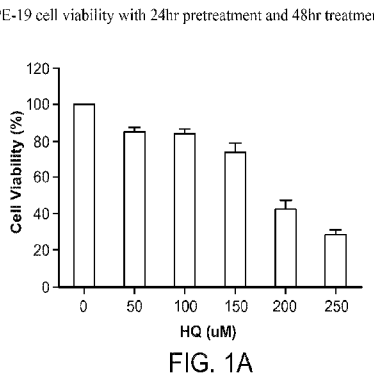


FIG. 1A

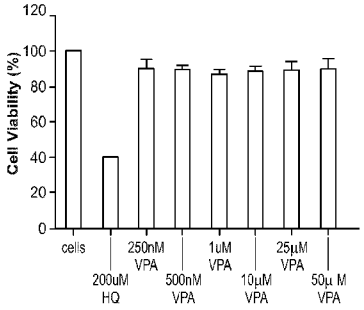


FIG. 1B

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COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING RETINAL DEGENERATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the following U.S. Provisional Application Nos.: 61/301,748, filed February 5, 2010, and 61/350,711, filed June 2, 2010, the entire contents of each of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Blindness or low vision affects 3.3 million Americans age 40 and over. Age-related macular degeneration is the leading cause of blindness, while retinitis pigmentosa affects over 100,000 people in the United States alone. Age-related macular degeneration (AMD) is a chronic disease associated with aging that gradually destroys central vision. The macula consists of millions of densely packed cones and rods. In the early stage of AMD, several small drusen or a few medium-sized drusen are detected on the macula in one or both eyes by ophthalmascope examination. At the intermediate stage, many medium-sized drusen or one or more large drusen are detected. This accumulation is typically characterized by perceptible changes in central vision. In the advanced stage, several large drusen and extensive breakdown of light-sensitive cells in the macula, are detected. These features cause a well-defined area of central vision loss, which typically expands and worsens over time. AMD is a leading cause of vision loss in Americans 60 years of age and older. Because current methods for treating AMD and retinitis pigmentosa are incapable of preventing or reversing vision loss, improved therapies are urgently required.

SUMMARY OF THE INVENTION

As described below, the present invention features ocular compositions comprising valproic acid and analogs thereof and methods of using such compositions for the treatment or prevention of retinal cell death, including cell death associated with age-related macular degeneration.

In one aspect, the invention provides a method for treating or preventing ocular cell death in a subject in need thereof, the method involves administering an

effective amount of valproic acid, valpromide, lithium or an analogue thereof to the subject.

In another aspect, the invention provides a method for preserving or enhancing visual function, the method involves administering an effective amount of valproic acid, valpromide, lithium or an analogue thereof to the subject.

In yet another aspect, the invention provides a method for increasing retinal regeneration, the method involves administering an effective amount of valproic acid, valpromide, lithium or an analogue thereof to the subject.

In various embodiments of any of the above aspects, the ocular cell is a retinal pigment epithelial cell. In various embodiments of any of the above aspects, the ocular cell death is retinal pigment epithelial cell death. In various embodiments of any of the above aspects, the method reduces retinal pigment epithelial cell apoptosis. In various embodiments of any of the above aspects, the method increases the number of retinal pigment epithelial cells. In various embodiments of any of the above aspects, the method increases retinal thickness. In various embodiments of any of the above aspects, the ocular cell death is associated with a disease that is any one or more of retinitis pigmentosa, age-related macular degeneration, glaucoma, corneal dystrophies, retinoschises, Stargardt's disease, autosomal dominant druzen, and Best's macular dystrophy. In various embodiments of any of the above aspects,, the disease is the wet or dry form of age-related macular degeneration. In another embodiment, the subject contains a mutation that affects opsin folding (e.g., the opsin contains a P23H mutation). In various embodiments of any of the above aspects, the method involves administering valproic acid and lithium or valpromide and lithium. In various embodiments of any of the above aspects, the valproic acid or valpromide and lithium are administered within ten days of each other. In various embodiments of any of the above aspects, the valproic acid or valpromide and lithium are administered within one, three, or five days of each other. In various embodiments of any of the above aspects, the valproic acid or valpromide and lithium are administered within twenty-four hours of each other. In various embodiments of any of the above aspects, the valproic acid or valpromide and lithium are administered simultaneously. In various embodiments of any of the above aspects, the valproic acid, valpromide, and/or lithium are administered to the eye (e.g., topical administration). In various embodiments of any of the above aspects, the valproic acid, valpromide, and/or lithium are administered topically by drop form to the surface of the eye. In various

embodiments of any of the above aspects, the administration is intra-ocular. In various embodiments of any of the above aspects, the valproic acid, valpromide, and/or lithium are each incorporated into a composition that provides for their long-term release. In various embodiments of any of the above aspects, the method further involves identifying the subject as having or having a propensity to develop ocular cell death, a disease associated with retinal pigmented epithelial cell death, or the wet or dry form of age-related macular degeneration.

In another aspect, the invention provides a pharmaceutical composition for treating or preventing ocular cell death in a subject in need thereof, the composition contains an effective amount of valproic acid, valpromide, or lithium and a pharmaceutically acceptable excipient formulated for ocular delivery. In one embodiment, the composition is labeled for the treatment of a disease that is any one or more of age-related macular degeneration, retinitis pigmentosa, glaucoma, coreal systrophy, retinoschises, Stargardt's disease, autosomal dominant druzen, or Best's macular dystrophy. In another embodiment, the ocular cell is a retinal pigment epithelial cell.

In another aspect, the invention features a method for treating or preventing the wet or dry form of age-related macular degeneration in a subject, the method involving administering an effective amount of valproic acid or a derivative thereof to said subject.

In another aspect, the invention features a method for treating or preventing retinitis pigmentosa in a subject, the method involving administering an effective amount of valproic acid or a derivative thereof to said subject. In one embodiment, the effective amount is between 250 mg/day and 3000 mg/day. In another embodiment, the effective amount is 500 mg/day, 750 mg/day, 1000 mg/day, 1500 mg/day, 2000 mg/day, 2500 mg/day, or 3000 mg/day. In yet another embodiment, the method increases best-corrected visual acuity, increases visual field, increases central retinal thickness, or increases subjective visual perception. In still another embodiment, valproic acid is administered orally, ocularly, topically, or intra-ocularly. In related embodiments, the valproic acid is administered topically by drop form to the surface of the eye. In still another embodiment, valproic acid is administered for at least 1-12 months, 12-24 months, 36-48 months, or for the life of the patient.

In another aspect, the invention provides a kit for the treatment or prevention of ocular cell death, the kit contains an effective amount of valproic acid, valpromide, and/or lithium and instructions for the use of the kit in the method of any of claims 1-31. In one embodiment, the kit contains valproic acid and lithium or valpromide and lithium.

The invention provides ocular compositions containing valproic acid and valproic acid analogs and methods of using such compositions to reduce retinal pigment epithelium cell death, particularly cell death associated with age-related macular degeneration. Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels. "

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a valproic acid analog retains the biological activity of valproic acid, while having certain biochemical modifications that enhance the analog's function relative to unmodified valproic acid.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean " includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include retinal cell death, such as age-related macular degeneration, retinal detachment, retinal vascular disease, retinitis pigmentosa, glaucoma, diabetic retinopathy, corneal dystrophy, and dry eyes and other diseases associated with retinal pigment epithelium (RPE) cell death.

By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

By "increasing cell survival" is meant positively altering cell viability. In one embodiment, methods that increase cell survival create a corresponding reduction in cell death. Assays for measuring cell viability are known in the art, and are described, for example, by Crouch et al. (J. Immunol. Meth. 160, 81–8); Kangas et al. (Med. Biol.62, 338–43, 1984); Lundin et al., (Meth. Enzymol.133, 27–42, 1986); Petty et al. (Comparison of J. Biolum. Chemilum.10, 29–34, .1995); and Cree et al. (AntiCancer Drugs 6: 398–404, 1995). Cell viability can be assayed using a variety of methods, including MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide) (Barltrop, Bioorg. & Med. Chem. Lett.1: 611, 1991; Cory et al., Cancer Comm. 3, 207–12, 1991; Paull J. Heterocyclic Chem. 25, 911, 1988). Assays for cell viability are also available commercially. These assays include but are not limited to CELLTITER-GLO® Luminescent Cell Viability Assay (Promega), which uses luciferase technology to detect ATP and quantify the health or number of cells in culture, and the CellTiter-Glo® Luminescent Cell Viability Assay, which is a lactate dehyrodgenase (LDH) cytotoxicity assay (Promega).

By "cell death" is meant necrotic, apoptotic, or any other mechanism resulting in the death of a cell. Assays for measuring cell death are known to the skilled artisan. Apoptotic cells are characterized by characteristic morphological changes, including chromatin condensation, cell shrinkage and membrane blebbing, which can be clearly observed using light microscopy. The biochemical features of apoptosis

include DNA fragmentation, protein cleavage at specific locations, increased mitochondrial membrane permeability, and the appearance of phosphatidylserine on the cell membrane surface. Assays for apoptosis are known in the art. Exemplary assays include TUNEL (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling) assays, caspase activity (specifically caspase-3) assays, and assays for fas-ligand and annexin V. Commercially available products for detecting apoptosis include, for example, Apo-ONE® Homogeneous Caspase-3/7 Assay, FragEL *TUNEL* kit (ONCOGENE RESEARCH PRODUCTS, San Diego, CA), the ApoBrdU DNA Fragmentation Assay (BIOVISION, Mountain View, CA), and the Quick Apoptotic DNA Ladder Detection Kit (BIOVISION, Mountain View, CA).

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

By "ocular cell" is meant a cell of the eye. An exemplary ocular cell is the retinal pigment epithelial cell.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "treat," treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E are graphs showing that valproic acid (VPA), valpromide (VPD), and (Li treatment reduced hydroquinone (HQ) induced cell death. Figure 1A is graph showing treatment of ARPE-19 cells with hydroquinone (HQ) resulted in reduced cell viability in a dose-dependent manner. Figure 1B is a graph showing VPA (250 nM to 50 uM) reduced RPE cell death in the MTT assay (B, right panel), when VPA doses were applied concurrently with HQ treatment. Figure 1C is a graph showing that valproic acid (VPA) protects ARPE-19 cells against cell death induced by 250 μM and 350 μM hydroquinone treatment. The bars at the far left indicate that few dead cells are observed in the absence of hydroquinone. VPD denotes valpromide and Li denotes lithium. Figure 1D is a graph showing that VPA protects RPE cells in a dose dependent manner against HQ mediated cell death. Figure 1E is a graph showing that VPD was not protective of RPE cell death at a range of 10nM-50μM (v. protective effect at 250μM in Figure 1C).

Figure 2 is a graph showing that valproic acid (VPA) prevented hydroquinone-induced apoptosis in ARPE-19 cells treated with 250 μM and 350 μM hydroquinone.

Figure 3 is a graph showing Tunel staining in ARPE-19 cells treated with valproic acid (VPA), valpromide (VPD) and/or Lithium.

Figures 4A-4D depict scotopic ERG and histology results from P23H mutant mice treated with VPA and vehicle. Figure 4A is a graph showing scotopic ERG a-wave amplitude from mice treated for 11 weeks (age = 14 weeks) . Figure 4B is a graph showing scotopic ERG b-wave amplitude from mice treated for 11 weeks Figure 4C depicts a cross-section of retina from a control mouse. Figure 4D depicts a cross-section of retina from a VPA-treated mouse treated for 20 weeks (age = 24 weeks). Staining for rhodopsin is represented in green, while DAPI was used as nuclear stain (blue).

Figures 5A and 5B are box and whiskers diagrams showing increases in central retinal thickness and photopic ERG b-wave amplitude from mer tk-/- mice treated with either with VPA (250 mg/kg). Figure 5A is a box and whiskers diagram depicting results for central retinal thickness as determined by ultra-high resolution OCT and measured at ~500 microns from the center of the optic disc either temporally (abbreviated "temp") or nasally (abbreviated "nas") at the end of a 4 week daily i.p. treatment with either VPA or vehicle (control group). Figure 5B is a box and whiskers diagram depicting amplitude of the photopic b-wave measured at the same time from the two groups. Statistical significance of a comparison between the VPA-treated and control group (Mann-Whitney U test) is shown for each comparison pair. Boxes extend from 25th to 75th percentile, while the line in the middle of the box is plotted at the median. Whiskers extend from minimum to maximum.

Figures 6A-6C are box and whiskers diagrams showing increases in central retinal thickness and photopic ERG b-wave amplitude after sodium iodate injection (20mg/kg; i.v.) in C57BL/6 mice treated with VPA. Figures 6A and 6B are box and whiskers diagrams depicting results for central retinal thickness as determined by ultra-high resolution OCT and measured at ~500 microns from the center of the optic disc either temporally (abbreviated "temp") or nasally (abbreviated "nas") at the end of Day 9 (Figure 6A) and Day 15 (Figure 6B) after sodium iodate injection for mice treated with either VPA or vehicle (control group). Figure 6C is a box and whiskers diagram depicting the amplitude of the photopic b-wave measured at Day 10 from the two groups mice of mice treated with VPA or vehicle (control group). Statistical significance of a comparison between the VPA-treated and control group (Mann-Whitney U test) is shown for each comparison pair.

Figures 7A-7D are box and whiskers diagrams (Figures 7A, 7C, 7D) or graphs (Figure 7B) showing a change in BCVA at follow-up visit compared to baseline visit (boxes in panels A,C, and D represent data from the 25th to the 75th percentile, whereas the whiskers represent the total range of the data [min to max]). Figure 7A shows BCVA results averaged for left eye and right eye: only patient data for both eyes was used; also expected BCVA value from the natural history of the disease is shown as a comparison. Figure 7B indicates the number of eyes showing improvement, no change, or worsening of BCVA at follow-up, by daily treatment dose of VPA. Figure 7C shows BCVA change as a function of daily dose and provides a comparison of observed results to the value expected based on the natural history of the disease; only patient data for both eyes was used. Figure 7D presents the same results as Figure 7C, but instead of an average of both eyes, only the results for the more improved eye were used. .

Figures 8A and 8B are box and whiskers diagrams showing the change in BCVA at a follow-up visit compared to a baseline visit for eyes with dry ARMD treated with 500 mg or 750 mg VPA per day, and compared to the value expected from the natural history of the disease. Figure 8A shows one result per patient included; in cases where two eyes had the same diagnosis, the result was averaged. Figure 8B presents the same results as Figure 8A, but when data for two eyes were available, only the result from the more improved eye was included.

Figures 9A-9C show treatment results for eyes with wet ARMD. Figure 9A shows the change in BCVA at a follow-up visit compared to a baseline visit for wet AMD patients treated with 500 mg or 750 mg VPA per day. One result per patient was included; in cases where two eyes had the same diagnosis, the results were averaged. The value expected from the natural history of the disease is also shown for comparison (Wong et al. 2007). Figure 9B shows the results for eyes with decreased or increased central retinal thickness as measured by OCT. Figure 9C shows a representative OCT from the left eye of patient #21 at baseline (upper panel) and at follow-up (lower panel).

Figures 10A and 10B show the effect on visual field in patients treated with VPA. Figure 10A provides Goldmann Kinetic Perimetry tracings from patient 6 at baseline (left) and after 6 months of VPA treatment (middle -red), overlap of baseline and follow-up (right). Figure 10B quantitates the observed change in Visual Field:

Baseline and follow-up VF areas are graphed for each individual patient, length of follow-up varied for each patient. Areas were analyzed by right eye, left eye and average of both eyes. Numbers correspond to the individual subjects.

Figure 11 shows an analysis of change in visual field in patients treated with VPA. Goldmann Kinetic Perimetry tracings (isopter V4e) from each eye were digitized and areas (mm2) were calculated as described in methods and log transformed. Scatterplots of change in log transformed VF over the course of treatment are shown for left eyes, right eyes and all eyes combined. Mean value is shown by thin bar and standard deviation is represented by upper and lower dark bars.

DETAILED DESCRIPTION OF THE INVENTION

The invention features compositions and methods that are useful for the treatment or prevention of ocular cell death.

The invention is based, at least in part on the discovery that valproic acid reduced cell death both *in vitro* and *in vivo* models of retinal pigment epithelium (RPE) damage. As well as on the discovery that valproic acid increased visual function in patients with retinitis pigmentosa and age-related macular degeneration. ARPE-19 cell death was induced by contacting the cells *in vitro* with 200-350 μM HQ. More than 60% of cells treated with 250 μM HQ or more died. VPA treatment (500nM – 250μM) significantly increased the number of viable cells at 48 hours. Annexin V staining showed that the number of apoptotic cells increased as the hydroquinone dosage increased. Treatment with 900 nm VPA significantly increased the number of viable cells (i.e., increased the number of cells that were not stained by Annexin V). Cleaved caspase-3 analysis revealed a drastic reduction in downstream caspase-3 activation in the presence of 900 nM VPA. VPA also protected the retinal pigment epithelium (RPE) cells from paraquat-induced RPE cell death in mice.

Age-related macular degeneration

ARMD, which is the leading cause of blindness in people over 60 in the developed world, is characterized by loss of central visual function, including visual acuity. ARMD is categorized into two major forms: an angiogenic, wet form and a non-exudative, dry form. Recent emerging technologies and novel therapeutics have inadequately targeted the physiological aspects and progression of wet ARMD, which has a faster disease progression and poorer visual prognosis than dry ARMD.

Treatment options have emerged to suppress choroidal neovascularization, the hallmark of wet ARMD, such as photocoagulation, photodynamic therapy, and anti-VEGF therapies, including monoclonal antibodies and corticosteroids. These treatments may temporarily stabilize and improve visual function in wet ARMD. Current nutritional treatment for dry ARMD has failed to stop disease progression or reverse vision loss. The Age-Related Eye Disease Study (AREDS) found that a combination of nutritional supplements (zinc and vitamins A, C, and E) slows the progression of ARMD from intermediate to advanced stages over the course of several years. Statins have been studied as another treatment option to clear subretinal deposits (drusen) associated with dry ARMD, but insufficient evidence exists regarding their efficacy. Photocoagulation failed to improve vision in patients with dry ARMD.

Most investigators agree that smoking and genetics are consistently associated with ARMD. Similar but less pronounced changes to Bruch's membrane and the retinal pigment epithelium (RPE) that occur in ARMD can be seen in normal aging. ARMD is a growing public health concern with an increasingly older population. Wet ARMD accounts for 10%-20% of ARMD patients and accounts for 90% of those with severe vision loss. Dry ARMD can also lead to significant vision loss. The fundamental characteristics of ARMD are an inflammatory reaction of the RPE and photoreceptor cells and subsequent cell death. Contributing to this process are photo-oxidative stress, complement activation, immune dysregulation, and inflammatory cell infiltration. Significantly, none of the currently available therapies address the underlying pathophysiology of dry or wet ARMD.

At present, few effective treatments exist for age-related macular degeneration (ARMD), the leading cause of blindness in the elderly in the developed world. As reported herein, valproic acid is a safe and effective therapy for non-exudative ("dry") or exudative ("wet") ARMD in humans that exerts its biological effect in the retina by protecting photoreceptor cells, reducing inflammatory pro-angiogenic cytokines in the RPE, limiting oxidative apoptotic injury and mitigating alternative complement pathway-activated cell death. Thus, VPA and analogs and derivatives thereof have a biological profile that is well suited for treatment of retinal diseases. As reported herein below, human studies in patients with ARMD

Retinitis pigmentosa

Retinitis pigmentosa (RP) is a severe neurodegenerative disease of the retina characterized initially by night blindness, with progression to tunnel vision and eventual loss of central vision and total blindness. Targeted therapies for RP are complicated by the identification of more than 40 genes linked to the dominant and recessive forms of this disease. While a few new approaches for RP treatment have recently been investigated, including nutritional supplementation, light reduction, and gene therapy, of these, vitamin A supplementation is the most promising, but its benefits are modest and side effects are problematic. Therefore, currently there is no significant treatment or cure for RP.

In a screen to determine whether known small molecules can increase the yield of properly folded RP mutant rhodopsins in heterologous cell culture valproic acid (VPA) was identified. VPA is a potent inhibitor of histone deacetylase (HDAC) (Gottlicher et al., Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J. 2001;20:6969-78) and the inflammatory response pathway via apoptosis of microglial cells (Dragunow et al., Neuroscience. 2006;140:1149-56; Chen et al., Neuroscience. 2007;149:203-12; Kim et al., J Pharmacol Exp Ther. 2007;321:892-901). In addition, VPA down-regulates complement proteins (Suuronen et al., Biochem Biophys Res Commun. 2007;357:397-401 and increases the levels of various neurotrophic factors (Yasuda et al., Mol Psychiatry. 2009;14:51-9. Thus, VPA has a unique biological profile suitable for treating retinal diseases. VPA and its derivative, divalproex sodium have also been used for chronic pain syndromes, cancer therapy and schizophrenia. As reported herein below, VPA is also useful for the treatment of patients with retinal dystrophies as shown in an analysis of the efficacy of VPA treatment on vision function of patients with RP.

Valproic acid and Analogs thereof

Regulation of gene expression is mediated by several mechanisms, including the post-translational modifications of histones by dynamic acetylation and deacetylation. The enzymes responsible for reversible acetylation/-deacetylation processes are histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Valproic acid is a histone deacetylate inhibitor. The invention provides for the use of valproic acid, valpromide, and analogs thereof to prevent or reduce RPE cell death in a subject at risk thereof (e.g., a subject diagnosed as having or having a

propensity to develop age-related macular degeneration). Valproic acid, valpromide, and analogs thereof may be administered alone or in combination with lithium to ameliorate RPE cell death, particularly cell death associated with the wet or dry form of age-related macular degeneration.

