Treatment of Geographic Atrophy With Subconjunctival Sirolimus: Results of a Phase I/II Clinical Trial

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Submitted: January 13, 2013
Accepted: March 25, 2013

Citation: Wong WT, Dresner S, Forooghian F, et al. Treatment of geographic atrophy with subconjunctival sirolimus: results of phase I/II clinical trial. *Invest Ophthalmol Vis Sci*. 2013;54;2941–2950. DOI:10.1167/iovs.13-11650

Purpose. To investigate the safety and effects of subconjunctival sirolimus, an mTOR inhibitor and immunosuppressive agent, for the treatment of geographic atrophy (GA).

Methods. The study was a single-center, open-label phase II trial, enrolling 11 participants with bilateral GA; eight participants completed 24 months of follow-up. Sirolimus (440 μg) was administered every 3 months as a subconjunctival injection in only one randomly assigned eye in each participant for 24 months. Fellow eyes served as untreated controls. The primary efficacy outcome measure was the change in the total GA area at 24 months. Secondary outcomes included changes in visual acuity, macular sensitivity, central retinal thickness, and total drusen area.

Results. The study drug was well tolerated with few symptoms and related adverse events. Study treatment in study eyes was not associated with structural or functional benefits relative to the control fellow eyes. At month 24, mean GA area increased by 54.5% and 39.7% in study and fellow eyes, respectively ($P = 0.41$), whereas mean visual acuity decreased by 21.0 letters and 3.0 letters in study and fellow eyes, respectively ($P = 0.03$). Substantial differences in mean changes in drusen area, central retinal thickness, and macular sensitivity were not detected for all analysis time points up to 24 months.

Conclusions. Repeated subconjunctival sirolimus was well-tolerated in patients with GA, although no positive anatomic or functional effects were identified. Subconjunctival sirolimus may not be beneficial in the prevention of GA progression, and may potentially be associated with effects detrimental to visual acuity. (ClinicalTrials.gov number, NCT00766649.)

Keywords: age-related macular degeneration, geographic atrophy, sirolimus, rapamycin, clinical trial

Geographic atrophy (GA), the advanced atrophic form of age-related macular degeneration (AMD), is characterized by the development of areas of outer retinal and retinal pigment epithelial (RPE) atrophy in the macula, which progressively enlarge in area,[1,2] leading to a loss in central vision.[3] No treatment is currently available to prevent the onset of GA, or to slow down the expansion of atrophic lesions once GA begins.[4,5] Pathogenic mechanisms involved in GA onset and progression are incompletely understood[6] and current animal models for the study of GA-related processes only partially recapitulate the human disease.[7] At the current state of knowledge, proof-of-concept clinical trials in humans that explore candidate treatment strategies[8–10] continue to be important in furthering understanding of the disease and in identifying viable therapeutic targets.[4,5,11]

Although the precise etiology of GA is unclear, there is accumulating evidence that chronic inflammation may play a causal role in AMD pathogenesis.[12,13] Genetic studies of AMD have revealed that the complement pathway of the immune system is involved in conferring susceptibility to advanced forms of AMD, including GA.[14–16] In histopathologic studies of GA, inflammatory changes in the forms of microglia/macrophage accumulation in the outer retina[17] and dysregulated expression of complement regulatory proteins in GA lesions[18] have been described. These observations suggest that therapeutic approaches that decrease the level of chronic inflammation in the retina may help slow GA progression.[13]

Sirolimus, also called rapamycin, is an immunosuppressive agent that has been approved as an oral medication to prevent organ rejection following renal transplantation[19] and as a coated stent to prevent coronary artery restenosis following balloon angioplasty.[20] In ocular diseases, oral sirolimus has also been investigated in clinical studies of refractory uveitis[21,22] and neovascular AMD.[23] Sirolimus, in forming a complex with immunophilin FK binding protein 12 (FKBP12), directly interacts with and inhibits the protein kinase known as mechanistic target of rapamycin (mTOR),[24,25] which regulates a wide range of cell functions including metabolism, growth, proliferation, and survival.[26] Sirolimus has demonstrated multiple potential uses in the treatment of cancer, metabolic diseases, and aging[27]; in addition, its immunosuppressive properties, exerted via the suppression of T- and B-cell

proliferation and antibody production,[28–31] has prompted its evaluation as a treatment for retinal diseases with an inflammatory component such as diabetic retinopathy.[32,33]

The current study used a proprietary, depot-forming formulation of sirolimus that has been developed for local ocular delivery either as a subconjunctival or as an intravitreal injection.[34] When injected into the subconjunctival space, this formulation forms a depot from which the drug can diffuse in a sustained manner into surrounding ocular compartments including the retina and choroid. Preclinical pharmacokinetic studies of subconjunctival sirolimus in rabbits reported measurable levels in the retina and choroid following administration (Investigators' Brochure, DE-109, unpublished data, 2012; Santen Pharmaceutical Co., Ltd., Osaka, Japan). The goal of this phase I/II, open-label, prospective, pilot study was to evaluate the safety and effects of subconjunctivally administered sirolimus for the treatment of GA. To the best of our knowledge, this study represents the first report of an immunosuppressive therapy for the treatment of GA. The nature of the results of this study would allow an evaluation of the feasibility of local immunosuppression as a therapeutic strategy for GA.

