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Impact of Prophylactic Ranibizumab to Prevent Neovascular Age-Related Macular Degeneration on Eyes With Intermediate Age-Related Macular Degeneration

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Purpose: The purpose of this study was to determine the impact of prophylactic ranibizumab (PR) injections given every 3 months in eyes with intermediate nonexudative age-related macular degeneration (AMD) on drusen volume, macular layer thicknesses, and progression of geographic atrophy (GA) area over 24 months in the PREVENT trial.

Methods: This post hoc analysis of the prospective PREVENT trial compared eyes with intermediate AMD randomized to PR versus sham injections to determine rates of conversion to neovascular AMD over 24 months. Drusen area and volume, macular thickness and volume, and retinal layer thicknesses were measured on spectral-domain optical coherence tomography images and analyzed. Masked grading of GA area and subretinal drusenoid deposits (SDDs) using fundus autofluorescence images was performed.

Results: There were no statistical differences in drusen area and volumes between groups, and similar reductions in central subfield thickness, mean cube thickness, cube volume, and retinal sublayer thickness from baseline to 24 months ($P = 0.018$ to < 0.001), with no statistical differences between groups in any of these anatomic parameters. These findings were not impacted by the presence or absence of SDD. Among the 9 eyes with GA in this study, mean GA growth rate from baseline to 24 months was $1.34 +/- 0.79$ mm^2/year after PR and $1.95 +/- 1.73$ mm^2/year in sham-treated eyes ($P = 0.49$), and similarly showed no statistical difference with square root transformation ($P = 0.61$).

Conclusions: Prophylactic ranibizumab given every 3 months did not appear to affect drusen volume, macular thinning, or GA progression in eyes with intermediate AMD.

Translational Relevance: This work investigates the impact of PR on progressive retinal degeneration in a clinical trial.

Introduction

Age-related macular degeneration (AMD), a leading cause of severe vision loss among individuals over 50 years of age in the developed world, is divided broadly into 2 types: non-neovascular or nonexudative AMD and neovascular or exudative AMD (nAMD).[1–7] Although nAMD constitutes less than 20% of AMD, it is responsible for a large proportion

of eyes with severe vision deficit associated with AMD. However, the non-neovascular form of AMD, which constitutes the majority of eyes with AMD, also leads to progressive vision loss, albeit in a much more gradual manner in comparison to nAMD. There are multiple stages associated with non-neovascular AMD, including early, intermediate, and advanced.[8] According to the Beckman Initiative for Macular Research Classification Committee, early AMD consists of eyes with a few medium drusen ($\geq$63 μm and <125 μm) and no pigmentary abnormalities, intermediate AMD consists of eyes with one or more large drusen ($\geq$125 μm) and/or pigmentary abnormalities with medium drusen, and advanced or late nonexudative AMD consists of eyes with geographic atrophy (GA).[8] Multiple studies have shown the correlation of progressive reduction in macular thickness and increased macular atrophy with the evolution of AMD from an earlier stage to a later stage, in the passage of time.[9–14] In recent years, rapid advancement of optical coherence tomography (OCT) technology and segmentation software have led to vastly improved resolution of in vivo imaging of retinal layers, correlating well with the actual histological anatomy.[15,16] Such studies have correlated the initial reduction in retinal thickness with loss of outer retinal layers consisting of the photoreceptor outer nuclear layer (ONL) and Henle fiber layers (HFLs), as well as loss of inner retinal layers in the passage of time.

PREVENT was a multicenter, prospective, randomized, controlled, participant-masked phase I/II comparative trial, in which eyes with intermediate AMD in patients with nAMD in contralateral eyes diagnosed within 5 years were randomized (1:1) to 0.5 mg ranibizumab versus sham injections given every 3 months for 24 months.[17] PREVENT's primary outcomes demonstrated no difference in the rate of neovascular conversion between the ranibizumab treatment and sham group, which was approximately 13% in both study arms. A previous report has shown increased GA in eyes after receiving photodynamic therapy followed by anti-VEGF injections for 5 years.[18] Similarly, secondary analyses of multiple prior pivotal multicenter clinical trials have also shown progressive development of macular atrophy after receiving anti-VEGF therapy over an extended period of time.[19–23] Thus, the objective of the current study is to compare changes in, drusen, macular dimensions, and GA between ranibizumab- and sham-treated eyes in the PREVENT trial over 24 months.