Pharmaceutical Compositions

The present invention features pharmaceutical preparations comprising valproic acid, valpromide, and/or lithium together with pharmaceutically acceptable carriers, where the compounds protect against cell death or provide for the generation of a mutant protein in a biochemically functional conformation. Such preparations have both therapeutic and prophylactic applications. In one embodiment, a pharmaceutical composition includes valproic acid or valpromide in combination with lithium. The valproic acid or valpromide and the lithium are formulated together or separately. Compounds of the invention may be administered as part of a pharmaceutical composition. The compositions should be sterile and contain a therapeutically effective amount of the polypeptides in a unit of weight or volume suitable for administration to a subject. The compositions and combinations of the invention can be part of a pharmaceutical pack, where each of the compounds is present in individual dosage amounts.

Pharmaceutical compositions of the invention to be used for prophylactic or therapeutic administration should be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 μm membranes), by gamma irradiation, or any other suitable means known to those skilled in the art. Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. These compositions ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution.

The compounds may be combined, optionally, with a pharmaceutically acceptable excipient. The term "pharmaceutically-acceptable excipient" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances that are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate administration. The components of the

pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction that would substantially impair the desired pharmaceutical efficacy.

Compounds of the present invention can be contained in a pharmaceutically acceptable excipient. The excipient preferably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetate, lactate, tartrate, and other organic acids or their salts; tris- hydroxymethylaminomethane (TRIS), bicarbonate, carbonate, and other organic bases and their salts; antioxidants, such as ascorbic acid; low molecular weight (for example, less than about ten residues) polypeptides, e.g., polyarginine, polylysine, polyglutamate and polyaspartate; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone (PVP), polypropylene glycols (PPGs), and polyethylene glycols (PEGs); amino acids, such as glycine, glutamic acid, aspartic acid, histidine, lysine, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, sucrose, dextrins or sulfated carbohydrate derivatives, such as heparin, chondroitin sulfate or dextran sulfate; polyvalent metal ions, such as divalent metal ions including calcium ions, magnesium ions and manganese ions; chelating agents, such as ethylenediamine tetraacetic acid (EDTA); sugar alcohols, such as mannitol or sorbitol; counterions, such as sodium or ammonium; and/or nonionic surfactants, such as polysorbates or poloxamers. Other additives may be included, such as stabilizers, anti-microbials, inert gases, fluid and nutrient replenishers (i.e., Ringer's dextrose), electrolyte replenishers, and the like, which can be present in conventional amounts.

The compositions, as described above, can be administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being treated and the desired outcome. It may also depend upon the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result.

With respect to a subject having a disease or disorder characterized by RPE cell death, an effective amount is sufficient to reduce cell death, increase cell viability,

and/or increase the level of a correctly folded protein in a cell. With respect to a subject having a disease or disorder characterized by RPE cell death, an effective amount is an amount sufficient to stabilize, slow, or reduce the a symptom associated with a pathology. Generally, doses of the compounds of the present invention would be from about 0.01 mg/kg per day to about 1000 mg/kg per day. It is expected that doses ranging from about 50 to about 2000 mg/kg will be suitable. Lower doses will result from certain forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of a composition of the present invention.

A variety of administration routes are available. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. In one preferred embodiment, a composition of the invention is administered intraocularly. Other modes of administration include oral, topical, intraocular, buccal, transdermal, within/on implants, or parenteral routes. The term "parenteral" includes subcutaneous, intrathecal, intravenous, intramuscular, intraperitoneal, or infusion. Compositions comprising a composition of the invention can be added to a physiological fluid, such as to the intravitreal humor.

Pharmaceutical compositions of the invention can comprise one or more pH buffering compounds to maintain the pH of the formulation at a predetermined level that reflects physiological pH, such as in the range of about 5.0 to about 8.0. The pH buffering compound used in the aqueous liquid formulation can be an amino acid or mixture of amino acids, such as histidine or a mixture of amino acids such as histidine and glycine. Alternatively, the pH buffering compound is preferably an agent which maintains the pH of the formulation at a predetermined level, such as in the range of about 5.0 to about 8.0, and which does not chelate calcium ions. Illustrative examples of such pH buffering compounds include, but are not limited to, imidazole and acetate ions. The pH buffering compound may be present in any amount suitable to maintain the pH of the formulation at a predetermined level.

Pharmaceutical compositions of the invention can also contain one or more osmotic modulating agents, i.e., a compound that modulates the osmotic properties (e.g, tonicity, osmolality and/or osmotic pressure) of the formulation to a level that is acceptable to the blood stream and blood cells of recipient individuals. The osmotic modulating agent can be an agent that does not chelate calcium ions. The osmotic modulating agent can be any compound known or available to those skilled in the art that modulates the osmotic properties of the formulation. One skilled in the art may empirically determine the suitability of a given osmotic modulating agent for use in the inventive formulation. Illustrative examples of suitable types of osmotic modulating agents include, but are not limited to: salts, such as sodium chloride and sodium acetate; sugars, such as sucrose, dextrose, and mannitol; amino acids, such as glycine; and mixtures of one or more of these agents and/or types of agents. The osmotic modulating agent(s) may be present in any concentration sufficient to modulate the osmotic properties of the formulation.

Compositions comprising a compound of the present invention can contain multivalent metal ions, such as calcium ions, magnesium ions and/or manganese ions. Any multivalent metal ion that helps stabilizes the composition and that will not adversely affect recipient individuals may be used. The skilled artisan, based on these two criteria, can determine suitable metal ions empirically and suitable sources of such metal ions are known, and include inorganic and organic salts.

Pharmaceutical compositions of the invention can also be a non-aqueous liquid formulation. Any suitable non-aqueous liquid may be employed, provided that it provides stability to the active agents (s) contained therein. Preferably, the non-aqueous liquid is a hydrophilic liquid. Illustrative examples of suitable non-aqueous liquids include: glycerol; dimethyl sulfoxide (DMSO); polydimethylsiloxane (PMS); ethylene glycols, such as ethylene glycol, diethylene glycol, triethylene glycol, polyethylene glycol ("PEG") 200, PEG 300, and PEG 400; and propylene glycols, such as dipropylene glycol, tripropylene glycol, polypropylene glycol ("PPG") 425, PPG 725, PPG 1000, PPG 2000, PPG 3000 and PPG 4000.

Pharmaceutical compositions of the invention can also be a mixed aqueous/non-aqueous liquid formulation. Any suitable non-aqueous liquid formulation, such as those described above, can be employed along with any aqueous liquid formulation, such as those described above, provided that the mixed aqueous/non-aqueous liquid formulation provides stability to the compound contained

therein. Preferably, the non- aqueous liquid in such a formulation is a hydrophilic liquid. Illustrative examples of suitable non-aqueous liquids include: glycerol; DMSO; PMS; ethylene glycols, such as PEG 200, PEG 300, and PEG 400; and propylene glycols, such as PPG 425, PPG 725, PPG 1000, PPG 2000, PPG 3000 and PPG 4000.

Suitable stable formulations can permit storage of the active agents in a frozen or an unfrozen liquid state. Stable liquid formulations can be stored at a temperature of at least -70°C, but can also be stored at higher temperatures of at least 0°C, or between about 0.1°C and about 42°C, depending on the properties of the composition. It is generally known to the skilled artisan that proteins and polypeptides are sensitive to changes in pH, temperature, and a multiplicity of other factors that may affect therapeutic efficacy.

In certain embodiments a desirable route of administration can be by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing polypeptides are well known to those of skill in the art. Generally, such systems should utilize components that will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily modify the various parameters and conditions for producing polypeptide aerosols without resorting to undue experimentation.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of compositions of the invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as polylactides (U.S. Pat. No. 3,773,919; European Patent No. 58,481), poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acids, such as poly-D-(-)-3-hydroxybutyric acid (European Patent No. 133, 988), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, K.R. et al., Biopolymers 22: 547-556), poly (2-hydroxyethyl methacrylate) or ethylene vinyl acetate (Langer, R. et al., J. Biomed. Mater. Res. 15:267-277; Langer, R. Chem. Tech. 12:98-105), and polyanhydrides.

Other examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems such as biologically-derived bioresorbable hydrogel (i.e., chitin hydrogels or chitosan hydrogels); sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the agent is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,667,014, 4,748,034 and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,832,253, and 3,854,480.

Another type of delivery system that can be used with the methods and compositions of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vessels, which are useful as a delivery vector *in vivo* or *in vitro*. Large unilamellar vessels (LUV), which range in size from 0.2 - 4.0 μm, can encapsulate large macromolecules within the aqueous interior and be delivered to cells in a biologically active form (Fraley, R., and Papahadjopoulos, D., Trends Biochem. Sci. 6: 77-80).

Liposomes can be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN™ and LIPOFECTACE™, which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications, for example, in DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88, 046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Liposomes also have been reviewed by Gregoriadis, G., Trends Biotechnol., 3: 235-241).

Another type of vehicle is a biocompatible microparticle or implant that is suitable for implantation into the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International application no. PCT/US/03307 (Publication No. WO 95/24929, entitled "Polymeric Gene Delivery System"). PCT/US/0307 describes biocompatible, preferably biodegradable polymeric matrices for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrices can be used to achieve sustained release of the exogenous gene or gene product in the subject.

The polymeric matrix preferably is in the form of a microparticle such as a microsphere (wherein an agent is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein an agent is stored in the core of a polymeric shell). Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Other forms of the polymeric matrix for containing an agent include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device is selected to result in favorable release kinetics in the tissue into which the matrix is introduced. The size of the polymeric matrix further is selected according to the method of delivery that is to be used. Preferably, when an aerosol route is used the polymeric matrix and composition are encompassed in a surfactant vehicle. The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material, which is a bioadhesive, to further increase the effectiveness of transfer. The matrix composition also can be selected not to degrade, but rather to release by diffusion over an extended period of time. The delivery system can also be a biocompatible microsphere that is suitable for local, site-specific delivery. Such microspheres are disclosed in Chickering, D.E., et al., Biotechnol. Bioeng., 52: 96-101; Mathiowitz, E., et al., Nature 386: 410-414.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the compositions of the invention to the subject. Such polymers may be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multivalent ions or other polymers.

Exemplary synthetic polymers which can be used to form the biodegradable delivery system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene, polyvinylpyrrolidone, and polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion.

Methods of Ocular Delivery

The compositions of the invention are particularly suitable for treating diseases characterized by retinal cell death, such as age-related macular degeneration, retinal detachment, retinal vascular disease, retinitis pigmentosa, glaucoma, diabetic retinopathy, corneal dystrophy, and dry eyes.

In one approach, the compositions of the invention are administered through an ocular device suitable for direct implantation into the vitreous of the eye. The

compositions of the invention may be provided in sustained release compositions, such as those described in, for example, U.S. Pat. Nos. 5,672,659 and 5,595,760. Such devices are found to provide sustained controlled release of various compositions to treat the eye without risk of detrimental local and systemic side effects. An object of the present ocular method of delivery is to maximize the amount of drug contained in an intraocular device or implant while minimizing its size in order to prolong the duration of the implant. See, e.g., U.S. Patents 5,378,475; 6,375,972, and 6,756,058 and U.S. Publications 20050096290 and 200501269448. Such implants may be biodegradable and/or biocompatible implants, or may be non-biodegradable implants. Biodegradable ocular implants are described, for example, in U.S. Patent Publication No. 20050048099. The implants may be permeable or impermeable to the active agent, and may be inserted into a chamber of the eye, such as the anterior or posterior chambers or may be implanted in the schlera, transchoroidal space, or an avascularized region exterior to the vitreous. Alternatively, a contact lens that acts as a depot for compositions of the invention may also be used for drug delivery. I

In a preferred embodiment, the implant may be positioned over an avascular region, such as on the sclera, so as to allow for transcleral diffusion of the drug to the desired site of treatment, e.g. the intraocular space and macula of the eye. Furthermore, the site of transcleral diffusion is preferably in proximity to the macula. Examples of implants for delivery of an a composition include, but are not limited to, the devices described in U.S. Pat. Nos. 3,416,530; 3,828,777; 4,014,335; 4,300,557; 4,327,725; 4,853,224; 4,946,450; 4,997,652; 5,147,647; 5,164,188; 5,178,635; 5,300,114; 5,322,691; 5,403,901; 5,443,505; 5,466,466; 5,476,511; 5,516,522; 5,632,984; 5,679,666; 5,710,165; 5,725,493; 5,743,274; 5,766,242; 5,766,619; 5,770,592; 5,773,019; 5,824,072; 5,824,073; 5,830,173; 5,836,935; 5,869,079, 5,902,598; 5,904,144; 5,916,584; 6,001,386; 6,074,661; 6,110,485; 6,126,687; 6,146,366; 6,251,090; and 6,299,895, and in WO 01/30323 and WO 01/28474, all of which are incorporated herein by reference.

Examples include, but are not limited to the following: a sustained release drug delivery system comprising an inner reservoir comprising an effective amount of an agent effective in obtaining a desired local or systemic physiological or pharmacological effect, an inner tube impermeable to the passage of the agent, the inner tube having first and second ends and covering at least a portion of the inner

reservoir, the inner tube sized and formed of a material so that the inner tube is capable of supporting its own weight, an impermeable member positioned at the inner tube first end, the impermeable member preventing passage of the agent out of the reservoir through the inner tube first end, and a permeable member positioned at the inner tube second end, the permeable member allowing diffusion of the agent out of the reservoir through the inner tube second end; a method for administering a compound of the invention to a segment of an eye, the method comprising the step of implanting a sustained release device to deliver the compound of the invention to the vitreous of the eye or an implantable, sustained release device for administering a compound of the invention to a segment of an eye; a sustained release drug delivery device comprising: a) a drug core comprising a therapeutically effective amount of at least one first agent effective in obtaining a diagnostic effect or effective in obtaining a desired local or systemic physiological or pharmacological effect; b) at least one unitary cup essentially impermeable to the passage of the agent that surrounds and defines an internal compartment to accept the drug core, the unitary cup comprising an open top end with at least one recessed groove around at least some portion of the open top end of the unitary cup; c) a permeable plug which is permeable to the passage of the agent, the permeable plug is positioned at the open top end of the unitary cup wherein the groove interacts with the permeable plug holding it in position and closing the open top end, the permeable plug allowing passage of the agent out of the drug core, through the permeable plug, and out the open top end of the unitary cup; and d) at least one second agent effective in obtaining a diagnostic effect or effective in obtaining a desired local or systemic physiological or pharmacological effect; or a sustained release drug delivery device comprising: an inner core comprising an effective amount of an agent having a desired solubility and a polymer coating layer, the polymer layer being permeable to the agent, wherein the polymer coating layer completely covers the inner core.

Other approaches for ocular delivery include the use of liposomes to target a compound of the present invention to the eye, and preferably to retinal pigment epithelial cells and/or Bruch's membrane. For example, the compound may be complexed with liposomes in the manner described above, and this compound/liposome complex injected into patients with an ocular PCD, using intravenous injection to direct the compound to the desired ocular tissue or cell. Directly injecting the liposome complex into the proximity of the retinal pigment

epithelial cells or Bruch's membrane can also provide for targeting of the complex with some forms of ocular PCD. In a specific embodiment, the compound is administered via intra-ocular sustained delivery (such as VITRASERT or ENVISION). In a specific embodiment, the compound is delivered by posterior subtenons injection. In another specific embodiment, microemulsion particles containing the compositions of the invention are delivered to ocular tissue to take up lipid from Bruch's membrane, retinal pigment epithelial cells, or both.

Nanoparticles are a colloidal carrier system that has been shown to improve the efficacy of the encapsulated drug by prolonging the serum half-life. Polyalkylcyanoacrylates (PACAs) nanoparticles are a polymer colloidal drug delivery system that is in clinical development, as described by Stella et al., J. Pharm. Sci., 2000. 89: p. 1452-1464; Brigger et al., Int. J. Pharm., 2001. 214: p. 37-42; Calvo et al., Pharm. Res., 2001. 18: p. 1157-1166; and Li et al., Biol. Pharm. Bull., 2001. 24: p. 662-665. Biodegradable poly (hydroxyl acids), such as the copolymers of poly (lactic acid) (PLA) and poly (lactic-co-glycolide) (PLGA) are being extensively used in biomedical applications and have received FDA approval for certain clinical applications. In addition, PEG-PLGA nanoparticles have many desirable carrier features including (**i**) that the agent to be encapsulated comprises a reasonably high weight fraction (loading) of the total carrier system; (**ii**) that the amount of agent used in the first step of the encapsulation process is incorporated into the final carrier (entrapment efficiency) at a reasonably high level; (**iii**) that the carrier have the ability to be freeze-dried and reconstituted in solution without aggregation; (**iv**) that the carrier be biodegradable; (**v**) that the carrier system be of small size; and (**vi**) that the carrier enhance the particles persistence.

Nanoparticles are synthesized using virtually any biodegradable shell known in the art. In one embodiment, a polymer, such as poly (lactic-acid) (PLA) or poly (lactic-co-glycolic acid) (PLGA) is used. Such polymers are biocompatible and biodegradable, and are subject to modifications that desirably increase the photochemical efficacy and circulation lifetime of the nanoparticle. In one embodiment, the polymer is modified with a terminal carboxylic acid group (COOH) that increases the negative charge of the particle and thus limits the interaction with negatively charge nucleic acid aptamers. Nanoparticles are also modified with polyethylene glycol (PEG), which also increases the half-life and stability of the particles in circulation. Alternatively, the COOH group is converted to an N-

hydroxysuccinimide (NHS) ester for covalent conjugation to amine-modified aptamers.

Biocompatible polymers useful in the composition and methods of the invention include, but are not limited to, polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetage phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethylmethacrylate), poly(butylmethacrylate), poly(isobutylmethacryla- te), poly(hexlmethacrylate), poly(isodecylmethacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), poly(vinyl acetate, poly vinyl chloride polystyrene, polyvinylpryrrolidone, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutylmethacrylate), poly(hexlmethacrylate), poly(isodecl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecl acrylate) and combinations of any of these. In one embodiment, the nanoparticles of the invention include PEG-PLGA polymers.

Compositions of the invention may also be delivered topically. For topical delivery, the compositions are provided in any pharmaceutically acceptable excipient that is approved for ocular delivery. Preferably, the composition is delivered in drop form to the surface of the eye. For some application, the delivery of the composition relies on the diffusion of the compounds through the cornea to the interior of the eye.

Those of skill in the art will recognize that the best treatment regimens for using compounds of the present invention to treat an ocular PCD can be straightforwardly determined. This is not a question of experimentation, but rather

one of optimization, which is routinely conducted in the medical arts. *In vivo* studies in nude mice often provide a starting point from which to begin to optimize the dosage and delivery regimes. The frequency of injection will initially be once a week, as has been done in some mice studies. However, this frequency might be optimally adjusted from one day to every two weeks to monthly, depending upon the results obtained from the initial clinical trials and the needs of a particular patient.

Human dosage amounts can initially be determined by extrapolating from the amount of compound used in mice, as a skilled artisan recognizes it is routine in the art to modify the dosage for humans compared to animal models. In certain embodiments it is envisioned that the dosage may vary from between about 1 mg compound/Kg body weight to about 5000 mg compound/Kg body weight; or from about 5 mg/Kg body weight to about 4000 mg/Kg body weight or from about 10 mg/Kg body weight to about 3000 mg/Kg body weight; or from about 50 mg/Kg body weight to about 2000 mg/Kg body weight; or from about 100 mg/Kg body weight to about 1000 mg/Kg body weight; or from about 150 mg/Kg body weight to about 500 mg/Kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, 3500, 4000, 4500, 5000 mg/Kg body weight. In other embodiments, it is envisaged that higher does may be used, such doses may be in the range of about 5 mg compound/Kg body to about 20 mg compound/Kg body. In other embodiments the doses may be about 8, 10, 12, 14, 16 or 18 mg/Kg body weight. Of course, this dosage amount may be adjusted upward or downward, as is routinely done in such treatment protocols, depending on the results of the initial clinical trials and the needs of a particular patient.

Kits

The invention provides kits for the treatment or prevention of RPE cell death. In one embodiment, the kit includes a pharmaceutical pack comprising an effective amount of valproic acid, valpromide, lithium, or combinations thereof. Preferably, the compositions are present in unit dosage form. In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic composition; such containers can be boxes, ampoules, bottles, vials, tubes, bags,

pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired compositions of the invention or combinations thereof are provided together with instructions for administering them to a subject having or at risk of developing RPE cell death. The instructions will generally include information about the use of the compounds for the treatment or prevention of RPE cell death. In other embodiments, the instructions include at least one of the following: description of the compound or combination of compounds; dosage schedule and administration for treatment of a disease characterized by RPE cell death (e.g., age-related macular degeneration) or symptoms thereof; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1: VpA, Valpromide (VpD), and Li treatment ameliorated HQ induced cell death

To determine the toxicity of hydroquinone (HQ) on ARPE-19 cells, an oxidative stress model of hydroquinone (HQ)-induced RPE cell death was used. HQ is an oxidative apoptotic agent that is enriched in cigarette smoke. Cell viability was determined by the MTT assay (i.e., conversion of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple dye is an indicator of live cells). The experiments for testing compounds in the oxidative stress model of hydroquinone (HQ)-induced RPE cell death involved pre-incubating with the test compound, exposure to HQ, change of media and recovery, and then performing the MTT assay.