Methods

This was a single-center, prospective, open-label, phase I/II study of subconjunctival sirolimus for the local treatment of GA. The study was conducted at the National Institutes of Health (NIH) Clinical Center, Bethesda, MD, and was supported by the National Eye Institute Intramural Research Program. The study protocol and Health Insurance Portability and Accountability Act (HIPAA)–compliant informed consent forms were reviewed and approved by the NIH CNS Institutional Review Board. Study oversight was provided by an independent external data and safety monitoring committee that reviewed study data every 3 months. Written informed consent was obtained from each participant before enrollment into the study. An investigational new drug application (IND) (102,516) was obtained from the U.S. Food and Drug Administration. The study was registered at www.clinicaltrials.gov under the identifier NCT00766649 (registration date, October 3, 2008). The study adhered to the tenets of the Declaration of Helsinki.

Study Population

Eligible participants were at least 55 years of age, and had a diagnosis of bilateral GA related to AMD. The primary eligibility criteria included: (1) the presence of GA in each eye of area $\geq$ half disc area (approximately 1 mm^2), (2) the presence of at least one large druse ($\geq$125 μm) in each eye, (3) best-corrected visual acuity (BCVA) of between 20/20 and 20/400 in each eye, and (4) the absence of evidence or history of exudative AMD. A complete listing of inclusion and exclusion criteria can be found in Supplementary Table S1. Eligibility for participation in the study was determined following a screening visit to the clinic. Clinical evaluation during screening included a brief physical examination, an ophthalmic history assessment, measurement of BCVA and intraocular pressure, dilated fundus examination, and fundus photography.

Study Medication

The study drug (MS-R001) comprised of a proprietary formation of a nonaqueous 22 μg/μL (2%) solution of sirolimus in a vehicle composed of PEG 400 and 4% ethanol. The study drug was synthesized by Santen Pharmaceutical Co., Ltd. (Osaka, Japan) and donated for use in the study. The study drug was supplied frozen in a 0.5 mL sterile injectable solution, thawed immediately prior to use, and drawn slowly into a sterile 0.3 mL syringe (Becton Dickinson, Frankling Lakes, NJ) with a 29-gauge, ½-inch-long needle. The study drug was protected from light before delivery and administered within 2 hours of being drawn up. The study drug was delivered as an injection into the subconjunctival space (20 μL injection volume, containing 440 μg sirolimus). Briefly, the inferior temporal conjunctiva of the treated eye was anesthesized with cotton-tipped applicators soaked in 0.5% proparacaine topical eye drops held at the injection site for approximately 1 minute, after which the study drug was slowly injected into the subconjunctival space at the anesthetized location.

Study Design

One eye in each participant was chosen to receive the study drug by random assignment using a computer-generated algorithm (this eye will be referred to hereafter as the study eye). The contralateral eye (referred to hereafter as the fellow eye) was assigned to observation without treatment. The study drug was administered in the study eye at the baseline visit and every 3 months thereafter. The rationale for this monocular study design was based on observed correlations between the rates of GA enlargement in fellow eyes of patients with bilateral GA,[35–37] thus allowing the use of the fellow eye as a control for the treated study eye.[9] Following the 24-month study visit, participants were given the option of stopping the study drug or continuing treatment in the study eye on the same schedule (injections every 3 months) until a common termination date.

Study Assessments

Study visits were scheduled at baseline and at every 3 months thereafter. Two additional safety visits were also scheduled during the study: (1) At 1 month following the first injection at study baseline, and (2) at 3 months following the final administration of the study drug. BCVA measurements using Early Treatment Diabetic Retinopathy Study (ETDRS) charts and protocols, slit-lamp and dilated fundus examinations, measurement of intraocular pressure, and assessment of adverse events and concomitant medications were performed at every visit. Laboratory assessments consisting of complete blood count, serum electrolytes, serum lipid profiles, and urine analysis were performed at least every 6 months; measurement of serum sirolimus levels was performed every 3 months. A physical examination was performed at baseline, at 12 months, and at 24 months, and at the final study visit. The following examination procedures were performed in both eyes at baseline and every 6 months thereafter (i.e., at months 6, 12, 18, and 24): (1) stereoscopic color fundus photography (CFP), (2) fundus autofluorescence (FAF) imaging using both a confocal scanning ophthalmoscope (HRA FAF; HRA2; Heidelberg Engineering, Vista, CA) and a modified fundus camera (Topcon 50-EX fundus camera; Topcon Medical Systems, Oakland, NJ) using band-pass filters for excitation (550–600 nm) and emission (660–800 nm) as previously described,[38] (3) spectral domain optical coherence tomography (SD-OCT) imaging (Cirrus HD-OCT, software ver. 6.0; Carl Zeiss Meditec, Inc., Jena, Germany), and (4) macular sensitivity assessment using microperimetry (MP-1 microperimeter; Nidek Technologies, Padova, Italy).

CFPs and HRA FAF images were sent to the Doheny Image Reading Center (University of Southern California, Los Angeles, CA) for digital manual grading by masked graders. The area of GA and the total area of drusen in field 2 (30° photographic field centered on the fovea) were determined by planimetry from color stereoscopic fundus images. For the quantification drusen area, borders of all drusen were manually delineated by graders. FAF images were graded according to a standardized protocol