Methods

This trial was conducted in accordance with the Health Insurance Portability and Accountability Act,

and also adhered to the tenets of the Declaration of Helsinki. Institutional review approval was obtained through Western Institutional Review Board (Puyallup, WA). Written informed consent was obtained from all study participants. Details of the inclusion and exclusion criteria are outlined in detail in the primary manuscript published recently.[17] The study protocol is also available as an online supplement. In brief, participants who were at least 50 years of age with established nAMD in the contralateral, non-study eye and presented with intermediate nonexudative AMD in the study eye were enrolled for this clinical trial. Study participants underwent: (1) standardized, Early Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) testing at 4 meters, (2) slit-lamp biomicroscopy and indirect ophthalmoscopy, (3) spectral-domain (SD) OCT (Cirrus, Carl Zeiss Meditec, Inc., Dublin, CA), (3) color fundus photography (Topcon TRC-50IX; Tokyo, Japan, at 2 centers, Topcon TRC-50EX; Tokyo, Japan at 1 center, and FF4; Carl Zeiss Meditec, Inc., Dublin, CA at 1 center), and (4) fundus autofluorescence (FAF) and fluorescein angiography (Heidelberg HRA, Heidelberg, Germany) at baseline and every 3 months through 24 months.

For this post hoc analysis, automated algorithms from Zeiss Cirrus review software were utilized to measure macular and drusen dimensions. Data were then exported and automated algorithms from Voxeleron Orion software (Austin, TX) were utilized to segment and quantify individual retinal layers and to measure the full thickness of the macula. The accuracy of the segmentation results was manually confirmed.

SD-OCT Segmentation Methodology

Zeiss Cirrus HD-OCT 512 × 128 macular cubes images were reviewed using Cirrus review software (Carl Zeiss Meditec, Inc., Dublin, CA) version 11.5.[24] Scans were reviewed for signal strength and excluded if below 7. The internal limiting membrane (ILM) and retinal pigment epithelium (RPE) segmentations were confirmed for accuracy. Macular thickness parameters including central subfield thickness (CST), mean cube thickness (MCT), and cube volume (CV) were recorded. Advanced RPE analysis segmentations of "Bruch's membrane" were confirmed for accuracy, and RPE elevation area and volume measurements were recorded, corresponding to drusen area and volume, respectively. Motion corrected cube data were exported for IMG analysis. Orion (Voxeleron, Austin, TX) version 3.1 was used to import IMG data,[25,26] and to perform sublayer segmentations. Segmentations from ILM, inner nuclear layer (INL), and RPE were reviewed for accuracy (Fig. 1). The INL segmenta-
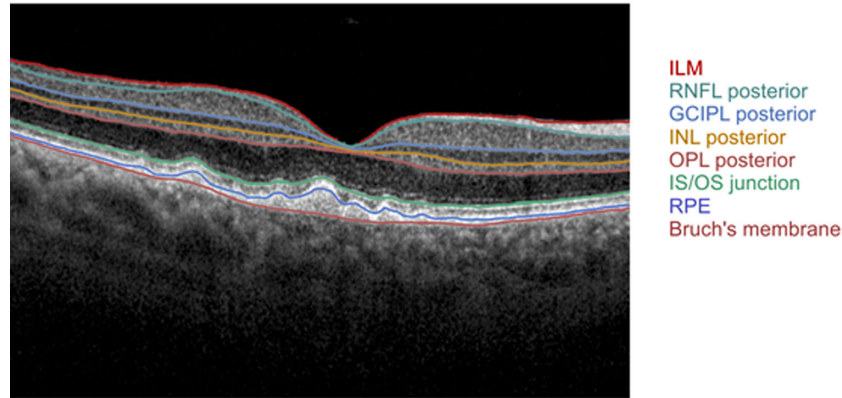
Figure 1. Example of retinal sublayer segmentation provided by the VOXELERON Orion software across the central 1-mm, 3-mm, and 6-mm subfields. Notice that segmentation measurement of the outer retina included the synaptic outer plexiform layer, Henle's fiber layer (HFL), outer nuclear layer (ONL), and the photoreceptor inner and outer segments.

tion was confirmed on analyzed scans to identify the INL/outer plexiform layer (OPL) border, thus defining the outer retinal layers (ORLs) as containing the synaptic OPL, HFL, the ONL, and photoreceptor inner and outer segments. The OPL and ONL segmentations were reviewed but their analysis as distinct layers were rejected because of variability of the associated HFL segmentation in off-axis scans and overlying drusen.[27] Instead, synaptic OPL, HFL, ONL, and inner/outer photoreceptor segments were grouped together for reliable assessment of the ORL thickness. Thus, analysis included total retinal thickness (TRT), inner retinal layer (IRL; ILM to junction of INL with synaptic OPL), and ORL within the 1-mm, 3-mm, and 6-mm circles.

