

SAFETY AND EFFECT ON ROD FUNCTION OF ACU-4429, A NOVEL SMALL-MOLECULE VISUAL CYCLE MODULATOR

RYO KUBOTA, MD, PhD,* NANCY L. BOMAN, MD, PhD,* ROBERT DAVID, MD,* SURESH MALLIKAARJUN, PhD, FCP,‡ SHIVA PATIL, PhD,‡ DAVID BIRCH, PhD†

Background: ACU-4429 is a first in class small-molecule visual cycle modulator that inhibits the isomerase complex and, in mouse models of retinal degeneration, prevents the accumulation of A2E.

The purpose of this study was to assess the tolerability, pharmacokinetics, pharmacodynamics, and safety of a single, orally administered dose of ACU-4429 in healthy subjects.

Methods: Sequential cohorts were administered single doses ranging from 2 mg to 75 mg. Full-field electroretinograms were recorded before and after exposure to full-field bleaching light. Pharmacokinetics samples were taken at predetermined times. Safety assessments included adverse events, vital signs, clinical laboratory assays, electrocardiograms, and ophthalmologic examination.

Results: After 45-minute dark adaptation, electroretinographic findings demonstrated a dose-related slowing of the rate of recovery that reached its maximum on Day 2 and returned to baseline by Day 7. Mean area under the concentration curve and peak plasma concentration increased proportionally with increasing doses. Median time to peak concentration was 4 hours postdose. Mean elimination mean half-life was 4 hours to 6 hours. Adverse events were mild and visual in nature (dyschromatopsia and alteration in dark adaptation), transient, and resolved within a few days. Adverse event frequency was dose dependent.

Conclusion: Oral administration of ACU-4429 produced a dose-dependent inhibition of the b-wave of the electroretinograms, was well tolerated up to 75 mg, and demonstrated linear pharmacokinetics across doses.

RETINA 32:183–188, 2012

Age-related macular degeneration (AMD) is a chronic degenerative disease of the retina and the most common cause of visual loss in the elderly in the developed world.¹ There are two types of AMD: exudative (wet) and nonexudative (dry). Dry AMD accounts for approximately 85% of all AMD cases.² There is no approved treatment for this form of the disease, which can progress through different stages

and in the advanced form, geographic atrophy, is associated with severe vision loss. Dry AMD creates a substantial disability in older individuals and is an important unmet medical need.

Age-related macular degeneration and other retinal degenerative diseases such as Stargardt disease have been associated with accumulation of lipofuscin in the retinal pigment epithelium.³ The lipofuscin that accumulates in the retinal pigment epithelium is heterogeneous and accumulates as a byproduct of visual cycling. It includes the bisretinoid compounds A2E, iso-A2E, all-*trans*-retinal dimer, all-*trans*-retinal dimer-ethanolamine, and all-*trans*-retinal dimer-phosphatidylethanolamine. Accumulation of A2E occurs with aging⁴ and may be associated with mutations in genes, such as *ABCA4*^{5–7} and *ELOVL4*,^{8–10} and in AMD.¹¹ Modulation of the visual cycle thus represents a rational therapeutic approach for degenerations associated with accumulations

From *Acucela, Inc, Seattle, Washington; †Retina Foundation of the Southwest, Dallas, Texas; and ‡Otsuka Pharmaceutical Development and Commercialization, Inc, Princeton, New Jersey.

Supported by Otsuka Pharmaceutical Development and Commercialization, Inc, and Acucela Inc.

Presented at the 8th International Symposium on Ocular Pharmacology and Therapeutics (ISOPT), December 5, 2009 Rome, Italy.

Reprint requests: Ryo Kubota, MD, PhD, Acucela Inc, 1301 2nd Avenue, Suite 1900, Seattle, WA 98101-3805; e-mail: fnolan@acucela.com

of A2E. A key marker for determinations of pharmacologic visual cycle modulation is the time course of recovery of rod sensitivity after exposure to a bleaching light.