Investigative Ophthalmology & Visual Science

by scoring the areas of decreased, increased, and abnormal FAF in the macula. Color and FAF images were graded and quantified independently. SD-OCT imaging was performed with the 512 × 128 scan pattern (covering a 6 × 6 mm area) centered over the anatomic fovea. A circular grid as defined in the Age Related Eye Disease Study (AREDS)[39] was superimposed on the scan field and centered on the fovea. Retinal thickness in the central subfield (1-mm-diameter circle) and total macular volume in the central and inner subfields (covering the central 3-mm-diameter circle) were computed using the device software (Cirrus HD-OCT, software ver. 6.0; Carl Zeiss Meditec, Inc.), following manual confirmation and correction of computer-rendered retinal segmentation. Microperimetry testing was performed using the MP-1 microperimeter (NAVIS software, version 1.7.1; Nidek Technologies). Assessments were performed as previously described.[9,40] Retinal sensitivity was calculated with a background luminance of four apostilibs (1.27 cd/m^2) using a grid of 68 testing loci that were evenly spaced within a circle of radius 18° centered on the center of the macula. The starting stimulus light attenuation was set at 10 dB. A 4-to-2 staircase strategy was used, using testing intensities ranging from 127 to 2.54 cd/m^2, which correspond to retinal sensitivities of 0 to 20 dB. The follow-up testing feature in the testing software (NAVIS software, version 1.7.1; Nidek Technologies) was used. Scotomatous (or nonresponding) points were defined as testing loci that elicited no participant response even at the highest intensity stimulus. Responding points were defined as all other testing loci for which a response was recorded after stimulus presentation (i.e., points for which a response was elicited within the entire range of stimuli intensities used by the testing algorithm). The following test parameters were tallied: (1) number of scotomatous loci (loci with sensitivity of <0 dB), and (2) macular mean sensitivity (dB) of all responding points.

Study Objectives

The overall study objective was to evaluate the safety and effects of the study drug in delaying the anatomic and functional progression of GA in patients with bilateral disease. The primary outcome measure of the study was the change in the total GA area from baseline to month 24. Other secondary outcome measures included the following: (1) change in BCVA, (2) change in mean retinal subfield thickness as measured using SD-OCT, (3) change in drusen area as measured from color fundus photographs, and (4) change in retinal sensitivity as measured by microperimetry.

Statistical Analysis

Commercial software (Prism, ver. 5.0; GraphPad, La Jolla, CA) was used to calculate summary statistics (mean and SD or SEM) for demographic, visual acuity, fundus image data, and microperimetry performance data. Paired *t*-tests were used to compare these parameters between study and fellow eyes. Correlations between study and fellow eyes were computed with the Pearson correlation coefficient. All *P* values are two-tailed. Error bars in graphical representations of data and reports of variation used in the text indicate SEM.

Results

Baseline Patient Demographics and Ocular Characteristics

A total of 11 participants were enrolled into the study between February 2009 and April 2010. Of these, eight participants completed at least 24 months of follow-up. Two participants (P2, P6) withdrew from the study after 6 months of follow-up for reasons unrelated to the study drug (e.g., relocation, inability to travel). One participant (P3) died 6 months after one study visit from complications of bowel incarceration following hernia repair surgery, which was unrelated to the study drug. Analyses of treatment effect outcome measures were limited to the eight participants who completed 24 months of follow-up. The analysis of safety data included all 11 enrolled participants.

Because the design of the study involved comparison of changes in treated study eyes to untreated fellow eyes, we evaluated characteristics of study and fellow eyes in individual participants at study baseline. Table 1 summarizes the baseline

Table 1. Baseline Demographic and Ocular Data for Participants Completing 24 Months of Follow-Up

Number of participants	8
Age, y, mean ± SD (range)	77.88 ± 8.15 (68–89)
Sex, female, *n* (%)	3 (37.5%)
Sex, male, *n* (%)	5 (62.5%)
Race, white, *n* (%)	8 (100%)
Assignment of study eye, right *n* (%)	4 (50%)
Lens status of study eye, pseudophakic, *n* (%)	
Study eye	3 (37.5%)
Fellow eye	3 (37.5%)
Baseline best-corrected visual acuity, letters, mean ± SD (range)	
Study eye	62.4 ± 12.7 (40–83)
Fellow eye	55.1 ± 20.6 (19–79)
Location of geographic atrophy lesion	
Subfoveal, involving center of fovea	12 eyes of 6 participants
Nonsubfoveal, not involving center of fovea	4 eyes of 2 participants
Total area of GA, mm^2, mean ± SD (range)	
Color fundus photography	
Study eye	6.96 ± 4.15 (1.16–13.87)
Fellow eye	7.29 ± 4.98 (2.19–14.38)
Modified fundus camera	
Study eye	12.84 ± 7.77 (1.94–25.69)
Fellow eye	13.81 ± 9.37 (4.10–27.25)
Confocal scanning ophthalmoscope	
Study eye	9.23 ± 5.59 (1.55–19.01)
Fellow eye	9.72 ± 6.69 (3.07–19.63)
Central subfield retinal thickness, μm, mean ± SD (range)	
Study eye	176 ± 37 (122–232)
Fellow eye	198 ± 32 (137–227)
Mean total drusen area, mm^2, mean ± SD (range)	
Study eye	0.643 ± 0.607 (0.06–1.50)
Fellow eye	0.661 ± 0.928 (0.06–2.66)
Microperimetry measurements, number of scotomatous points (i.e., sensitivity < 0 dB), mean ± SD (range)	
Study eye	6.5 ± 4.9 (1–15)
Fellow eye	7.6 ± 6.2 (2–18)
Mean overall sensitivity of nonscotomatouspoints, dB, mean ± SD (range)	
Study eye	8.83 ± 2.87 (6.34–14.60)
Fellow eye	8.49 ± 2.87 (3.68–12.39)
Mean overall sensitivity of all points, dB, mean ± SD (range)	
Study eye	7.95 ± 2.98 (5.17–13.94)
Fellow eye	7.57 ± 3.15 (2.96–11.64)