SD-OCT segmentation was performed on all patients with the exception of the 14 eyes in 14 patients who developed neovascular conversion, and the 18 patients who did not complete the studies, in order to avoid their confounding effects on the analysis. In addition, regarding Zeiss Cirrus segmentation, 7.2% of the segmented scans (58 out of 807 scans) were excluded from analysis due to their signal strength index (SSI) below 7. Regarding Voxeleron segmentation, two scans corresponding to two patients were excluded from analysis due to segmentation errors that could not be adequately corrected.

Grading of AMD Severity

The 9-step Age-Related Eye Disease Study (AREDS) severity scale was graded from digital color fundus photographs (CFPs) obtained using various fundus camera platforms, as outlined above, with images calibrated using the standard disc diameter of 1,800 microns (µm).[28] Images were graded by two independent masked, trained graders at the University of California, Davis Reading Center, with discrep-

ancies adjudicated by a senior image grader (author G.Y.). Non-neovascular or atrophic AMD features, including drusen size, type, and area, as well as hyper- and hypopigmentation were assessed to calculate the detailed 9-step AMD severity scale as described in the AREDS2 study.[29]

Measurement of GA Characteristics and Grading of Subretinal Drusenoid Deposits

Geographic atrophy (GA) areas were measured from 30 degrees × 30 degrees FAF images captured using Spectralis OCT + SLO devices (Heidelberg Engineering, Heidelberg, Germany). Images were graded by two independent masked, trained graders at the University of California, Davis Reading Center. GA was defined as sharply demarcated, homogenously dark region(s), larger than 433 µm in diameter and not including areas of peripapillary atrophy.[30,31] GA lesions were classified as either single or multifocal, as foveal or non-foveal, and in terms of autofluorescence pattern, including (1) none, (2) focal, (3) banded, or (4) diffuse as previously described.[32–34] GA areas were measured by manual tracing by two graders using the Heidelberg Eye Explorer software (version 1.9.13.0), summed to a single value in cases with multifocal GA, with or without square root transformation of the GA area.[35] The presence of subretinal drusenoid deposits (SDDs; or reticular pseudodrusen), as seen on FAF images, was defined as clusters of five or more discrete round lesions of hypoautofluorescence, often similar in size and with a confluent ribbon-like pattern.[36,37] Infrared reflective and SD-OCT images were used when available to help confirm the presence of SDDs. GA areas were reported as the mean of the values recorded by the two graders, whereas discrepancies for grading of other GA characteristics or SDD

presence were adjudicated by a senior image grader (author G.Y.). Images where poor quality precluded grading of GA characteristics or SDDs were labeled as ungradable and excluded from analyses.

All of the image graders were masked to patient's identity, patient's demographics, and treatment arm. Each patient was assigned a unique alpha numeric code. To allow proper tracking of the numerous images and to avoid confusion and errors on image processing for assessment between the study sites and reading sites, an image date was attached to each image, without specifying the visit number.

Statistical Methods

Within-group change from baseline to 24 months was analyzed with the Wilcoxon rank sum test.[38] Between-group change from baseline to 24 months was analyzed with analysis of covariance including adjustment for baseline level.[39] Outlying values were truncated at three standard deviations from the overall mean. The *P* values less than 0.05 were considered significant. Given these secondary analyses were hypothesis generating, they were carried out without adjustment for multiplicity; conclusions will require confirmation in future, prospective studies. Analyses were performed using R software.[40]

Results

Baseline AMD Severity Score

Table 1A outlines the baseline AMD 9-step Severity Scale. The median AREDS Severity Scale level for eyes receiving intravitreal ranibizumab (IVR) was 7, and the

Table 1A. Baseline AREDS Severity Level

AREDS Severity Level, No. (%)	IVR [No (%)]	SHAM [No (%)]
0	1 (2)	1 (2)
1	1 (2)	0
2	2 (5)	2 (5)
3	1 (2)	0
4	2 (5)	2 (5)
5	3 (7)	4 (9)
6	5 (11)	9 (21)
7	10 (23)	10 (23)
8	18 (41)	14 (33)
9	1 (2)	1 (2)

AREDS, Age-related Eye Disease Study; IVR, intravitreal ranibizumab; no, number; SHAM, sham injection.