In animal models, compounds that inhibit the visual cycle have been shown to slow the time course of rod recovery of function after a bleaching light exposure^{12,13} and reduce accumulation of A2E in the retinal pigment epithelium.^{5,14} Similarly, in human subjects, synthetic retinoids such as fenretinide not only cause delayed dark adaptation, elevations in dark-adapted thresholds, and electroretinographic (ERG) abnormalities^{15–23} but also have many additional adverse effects.^{20,24} ACU-4429 is a small molecule that inhibits retinal pigment epithelium 65 (Bavic C, 2008, unpublished data), the *trans* to *cis* retinal isomerase. In the present study, we evaluated the safety and tolerability after administration of single doses of ACU-4429 in healthy volunteers, and the effect on rod and cone ERG responses before and after exposure to an intense bleaching light.

Materials and Methods

This was a single-center, randomized, double-masked, placebo-controlled, dose escalation study. Healthy male and female subjects between the ages of 55 and 80 years were randomized to receive single doses of either ACU-4429 or placebo (in a 5:1 ratio, except for the 3 subjects in the 10-mg cohort with no matching placebo) in dose-escalating cohorts (2, 7, 10, 20, 40, 60, and 75 mg). Study drug was administered on an empty stomach in the morning (from 07:30 to 10:30) after an overnight fast. Escalation to the next dose level occurred only after review of safety data by an independent data monitoring committee.

Study participants weighed between 50 kg and 110 kg, had normal clinical laboratory test results and electrocardiogram, were free of ocular conditions (cataracts, glaucoma, uveitis, diabetic retinopathy, and active conjunctivitis), received no retinoids during the previous 12 months, received no treatment with phosphodiesterase Type 5 inhibitors within the last week, had a visual acuity better than 0.3 logarithm of the minimum angle of resolution as tested on the Early Treatment of Diabetic Retinopathy Study charts, had normal results on D-28 color vision testing, and no clinically significant fundoscopic abnormalities. Subjects did not use cigarettes, alcohol, or drugs and had not received concomitant treatment with hypnotics, antidepressants and psychoactive substances, digitalis glycosides (digoxin, ouabain, digitoxin), L-DOPA, chloroquine or hydroxychloroquine, systemic corticosteroids, topical antiglaucoma medication, or

medications for treatment of “wet” AMD. The institutional review board approved the protocol. All study participants gave written informed consent.

The primary objective of this study was to provide an initial evaluation of the safety and tolerability of single-dose oral administration of ACU-4429 in healthy volunteers in dose-escalating cohorts. Safety assessments included incidence of adverse events (AEs), vital signs, clinical laboratory assays, electrocardiograms, and ophthalmologic examinations.

The pharmacokinetic (PK) profile of ACU-4429 was assessed predose and at 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after dosing. The PK parameters were derived using noncompartmental methods. Calculations were performed, if appropriate, using WinNonlin (Version 5.1; Pharsight Corporation, Mountain View, CA). Calculated PK parameters included area under the concentration curve, peak plasma concentration (C_{max}), time to peak concentration (T_{max}), and mean half-life ($T_{1/2}$).

For dose cohorts of ≥ 10 mg, full-field ERG measurements were recorded after pupil dilation using 10% tropicamide and 30 minutes of dark adaptation at baseline and on Day 1 (approximately 4 hours postdose), Day 2 (24–36 hours postdose), and Day 4 (60- and 75-mg cohorts only). Responses were obtained from both eyes simultaneously and included the International Society for Clinical Electrophysiology of Vision (ISCEV) standard rod response ($0.03 \text{ cd/m}^2\text{-seconds}$) and combined response ($1.5 \text{ cd}\cdot\text{s}\cdot\text{m}^{-2}$) in the dark, and the 31-Hz flicker response ($2.25 \text{ cd/m}^2\text{-seconds}$) and 1-Hz cone response ($2.25 \text{ cd/m}^2\text{-seconds}$) in the presence of a background illumination (34 cd/m^2). After a 10-minute exposure to a full-field bleaching light (556 cd/m^2), recovery of the ERG was measured for 60 minutes at 10-minute intervals. Data from participants receiving placebo study were pooled across cohorts.