Investigative Ophthalmology & Visual Science

Table 2. Summary of All Adverse Events by Category and Severity for All Enrolled Participants ($n = 11$)

	Severity											
	Mild/Grade 1				Life-Threatening/Grade 4				Total			
Adverse Event Category	*n*	Possibly Related	%	% Related	*n*	Possibly Related	%	% Related	*n*	Possibly Related	%	% Related
Allergic, immuno logic	1	0	1.6%	0.0%	0	0	0.0%	0.0%	1	0	1.6%	0.0%
Blood, bone marrow	28	0	45.9%	0.0%	0	0	0.0%	0.0%	28	0	45.2%	0.0%
Musculo skeletal	1	0	1.6%	0.0%	0	0	0.0%	0.0%	1	0	1.6%	0.0%
Genito urinary	7	0	11.5%	0.0%	0	0	0.0%	0.0%	7	0	11.3%	0.0%
Ocular	9	4	14.8%	80.0%	0	0	0.0%	0.0%	9	4	14.5%	80.0%
Gastro intestinal	0	0	0.0%	0.0%	1	0	100.0%	0.0%	1	0	1.6%	0.0%
Dermatologic	5	0	8.2%	0.0%	0	0	0.0%	0.0%	5	0	8.1%	0.0%
Back pain	2	0	3.3%	0.0%	0	0	0.0%	0.0%	2	0	3.2%	0.0%
Auditory	1	0	1.6%	0.0%	0	0	0.0%	0.0%	1	0	1.6%	0.0%
Lymphatics	1	0	1.6%	0.0%	0	0	0.0%	0.0%	1	0	1.6%	0.0%
Endocrine	1	0	1.6%	0.0%	0	0	0.0%	0.0%	1	0	1.6%	0.0%
Other	5	1	8.2%	20.0%	0	0	0.0%	0.0%	5	1	8.1%	20.0%
Total	61	5	100.0%	100.0%	1	0	100.0%	0.0%	62	5	100.0%	100.0%

demographic and ocular data for the eight participants completing the study follow-up. In the study and fellow eye, respectively, mean baseline GA area (on CFP) was 6.96 and 7.29 mm^2, whereas mean baseline BCVA was 62.4 letters (≈20/62.5) and 55.1 letters (≈20/80). We found that baseline total GA area, as measured on all three imaging modalities, was well correlated to the $Y = X$ line (R^2 values = 0.73–0.76, Supplementary Fig. S1), indicating that random assignment has been successful in the pairing of study and fellow eyes in terms of the primary outcome measure.

Ocular and Systemic Safety of Investigational Agent

A total of 62 adverse events were recorded in all enrolled participants ($n = 11$) for all available follow-up (Table 2). Sixty-one events were recorded as mild in severity and one event, which involved the death of the participant (P3), was recorded as severe. This death, resulting from complications of hernia surgery, was judged to be unrelated to the study drug. There were a total of nine ocular adverse events, seven involving the study eye and two involving the fellow eye (Table 3). Four of the nine ocular adverse events and one of the nonocular events were judged as being related to the study drug. All study drug–related events were mild in severity, and resolved in a few days; they included ocular discharge (one event), ocular discomfort (two events), conjunctival bleeding (one event), and periorbital headache ipsilateral to the study injection (one event, nonocular event). The study drug was well tolerated by study participants and all participants received scheduled study injections at all the specified time points. Monitoring of serum sirolimus levels performed every 3 months yielded measurements that were below the level of detection (<2.0 ng/mL) for all participants at all time points.

Table 3. Ocular Adverse Events in Study and Fellow Eyes for All Enrolled Participants ($n = 11$)

Ocular Events	Study Eye	Fellow Eye	Total
Small subretinal/intraretinal hemorrhages	1	0	1
Elevated IOP	1	1	2
Irritation	1	0	1
Gritty	1	0	1
Discharge	1	0	1
Bleeding postinjection	1	0	1
Cataract extraction	1	1	2
Total	7	2	9

Effect of Study Drug on Area of Geographic Atrophy

Color fundus photographs (CFPs) and fundus autofluorescence (FAF) images, obtained separately with a modified fundus camera (mFC) and a confocal scanning ophthalmoscope (SLO), were captured at baseline and at every 6 months up to month 24. The total area of GA lesion was quantified by masked readers at a reading center using planimetric techniques on all three imaging modalities. Figure 1A shows the percentage increase in GA area from baseline to month 24 for each of the eight participants completing follow-up as measured using CFP images. All study and fellow eyes demonstrated an increase in total GA area from baseline; six of eight participants showed a percentage increase in GA area that was greater in the study eye than that in the fellow eye. Figures 1B, 1C show that in terms of the mean absolute increase (Fig. 1B) and the mean percentage increase (Fig. 1C) in GA area, study eyes demonstrated slightly greater mean increases compared with fellow eyes at all time points examined (months 6, 12, 18, and 24; paired *t*-test, $P > 0.05$ in all comparisons). The comparisons between study and fellow eyes obtained from the grading of autofluorescence images obtained using mFC and SLO imaging were similar to those obtained with CFP (Supplementary Fig. S2), indicating that GA areas quantified using these different imaging modalities were highly correlated. Square-root transformations of GA area measurements on all three modalities (CFP, mFC FAF, and SLO FAF) were performed as previously described[41,42] (Supplementary Fig. S3); trends similar to those found with untransformed area measurements were observed.