Table 1B. Baseline Subretinal Drusenoid Deposits Subretinal Drusenoid Deposits, no. (%)

Absent	22 (52)	30 (71)
Present	18 (43)	10 (24)
Uncertain	2 (5)	2 (5)

mode was 8. In comparison, the median AMD severity scale level for eyes receiving sham injections (sham) was also 7, and the mode was also 8. Therefore, there was balance in the baseline AREDS Severity Scale levels between groups.

Baseline Subretinal Drusenoid Deposits

The majority of each study group was found to have SDDs (Table 1B), with 22 (52%) of IVR eyes versus 30 (71%) of SHAM eyes without SDD, 18 (43%) of IVR eyes versus 10 (24%) of SHAM eyes with SDD. There was uncertainty in the presence or absence of SDD in two (5%) of the eyes in each group (see Table 1B).

Baseline Central Subfield Thickness, Mean Cube Thickness/Volume, Drusen Area/Volume

Baseline comparisons showed no statistical difference in mean CST (262 ± 36 μm vs. 250 ± 25 μm), MCT (273 ± 18 μm vs. 272 ± 17 μm), and CV (9.84 ± 0.66 mm^3 vs. 9.81 ± 0.63 mm^3), median drusen area (interquartile range [IQR]) (0.2, IQR = 0.022 to 0.625 mm^2 vs. 0.2, IQR = 0.022 to 0.625)mm^2, median drusen volume (IQR) (0.01, IQR = 0.000 to 0.022 mm^3 vs. 0.01, IQR = 0.000 to 0.022)mm^3 (Table 2).

Comparison of Results at 24 Months with Baseline and Between Groups (ZEISS Cirrus SD-OCT Assessment)

Table 3 summarizes the results at 24 months in comparison to baseline, utilizing the ZEISS Cirrus Review software. For both study groups, there were minimal differences in drusen area and volume at 24 months in comparison to baseline ($P = 0.046$ for IVR and 0.54 for SHAM in the drusen area; $P = 0.052$ for IVR and 0.32 for SHAM in the drusen volume; Figs. 2A, 2B). In contrast, there were significant reductions in central subfield thickness ($P = 0.006$ for IVR and 0.018 for SHAM), mean cube thickness ($P < 0.001$ for IVR and $= 0.001$ for SHAM), cube volume ($P < 0.001$ for IVR and $= 0.003$ for SHAM),

Table 2. Comparison of Baseline Variables Between Groups (Zeiss, Cirrus Assessment)

Variable	IVR ($n = 44$)	SHAM ($n = 44$)
Central subfield thickness, mean (SD), μm	262 (36)	250 (25)
Mean cube thickness, mean (SD), μm	273 (18)	272 (17)
Cube volume, mean (SD), mm^3	9.84 (0.66)	9.81 (0.63)
Drusen area, median (IQR), mm^2	0.200 (0.022 to 0.625) [$n = 36$]	0.200 (0.022 to 0.625) [$n = 36$]
Drusen volume, median (IQR), mm^3	0.010 (0.000 to 0.022) [$n = 36$]	0.010 (0.000 to 0.022) [$n = 36$]

IQR, interquartile range; IVR, intravitreal ranibizumab; SD, standard deviation; SHAM, sham injections.

Table 3. Comparison of Results at 24 Months With Baseline, and Between Groups (Zeiss, Cirrus)

Variable	IVR ($n = 31$)		SHAM ($n = 35$)		Adjusted Difference (95% CI)[‡]	P Value[‡]
	Mean (SD)	P Value[†]	Mean (SD)	P Value[†]		
Central subfield thickness, μm	−5 (9)	0.006	−8 (16)	0.018	3 (−4 to 10)	0.42
Mean cube thickness, μm	−5 (6)	<0.001	−3 (5)	0.001	−1 (−4 to 1)	0.32
Cube volume, mm^3	−0.17 (0.22)	<0.001	−0.10 (0.20)	0.003	−0.06 (−0.16 to 0.05)	0.28
Drusen area, mm[*]	0.092 (0.277) [n=30]	0.046	0.024 (0.354)	0.54	0.046 (−0.104 to 0.195)	0.55
Drusen volume, mm[*]	0.037 (0.109) [n=30]	0.052	0.014 (0.108)	0.32	0.017 (−0.034 to 0.069)	0.51

CI, confidence interval; IVR, intravitreal ranibizumab; SD, standard deviation; SHAM, sham injections.