A cell viability assay was performed with a variety of HQ concentrations (Figure 1A). Significant cell death was observed at 250μM HQ with cell mortality rapidly increasing at successively higher concentrations. Incubation with 900nM VpA, 250μM VpD, and 4mM Li lead to a significant reduction in cell death with an even greater increase in the Li and VpA combination treatment at 250μM HQ (Figure 1C). At 350μM HQ, all four interventions significantly increased cell survival. 250μM valpromide (VpD), and the combination of Li + VpA treatment showed slightly greater cell survival that valproic acid alone. At 450μm HQ, none of the treatments showed any cytoprotective effects. VPA protected RPE cells in a dose dependent manner against HQ mediated cell death with a significant increase in cell viability at 250nM VPA (Figure 1D). VPD was not protective of RPE cell death at a range of 10nM-50μM in the oxidative stress model of hydroquinone (HQ)-induced RPE cell death (Figure 1E), although a protective effect was observed at 250μM VPD (Figure 1C). Thus, these results show that valproic acid (VPA), valpromide (VPD), and (Li treatment reduced hydroquinone (HQ) induced cell death.

Example 2: VpA, VpD, and Li treatment ameliorate HQ induce apoptosis

To determine the apoptotic status of ARPE-19 cells treated with HQ, annexin V staining was performed (Figure 2). The data suggests that HQ treatment results in primarily apoptotic cell death with late apoptotic cells dominating the total cellular fraction as HQ concentration increases. At 250μM HQ, the previously mentioned four interventions show a significant reduction in the apoptotic population with an

overall increase in the unstained fraction of cells. At 350μm HQ, all four interventions significantly reduce the annexin V positive (early apoptotic) fraction and reduce the overall amount of apoptosis. When incubated with 450μM HQ, the interventions show no anti-apoptotic effects.

Example 3: VpA treatment reduced cleaved caspase-3 levels

To examine the inhibitory effects of VpA in the apoptotic pathways, *in situ* cleaved caspase-3 levels were evaluated using a fluorescence based substrate cleavage assay. These results showed that when pre-incubated with 900nM VpA, fewer ARPE-19 cells test positive for cleaved caspase-3 at 250 and 350μm HQ. At 450μM HQ, VpA intervention was not protective. To evaluate the proposed role of the heat shock response (hsr), the same assay was performed on WT and HSF1 knockout MEF cells. The results from WT MEF cells were similar to those of ARPE-19 cells. VpA pre-treatment reduced cleaved caspase-3 positive cells in both systems at 250 and 350μM HQ. However, in HSF1 knockout MEFs, VpA pre-treatment exerted no anti-apoptotic activity at any concentration of HQ. The VpA treated cells displayed a similar apoptotic profile to the HQ treated cells in HSF1 deficient MEFs.

Example 4: VpA, VpD, and Li treatment exhibited multipotent anti-apoptogenic effects

To evaluate the anti-apoptotic effects of VpA, VpD, and Li treatment upstream of nuclear DNA destruction, TUNEL staining was performed (Figure 3). At 250μM HQ, 900nM Vpa, 2mM Li, and 250μM VpD reduced the TUNEL stain intensity by about half. At 350μM HQ, the same three interventions significantly reduced the level of TUNEL staining. At 450μM HQ, there were no significant anti-apoptotic effects observed in response to VpA, VpD, or Li pre-treatment.

Example 5: VPA reduces cytokine levels in RPE cell monolayer.

To evaluate the effect of VPA on cytokine levels, cytokines and chemokines from conditioned media were assayed at fixed time points from apical and basolateral surfaces in RPE cultures. Treatment with IL-1β, IFN-γ, TNF-α (ICM) was used to induce a cytokine response. VPA was tested at 10μM. Sandwich Enzyme-linked immunosorbent assay (ELISA) was performed to detect cytokine levels using a commercially available sensitive, fluorescent detection system (Searchlight).

Cytokine release from the apical and basloateral surfaces of an RPE cell monolayer at different time points after treatment with VPA, VPA and IL-1β, IFN-γ, TNF-α (ICM), or medium alone (SF) are shown in Table 1 (below):

Table 1. Cytokine levels in RPE cell monolayer

				hIL6	hIL8	hRANTES	hMCP1	hVEGF	hSDF1b
Time (hrs)	Sample	Ap/Ba		pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
8	SF	Ba	Av	20	3	0	413	2609	0
			St Dev	18	2	0	262	506	1
8	ICM	Ba	Av	2770	1044	48	35725	2149	0
			St Dev	1358	176	43	17071	1011	1
8	ICM + VPA	Ba	Av	3036	1385	25	27518	2310	0
			St Dev	1865	121	9	8797	528	1
8	VPA	Ba	Av	19	2	0	525	2414	0
			St Dev	19	4	0	459	625	1
24	SF	Ba	Av	90	8	1	2644	5477	3
			St Dev	145	10	0	2718	1334	3
24	ICM	Ba	Av	152812	114164	7431	363914	7288	0
			St Dev	42341	26170	4663	58373	931	1
24	ICM+VPA	Ba	Av	71694	23669	7255	241183	5642	3
			St Dev	28831	10996	3561	120291	1215	3
24	VPA	Ba	Av	48	3	1	1247	6230	5
			St Dev	42	5	1	816	417	3

At 8 hours, treatment with VPA or VPA and ICM showed minimal increases in cytokine levels compared to the control. However, at 24 hours, ICM induced significant increases in cytokines (IL-6, IL-8, MCP-1, VEGF, MCP-2, hGROα, and RANTES), which are associated with recruitment of systemic immune cells. In particular, a dominant effect was observed on the basolateral side of the RPE monolayer. Treatment with VPA reduced levels of the basolateral ICM-induced cytokines (IL-6, IL-8, MCP-1, VEGF, MCP-2, hGROα, and RANTES). Thus, these results show that VPA inhibits the release of cytokines and can inhibit recruitment of systemic immune cells and reduce inflammation.

Example 6: VPA increases retinal formation in mouse models of retinal disease or retinal damage.

To assess the effect of systemic VPA administration on the course of the retinal degeneration, experiments were conducted in mouse models of retinal disease, including an opsin mutant (P23H) mouse and a Mer -/- mouse, and retinal damage, including sodium-iodate induced retinal damage.

For experiments conducted on P23H mice, a mouse expressing a mutant opsin (hP23H RHO+/-, mRHO +/+) was generated and used in several published studies (White et al. 2007. Increased sensitivity to light-induced damage in a mouse model of autosomal dominant retinal disease. Invest Ophthalmol Vis Sci. 48:1942-1951; Krebs M.P., White D.A., Kaushal S. 2009. Biphasic photoreceptor degeneration induced by light in a T17M rhodopsin mouse model of cone bystander damage. Invest Ophthalmol Vis Sci. 50:2956-2965; Mao et al. 2010. AAV delivery of wild-type rhodopsin preserves retinal function in a mouse model of autosomal dominant retinitis pigmentosa. Hum Gene Ther. Dec 3, 2010). P23H transgenic mice (line 37) contain a human P23H RHO transgene including the entire rhodopsin gene transcriptional unit plus 4.2 kb of upstream and 8.4 kb of downstream DNA. Founder P23H rhodopsin mice (on an FVB background) were backcrossed with C57BL/6J mice for 10 generations to obtain human transgenic mice on a uniform B6 genetic background. This line contains an equal number of copies of the human rhodopsin transgene and the endogenous mouse rhodopsin gene.

Male and female P23H mice were treated for 11-12 weeks (with a subgroup treated for 20 weeks) with an i.p. injection of VPA (250 mg/kg) or vehicle once every two days starting from age P25-P30. Full-field ERGs were recorded at baseline and after 11 weeks of treatment. Vision function was evaluated by ERG recordings (Espion E2 with ColorDome, Diagnosys LLC, Lowell, MA). Mice were dark-adapted overnight. Subdermal needle electrodes served as reference and ground, while a contact lens electrode served as active. Full-field ERG recordings were obtained from both eyes from white flashes of increasing intensities (0.02, 0.26, 2.8, 28 and 100 cd.s/m2) and b-waves of the recorded traces were measured and analyzed. The bandpass filter was set between 0.3 and 300 Hz. The averaged responses were measured in a conventional way (from the trough of the a-wave) to obtain b-wave amplitude.

Scotopic ERG a- and b-wave amplitudes were significantly larger in P23H mice after 11 weeks of dosing with VPA compared to control mice ($p<0.05$ and $p<0.001$, two-way ANOVA, see also Figures 4A and 4B). Preservation of the retinas of mice treated with VPA was also demonstrated at the end of the 20-week period by immunohistochemistry (IHC) (Figures 4C and 4D). Consistently, outer nuclear layer (ONL) thickness was observed as 2-3 rows of nuclei in VPA-treated mice (Figure 4D) and only one row of nuclei in the retina of vehicle-treated mice (Figure 4C).

Disorganization and thinning of INL in the control treated retina is notable compared to the VPA-treated retina. Thus, the experiments show that VPA had a regenerative effect on retinal degeneration in a P23H mouse model of autosomal dominant retinal disease.

For experiments conducted on mer -/- mice, ablation of Mer tyrosine kinase function in mer knockout mice results in a retinal phenotype (rapid, progressive degeneration of the photoreceptors (PRs)) virtually identical to that in the Royal College of Surgeons/RCS rat model of retinal degeneration (Duncan et al. An RCS-like retinal dystrophy phenotype in mer knockout mice. Invest Ophthalmol Vis Sci. 2003 Feb;44(2):826-38.). Mer is a member of the Axl/Mer/Tyro3 receptor tyrosine kinase subfamily. Mer is the designation for the murine gene that is orthologous to rat and human Mertk. Mutations in human MERTK have been identified in patients with retinitis pigmentosa (Gal et al. 2000. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa Nat Genet 26,270-271). The similarity in phenotypes between the two rodent models suggests that an RPE phagocytic defect is a feature of all types of retinal degeneration caused by loss of function of Mer tyrosine kinase, perhaps including mutations in human MERTK.

Male and female mertk-/- mice (n=12) were treated daily with an intraperitoneal injection of valproic acid solution (250 mg/kg) from P25-P30 until P65-P67. Mice of the same age dosed in the same manner with saline served as a control group (n=12). At the end of the treatment, OCT measurements and ERG recordings were performed on both groups. In ultra-high resolution OCT retinal thickness measurement experiments, mice were anesthetized (ketamine 100 mg/kg and xylaizine 10 m/kg mixture) and pupils dilated with 1 drop of phenylephrine hydrochloride 2.5% and tropicamide 1%. Full retinal thickness at ~500 microns nasally and temporally from the optic disc was measured with ultrahigh resolution OCT (Bioptigen Inc., Durham, NC). The nasal and temporal measurements from both eyes were averaged across the eyes and analyzed separately.

For ERG recording, mice were anesthetized and pupil dilated in a similar manner as for OCT recording. Vision function was evaluated by full-field ERG recordings (Espion E2 with ColorDome, Diagnosys LLC, Lowell, MA). Specifically, after 9 minutes of light adaptation, photopic ERG traces were obtained from both eyes as a result of a white flash stimulation with an intensity of 10 phot cd.s/m2 , at 1Hz frequency presented on a 34 cd/m2 white background. The bandpass filter was set

between 0.3 and 300 Hz. Responses were averaged (n=30) to enhance the signal-to-noise ratio and the averaged response was measured in a conventional way (from the trough of the a-wave) to obtain b-wave amplitude. Responses from the left and the right eye were averaged to obtain a single measure per animal.

Both temporal and nasal central retinal full-thickness measurements demonstrated significantly thicker retina in the VPA-treated group (~33% difference; $p<0.001$; Mann-Whitney test, Figure 5A). The presence of outer nuclear layer (ONL) containing more than 1 row of nuclei was observed in the majority of the VPA-treated mice (21 out of 24 eyes, or 87.5%), and in a few of the control mice (7 out of 24 eyes, or 29.2%, data not shown). Correspondingly, photopic ERG b-wave amplitude was ~80% larger in the VPA-treated group ($p<0.05$, Figure 5B) at P65, while photopic b-wave peak time was ~16% faster with VPA treatment (mean time 74 msec vs. 87 msec, $p<0.05$).

For experiments on mice with induced retinal damage, intravenous injection of sodium iodate solution (20-40 mg/kg or more) in mice resulted in a rapid loss of RPE and photoreceptors either in the central part of the retina or in the whole retina depending on the dose used (Franco et al. 2009. Decreased visual function after patchy loss of retinal pigment epithelium induced by low-dose sodium iodate. Invest Ophthalmol Vis Sci. 50:4004-4010; Machalińska et al. 2010. Sodium iodate selectively injuries the posterior pole of the retina in a dose-dependent manner: morphological and electrophysiological study. Neurochem Res. 35:1819-1827). Sodium iodate induced retinal damage in mice is a model of Age related macular degeneration (ARMD). The RPE is the initial site of the toxic action of sodium iodate, with secondary effects exerted on photoreceptor changes. ERG response disappears by Day 3 after dosing at 40 mg/kg, however the response is preserved at ~50% level and stays relatively stable for a couple of weeks after dosing with 20 mg/kg. Similarly, the process of ongoing retinal apoptosis is most pronounced at Day 3 and the intensity of the process rapidly declines, with very little apoptosis present at Day 28. (Machalińska et al., 2010).

Adult C57BL/6 mice were pre-treated with either VPA (250 mg/kg i.p.) or vehicle for two days. Following pre-treatment, a single tail vein injection of 20 mg/kg sodium iodate was performed on all mice. Mice were then injected i.p daily with either VPA or vehicle for 10 days. For evaluation of retinal health, optical coherence tomography (OCT) retinal thickness measurements and ERG recordings were used.

Specifically, full retinal thickness at ~500 microns nasally and temporally from the optic disc was measured with ultra-high resolution OCT (Bioptigen Inc., Durham, NC). The nasal and temporal measurements from both eyes were averaged across the eyes and analyzed separately. For full-field ERG, equipment, bandpass settings, electrodes, anesthesia and pupil dilation were the same as described in the P23H section above. Mice underwent 10 minutes of light adaptation (34 cd/m2 white background), and at the end of this period, photopic ERG traces were obtained from both eyes as a result of a white flash stimulation with an intensity of 10 phot cd.s/m2 at 1Hz frequency. Responses were averaged to enhance the signal-to-noise ratio and the averaged response was measured in a conventional way (from the trough of the a-wave) to obtain b-wave amplitude. Responses from the left and the right eye were averaged to obtain a single measure per animal.

OCT measurements showed a thicker central retina in the VPA-treated group at both Day 9 and Day 15 compared to the control group (Figure 6A). This preservation of thickness in the VPA-treated group compared to the control group was very similar at the second time point (Day 15; Figure 6B), indicating persistence of the protective effect after discontinuation of treatment, in the number of animals tested (n=3/group). Photopic b-wave amplitude also showed preservation at Day 10 (Figure 6C). Thus, VPA had a protective effect on cone and rod survival in the sodium iodate model of ARMD.

Example 7: VPA provides an effective treatment for human subjections with ARMD

Virtually no effective treatments exist for age-related macular degeneration (ARMD), the leading cause of blindness in the elderly in the developed world. The following study was conducted to assess the efficacy and safety of valproic acid (VPA) as a treatment for non-exudative ("dry") or exudative ("wet") ARMD. Fourteen patients with dry (18 eyes) and wet (10 eyes) ARMD were treated off-label with VPA at 500 or 750 mg/day for an average of 22.7 weeks. Treatment efficacy was assessed by detecting a change in best-corrected visual acuity (BCVA) and an improvement in central retinal thickness measured by optical coherence tomography (OCT). Additionally, a subjective change in visual perception after treatment was assessed. Overall, BCVA improved in 12 eyes, remained unchanged in 11 eyes and worsened in five eyes. However, in patients taking 750 mg/day of VPA, BCVA

improved in 11 eyes (61.1%), was unchanged in seven eyes (38.9%) and did not worsen in any eyes. Eleven patients (73.3%) reported subjective improvement in visual perception, mirroring BCVA improvement in at least one eye. For nine wet ARMD eyes for which central retinal thickness was measured, six eyes (67%) improved.

This is the first report to describe improved BCVA and subjective visual perception in patients with dry ARMD, and improved BCVA and retinal thickness in patients with end-stage wet ARMD, after VPA treatment. Oral VPA at the doses used was well-tolerated in most patients. This study served as the basis for the design of a randomized, placebo-controlled clinical trials for both wet and dry ARMD.

Outcome measures for dry and wet ARMD eyes are described in Table 2 (below).

Table 2. Clinincal and Treatment Characteristics of Study Patients. Eyes with non-exudative form of ARMD coded as "DRY"; eyes with exudative form of AMD coded as "WET".

Patent ID	Age at baseline	Average VP daily dose	Length of treatment	Eye	Type of ARMD	BCVA change at	Subjective assessment
# 1	69.0	750	35.0	OD	DRY	0	Improved
# 2	79.7	500	6.0	OD	WET	0	Stable
				OS	WET	0.079	
# 3	77.7	500	16.0	OD	DRY	0.301	Improved
				OS	DRY	0	
# 4	81.6	750	38.0	OD	DRY	-0.097	Improved
				OS	DRY	-0.079	
# 5	73.5	750	11.0	OD	DRY	-0.125	Improved
				OS	DRY	0	
# 6	74.3	750	38.0	OD	DRY	0	Stable
				OS	DRY	-0.079	
# 7	87.2	500	21.0	OD	DRY	0	Improved
				OS	WET	0.382	
# 8	83.8	750	7.5	OD	DRY	0	Improved
# 9	84.9	750	20.1	OD	DRY	-0.219	Improved
				OS	WET	-0.477	
# 10	87.2	500	9.6	OD	DRY	-0.097	Improved
				OS	WET	0.067	
# 11	76.4	750	17.0	OD	DRY	0	Stable
				OS	WET	-0.097	
# 14	91.2	750	19.0	OD	DRY (GA)	0	Improved
				OS	WET	0	
# 17	84.1	500	29.3	OD	WET	0.113	Stable
				OS	DRY	0	
# 21	56.5	750	33.0	OD	DRY	-0.079	Improved
				OS	WET	-0.301	
# 24	68.7	750	39.3	OD	DRY (GA)	-0.277	Improved
				OS	DRY (GA)	-1.000	

Nine Caucasian females and six Caucasian males were studied, ranging in age from 56 to 91 years. The average baseline visual acuity for the study cohort was 0.785 (±0.61) logMAR units, corresponding to a Snellen Acuity of approximately 20/122. The average change in BCVA at the time of follow-up for all eyes included in this analysis (n=28) was an improvement of -0.071 logMAR (±0.25) units, equivalent to 0.7 lines (±2.5 lines). When results from both eyes of a patient were available and averaged, the change was an improvement of -0.077 logMAR units, which was significantly different ($p<0.01$) from the expected value based on published natural history data (Figure 7A). However, the two treatment groups (500 mg/day and 750 mg/day) varied in their response to treatment. None of the eyes in patients treated

with 750 mg/day demonstrated a decrease in BCVA and 11 of these 18 eyes improved (Figure 7B). Averaging both eyes, in the 500 mg/day group visual acuity worsened 0.085 (±0.084) logMAR units, while in the 750 mg/day group visual acuity improved -0.182 (±0.221) logMAR units, and this difference was statistically significant ($p<0.05$, Figure 7C). Additionally, a one-sample t-test between the treatment group taking 750 mg/day and a theoretical value of 0.147 logMAR units based on natural history yielded results indicating significant BCVA improvement ($p<0.01$). This difference remained significant when, instead of the average, the better (more improved) eye was used in the analysis (Figure 7D).

In eyes with dry ARMD in the 500 mg/day group, visual acuity worsened 0.013 (±0.10) logMAR units, while those in the group treated with 750 mg/day had an improvement in average visual acuity of -0.15 (±0.23) logMAR units (Figure 8A). The difference between the two groups was not statistically significant. However, a one-sample t-test between the group treated with 750 mg/day and a theoretical value expected from the natural history data was statistically significant ($p=<0.01$, Figure 8A). The difference was also significant when the better eye was used in the analysis (Figure 8B). When the most improved eye from each patient was used for analysis, even eyes in the group treated with 500 mg/day showed no worsening in BCVA.

Eyes with wet ARMD treated with 500 mg/day had a slight worsening in visual acuity of 0.150 (±0.157) logMAR units, while the group treated with 750 mg/day had an improvement on average of -0.266 (±0.337) logMAR units, equivalent to an improvement of 2.7 lines. The difference between the two groups was statistically significant ($p<0.05$, Figure 9A). The result of a one-sample t-test between the group taking750 mg/day and a theoretical value expected from the natural history data17was significant ($p < 0.05$).

Central retinal thickness measurements by OCT were available in nine eyes of eight patients with wet ARMD. Six of these eyes (67%) had improvement in central retinal thickness compared to baseline, whereas one eye (11%) remained stable (Figure 9B). A representative OCT scan from patient #21 is presented in Figure 9C. Resorption of the cystic fluid in the inferior foveal region is evident. Subjective assessment of visual function correlated with BCVA changes in most patients. Eleven of 15 patients included in the study (73%) reported improvement in visual functioning. Improvement in BCVA in at least one eye was found in six of those eleven patients (54%) (Table 2). Two patients reported the onset of fatigue during

VPA treatment. Patients also reported episodes of heartburn, weight loss, tremor, upset stomach, diarrhea, and dizziness while on VPA. Of those patients who reported a side effect, each patient experienced only that single side effect. One patient discontinued treatment due to fatigue. This patient did not have any co-morbid conditions and was not taking any medication that could account for the reported fatigue.