Results

Forty-six study participants were enrolled in the randomized study between May 2008 and June 2009. Thirty-eight were randomly assigned to receive ACU-4429 and 8 were randomly assigned to receive placebo. All study participants completed the study.

The baseline demographics and clinical characteristics were generally comparable between ACU-4429-treated and placebo-treated study participants and among the different dose levels of ACU-4429. Mean age was 62 years and 60 years in ACU-4429-treated and placebo-treated study participants, respectively. Sixty-one percent of study participants were women (63% in the ACU-4429 group and 50% in the placebo

group) and most (80%) were white (76% in the ACU-4429 group and 100% in placebo group).

Pharmacokinetics

Plasma concentrations of ACU-4429 were near or below the assay's lower limit of quantitation after a single oral dose of 2 mg of ACU-4429. For all other dose levels, C_{max} was attained 4 hours after oral administration of ACU-4429. Time to peak concentration (T_{max}) was not dose dependent. Mean C_{max} and area under the concentration curve increased with each dose escalation. Mean area under the concentration curve increased from 12.87 ng-hours/mL to 172.43 ng-hours/mL and mean C_{max} from 1.42 ng/mL to 17.16 ng/mL in the 7-mg to 75-mg cohorts, respectively. The decline from C_{max} was monophasic. ACU-4429 was eliminated systemically with a mean half-life ranging from 4–6 hours. There were no significant gender or race differences found in the PK characteristics of ACU-4429. Oral PKs of ACU-4429 was linear with dose. Increases in ACU-4429 dose led to proportional increases area under the concentration curve and C_{max} . Figure 1 displays the median concentration versus time profile after administration of single oral doses of ACU-4429. Table 1 summarizes the mean PK parameters after administration of a single dose of ACU-4429.

Pharmacodynamic Effect (Electroretinographic Measurements)

Electroretinographic findings for all 6 dose groups (placebo and 10, 20, 40, 60, and 75 mg) are summarized in Figure 2. Amplitudes are expressed as the percentage of the mean dark-adapted amplitude from the two

pretreatment evaluations. For subjects in all groups, the bleaching light exposure led to a reduction in ERG amplitude to <10% of baseline. For subjects given placebo, there was a rapid rise in amplitude such that the response was 90% recovered by 20 minutes. For subjects given ACU-4429, there was a clear dose-related slowing of the rate of recovery; that is, the slope of the recovery function became slower with increasing dose. At the highest dose, the amplitude was still <10% of the baseline response after 60 minutes. The degree of suppression can be quantified from the slopes of the recovery functions (Table 2).

Also shown in Figure 2 is the dark-adapted (prebleach) amplitude expressed as a percentage of the mean dark-adapted amplitude on the 2 pretreatment evaluations. Note that the prebleach amplitude is indistinguishable from the pretreatment value for the placebo, 10-mg, and 20-mg groups; that is, 40 minutes of dark adaptation was sufficient to produce a comparable ERG with the pretreatment dark-adapted value. However, for the 40-mg dose, the prebleach amplitude on Day 2 was <50% of the dark-adapted amplitude before treatment, that is, 40 minutes of dark adaptation after normal room light exposure was not sufficient to produce the normal dark-adapted amplitude. Higher doses had progressively more affect on the prebleach amplitude; at the 75-mg dose, the amplitude was 11% baseline. An analysis of variance indicated that both day of testing ($F_{2,69} = 14$, $P < 0.001$) and dose ($F_{5,69} = 3.36$, $P < 0.005$) had a significant effect on relative sensitivity. The interaction between day and dose was also significant ($F_{10,69} = 2.9$, $P < 0.005$), such that doses of ≥ 40 mg only reduced prebleach sensitivity on Day 2.