[*]To satisfy the assumption of normality for statistical analysis, the square root transformation was applied to drusen area (original units = square mm) and the cube root transformation was applied to RPE volume (original units = cubic mm).

[†]Within-group *P* value testing the null hypothesis of no change from baseline (Wilcoxon rank sum test).

[‡]Between-group *P* value testing the null hypothesis of no difference between groups adjusted for baseline level (analysis of covariance).

when comparing the results at 24 months with baseline (Figs. 3A, 3B, 3C). Despite the above results, there were no differences of the results between the two study groups in any of the variables outlined above (*P* values ranging from 0.28 to 0.55; see Table 3). Due to the potential confounding effects, eyes that developed neovascular conversion and eyes that did not complete the study were eliminated from the analysis.
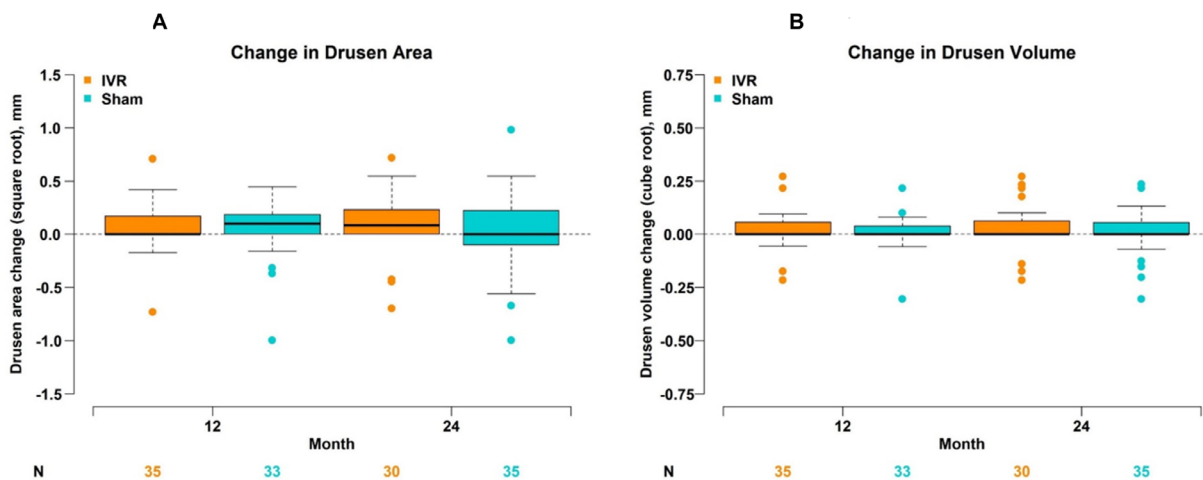

Figure 2. (**A, B**) There were minimal differences in the drusen area and volume from baseline to 24 months (*P* = 0.046 for IVR and 0.54 for SHAM in the drusen area; *P* = 0.052 for IVR and *P* = 0.32 for SHAM in the drusen volume).
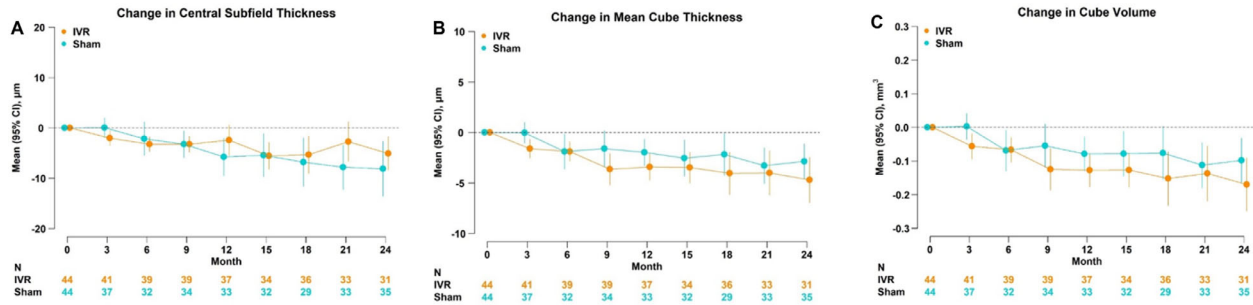
Figure 3. (A, B, C) There were significant reductions in the central subfield thickness ($P = 0.006$ for IVR and $P = 0.018$ for SHAM), mean cube thickness ($P < 0.001$ for IVR and $P = 0.001$ for SHAM), cube volume ($P < 0.001$ for IVR and $P = 0.003$ for SHAM from baseline to 24 months).