These results indicate that VPA should be considered as a treatment for both wet and dry ARMD. At 500 mg/day, VPA had minimal effect on BCVA and retinal thickness. However, at 750 mg/day, VPA was associated with a subjective improvement in visual functioning and a statistically significant improvement in BCVA. In patients with dry ARMD, the median visual acuity improved approximately one line with no patient worsening. Certain patients had considerable improvement in BCVA (up to 10 lines). In patients taking 750 mg/day of VPA with end-stage wet ARMD who failed to improve with multiple injections of bevacizumab and/or ranibizumab, one line and up to 7.5 lines of improvement was achieved. Additionally, central retinal thickness improved in the majority of treated patients. In sum, these studies showed a clear dose-dependent evidence of efficacy. Such an improvement in end-stage patients is virtually unprecedented.

Typically in the context of clinical trials, significant changes in visual acuity are reported as the rate of loss of three or more lines of visual acuity (by ETDRS or Snellen chart). In ARMD, any measurable improvement or stabilization of this progressive blinding disease is clinically relevant. From published natural history data of dry and wet ARMD patients, the observed loss on average is 1.5 lines (0.147 logMAR) over a 5.5 month period. VPA was administered in an attempt to mitigate this loss. These data demonstrated that patients taking 750 mg/day of VPA actually showed improved vision regardless of the form of ARMD (i.e., both dry and wet ARMD). Among patients reporting subjective improvement in their visual functioning, 75% had improved BCVA. Patients in this study tolerated VPA well.

Side effects reported by patients in this study are consistent with the side effect profile reported in studies using VPA for other indications. VPA provides a promising new treatment for the dry and wet forms of ARMD consistent with current understanding of the pathophysiology of the disease and the known biological properties of existing therapies. A single patient reported a side effect (severe fatigue) that was not transient. Without exception, all transient side effects subsided after

changing the time of administration to after meals or without intervention. There were no co-morbidities in the patient who discontinued treatment that could account for severe fatigue. These data are retrospective and non-randomized. The ARMD in these eyes was not graded, and neither patients nor researchers were masked to the type of treatment received, which could potentially introduce a placebo-effect bias. However, measurements of central retinal thickness conducted using objective OCT instruments have no such bias, and these measurements showed significant and profound improvement. Another limitation is that this small subject pool was selected from two effects reported by patients in this study are consistent with the side effect profile reported in studies using VPA for other indications.

Given the huge costs and significant time associated with the preclinical and clinical phases of new drug development, it is valuable to rapidly and economically evaluate the repurposing of already FDA-approved drugs for new indications such as ARMD. We present here a potential new treatment for the dry and wet forms of ARMD based on our understanding of the pathophysiology of the disease and the known biological properties of existing therapies. There are several major advantages to the use of VPA for ARMD. First, since the bulk of the preclinical, pharmacokinetic, manufacturing, safety and tolerability analysis is complete, clinical trials can generally begin for such FDA-approved drugs along the Phase II spectrum. The huge costs associated with discovering and testing new medications is avoided, effectively allowing translation of basic science discovery via investigator-initiated studies. Second, the time-to-treatment in humans is expedited, fostering delivery of effective medications in a shorter time. Third, the relatively inexpensive cost of "relabeling" existing formulations for new indications motivates pharmaceutical companies to invest in therapies for common diseases. A single patient reported a side effect (severe fatigue) that was not transient. Without exception, all transient side effects subsided after changing the time of administration to after meals or without intervention. There were no co-morbidities in the patient who discontinued treatment that could account for severe fatigue.

These data are retrospective and non-randomized. The ARMD in these eyes was not graded, and neither patients nor researchers were masked to the type of treatment received, subjecting results to potentially significant placebo-effect bias. However, measurements of central retinal thickness conducted using objective OCT instruments have no such bias, and these measurements showed significant and

profound improvement. The subject pool was selected from two clinical sites and was not population-based.

Example 6: VPA provides an effective treatment for human subjections with Retinitis Pigmentosa

To examine the efficacy and safety of Valproic Acid (VPA) in patients with Retinitis Pigmentosa (RP) thirteen eyes were examined before and after brief treatment (average 4 months) with VPA. Visual fields (VF) for each eye were defined using digitized Goldmann Kinetic Perimetry tracings. VF areas were log transformed and VF loss/gain relative to baseline was calculated. Visual acuity was measured using a Snellen chart at a distance of twenty feet. Values were converted to a logarithm of minimum angle of resolution (logMAR) score.

Nine eyes had improved visual field with treatment, two eyes had decreased visual field and two eyes experienced no change, with an overall average increase of 11%. Assuming typical loss in VF area without treatment, this increase in VF was statistically significant ($p<.02$). An average decrease (0.172) in the logMAR scores was seen in these 13 eyes, which translates to a positive change in Snellen score of approximately 20/47 to 20/32, which was significant ($p<.02$) assuming no loss in acuity without treatment. Side effects were mild and well tolerated. These results indicate that VPA treatment is beneficial for patients with RP.

Table 3 summarizes the average characteristics of the RP patients included in this analysis.

Table 3

Patient #	1		2		3		4		5		6		7		Avg ± sd	
Gender	M		M		M		F		M		F		M			
Race/Ethnicity	Caucasian		African American		Caucasian		Caucasian		Caucasian		Caucasian		Caucasian			
Age	56		21		46		16		23		31		52		36±16	
Autosomal Dominant RP	Y				Y		Y		Y		Y		Y			
Length of VPA treatment (mths)	2		2		4		4		4		6		5		4± 1	
Avg. Dose of VPA (mg/day)	750		750		500		500		500		500		750		643±133	
Eye	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
Visual Field at baseline (mm^2) isopter V4e	192	165	148	107	38	201	562	558	504	-	278	450	442	434	309 ± 197	319±184
Visual Field at Follow up (mm^2)	210	187	165	118	96	200	586	594	452	-	517	575	448	385	353±192	343±207
Visual Acuity at baseline (logMAR)	1	1	.544	.301	.176	.301	0	0	0	.097	1.301	.097	.176	.301	.456± .515	.300±.332
Visual Acuity at follow-up (logMAR)	.176	.301	.544	.176	.097	.301	0	0	0	.097	1	.097	0	.097	.260±.380	.153±.113

Patients' ages ranged from 16 to 56 (average = 36), with five males included. The majority of patients (n=6, 86%) had a family history or reported genotyping suggestive of an autosomal dominant form of RP (ADRP). The length of treatment on VPA was short, with a range from 2 to 6 months. Overall, average visual acuity was 20/47 per eye (range 20/20 to count fingers at 3' or 20/4000).

Visual fields were measured using kinetic perimetry; tracings were digitized and the areas determined by ispoter V were converted into areas of functioning retina. Examples of baseline and follow-up perimeter tracings are shown in Figure 10A. The patient whose visual field is shown on Figure 10A (patient #6, Table 3) had two follow-ups within a short period of time (4 weeks) and VF results were stable, suggesting stability in vision function gain as a result of treatment. Line graphs of baseline and follow-up values of visual field areas for all seven study subjects, for each eye and the average values per patient are shown in Figure 10B. Baseline and follow-up data are plotted according to their time of measurement, thus accounting for duration of treatment; these graphs depict the individual slopes of change in visual field. Using a difference from baseline of ± 2% as a criterion for change, nine eyes (5 right eyes and 4 left eyes) had improved intact visual fields after brief treatment with VPA, while two eyes lost VF area (one right eye and one left eye) and two eyes (one right and one left eye) had no change in VF. Table 4 lists the values for percent change from baseline and difference in logVF as described below.

Table 4: Percent Change in Visual Field

Patient	Eye	% Change in VF Area	Δ logVF
1	OD	9.4	.092
	OS	13.9	.130
2	OD	11.8	.112
	OS	10.2	.097
3	OD	156.4	.942
	OS	-0.4	-.004
4	OD	4.4	.043
	OS	6.5	.063
5	OD	-10.2	-.107
	OS	*	*
6	OD	86.3	.622
	OS	27.7	.246
7	OD	1.3	.013
	OS	-11.2	-0.120
Average±SD		23.5±46.8	.164±.298
Median		9.4	0.09

* - VF results excluded due to poor quality

Some studies estimated an average visual loss of 0.10 $\log_e$ units per year (equivalent to ~10.5%/year) or about 0.033 $\log_e$ units (~3.52%) for 4 months (16), which is the average length of VPA treatment in this study. The change in logVF (ΔlogVF) from baseline is presented in Table 3 and Figure 11. The average change for all 13 eyes over the course of treatment was +0.164 (SD .298) $\log_e mm^2$, (range -0.012 to +0.942) corresponding to an average increase of about 35 mm^2. Assuming a baseline area of 314 mm^2 (the average baseline of subjects in this study), this translates to an 11% increase in area of functioning retina.

To determine if this effect was significant, the change in VF for eyes on VPA was compared to two theoretical rates of vision loss with no treatment. The first assumption was that there would be no loss of VF without treatment, while the second assumption was that eyes would lose an average of 0.033 $\log_e$ units or 3.5% (16). The third assumption was for a more conservative estimate of visual field loss based on Berson etal.(18). Significance levels were calculated using the Wilcoxon signed rank test (Table 5). A significant difference ($p<.006$) in VF loss was seen for eyes on VPArelative to the natural history of the disease (16). Even if one assumes a slower rate of decline in visual field loss of 0.011 logVF unit or 1.5% for 4 months

duration of VPA treatment (based on data from Berson et al.(18)), the difference is statistically significant ($p<0.02$).

Table 5: Statistical Analysis of Changes in FV on VPA Relative to Typical Loss in RP Patients

Null Hypothesis Mean Change with no treatment	Significance (Two Sided) of signed rank test		
	Left eyes	Right eyes	All eyes combined
0	.08	.09	.09
-0.011	.22	.08	.02
-0.033	.09	.05	.006

An overall increase in visual acuity on VPA treatment was observed (Table 3). A decrease in logMAR units frombaseline to follow-up is indicative of improved BCVA. When analyzed by eye, average logMAR for all right eyes decreased at baseline from 0.457 (SD 0.515) to 0.260 (SD 0.380) at follow-up while average logMAR values for the left eyes decreased from 0.300 (SD 0.332) to 0.153(SD0.113). The average change in logMAR across all eyes was a loss of 0.172 (SD 0.269) log units(range -0.824 to 0), which translates to a positive change in Snellen scoreof approximately 20/47 to 20/32. Assuming no loss in visual acuity without treatment this changein acuity was significant ($p <0.02$).

A conservative analysis was performed to explore any potential negative effects of VPA on patient's visual field. For purposes of this analysis, an event of negative effect of VPA was indicated by net loss in visual field from baseline greater than 2%. This is a conservative approach because, absent any treatment, the natural progression of RP includes a potential for significant short-term deterioration in visual field (Dagnelie et al., Clin Vision Sci. 1990;5:1-26; Merin et al., Ocul Pharmacol Ther. 2008;24:80-6; Berson et al., Invest Ophthalmol Vis Sci. 2002;43:3027-36). Of the 13 eyes examined, two experienced worsening of their visual field (Figure 10). No abnormal liver function or blood chemistries were noted in the study sample. The most common side effects were mild and included tiredness (10%) and stomach irritation (13%).

Retinitis Pigmentosa is a blinding disease with no robust treatment options. The visual field areas of five of seven RP patients increased with a short treatment of

valproic acid. Encouragingly, in one case(patient # 6), the significant improvement in functioning retinal area was confirmed at two time points (23 and 27 weeks). While visual acuity is not always a reliable outcome measure for RP given that photoreceptor degeneration typically begins in the periphery and progresses to the central macula in only the latest stages of disease, nevertheless an overall improvement in acuity was observed while patients were treated with VPA. These positive results are encouraging given that the VPA dose used in this study was about 60% lower than the typical dose for epilepsy or the dose used in a recently published clinical trial for amyotrophic lateral sclerosis (Piepers et al., Ann Neurol. 2009;66:227-34).

While prior accounts of limited delay of progression of photoreceptor loss in RP patients has been reported with nutritional supplementation such as vitamin A or treatments such as hyperbaric oxygen therapy, this is the first reported case of improvement of vision function in patients with RP as a result of pharmacologic treatment.

Valproic acid is widely used as an anti-convulsant and mood stabilizer and its efficacy in these capacities is likely mediated via its ability to affect GABA levels through glutamic acid decarboxylase and GABA transaminase modulation. VPA was identified using a heterologous cell culture screen for small molecules that increase the yield of properly folded RP mutant rhodopsins. Without wishing to be bound by theory, a variety of evidence indicates that VPA likely works at the level of cell death protection or inflammatory mediation as its neuroprotective properties have been well documented (Feng et al., Neuroscience. 2008;155:567-72; Leng et al., J Neurosci. 2008;28:2576-88) and it can down-regulate the photoreceptor-specific inflammatory response pathway via apoptosis of microglial cells (Dragunow et al., Neuroscience. 2006;140:1149-56; Chen et al., Neuroscience. 2007;149:203-12; Kim et al., J Pharmacol Exp Ther. 2007;321:892-901). Furthermore, VPA is known to be a potent inhibitor of histone deacetylase (HDAC) (Gottlicher et al., Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J. 2001;20:6969-78). A particularly exciting property of VPA has recently been documented that indicates that it has the unique ability to reverse photoreceptor damage: VPA can induce cells to differentiate in culture (Gottlicher et al., Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed

cells. EMBO J. 2001;20:6969-78); moreover, it has been shown to stimulate glial cells to differentiate into photoreceptor-like cells.

While the results presented here are promising, only seven patients were analyzed and the length of follow-up was brief (an average of 4 months). Because patients in this analysis were not thoroughly genetically characterized, genetic variation in known RP genes might account for the variability in therapeutic response to VPA.

In summary, VPA provides a safe and effective therapy for RP, a tragic blinding disease for which no effective therapies currently exist. The results of the clinical analysis reported herein in conjunction with the *in vitro* data provided above indicate that VPA is an effective treatment for photoreceptor loss associated with RP. This study provides the basis for a placebo-controlled clinical trial with patients with well characterized RP genotypes to more fully evaluate the efficacy and safety of VPA as a treatment for RP.

The results described above were obtained using the following materials and methods.

Cell Culture

ARPE-19 cells were obtained from the American Type Culture Collection (ATCC CRL2302, Manassas, VA) and grown in high glucose Dulbecco's Modified Eagle Medium (DMEM, Cellgro/Mediatech Inc., Manassas, VA) supplemented with 10% heat-inactivated fetal calf serum (FCS, Sigma-Aldrich, St.Louis, MO) and 1% penicillin/streptomycin (Gibco, Grand Island, NY) at 37°C in presence of 5% CO_2. Cells were routinely subcultured or harvested for experiments using Tryple Express (Gibco). In all subsequent experiments, cells were grown from frozen aliquots to ensure that all experiments were conducted on similar cells between passage (P)6 to P9. The media was changed 24 hours prior to the start of each experiment. Addition of hydroquinone (Sigma-Aldrich) was accomplished by solubilizing the HQ in serum-free media and subsequent filter sterilization before adding HQ to the culture. Wild-type and heat shock factor 1 (HSF1) knockout mouse embryonic fibroblasts, referred to as $MEF^{+/+}$ and $MEF^{-/-}$ respectively, were generously provided by Dr. Benjamin's lab (McMillan et al., The Journal of Biological Chemistry, 273, 7523-7528). MEF's were cultured in high glucose DMEM supplemented with 0.1mM non-essential amino

acids (Gibco), 1% penicillin/streptomycin, 0.1mM β-mercaptoethanol (Sigma), and 10% FCS at 37°C in presence of 5% CO_2.

Cell Viability

ARPE-19 and MEF cells were grown to confluency on 100mm plates before being subjected to hydroquinone treatment at various concentrations for 48 hours or 72 hours in the presence of valproic acid. After addition of hydroquinone, the media and PBS wash were collected and spun at 3500 rpm for 5 min along with the trypsinized cells. The cell pellet was resuspended in ice-cold PBS and cell viability was evaluated by the Live/Dead mammalian cell viability kit (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Briefly, cell number was adjusted to 1 x 10^6/mL PBS before adding calcein AM and ethidium homodimer and after 15 minutes incubation at room temperature in the dark; the samples were analyzed by FACSCalibur (BD Biosciences, San Jose, CA). Ubiquitous esterase activity within cells was required to generate the calcein AM fluorescence while dead cells with compromised membranes were labeled by ethidium homodimer. Results are presented as a percentage of total cellular fractions stained with the appropriate fluorophore.

Annexin V labeling

ARPE-19 and MEF cells were grown, treated, and collected as previously described (McMillan et al., The Journal of Biological Chemistry, 273, **7523-7528)** and were then subjected to Annexin V-fluorescein isothiocyanate (AV-FITC) staining (Calbiochem, San Diego, CA) according to the manufacturer's instructions. Briefly, the cell pellet was re-suspended in fresh media, with media-binding reagent added to the suspension followed by Annexin V-FITC. After a 15 minute dark incubation at room temperature, the cells were spun at 1000 x g for 5 minutes. The pellet was resuspended in cold 1X binding buffer and then propidium iodide (PI) was added. The samples were analyzed by FACSCalibur (BD Biosciences) and a portion of the suspension was also placed on slides for immediate observation using a Leica DMI6000B fluorescence microscope. One of the earliest markers for apoptosis is indicated by the re-localization of phosphatidylserine (PS) in the plasma membrane from the cytosolic side to the extracellular side. FITC conjugated Annexin V was used to detect PS as an early marker of apoptosis. Propidium iodide is normally

impermeable to cells until the loss of membrane integrity and serves as a marker of necrosis/late apoptosis and cell death. Results are presented as a percentage of total cellular fraction stained with the appropriate fluorophore(s).

In Situ Cleaved Caspase 3 Assay

ARPE-19 and MEF cells were grown, treated, and collected as described above and were then subjected to cleaved caspase-3 staining (Calbiochem, San Diego, CA) according to the manufacturer's instructions. The cell pellet was resuspended in ice-cold PBS and cell number was adjusted to 1 x 10^6/mL. 1µL of FITC-DEVD-FMK was added to 300µL of each sample which was then incubated in a 37°C incubator with 5.0% CO_2 for 60 minutes. The cells were then washed three times by centrifugation at 3000 rpm for 5 minutes before being re-suspended in wash buffer. For analysis by flow cytometry, 300µL of prepared sample was placed on ice until evaluation in FACSCalibur (BD Biosciences) using FL-1 channel. Results are presented as a histogram of cell count and log FL-1 (FITC) channel fluorescence. Negative controls were treated with a caspase-3 inhibitor (Z-VAD-FMK) as instructed by the kit. The marker indicates percentage of cells with greater than a predetermined level of fluorescence from control samples indicated as cells positive for cleaved caspase-3 by the kit.

Immunofluoresence

ARPE-19 cells were grown on sterile coverslips to about 80-90% confluency before drug/stressor additions as described above. After 72 hours, the media was removed, the cells were washed with PBS before immediate addition of 4% paraformaldehyde in PBS for 15 minutes at room temperature. After washing the fixed cells with PBS, -20°C methanol was added for 10 minutes. Blocking was accomplished by addition of 5% normal serum (Sigma-Aldrich) from the same species that the secondary antibody is derived from. After 60 minutes blocking at room temperature, a 1:200 dilution of mouse-derived anti cleaved caspase-3 antibody (Cell Signal, Danvers, MA) was added to the coverslips. After 60 minutes of incubation, the coverslips were washed three times in PBS before being incubated in 1:200 anti-mouse FITC antibody (Jackson Immunoresearch, West Grove, PA) for 90 min. After three 5 minute washes with PBS, the coverslips were mounted to slides with anti-fade Vectashield (Vector Labs, Burlingame, CA) containing 4',6-diamidino-

2-phenylindole (DAPI). Slides were then viewed by a Leica DMI6000B fluorescence microscope and images were acquired by Image ProPlus (Media Cybernetics, Bethesda, MD).

TUNEL Staining

ARPE-19 cells were grown, and treated on sterile coverslips as described above. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining (Roche, Indianapolis, IN) was carried out according to manufacturers instructions. After treatment, the media was removed and the cells were washed with PBS before immediate addition of 4% paraformaldehyde in PBS for 60 minutes. After rinsing with PBS, the coverslips were then incubated in 0.1% Triton X-100 in 0.1% sodium citrate for 2 minutes at 4°C. Coverslips were rinsed twice with PBS and were then incubated with the TUNEL reaction mixture for 60 minutes at 37°C. After rinsing three times with PBS, the coverslips were mounted with Vectashield containing DAPI and visualized by fluorescence microscopy as previously mentioned. TUNEL image intensity was quantified using Cellprofiler, a free MATLAB based modular image analysis software available online (http://cellprofiler.org/download.htm) for download. In Cellprofiler, image analysis steps (modules) can easily be linked together to form a pipeline ideal for high throughput image analysis. Briefly, the corresponding DAPI images were analyzed and nuclei were identified and a mask containing nuclei outlines was applied to the TUNEL-FITC image. The pixel intensity within the applied nuclei outlines was then quantified and recorded. The results are reported as the intensity of the TUNEL staining as quantified by cell profiler in arbitrary units.

Western Blotting

ARPE-19, $MEF^{+/+}$ and $MEF^{-/-}$ cells were grown to confluency on 100mm plates before incubation with valproic acid. The cells were then enzymatically detached and pelleted after washing with PBS. The pellet was then lysed (0.1% dodecyl maltoside, 1X protease inhibitor cocktail (Roche)) for 60 minutes at 4°C on a tube rotator. The suspension was then spun at 13000 rpm for 10 min at 4°C and the resulting supernatant was mixed with an equal volume of 2X lamelli SDS-PAGE buffer (Invitrogen). Equal volumes were loaded onto a 4-20% Tris-HCl gem (Bio-Rad, Hercules, CA) and separated at 100V for 15 min followed by 150V for 60 min.