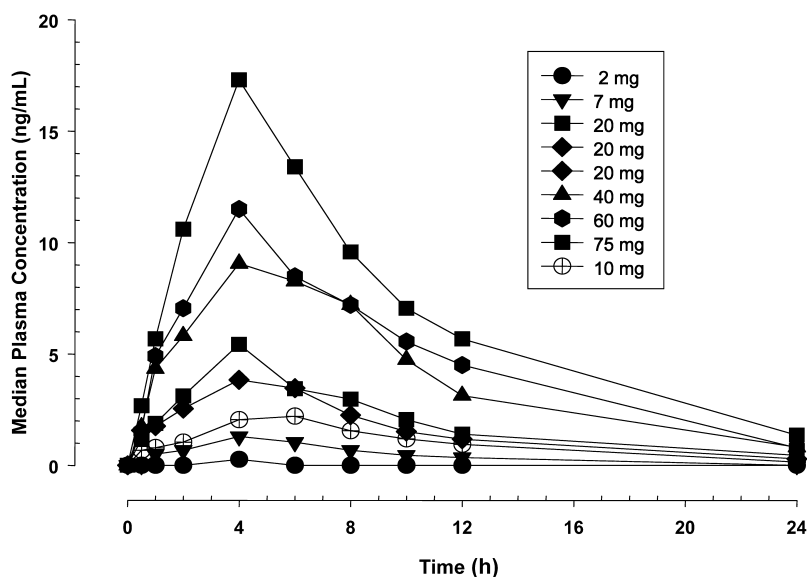


Fig. 1. ACU-4429 plasma concentration versus time. Median concentration versus time profiles after single oral doses of ACU-4429.

Table 1. Pharmacokinetic Parameters After Administration of a Single Dose of ACU-4429

PK Parameter	Mean (SD)					
	Dose of ACU-4429					
	7 mg (N = 5)	10 mg (N = 3)	20 mg (N = 10)	40 mg (N = 5)	60 mg (N = 5)	75 mg (N = 5)
AUC ₀₋₂₄ (ng·hours/mL)	12.96 (4.92)	27.63 (7.14)	46.37 (28.5)	90.6 (31.48)	117.56 (52.87)	162.66 (22.04)
AUC _{0-inf} (ng·hours/mL)	12.87 (5.12)	29.94 (7.33)	52.14 (34.24)	96.3 (35.79)	125.72 (58.51)	172.43 (22.95)
C _{max} (ng/mL)	1.42 (0.5)	2.67 (0.60)	4.46 (2.2)	9.7 (2.1)	12.22 (4.26)	17.16 (2.39)
T _{max} (hours)	ND (ND)	ND (ND)	ND (ND)	ND (ND)	ND (ND)	ND (ND)
T _{1/2λz} (hours)	3.93 (0.37)	5.88 (0.30)	6.26 (1.2)	5.42 (0.97)	5.26 (0.89)	5.41 (0.56)

AUC, area under the concentration curve; T_{max}, time to peak concentration; T_{1/2}, mean half-life; ND, not determined.