Of the eight participants completing 24 months of study follow-up, the location of GA at study baseline was graded as involving the fovea in both eyes of six of eight participants, and as sparing the fovea in both eyes of two of eight participants (P9 and P10). In participant 9, the location of GA progressed to involve the fovea in the study eye at month 12 and in the fellow eye at month 18. In participant 10, the locations of GA progressed to involve the fovea at month 6 in

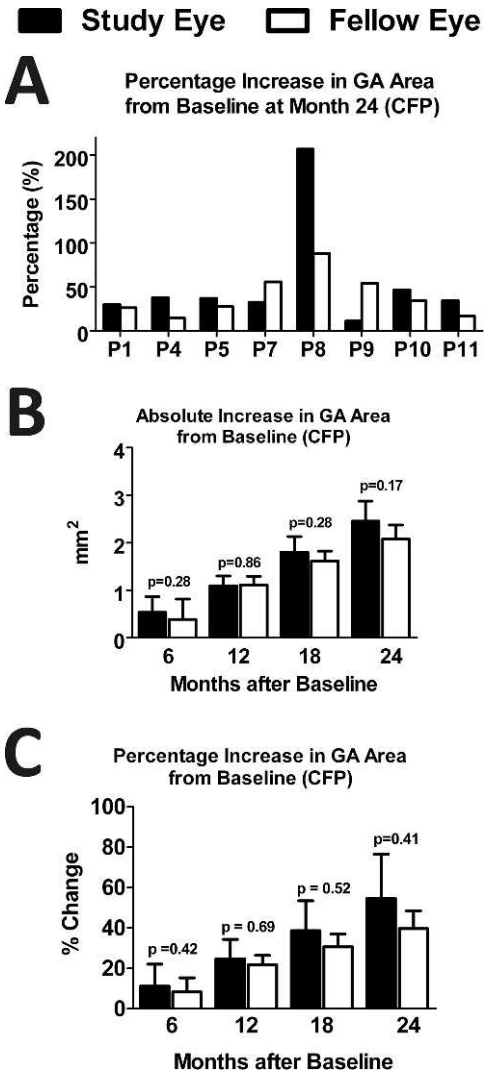

FIGURE 1. Change in GA area measurement on color fundus photography (CFP) in the study and fellow eyes from baseline for all participants completing 24 months of study follow up ($n = 8$). (**A**) Change in GA area from baseline to month 24 for each participant (as indicated by P1, P4, P5, and so forth) in study and fellow eyes. (**B**) Mean absolute increase in GA area from baseline at 6, 12, 18, and 24 months. (**C**) Mean percentage increase in GA area from baseline at 6, 12, 18, and 24 months. *P* values indicate results of a two-tailed paired *t*-test.

the study eye and continued to spare the fovea in the fellow eye.

Effect of Study Drug on Visual Acuity

Best-corrected visual acuity was monitored throughout the study and changes in visual acuity from baseline were evaluated and compared between study and fellow eyes. Figure 2A shows the changes in visual acuity from baseline to month 24 for each of the eight participants completing follow-up; seven of eight participants lost more letters or gained fewer letters in the study eye compared with the fellow eye. Figure 2B shows the mean change in visual acuity from baseline to months 6, 12, 18, and 24. At months 6, 12, and 18, decreases in mean visual acuity from baseline in study eyes were slightly greater than those in the fellow eyes. At month 24, mean visual acuity in study eyes decreased by −21.0 ± 21.5 letters, whereas that in fellow eyes decreased by −3.0 ± 8.1 letters, a difference of 18 letters ($P = 0.03$; 95% confidence interval, 0.9 to 25 letters). The proportions of participants losing ≥ 5 (Fig. 2C) or ≥10 letters (Fig. 2D) compared with baseline were also higher in the study eye than in the fellow eye for all time points. At 24 months, four of eight study eyes lost ≥ 10 letters of visual acuity from baseline compared to one of eight fellow eyes. No study or fellow eye developed exudative neovascular AMD during the study.

Effect of Study Drug on Central Subfield Retinal Thickness and Macular Volume

Mean retinal thickness in the central subfield (within a 1-mm-diameter circle centered on the fovea) and macular volume (within a 3-mm-diameter circle centered on the fovea) were evaluated using SD-OCT at baseline and at every 6 months thereafter. Changes in central subfield retinal thickness between baseline and month 24 were not systematically distinct in magnitude between study and fellow eyes of study participants (Fig. 3A). Mean central subfield retinal thickness and mean macular volume decreased progressively with follow-up time in both study and fellow eyes; comparisons between study and fellow eye at months 6, 12, 18, and 24 did not reveal any substantial differences (paired *t*-test, $P > 0.05$ in all comparisons) (Figs. 3B, 3C).

Effect of Study Drug on Total Drusen Area

The total area occupied by drusen was manually quantified by planimetry at the reading center as a secondary outcome measure at the baseline, 12-month, and 24-month visits. Changes in the total drusen area between baseline and the 24-month visit were variable between individual eyes and did not demonstrate a consistent difference between study and fellow eyes (Supplementary Fig. S4A). Mean changes in total drusen area also did not demonstrate an obvious difference between study and fellow eyes at the 12-month and the 24-month time points (Supplementary Fig. S4B).