Proteins were transferred to a PVDF immobilon membrane (Millipore, Billerica, MA) for 60 min at 100V. The membrane was blocked for 45 min with a 1:1 mix of PBS and Licor blocking buffer (LICOR, Lincoln, NE). The membrane was then probed with a 1:1000 dilution of primary antibody (HSP-40, -70, -90, and HSF1, Stressgen, Ann Arbor, MI). Target protein was standardized against actin (Abcam, Cambride, MA) levels. After three 5 minute washes with PBS-T (Tween), the membrane was incubated in secondary antibody (1:5000, LICOR). After washing, the proteins were visualized and quantified by the LICOR Odyssey imaging system.

Fluid transport

Human fetal eyes (16-18 weeks gestation) were obtained from Advanced Bioscience Resources (Alameda, CA). Primary cell cultures of hfRPE cells were prepared from human fetal eyes and cultured as described previously. Cells for fluid transport experiments (J_V) were cultured in transwells to confluence for 3 – 4 weeks for pigmentation and total tissue resistance (R_T) ~ 400 $\Omega \cdot cm^2$. For J_V measurements, a modified Üssing chamber was used to mount confluent monolayers of hfRPE using MEM-alpha (Sigma M4526) media and a capacitance probe technique as previously described. A nylon mesh supporting hfRPE was placed in a Kel-F chip in the fluid transport apparatus, and a capacitance probe measured changes in liquid level height as water moved across the RPE. Transepithelial potential (TEP) was measured using Ag/AgCl pellet electrodes. Total tissue resistance (R_T) was used to determine tissue viability. Fast changes in J_V, TEP and R_T were unable to be recorded since the solution composition changes in this chamber were relatively slow (≈1-2 chamber volumes per min). In these experiments, J_V, TEP, and R_T were recorded for 20 to 30 minutes before addition of valproic acid (10μM, 100 M, 250 μM, 500 μM). Valproic acid was perfused into both apical and basal bathing solutions of the hfRPE in untreated (control) transwells or in 24 hour valproic acid pre-treated (1 μM and 5 μM) transwells and the recordings continued for another 30-60 minutes until steady state J_V was measured. Valpromide (100 μM, 400 μM, 800 μM) was perfused into both apical and basal solutions of the hfRPE and J_V, TEP and R_T were measured as a control.

Statistics

Differences between damaged and drug-treated samples for the viability, and annexin-V assays were analyzed by two-way ANOVA, followed by Tukey's correction test when appropriate. A one-way ANOVA with Tukey's comparison was used to assess the TUNEL image intensity values. The Boniferroni correction was used to compare western samples. Statistical significance was set at $p<0.05$, and all statistical analysis was conducted with GraphPad Prism 5 (San Diego, California) software. Data are shown as mean ± SEM.

Human age-related macular degeneration studies

The appropriate Institutional Review Board approved the retrospective chart review for this study. Most patients (11) were treated at the Department of Ophthalmology, University of Florida–Gainesville from December 2007 to December 2008. The remaining four patients were treated at the Department of Ophthalmology, University of Massachusetts Medical School in Worcester, Massachusetts.

This analysis included males and females over 50 years old with ARMD in at least one eye. Patients with any condition prohibiting serial observation, photography and OCT analysis of their retina were excluded. Patients with macular or choroidal disorders other than ARMD were excluded. Additionally, patients with uncontrolled or advanced glaucoma as defined by intraocular pressure (IOP) greater than 25 mmHg or cup-to-disc ratios of 0.8 or greater were excluded. Patients with a history of vitreo-retinal surgery within three months prior to their baseline visit were also excluded.

Wet ARMD patients were included whose visual acuity did not improve after a minimum of six months of treatment with serial intravitreal injections of bevacizumab or ranibizumab. Six weeks of uninterrupted VPA treatment was required for inclusion in this analysis. Tweny-five patients with ARMD in both eyes received a prescription for off-label VPA, 15 of whom met the inclusion and exclusion criteria and were included in the subsequent analysis. The study cohort included six males and nine females with a mean age of 78.4 (± 9) years. Eight patients presented with dry ARMD in one eye and wet ARMD in the other eye. One patient had wet ARMD in both eyes. The remaining six patients had dry ARMD in one or both eyes. Visual acuity data from 28 eyes were available and included 18 eyes with dry ARMD and 10 eyes with wet ARMD (Table 2). Among the dry ARMD

eyes, three eyes presented with the geographic atrophy form of ARMD and were kept in the analysis.

Fourteen human subjects with dry (18 eyes) and wet (10 eyes) ARMD were treated with VPA. VPA dosage was 500 or 750 mg/day, a lower dose compared to that typically prescribed as a maintenance dose for epilepsy, which is the primary indication for VPA. All patients began on a dose of 500 mg/day. For eight patients who tolerated the 500 mg/day dose well, the daily dose was increased to 750 mg/day. Length of treatment varied from eight to 39 weeks (mean = 22.7 ± 11.8). The following data were extracted: patient demographics, diagnosis, BCVA, reported systemic side effects and subjective improvement of vision at follow-up. Hepatotoxicity was monitored with serum liver function tests.

Treatment efficacy was assessed by change in best-corrected visual acuity (BCVA) and improvement in central retinal thickness measured by optical coherence tomography (OCT). Additionally, subjective change in visual perception after treatment was assessed. BCVA was measured using a Snellen chart at 20 feet. Values were converted to a logarithm of minimum angle of resolution (logMAR) score for statistical analysis. A decrease in logMAR score is indicative of improved visual acuity. To detect dose-dependent changes in BCVA, patients were stratified by daily dose of VPA: those taking 500 mg/day and those taking 750 mg/day. All but one of the nine patients with wet AMD in at least one eye had OCT performed at baseline and at follow-up. Different OCT instruments were used at the two centers: Stratus OCT (Carl Zeiss Meditec, Dublin, CA) in Gainesville, FL and Spectralis OCT (Heidelberg Engineering, Vista, CA) in Worcester, MA. Because of the different axial resolutions of the two instruments used, criteria for a significant decrease/increase in retinal thickness were set at 10 μm for the Stratus OCT and 4 μm for the Spectralis OCT. Comparable regions of the central retina were analyzed at baseline and follow-up.

To compare visual acuity change to the natural history of the disease, data from two studies were used. For dry ARMD, a study by Cangemi et al., 200716 was used. For wet ARMD, a meta-analysis of several recent studies by Wong et al., 2007 was used.17 In both cases, the visual loss at 5.5 months was extrapolated from published values at six months. Additionally, when combined BCVA results from dry and wet ARMD eyes were compared to natural history data, a weighted average was

used. That is, expected 5.5-month values from the natural history data were weighted according to the proportion of dry versus wet patients in our sample.

Human retinitis pigmentosa studies

This study is a retrospective chart review of patients with RP who were treated off label with VPA at the University of Florida Ophthalmology Department clinic between December 2007 and January 2009. This analysis was approved by the appropriate Institutional Review Board. Fourteen RP patients were identified; of these seven had adequate baseline and follow-up visual fields. The visual fields from one eye of subject 5 were excluded due to poor quality. The length of treatment included in this analysis was based on the data available in the record and varied from 2 to 6 months. The dosage of VPA varied from 500 to 750 mg a day, which is lower than the dosage typically used for anti-convulsant therapy. A daily dosage of 500 mg was originally chosen as it is approximately half the dosage prescribed for other indications; as this was well tolerated, several patients were prescribed 750 mg. Patient demographics, diagnosis, family history, genotype, best corrected visual acuity (BCVA - which was converted to the logarithm of minimal angle of resolution (logMAR)), dosage of VPA, length of treatment, blood chemistries including ALT, AST, ammonia, electrolyte and blood cell panels including Na, K, Cl, bicarbonate, creatinine, white blood cells with differential, red blood cells and platelets) and reported side effects were all recorded.

For each patient, intact baseline and follow-up visual field areas were defined using the existing Goldmann Kinetic Perimetry tracings (isopter V4e) from each eye. The tracings were digitized and the corresponding areas of functioning retina (in mm 2) were calculated based on the method used by Dagnelie (Dagnelie G. Conversion of Planimetric Visual-Field Data into Solid Angles and Retinal Areas. Clinical Vision Sciences. 1990;5:95-100).

The change in visual field (mm^2) was defined as a simple measure of percent change from baseline:

Percent change from baseline = 100 x ((Follow-up mm^2 – Baseline mm^2)/Baseline mm^2)

Improvement in visual field (VF) was defined as a greater than 2% increase, a loss in VF area was defined as greater than a 2% decrease, while VF was considered unchanged if the follow-up value was within 2% of baseline.

Visual field loss in RP does not occur at a linear rate, and Massoff et al demonstrated it may decline exponentially, with an estimated loss of about 0.10 $\log_e$ VF areas (mm^2 units per year (16). VF areas (mm^2) were $\log_e$ transformed (logVF) and the difference between follow-up and baseline was calculated (ΔlogVF). To calculate average percent change in VF over the course of treatment the average difference across all eyes was calculated and converted to area (mm^2). This value was used as the follow-up value in the above formula, and the baseline value used was 314 mm^2 (which was the average baseline from all subjects).

Using varying estimates from the literature regarding the natural history of visual field loss in RP patients (Dagnelie et al., Clin Vision Sci. 1990;5:1-26; Merin et al., Ocul Pharmacol Ther. 2008;24:80-6; Berson et al., Invest Ophthalmol Vis Sci. 2002;43:3027-36), it was hypothesized that patients without treatment would lose either 0.0, 0.011 or 0.033 logVF over the average length of treatment (4 months). Data was not assumed to be distributed normally and significance levels were calculated using the Wilcoxon signed rank test. Statistical analysis was performed using Graph Pad Prism (La Jolla, CA).

Visual acuity was measured using a Snellen chart at a distance of twenty feet. Values were converted to a logarithm of minimum angle of resolution (logMAR) score for statistical analysis. Significance levels were calculated using the Wilcoxon signed rank test assuming that visual acuity would not change without treatment.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes

that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A method for preserving or enhancing visual function, the method comprising contacting an ocular cell with an effective amount of valproic acid, valpromide, or an analog or derivative thereof, thereby preserving or enhancing visual function.

2. A method for increasing ocular cell survival or reducing ocular cell death, the method comprising contacting an ocular cell at risk of cell death with an effective amount of valproic acid, valpromide, or an analog or derivative thereof, thereby increasing ocular cell survival or reducing ocular cell death.

3. The method of claim 2, wherein the method reduces retinal pigment epithelial cell apoptosis.

4. A method for increasing retinal regeneration, the method comprising contacting an ocular cell with an effective amount of valproic acid, valpromide, or an analog or derivative thereof, thereby increasing retinal regeneration.

5. The method of claim 4, wherein the method increases the number of retinal pigment epithelial cells or increases retinal thickness.

6. The method of any of claims 1-4, wherein the ocular cell is a retinal pigment epithelial cell.

7. A method for treating or preventing ocular cell death in a subject in need thereof, the method comprising administering an effective amount of valproic acid, valpromide, or lithium to the subject.

8. The method of claim 7, wherein the ocular cell death is retinal pigment epithelial cell death.

9. The method of claim 2 or 7, wherein the ocular cell death is associated with a disease selected from the group consisting of retinitis pigmentosa, age-related macular

degeneration, glaucoma, corneal dystrophi
autosomal dominant druzen, and Best's macular dystrophy.

10. The method of claim 9, wherein the disease is the wet or dry form of age-related macular degeneration.

11. The method of claim 1, wherein the subject comprises a mutation that affects opsin folding.

12. The method of claim 5, wherein the opsin comprises a P23H mutation.

13. The method of any of claims 1-7, wherein the method further comprises administering lithium to an ocular cell or subject.

14. The method of claim 13, wherein the method comprises administering valproic acid and lithium or valpromide and lithium.

15. The method of claim 14, wherein valproic acid or valpromide and lithium are administered within ten days of each other.

16. The method of claim 14, wherein the valproic acid or valpromide and lithium are administered within five days of each other.

17. The method of claim 14, wherein the valproic acid or valpromide and lithium are administered within twenty-four hours of each other.

18. The method of claim 14, wherein the valproic acid or valpromide and lithium are administered simultaneously.

19. The method of claim 7, wherein the valproic acid, valpromide, and/or lithium are administered systemically or administered directly to the eye.

20. The method of claim 19, wherein the administration is ocular, intra-ocular, or tc

21. The method of claim 13, wherein the topical administration is by drop form to the surface of the eye.

22. The method of claim 7, wherein the valproic acid, valpromide, and/or lithium are each incorporated into a composition that provides for their long-term release.

23. The method of claim 7, further comprising identifying the subject as having or having a propensity to develop ocular cell death, a disease associated with retinal pigmented epithelial cell death, retinitis pigmentosa, or the wet or dry form of age-related macular degeneration.

24. A method for treating or preventing the wet or dry form of age-related macular degeneration in a subject, the method comprising administering an effective amount of valproic acid or a derivative thereof to said subject.

25. A method for treating or preventing retinitis pigmentosa in a subject, the method comprising administering an effective amount of valproic acid or a derivative thereof to said subject.

26. The method of claim 24 or 25, wherein the effective amount is between 250 mg/day and 3000 mg/day.

27. The method of claim 24 or 25, wherein the effective amount is 500 mg/day, 750 mg/day, 1000 mg/day, 1500 mg/day, 2000 mg/day, 2500 mg/day, or 3000 mg/day.

28. The method of claim 24 or 25, wherein the method increases best-corrected visual acuity, increases visual field, increases central retinal thickness, or increases subjective visual perception.

29. The method of claim 24 or 25, wherein valproic acid is administered orally, ocularly, topically, or intra-ocularly.

30. The method of claim 29, wherein t
administered topically by drop form to the surface of the eye.

31. The method of claim 24 or 25, wherein valproic acid is administered for at least 1-12 months.

32. A pharmaceutical composition for treating or preventing ocular cell death in a subject in need thereof, the composition comprising an effective amount of valproic acid, valpromide, or lithium and a pharmaceutically acceptable excipient formulated for ocular delivery.

33. The pharmaceutical composition of claim 32, wherein the composition is labeled for the treatment of a disease selected from the group consisting of age-related macular degeneration, retinitis pigmentosa, glaucoma, coreal systrophy, retinoschises, Stargardt's disease, autosomal dominant druzen, or Best's macular dystrophy.

34. The pharmaceutical composition of claim 33, wherein the ocular cell is a retinal pigment epithelial cell.

35. A pharmaceutical composition for treating or preventing age-related macular degeneration or retinitis pigmentosa in a subject in need thereof, the composition comprising between 500 and 750 mg of valproic acid or a derivative thereof and a pharmaceutically acceptable excipient.

36. A kit for the treatment or prevention of ocular cell death, the kit comprising an effective amount of valproic acid, valpromide, and/or lithium and instructions for the use of the kit in the method of any of claims 1-31.

37. The kit of claim 36, wherein the kit comprises valproic acid and lithium or valpromide and lithium.

ARPE-19 cell viability with 24hr pretreatment and 48hr treatment with HQ

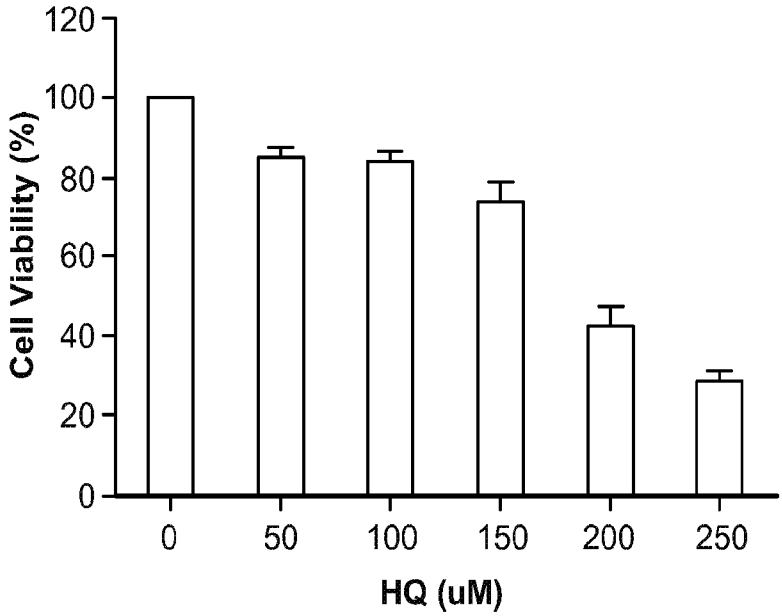


FIG. 1A

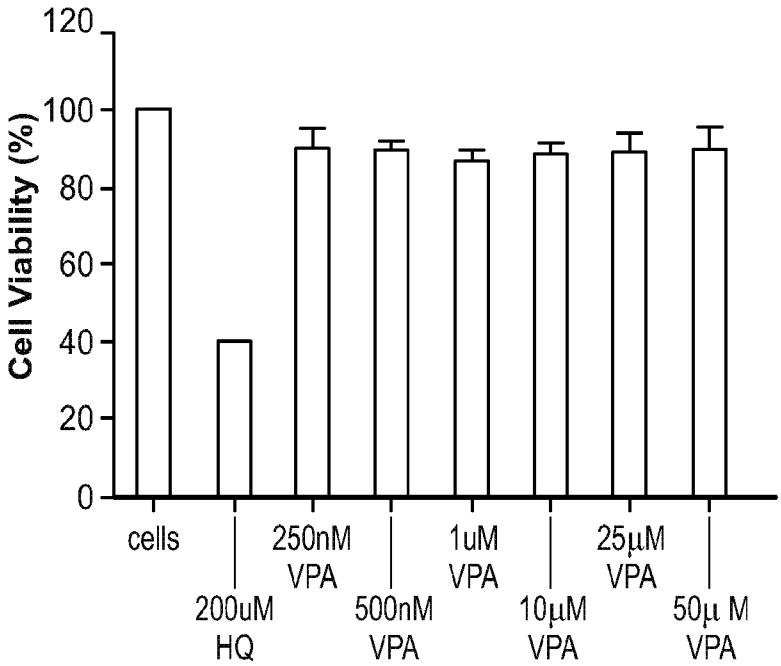


FIG. 1B

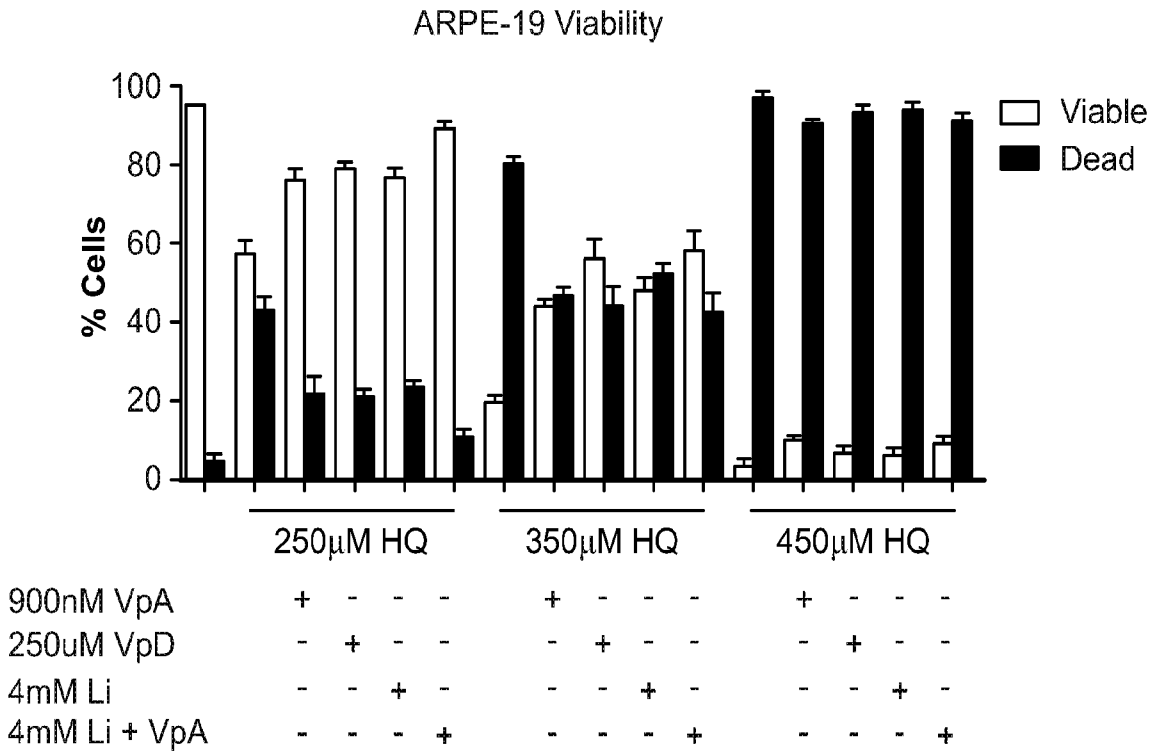

ARPE-19 Viability
% Cells
100
80
60
40
20
0
Viable
Dead
250μM HQ
350μM HQ
450μM HQ
900nM VpA
250uM VpD
4mM Li
4mM Li + VpA