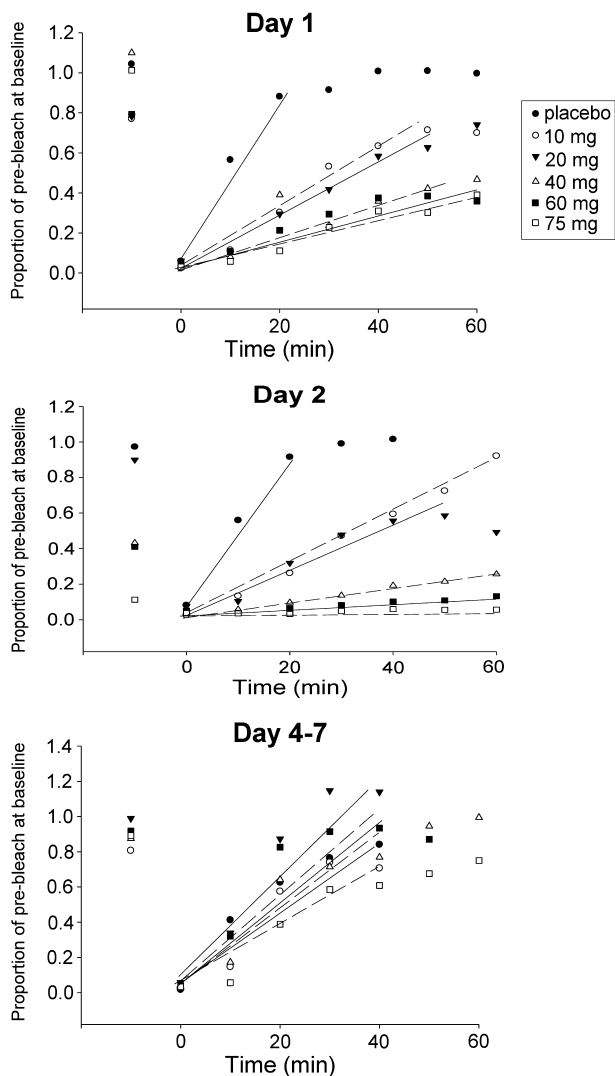


Fig. 2. Rod ERG suppression: proportion of prebleach baseline. Electroretinographic findings at Day 1, Day 2, and Days 4-7 for 6 dose groups (placebo and 10, 20, 40, 60, and 75 mg of ACU-4429) are summarized. Amplitudes are expressed as the percentage of the mean dark-adapted amplitude from the two pretreatment days. The solid and dashed lines are linear fits to the initial rising portion of the recovery function. Data points before 0 minutes represent the dark-adapted (prebleach) amplitude expressed as a percentage of the mean dark-adapted amplitude on the two pretreatment days.

Figure 3 summarizes the prebleach data in a different way. This figure shows the Day 2 rod amplitude (after 40 minutes of dark adaptation) expressed as a percentage of the pretreatment amplitude for each dose. Note that there was no effect on dark-adapted amplitude for placebo and for doses of 10 mg and 20 mg. Beginning at 40 mg, however, there was a dose-related suppression of rod amplitude, with the greatest suppression at the 75-mg dose, where the dark-adapted amplitude was 11% of baseline.

Figure 3 also shows the cone responses (flicker and single-flash) plotted in the same way (expressed as a percentage of the pretreatment amplitude for each dose). Unlike rod amplitudes, cone amplitudes remain within 20% of the pretreatment amplitude for all doses.

Adverse Events

Twenty-five (66%) subjects who received ACU-4429 and 2 (25%) who received placebo experienced at least 1 AE during the study. The most common AEs were visual in nature (50% ACU-4429; 0% placebo) and headache (ACU-4429 18%; placebo 13%). Visual AEs included dyschromatopsia (32%), unspecified visual disturbance (29%), night blindness (18%), blurred vision (11%), and photophobia (8%). Dyschromatopsia was observed in all study participants who received 60 mg or 75 mg of ACU-4429. All AEs were mild or moderate in intensity and were transient in nature, resolving within a few days after onset. Table 3 shows the number of visual AEs by dose group.

Discussion

Among the newer compounds in development for treating dry AMD is ACU-4429, a small-molecule,

Table 2. Degree of Rod ERG Suppression, Day 2

	Placebo	Dose of ACU-4429 (mg)				
		10	20	40	60	75
Slope	4.18	1.5	0.88	0.38	0.15	0.04
% Suppression	0	64	79	91	96	99

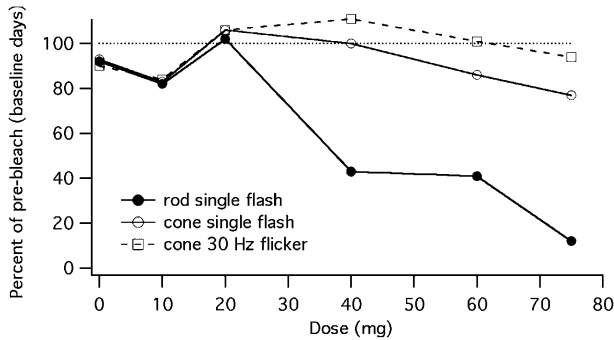


Fig. 3. Rod and cone response on Day 2. Prebleach amplitude after 40 minutes of dark adaptation on Day 2. Amplitudes are expressed as a percentage of the prebleach amplitude for each dose on the two pretreatment days.