Effect of Study Drug on Retinal Sensitivity as Measured by Microperimetry

Based on microperimetry testing for each eye at study baseline, testing loci were divided into scotomatous points (i.e., testing points for which no response was elicited at the highest stimulus level) and nonscotomatous or responding points (i.e., testing points for which a response was obtained at some stimulus level). At subsequent evaluations at 6, 12, 18, and 24 months, microperimetry measurements were used to calculate (1) the changes in the total number of scotomatous points from baseline values and (2) the changes in the mean sensitivity (in dB, on a scale from 0 to 20 dB) among points designated as nonscotomatous at baseline. Testing loci that transitioned to become scotomatous points during the study were assigned a sensitivity of 0 dB. Figure 4A shows that, although the mean number of scotomatous points tended to increase as a function of time, comparisons between study and fellow eyes did not reveal a significant difference. Figure 4B shows that the mean changes in macular sensitivity tended to decrease from baseline, but again these were not different between study and fellow eyes at any time point during the study. These parameters indicate that retinal sensitivity as measured using the MP-1 microperimeter (Nidek Technologies) were not measurably distinct between study and fellow eyes.

Investigative Ophthalmology & Visual Science

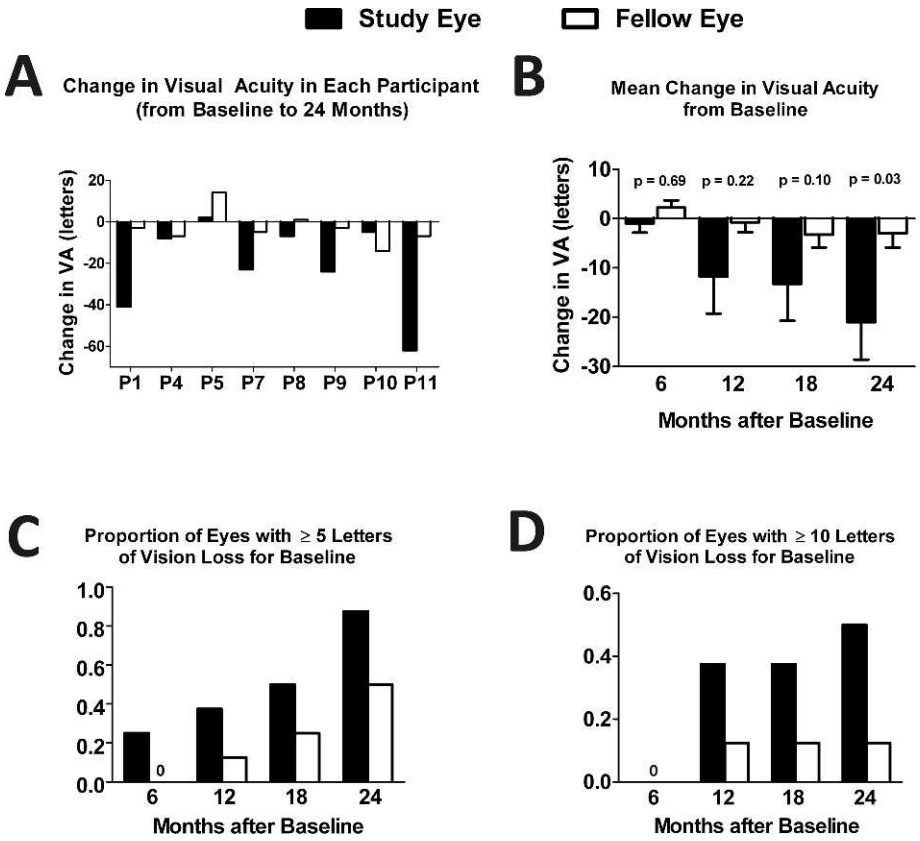

Figure 2. Visual acuity changes in the study and fellow eyes in participants completing 24 months of follow-up ($n = 8$). (**A**) Change in visual acuity (in ETDRS letters) from baseline to 24 months for each participant (indicated by number) for study eye and fellow eye. (**B**) Mean change in visual acuity in study and fellow eyes from baseline at 6, 12, 18, and 24 months. *P* values indicate the results of two-tailed paired *t*-tests between pairs of study and fellow eyes. (**C**) Proportions of the study and fellow eyes experiencing a >5-letter loss in visual acuity from baseline at 6, 12, 18, and 24 months. (**D**) Proportions of the study and fellow eyes experiencing a >10-letter loss in visual acuity from baseline at 6, 12, 18, and 24 months.

Discussion

We found in this study that repeated administration of subconjunctival sirolimus every 3 months was not associated with any substantial safety issues in participants with GA. There were few related ocular adverse events and systemic exposure to sirolimus was minimal as indicated by serum monitoring. The safety data in the current study corroborated those obtained in previous studies of this same drug preparation in diabetic macular edema[32,33] and chronic anterior uveitis.[43]

Potential effects of study drug on anatomic measures of GA were evaluated using measurements of GA area, central retinal thickness and volume, and total drusen area. In terms of area of GA, the different photographic and autofluorescence imaging modalities used (CFP, mFC, SLO) yielded highly congruent comparisons between study and fellow eyes.[9,44] We did not find evidence here that indicated that the study drug decreased the rate of growth of GA area. At all study time points and on all three imaging modalities, absolute and percentage increases in GA area were similar between study and fellow eyes. Although mean increases in study eyes were slightly larger than those in fellow eyes at all time points analyzed, based on the large *P* values computed and the small size of the study, we conclude that the study drug was unlikely to have exerted a large positive or negative effect on GA enlargement.