FIG. 1C

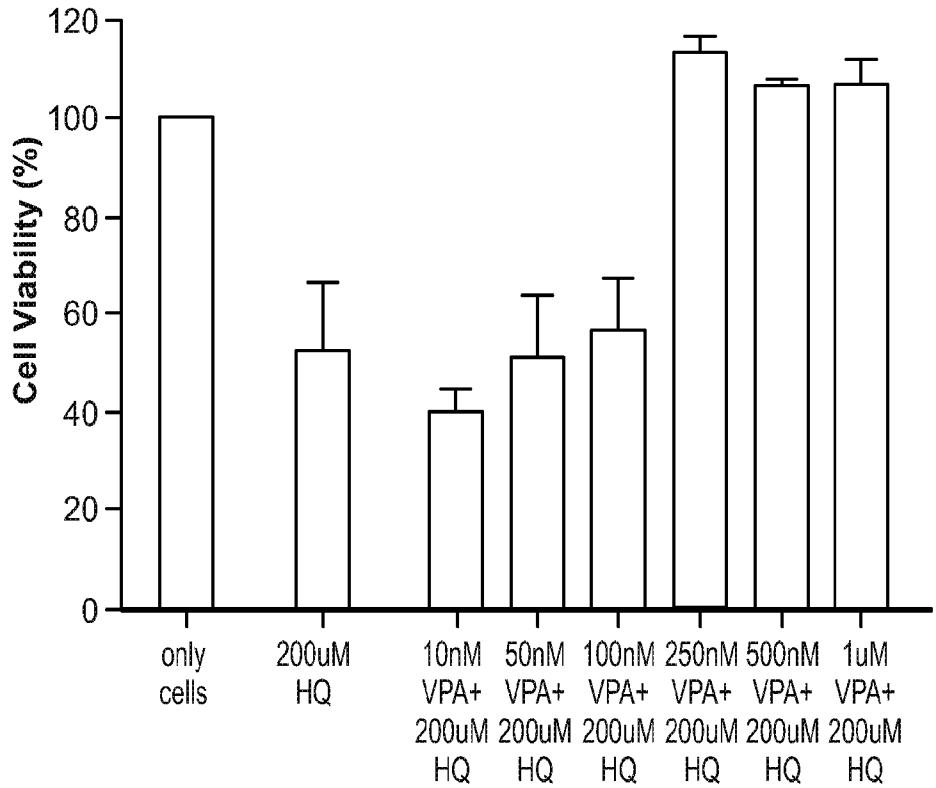

Cell Viability (%)
120
100
80
60
40
20
0
only cells
200uM HQ
10nM VPA+ 200uM HQ
50nM VPA+ 200uM HQ
100nM VPA+ 200uM HQ
250nM VPA+ 200uM HQ
500nM VPA+ 200uM HQ
1uM VPA+ 200uM HQ

FIG. 1D

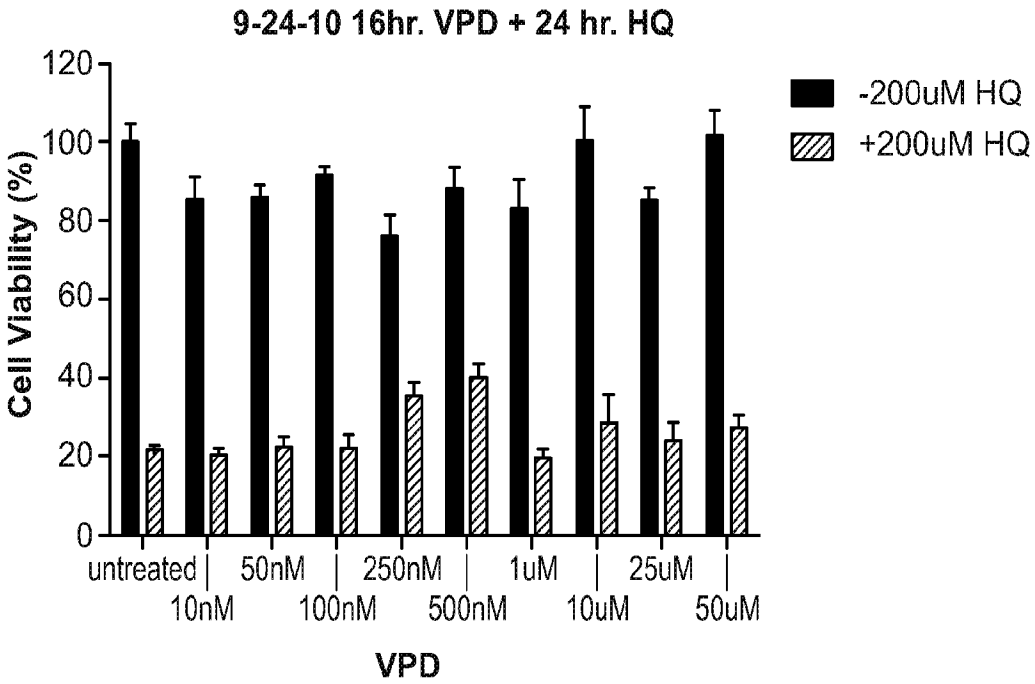

9-24-10 16hr. VPD + 24 hr. HQ
Cell Viability (%)
120
100
80
60
40
20
0
-200uM HQ
+200uM HQ
untreated
10nM
50nM
100nM
250nM
500nM
1uM
10uM
25uM
50uM
VPD

FIG. 1E

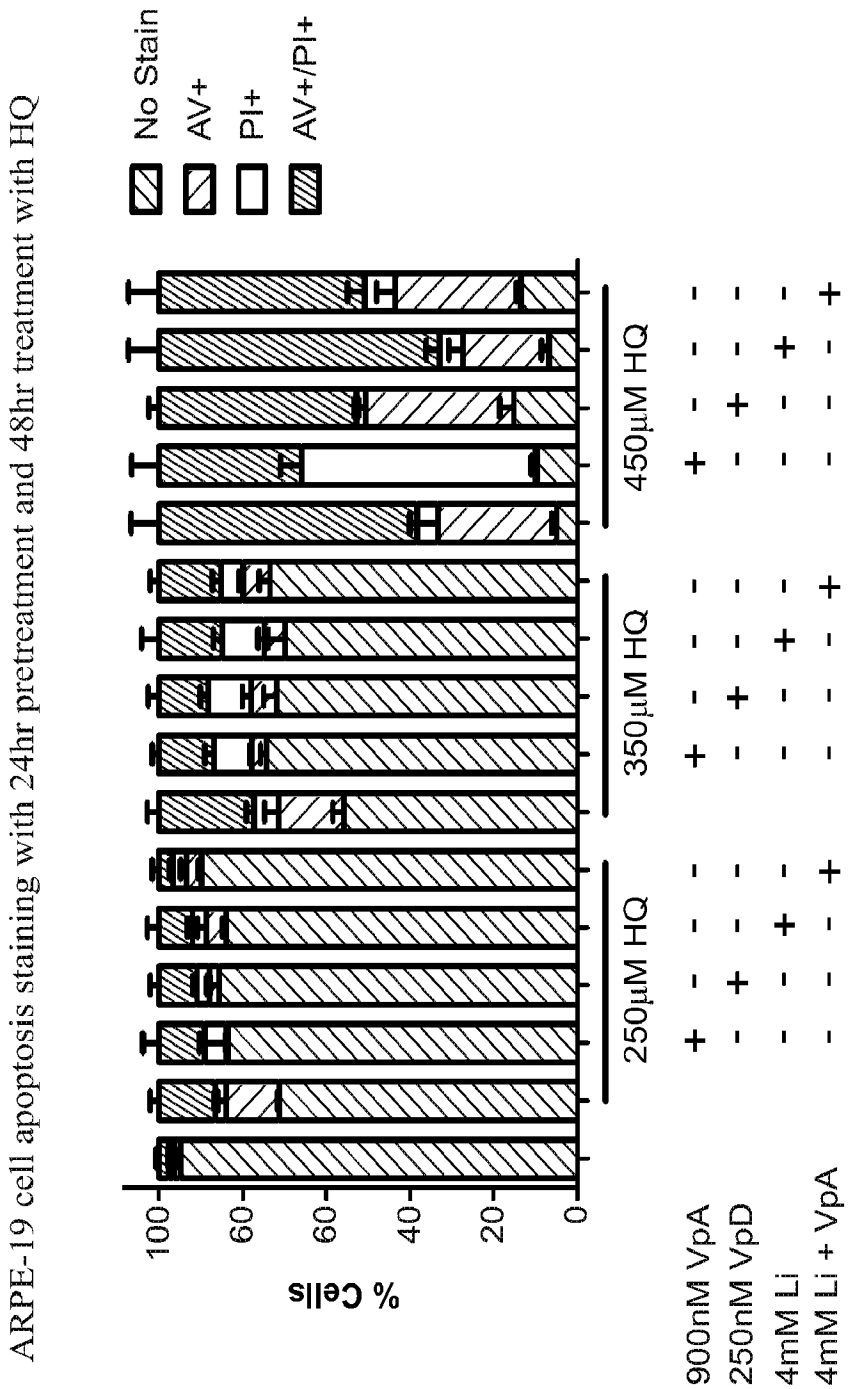

ARPE-19 cell apoptosis staining with 24hr pretreatment and 48hr treatment with HQ
No Stain
AV+
PI+
AV+/PI+
% Cells
100
60
60
40
20
0
250μM HQ
350μM HQ
450μM HQ
900nM VpA
250nM VpD
4mM Li
4mM Li + VpA

FIG. 2

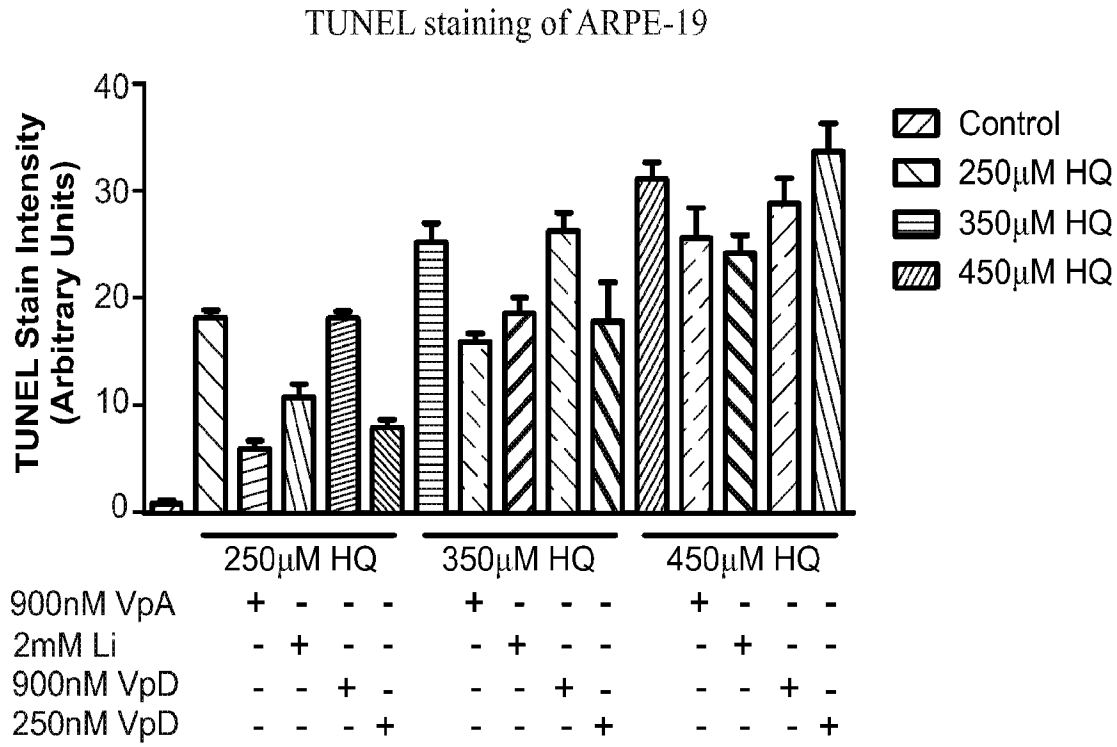

TUNEL staining of ARPE-19
TUNEL Stain Intensity (Arbitrary Units)
40
30
20
10
0
Control
250μM HQ
350μM HQ
450μM HQ
250μM HQ
350μM HQ
450μM HQ
900nM VpA + - - - + - - - + - - -
2mM Li - + - - - + - - - + - -
900nM VpD - - + - - - + - - - + -
250nM VpD - - - + - - - + - - - +

FIG. 3

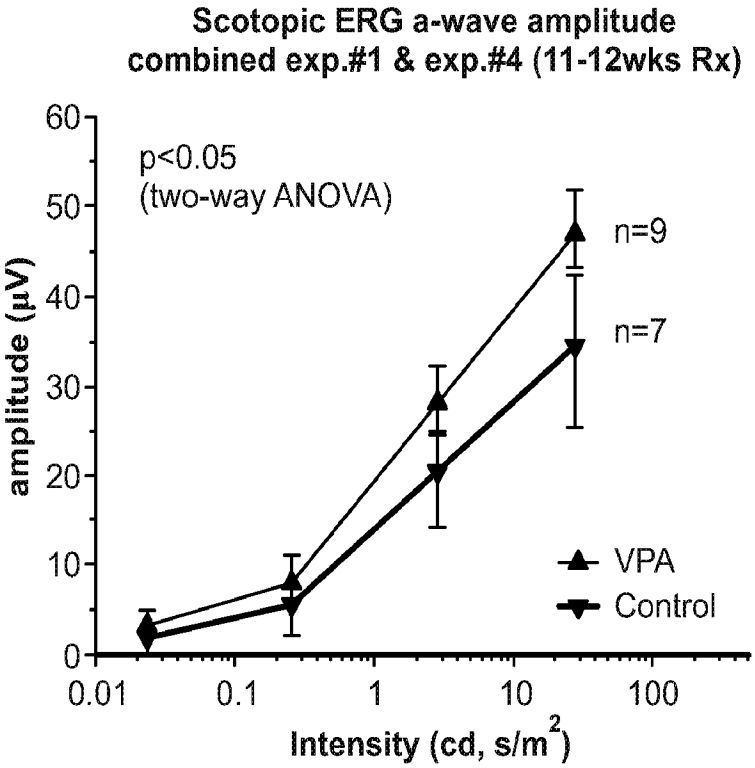

Scotopic ERG a-wave amplitude
combined exp.#1 & exp.#4 (11-12wks Rx)
p<0.05
(two-way ANOVA)
n=9
n=7
VPA
Control
amplitude (μV)
Intensity (cd, s/m2)
0
10
20
30
40
50
60
0.01
0.1
1
10
100

FIG. 4A

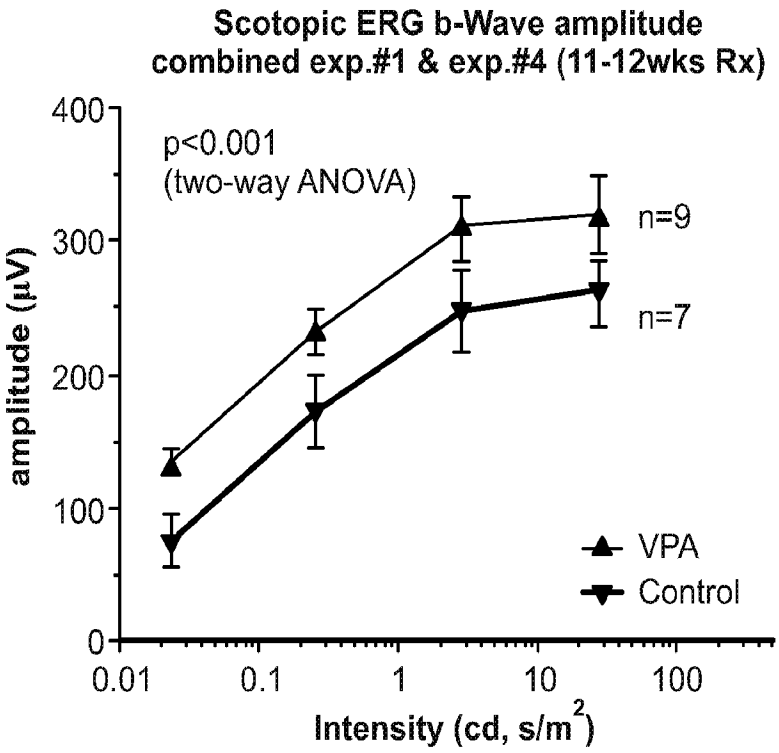

Scotopic ERG b-Wave amplitude
combined exp.#1 & exp.#4 (11-12wks Rx)
p<0.001
(two-way ANOVA)
n=9
n=7
VPA
Control
amplitude (μV)
Intensity (cd, s/m2)
0
100
200
300
400
0.01
0.1
1
10
100

FIG. 4B

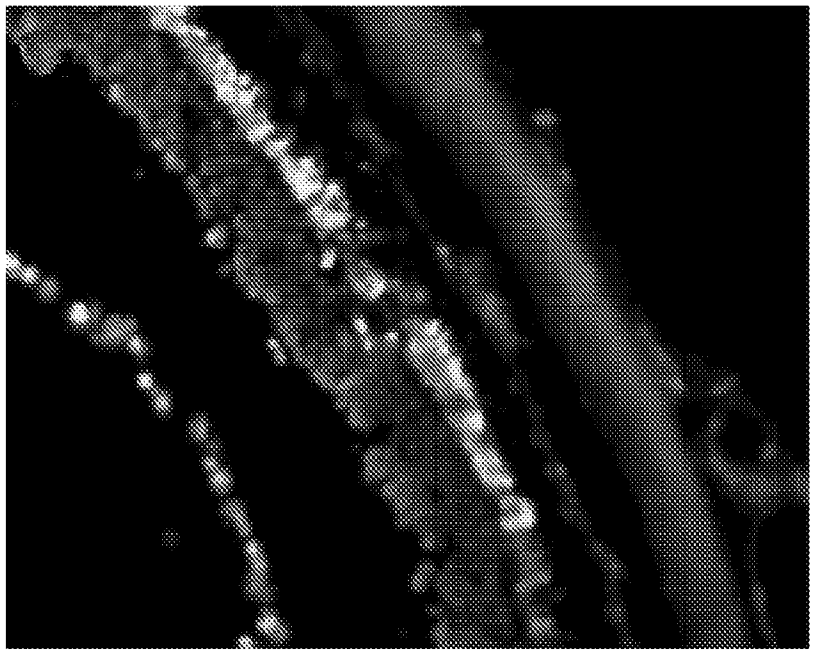


FIG. 4C

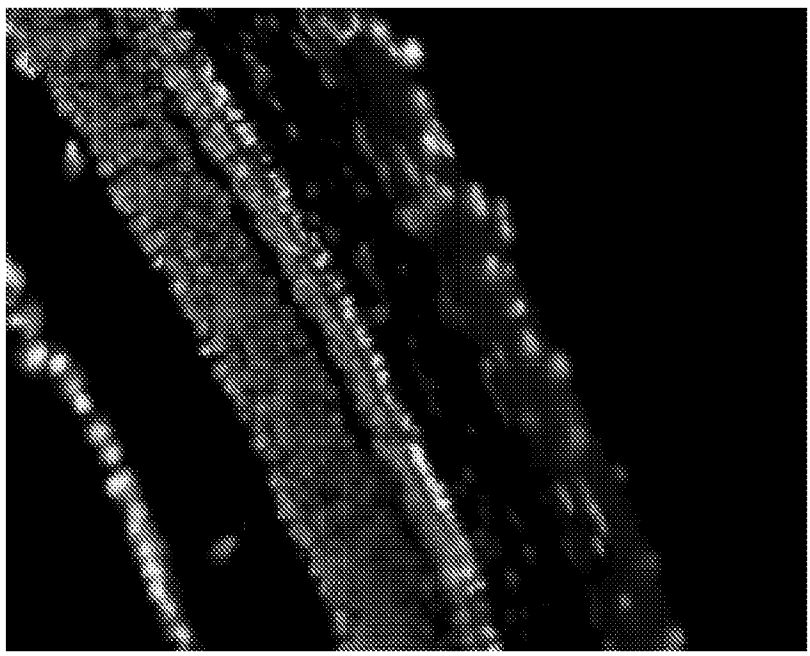


FIG. 4D

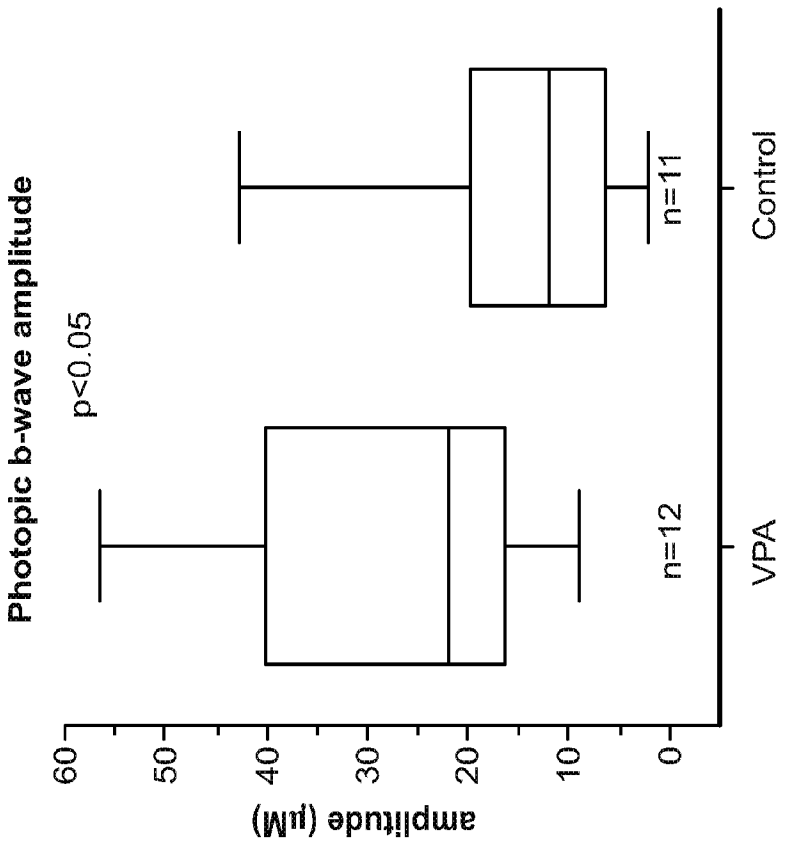

Photopic b-wave amplitude
p<0.05
amplitude (μM)
60
50
40
30
20
10
0
n=12
n=11
VPA
Control