nonretinoid isomerase inhibitor, which has shown significant potency in experimental studies, which led to the current first in human clinical trial. ACU-4429, in doses ranging from a low, no-effect 2 mg to the highest dose of 75 mg, was tested in a dose-escalating fashion in consecutive cohorts of healthy, elderly volunteers. As shown in the results, all doses were well tolerated and no serious AEs were noted. Reports of mild abnormalities of dark adaptation were seen in increased frequency at the higher doses, as expected. All symptoms were transient.

The PK data indicate that mean C_{max} and area under the concentration curve of ACU-4429 increased in a dose-proportional manner within the studied dose range of 2 mg to 75 mg. Mean terminal elimination half-life was short, ranging from 4 hours to 6 hours. However, the effect on ERG was long lasting, thus supporting a once-daily regimen for ACU-4429.

The ERG data show a dose-dependent effect of ACU-4429 on the visual cycle and are consistent with an ability to intervene in the processes that produce A2E. This suppression is similar to that seen with fenretinide.^{18,19} These findings document the potential of ACU-4429 to reduce the accumulation of A2E in pathologic conditions such as dry AMD.

The alteration in dark adaptation should be expected, given the mechanism of action of ACU-4429. Given the fact that the effect of ACU-4429 was found to be limited to the rod ERG and not seen in the cone ERG, the dyschromatopsia encountered at the high doses may indicate some “collateral” rod effect on chromatic pathways.

In aggregate, these initial human studies demonstrate that single doses of ACU-4429 cause a dose-dependent modulation of the visual cycle and the single doses are well tolerated.

Key words: A2E, ACU-4429, dark adaptation, dry AMD, ERG, geographic atrophy, lipofuscin, retinal degeneration, visual cycle modulator.

Table 3. Treatment-Emergent AEs Occurring in >1 ACU-4429-Treated Subject, Randomized Safety Population

Preferred Term	Dose of ACU-4429							ACU-4429 Overall, N = 38, n (%)	Placebo Overall, N = 8, n (%)
	2 mg, N = 5, n (%)	7 mg, N = 5, n (%)	10 mg, N = 3, n (%)	20 mg, N = 10, n (%)	40 mg, N = 5, n (%)	60 mg, N = 5, n (%)	75 mg, N = 5, n (%)		
Any adverse event	3 (60)	2 (40)	1 (33)	5 (50)	4 (80)	5 (100)	5 (100)	25 (66)	2 (25)
Chromatopsia	0	0	0	0	2 (40)	5 (100)	5 (100)	12 (32)	0
Visual disturbance	0	0	0	0	4 (80)	3 (60)	4 (80)	11 (29)	0
Night blindness	0	0	0	2 (20)	1 (20)	1 (20)	3 (60)	7 (18)	0
Headache	2 (40)	1 (20)	0	1 (10)	1 (20)	2 (40)	0	7 (18)	1 (13)
Vision blurred	0	0	0	0	1 (20)	1 (20)	2 (40)	4 (11)	0
Photophobia	0	0	0	1 (10)	0	1 (20)	1 (20)	3 (8)	0
Nausea	1 (20)	0	0	0	0	1 (20)	0	2 (5)	0
Ecchymosis	0	1 (20)	0	1 (10)	0	0	0	2 (5)	1 (13)