We similarly did not find from OCT-based measurements of central subfield retinal thickness and macular volume evidence that study drug delayed the course of retinal atrophy in the natural history of GA. The time-dependent and progressive decreases in central measures of retinal thickness and volume documented in the study suggest that these measures may constitute suitable outcome measures for studies of GA. On the other hand, the outcome measure of change in total drusen area was highly variable, did not demonstrate progressive change over time, and was relatively difficult to measure by planimetry, indicating it may be less useful in this regard. In addition, total drusen area measurements are confounded by the potential spread of atrophy into drusen-bearing retinal areas, which contributes to their loss or obscuration.

We examined study data for evidence that study drug demonstrated potential effects on visual function on visual acuity and macular sensitivity testing. In terms of visual acuity, we did not find evidence that indicated that the study drug provided benefit in GA. At all study time points, study eyes lost more letters on average than fellow eyes, with the greatest difference found at month 24 ($P = 0.03$). The proportions of study eyes losing >5 letters and >10 letters of visual acuity were also greater than those of fellow eyes for all time points. These differences may have been potentially confounded by the slightly better mean visual acuity in study eyes compared with fellow eyes at baseline (i.e., study eyes have "more letters to lose"). Also, the functional interaction between fellow eyes in a single patient previously described for GA[45] may have exerted a confounding influence.

Our study utilized a monocular treatment design that enrolls only participants with bilateral GA as previously described[9]; only one eye in each participant was randomly assigned to receive the study drug and compared with the untreated fellow eye in a paired analysis. This design relies on

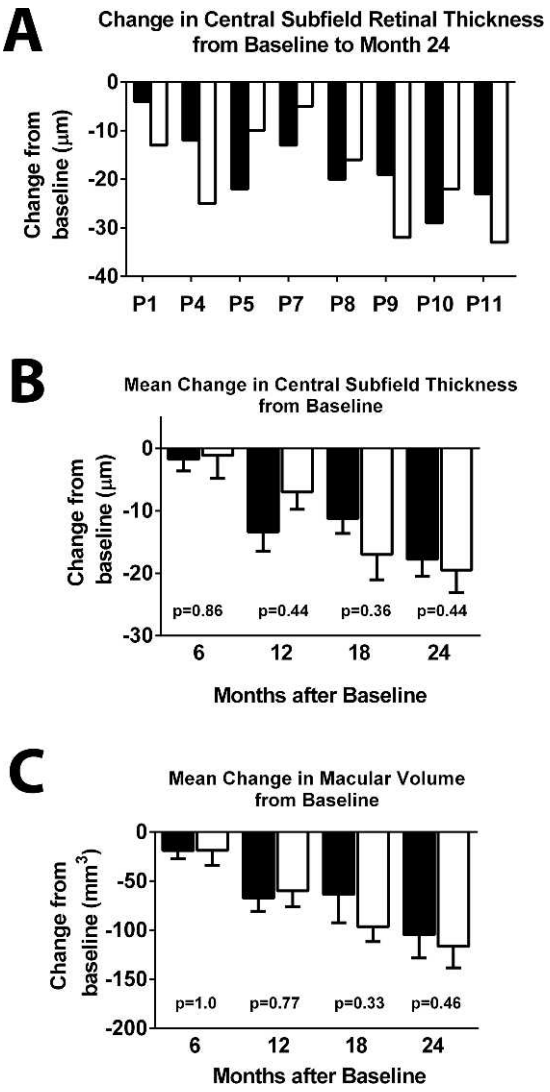

Figure 3. Change in central retinal subfield thickness as measured by SD-OCT in the study and fellow eyes from baseline for all participants completing 24 months of study follow-up ($n = 8$). (**A**) Change in central retinal subfield thickness from baseline to month 24 for each participant in study and follow eyes. (**B**) Mean change in central retinal subfield thickness from baseline at 6, 12, 18, and 24 months. (**C**) Mean change in macular volume from baseline at 6, 12, 18, and 24 months. *P* values indicate results of a two-tailed paired *t*-test.

(1) a balance in baseline features between fellow eyes, (2) the correlated nature of GA progression between fellow eyes in the natural history of disease,[35–37] and (3) an absence of "cross-over" treatment effects between eyes. The main limitation of this preliminary prospective study is the enrollment of only a small number of participants; as such, the study aims only to detect the potential presence of large treatment effects and is not sufficiently powered to allow for smaller differences in outcome measures to be statistically analyzed.

Although it is possible that the prevalence of GA may be influenced by sex,[46] we did not find consistent differences in study drug effects that segregated according to the sex of the participants (five male, three female). Sex-specific differences in sirolimus effects have been investigated in both animal[47,48] and human[49–51] studies, and may have been contributed to by differences in metabolism[48] or hormonal signaling[52,53]; however, how these effects relate to ocular immune function is unknown.