FIG. 5B

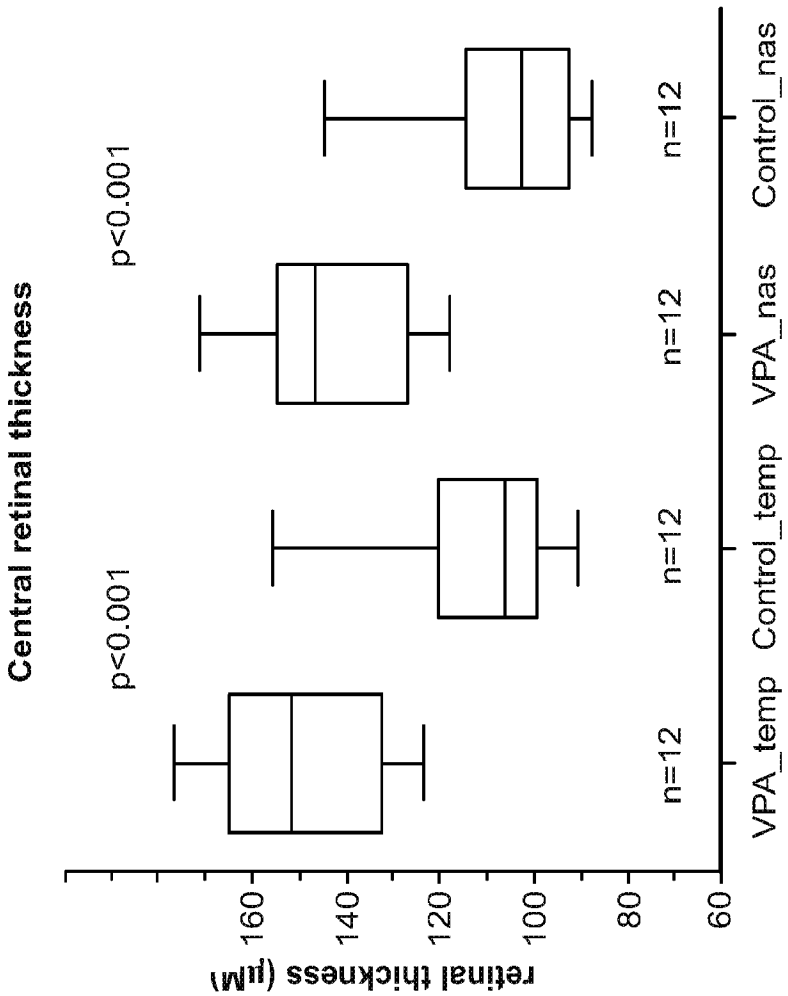

Central retinal thickness
p<0.001
p<0.001
retinal thickness (μM)
160
140
120
100
80
60
n=12
n=12
n=12
n=12
VPA_temp
Control_temp
VPA_nas
Control_nas

FIG. 5A

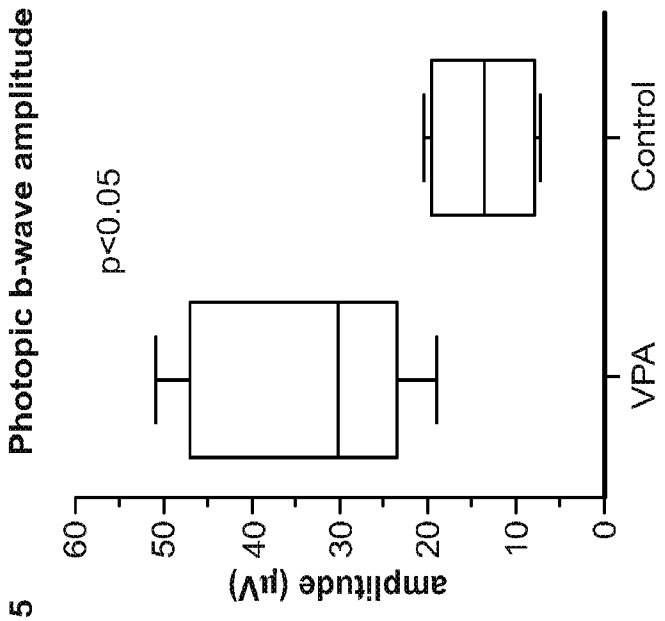


FIG. 6C

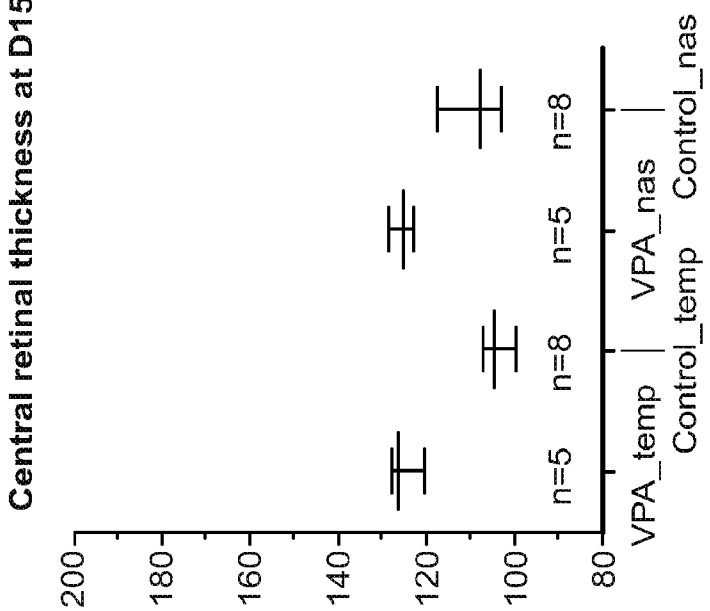


FIG. 6B

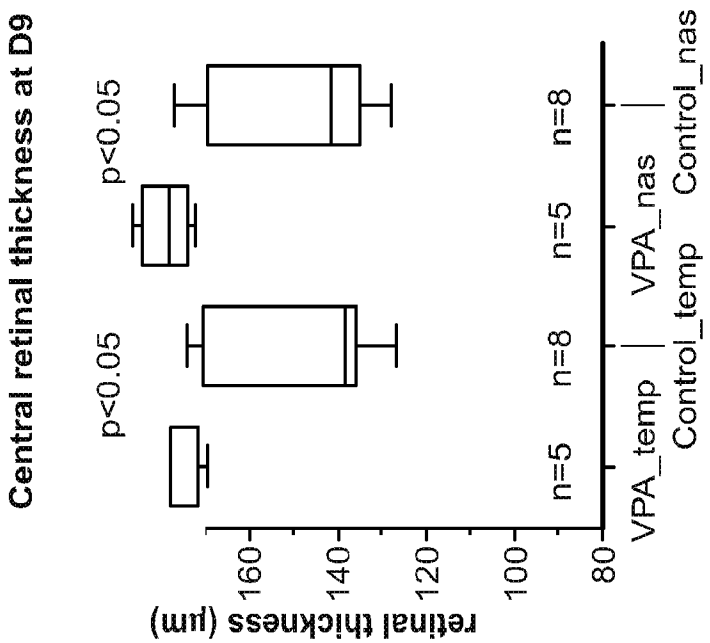


FIG. 6A

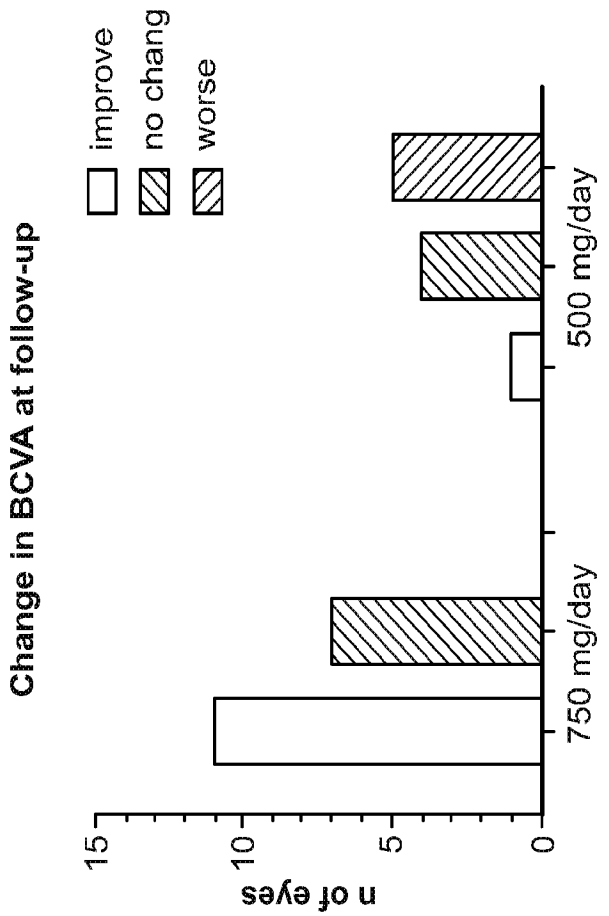

Change in BCVA at follow-up
improve
no chang
worse
n of eyes
15
10
5
0
750 mg/day
500 mg/day

FIG. 7B

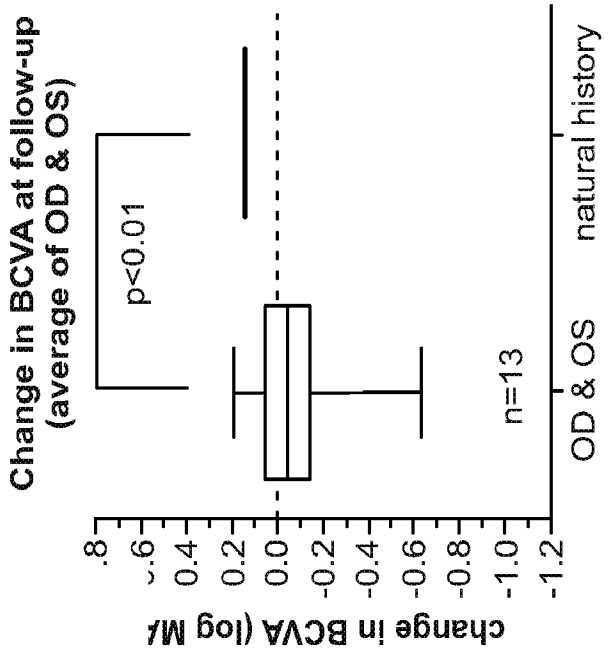

Change in BCVA at follow-up
(average of OD & OS)
change in BCVA (log MA
.8
.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
p<0.01
n=13
OD & OS
natural history

FIG. 7A

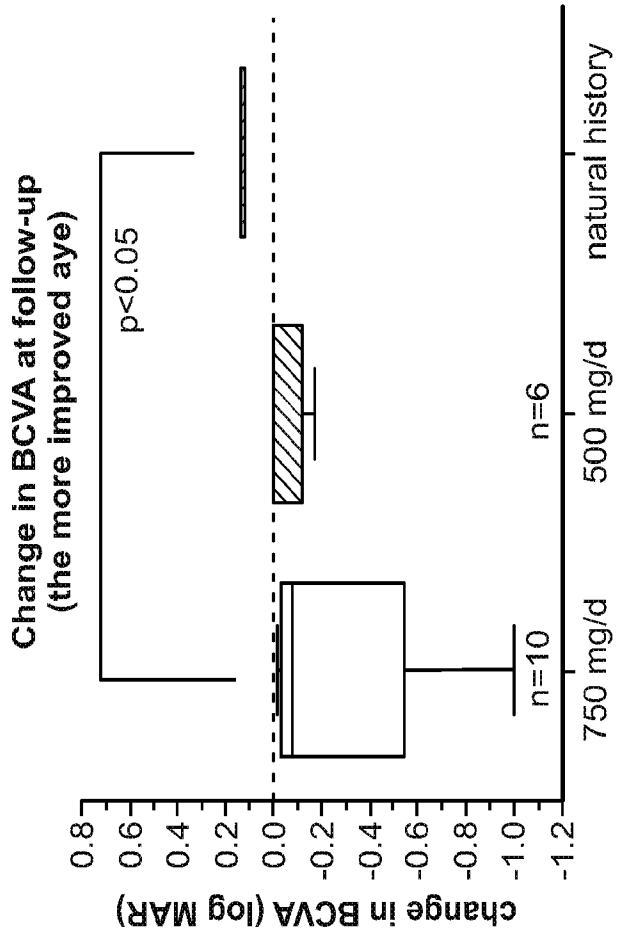

Change in BCVA at follow-up
(the more improved aye)
p<0.05
n=10
n=6
750 mg/d
500 mg/d
natural history
change in BCVA (log MAR)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2

FIG. 7D

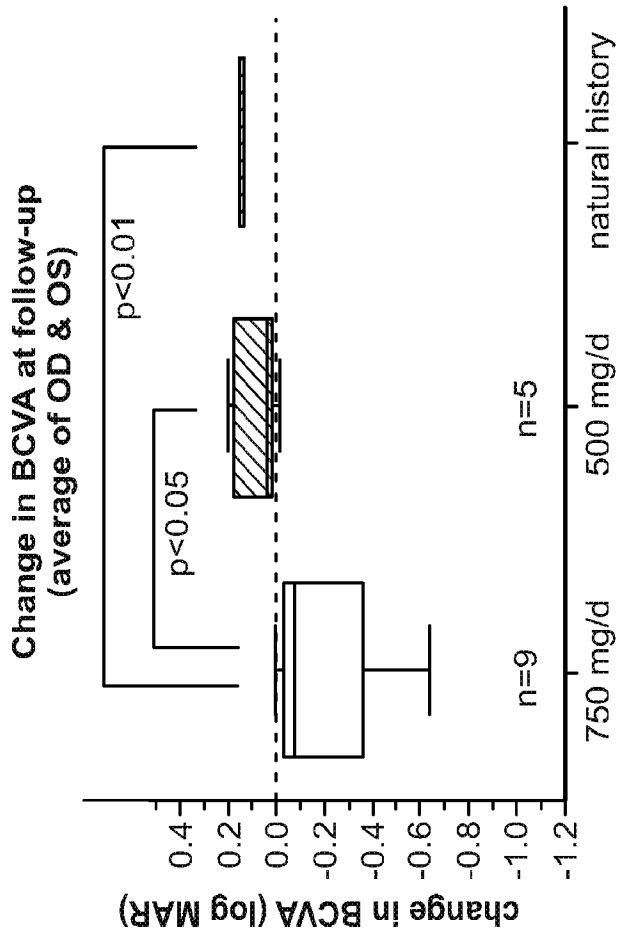

Change in BCVA at follow-up
(average of OD & OS)
p<0.01
p<0.05
n=9
n=5
750 mg/d
500 mg/d
natural history
change in BCVA (log MAR)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2

FIG. 7C

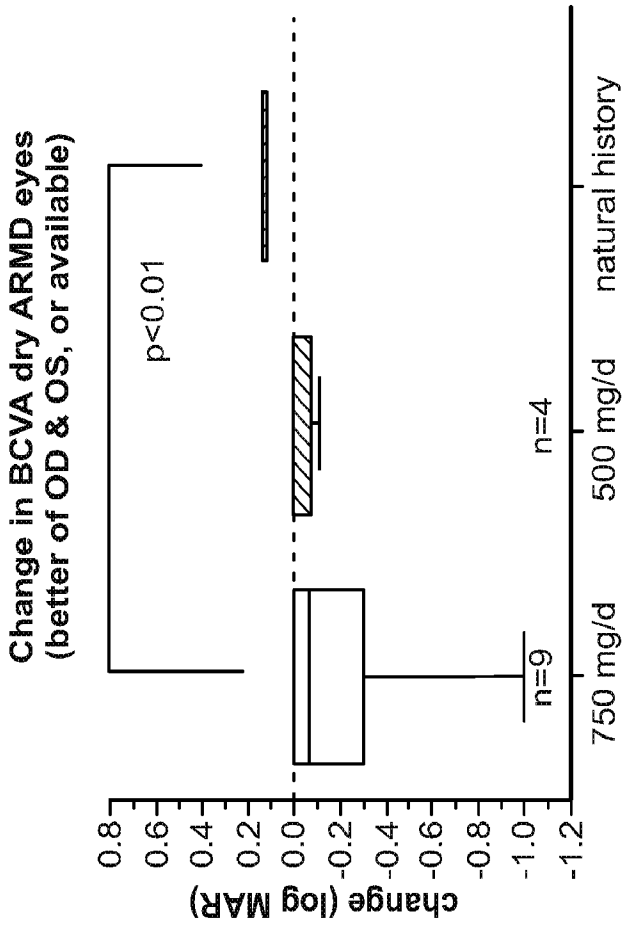

Change in BCVA dry ARMD eyes
(better of OD & OS, or available)
p<0.01
n=9
n=4
750 mg/d
500 mg/d
natural history
change (log MAR)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2

FIG. 8B

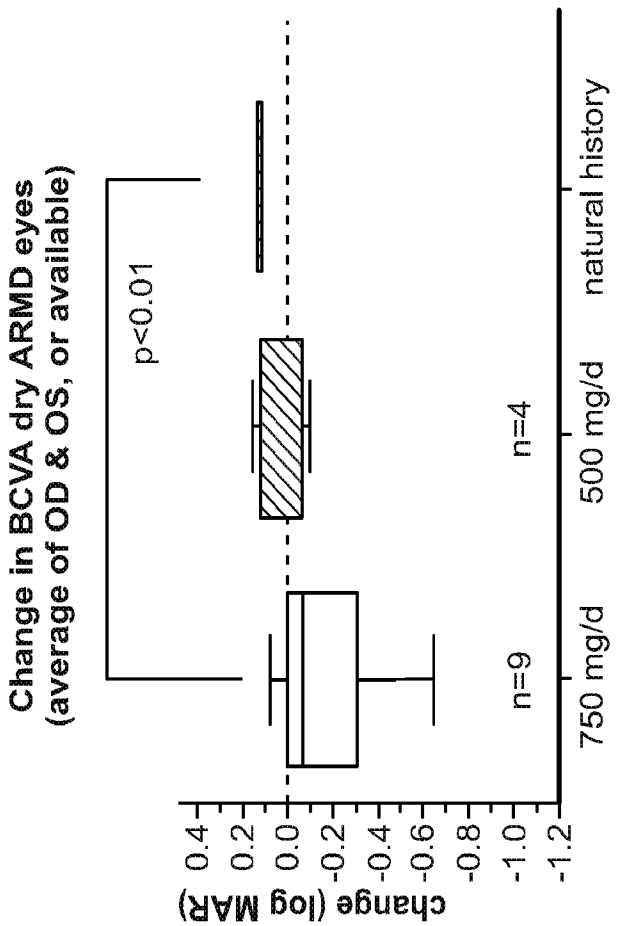

Change in BCVA dry ARMD eyes
(average of OD & OS, or available)
p<0.01
n=9
n=4
750 mg/d
500 mg/d
natural history
change (log MAR)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2

FIG. 8A

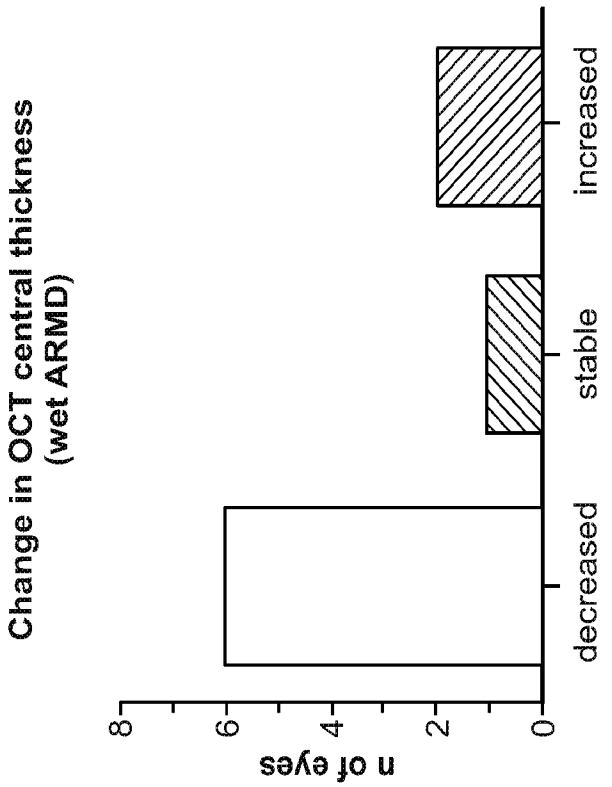

Change in OCT central thickness
(wet ARMD)
n of eyes
0
2
4
6
8
decreased
stable
increased