References

1. VanNewkirk MR, Nanjan MB, Wang JJ, et al. The prevalence of age-related maculopathy: the visual impairment project. *Ophthalmology* 2000;107:1593–1600.
2. Seddon J. Epidemiology of age-related macular degeneration. In: Schachat A, Ryan S, eds. *Retina*. 3rd ed. St. Louis, MO: Mosby; 2001.
3. Travis GH, Golczak M, Moise AR, Palczewski K. Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. *Annu Rev Pharmacol Toxicol* 2007;47:469–512.
4. Dorey CK, Wu G, Ebenstein D, et al. Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci* 1989;30:1691–1699.
5. Mata NL, Weng J, Travis GH. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc Natl Acad Sci U S A* 2000;97:7154–7159.
6. Mata NL, Tzekov RT, Liu X, et al. Delayed dark-adaptation and lipofuscin accumulation in *abcr* ± mice: implications for involvement of ABCR in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2001;42:1685–1690.
7. Weng J, Mata NL, Azarian SM, et al. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in *abcr* knockout mice. *Cell* 1999;98:13–23.
8. Bernstein PS, Tammur J, Singh N, et al. Diverse macular dystrophy phenotype caused by a novel complex mutation in the *ELOVL4* gene. *Invest Ophthalmol Vis Sci* 2001;42:3331–3336.
9. Karan G, Lillo C, Yang Z, et al. Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant *ELOVL4* transgenic mice: a model for macular degeneration. *Proc Natl Acad Sci U S A* 2005;102:4164–4169.
10. Zhang K, Kniazeva M, Han M, et al. A 5-bp deletion in *ELOVL4* is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet* 2001;27:89–93.
11. Eldred GE. Lipofuscin fluorophore inhibits lysosomal protein degradation and may cause early stages of macular degeneration. *Gerontology* 1995;41:S15–S28.
12. Messias A, Zrenner E, Tzekov R, et al. Single doses of all-trans-N-retinylacetamide slow down the ERG amplitude recovery after bleaching in rats. *Doc Ophthalmol* 2010;120:165–174.
13. Sieving PA, Chaudhry P, Kondo M, et al. Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in isotretinoin therapy. *Proc Natl Acad Sci U S A* 2001;98:1835–1840.
14. Radu RA, Mata NL, Nusinowitz S, et al. Treatment with isotretinoin inhibits lipofuscin accumulation in a mouse model of recessive Stargardt's macular degeneration. *Proc Natl Acad Sci U S A* 2003;100:4742–4747.
15. Caruso RC, Zujewski J, Iwata F, et al. Effects of fenretinide (4-HPR) on dark adaptation. *Arch Ophthalmol* 1998;116:759–763.
16. Conley B, O'Shaughnessy J, Prindiville S, et al. Pilot trial of the safety, tolerability, and retinoid levels of N-(4-hydroxyphenyl) retinamide in combination with tamoxifen in patients at high risk for developing invasive breast cancer. *J Clin Oncol* 2000;18:275–283.
17. Denman S, Weleber R, Hanifin JM, et al. Abnormal night vision and altered dark adaptometry in patients treated with isotretinoin for acne. *J Am Acad Dermatol* 1986;14:692–693.
18. Kaiser-Kupfer MI, Peck GL, Caruso RC, et al. Abnormal retinal function associated with fenretinide, a synthetic retinoid. *Arch Ophthalmol* 1986;104:69–70.
19. Marmor MF, Jain A, Moshfeghi D. Total rod ERG suppression with high dose compassionate Fenretinide usage. *Doc Ophthalmol* 2008;117:257–261.
20. Modiano MR, Dalton WS, Lippman SM, et al. Ocular toxic effects of fenretinide. *J Natl Cancer Inst* 1990;82:1063.
21. Ribatti D, Alessandri G, Baronio M, et al. Inhibition of neuroblastoma-induced angiogenesis by fenretinide. *Int J Cancer* 2001;94:314–321.
22. Weleber RG, Denman ST, Hanifin JM, Cunningham WJ. Abnormal retinal function associated with isotretinoin therapy for acne. *Arch Ophthalmol* 1986;104:831–837.
23. Zujewski J, Pai L, Wakefield L, et al. Tamoxifen and fenretinide in women with metastatic breast cancer. *Breast Cancer Res Treat* 1999;57:277–283.
24. Camerini T, Mariani L, De PG, et al. Safety of the synthetic retinoid fenretinide: long-term results from a controlled clinical trial for the prevention of contralateral breast cancer. *J Clin Oncol* 2001;19:1664–1670.