In considering the study data, the alternative hypothesis that the study drug may have had a deleterious effect on visual acuity cannot be completely ruled out. The central position of mTOR in pathways regulating transcription, cytoskeletal organization, cell proliferation, growth, and survival indicates that the effects of mTOR inhibition may be varied and potentially dose dependent.[27,54] Although the rationale for using sirolimus is related to the goal of decreasing chronic retinal inflammation, owing to the complex nature of mTOR-mediated pathways, it is possible that mTOR inhibition may induce pleiotropic effects,[26] some of which may negatively affect photoreceptor or RPE in GA patients. On one hand, there is evidence that sirolimus may have favorable effects in degenerative disease and in general aging.[55] Sirolimus has been associated with improving RPE dedifferentiation and hypertrophy and photoreceptor degeneration in a genetic mouse model of oxidative phosphorylation deficiency in RPE cells that recapitulates features of nonneovascular AMD.[56,57] In mouse models of Parkinson's disease, sirolimus administration rescued neuron cell death by inhibiting Akt phosphorylation and/or increasing autophagy.[58,59] On the other hand, mTOR inhibition through sirolimus has been associated with exacerbating the apoptotic effects of oxidative stress,[60] and augmenting CNS neuronal atrophy and cognitive decline in mouse models of Alzheimer's disease.[61,62] Relevant to the therapeutic strategy in this study, the use of sirolimus in treating ocular inflammation in a mouse model of uveitis has also demonstrated striking dose-dependent effects that have raised concern; whereas high-dose systemic sirolimus was found to attenuate ocular inflammation, low-dose rapamycin paradoxically exacerbated it, possibly by amplifying and prolonging T-cell responses.[63] In a recent study, long-term oral sirolimus in normal male mice was found to decrease visual function as measured by optokinetic tracking relative to control-fed mice (Renteria RC, et al. *IOVS* 2012;53:ARVO E-Abstract 3290). These findings, taken together, reflect the possibility that sirolimus may also have the potential to exert unfavorable effects, in addition to therapeutic ones.

Recent studies have revealed that sirolimus can exert differential inhibitory effects on two distinct protein complexes involving mTOR, mTORC1, and mTORC2, each of which mediates separate signaling pathways and functions.[64] The interpretation of the study data here potentially involves how the balances of these forms mTOR-mediated signaling may be altered in the retina. Although sirolimus can broadly inhibit mTORC1, its long-term effects on mTORC2 inhibition can vary according to cell-type.[65,66] Because RPE cells possess both mTORC1 and mTORC2 signaling complexes,[67] the net effect of sirolimus at a particular dose[68] may depend on the balance between positive effects on inhibiting RPE dedifferentiation[56] and senescence[67] mediated by mTORC1 inhibition and possible negative effects of altered glucose utilization[64] mediated by mTORC2 inhibition. Future studies examining the roles of mTORC1 and mTORC2 signaling in retinal cells would be instructive in this regard.

In conclusion, our results indicate that whereas subconjunctival sirolimus was well tolerated by study participants with GA, it was not associated with any detectable functional benefit, as measured by visual acuity and microperimetry, or with a deceleration of anatomic disease progression, as measured by GA lesion area or central retinal thickness. Whether this lack of beneficial effect is due to a paradoxical unfavorable effect of the drug, or to insufficient drug concentrations reaching the necessary areas of the retina/choroid complex cannot be determined from this study. Studies involving intravitreally delivered sirolimus that produce higher drug levels within the retina[34,69] are currently ongoing for the treatment of GA (NCT01445548, NCT 01675947).

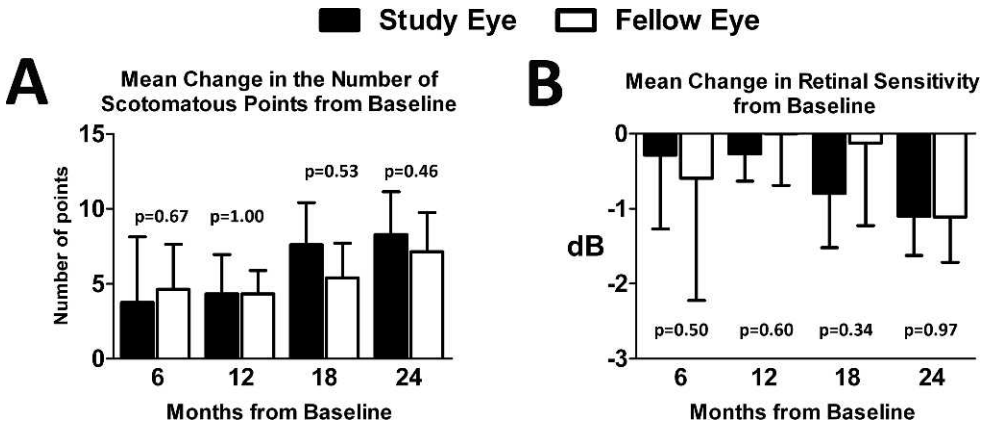

FIGURE 4. Change in microperimetry test parameters in the study and fellow eyes from baseline in all participants completing study follow-up ($n = 8$). (**A**) Mean change in the number of scotomatous points from baseline at 6, 12, 18, and 24 months. (**B**) Mean change in retinal sensitivity of nonscotomatous points from baseline at 6, 12, 18, and 24 months. All comparisons between study and fellow eyes at each time point were nonsignificant ($P > 0.05$, as computed by a two-tailed paired *t*-test).

Alternatively, it is possible that the immune etiology of GA may be more related with the initiation of GA than with its progression. As a result, immunosuppressive therapeutic strategies imposed at a later stage of the disease may not successfully arrest or alter disease course. The data in the current study are therefore relevant for further evaluation of ongoing studies involving sirolimus for GA and for uveitis (NCT00908466) in particular, and for the general approach toward immunosuppression as a therapeutic strategy in GA.

Acknowledgments

Supported by the Intramural Research Program of the National Eye Institute, National Institutes of Health.

Disclosure: **W.T. Wong**, None; **S. Dresner**, None; **F. Forooghian**, None; **T. Glaser**, None; **L. Doss**, None; **M. Zhou**, None; **D. Cunningham**, None; **K. Shimel**, None; **M. Harrington**, None; **K. Hammel**, None; **C.A. Cukras**, None; **F.L. Ferris**, None; **E.Y. Chew**, None

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Investigative Ophthalmology & Visual Science

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