FIG. 9B

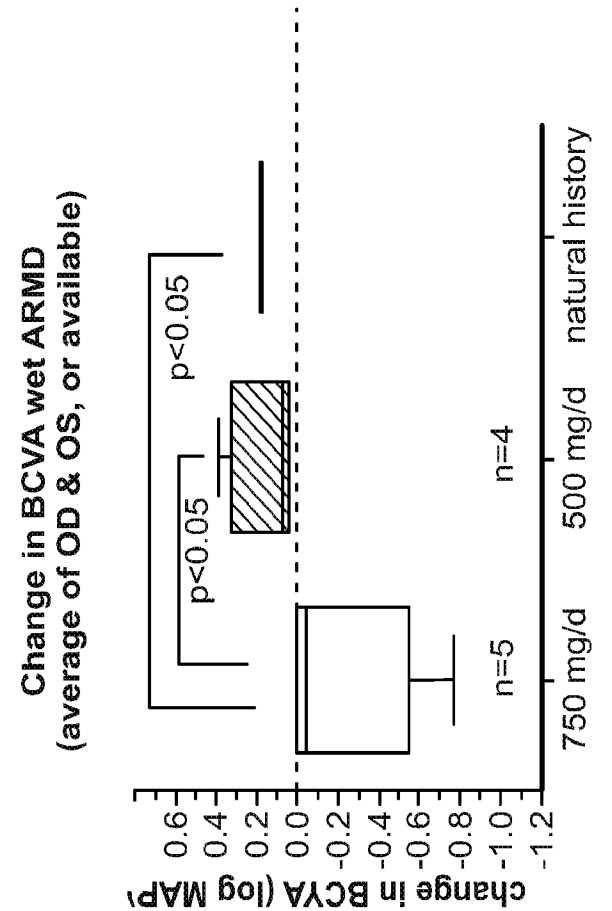
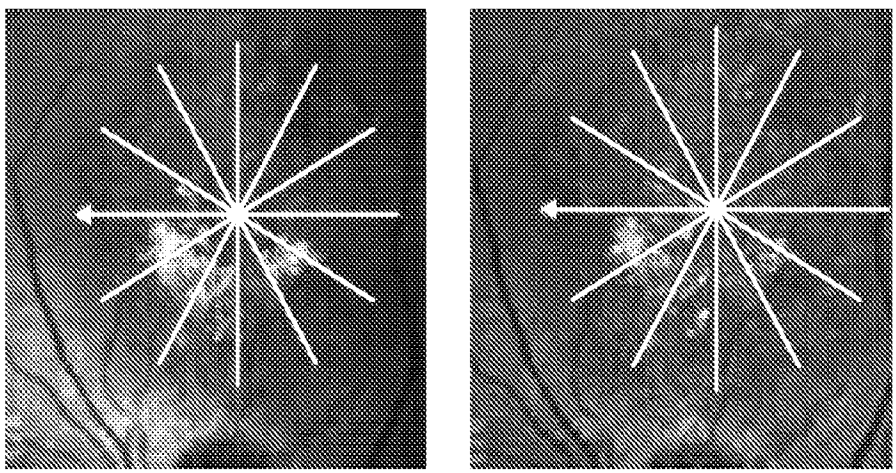

Change in BCVA wet ARMD
(average of OD & OS, or available)
change in BCVA (log MAR)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
p<0.05
p<0.05
n=5
n=4
750 mg/d
500 mg/d
natural history

FIG. 9A



FIG. 9C



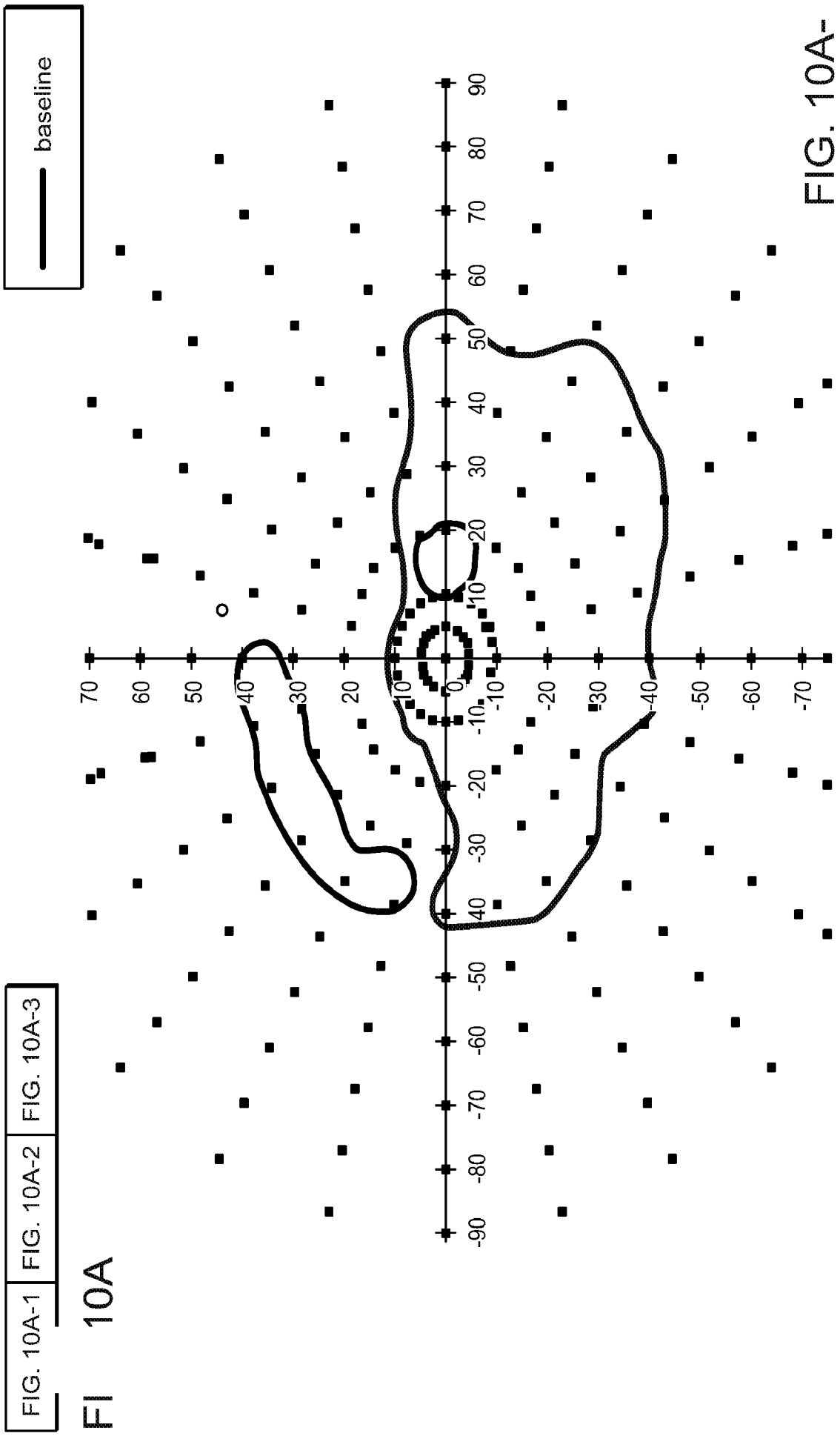


FIG. 10A-

FIG. 10A

FIG. 10A-1 | FIG. 10A-2 | FIG. 10A-3

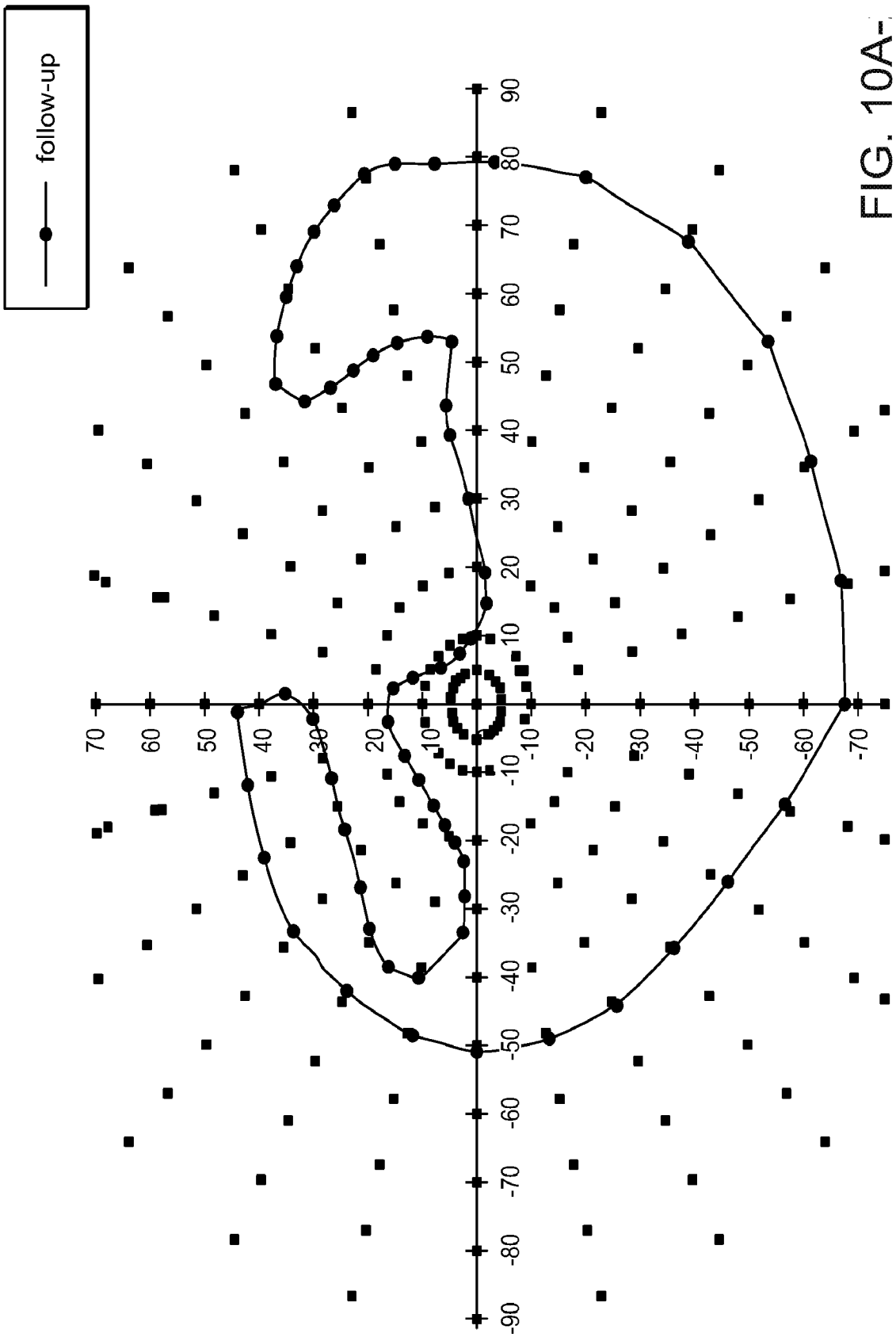

follow-up
90
80
70
60
50
40
30
20
10
-10
-20
-30
-40
-50
-60
-70
-80
-90
70
60
50
40
30
20
10
-10
-20
-30
-40
-50
-60
-70

FIG. 10A-

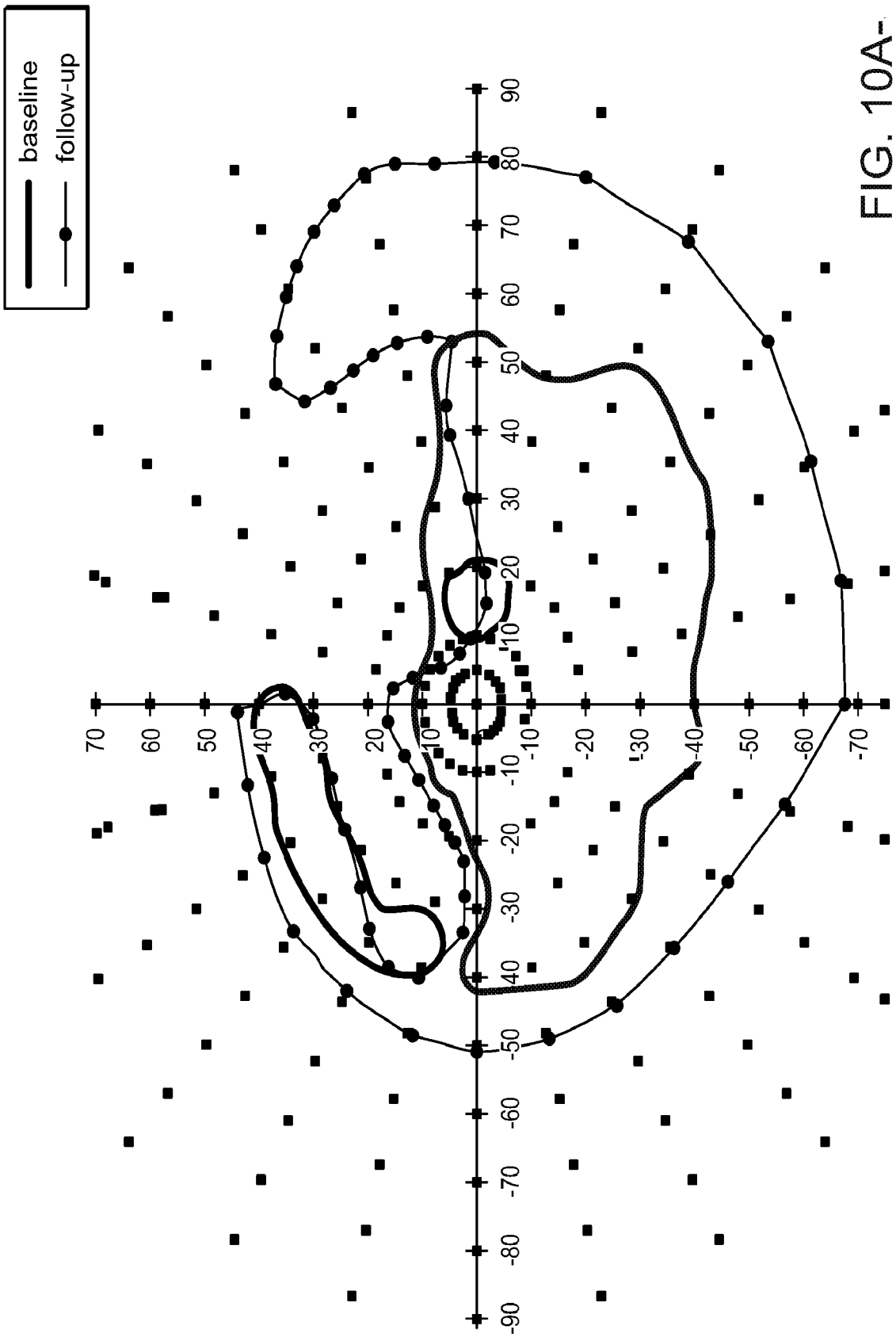
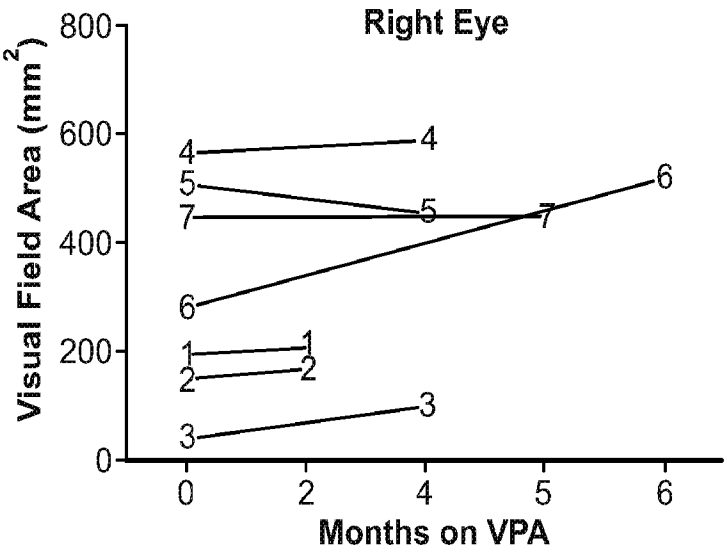
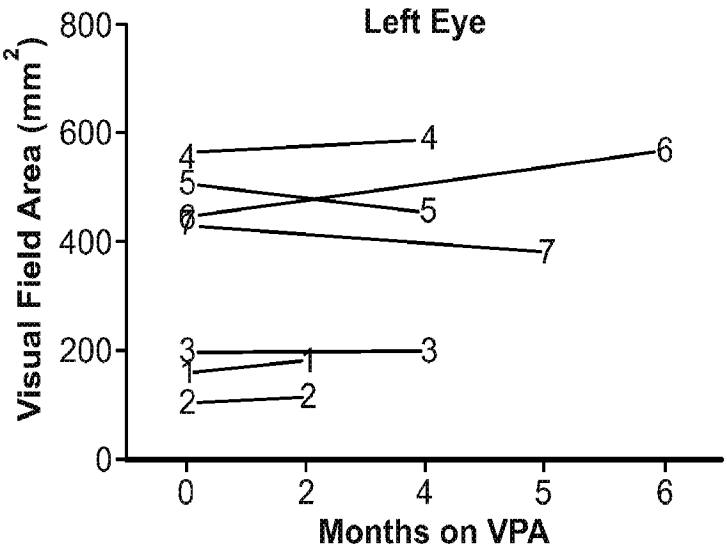
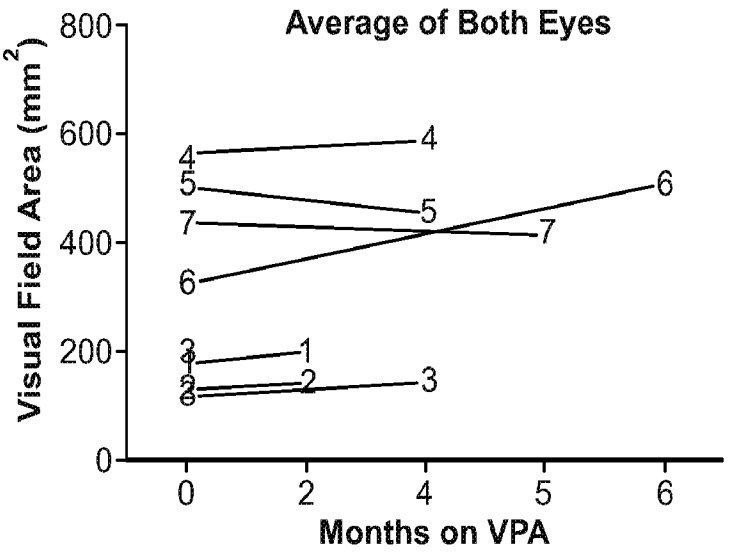

baseline
follow-up
90
80
70
60
50
40
30
20
10
-10
-20
-30
-40
-50
-60
-70
-80
-90
70
60
50
40
30
20
10
-10
-20
-30
-40
-50
-60
-70

FIG. 10A-


Right Eye
Visual Field Area (mm2)
800
600
400
200
0
0
2
4
5
6
Months on VPA


Left Eye
Visual Field Area (mm2)
800
600
400
200
0
0
2
4
5
6
Months on VPA


Average of Both Eyes
Visual Field Area (mm2)
800
600
400
200
0
0
2
4
5
6
Months on VPA

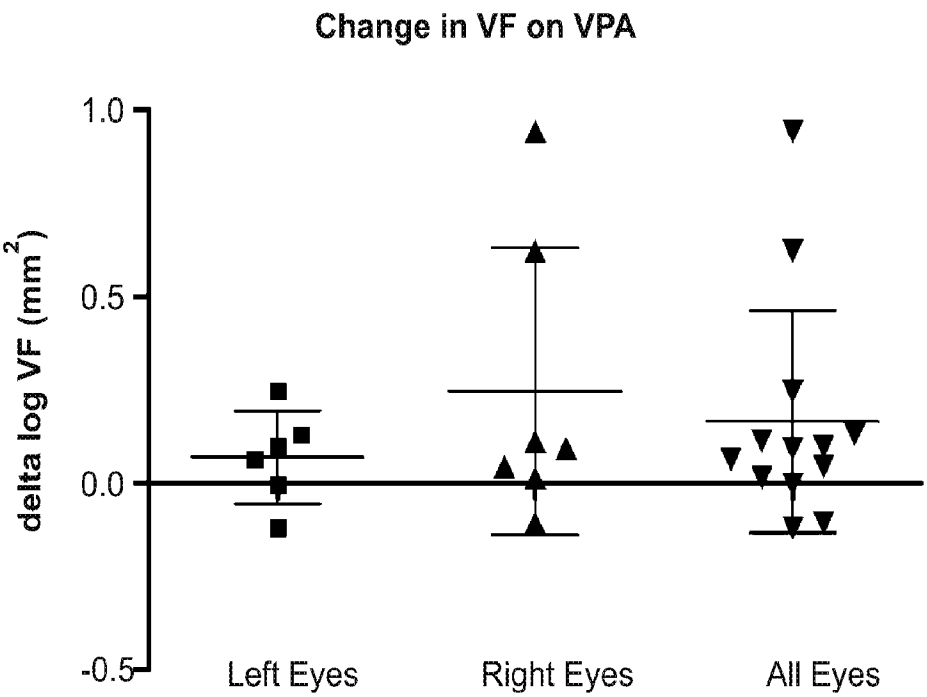

Change in VF on VPA
delta log VF (mm^2)
1.0
0.5
0.0
-0.5
Left Eyes
Right Eyes
All Eyes

FIG. 11