Table 4. Comparison of Baseline Variables Between Groups (Voxeleron, Orion Assessment)

Variable, Mean in Microns (SD), $mm^3 \times 10^3$	IVR ($n = 34$)	Sham ($n = 34$)
TRT 1 mm circle	203 (25)	197 (19)
TRT 3 mm circle	2148 (176)	2133 (154)
TRT 6 mm circle	7884 (532)	7952 (499)
IRL 1 mm circle[*]	105 (16)	102 (12)
IRL 3 mm circle[*]	1350 (121)	1358 (89)
IRL 6 mm circle[*]	5098 (385)	5186 (306)
ORL 1 mm circle[†]	98 (12)	95 (13)
ORL 3 mm circle[†]	798 (71)	775 (70)
ORL 6 mm circle[†]	2786 (204)	2766 (213)

[*]IRL, inner retinal layers (internal limiting membrane to junction of inner nuclear layer with synaptic outer plexiform layer); IVR, intravitreal ranibizumab.

[†]ORL, outer retinal layers (synaptic outer plexiform layer + Henle fiber layer + outer nuclear layer + inner and outer segments of photoreceptors); SD, standard deviation; TRT, total retinal thickness.

Comparison of Baseline Variables With Full-Thickness and Sublayer Segmentation (Voxeleron, Orion)

The Orion Software of Voxeleron was also utilized for both retinal full-thickness and sublayer segmentation. Table 4 summarizes the comparison of the multiple baseline variables on the Orion-generated OCT data (1-, 3-, and 6-mm subfields of TRT, IRL, and ORL. As shown in Table 4, the baseline variables were balanced between groups.

Comparison of Full-Thickness and Sublayer Segmentation at 24 Months With Baseline and Between Group Analysis (Voxeleron, Orion)

Consistent with the results generated by the Zeiss Cirrus Review software, the Orion Software also showed substantial reduction of TRT when comparing results at 24 months with baseline for both the IVR as well as the SHAM study groups (Table 5). Regarding TRT, the P values were 0.009, <0.001, and 0.002 for central 1-mm, 3-mm, and 6-mm subfield, respectively, of the IVR group, and the P values were 0.070, <0.001, and <0.001 for central 1-mm, 3-mm, and 6-mm subfield, for the SHAM group. Regarding IRLs, the P values were 0.85, 0.002, and 0.008 for the central 1-mm, 3-mm, and 6-mm subfield, respectively, of the IVR group, and 0.45, <0.001, and <0.001 for the central 1-mm, 3-mm, and 6-mm subfield, respectively, of the SHAM group. Regarding ORL, the P values were <0.001, <0.001, and 0.002 for central 1-mm, 3-mm, and 6-mm subfield, respectively, of the IVR group, and 0.031, 0.001, and 0.009 for central 1-mm, 3-mm, and 6-mm subfield, respectively, of the SHAM group.

Changes of Subretinal Drusenoid Deposits From Baseline to 24 Months

At baseline, there were significantly thinner MCT and MCV in eyes with SDD than eyes without SDD (Table 6). There were no differences in CST and drusen dimensions between eyes with and eyes without SDD at baseline.

Regarding changes in drusen sizes, there were no substantial changes in drusen area and drusen volume from baseline to 24 months for both eyes with SDD (20) and eyes without SDD (34), ($P = 0.66$ and 0.556, and $P = 0.189$ and 0.069, respectively). There were also no differences between groups for drusen area and drusen volume ($P = 0.838$ and $P = 0.816$, respectively). In contrast, there were substantial reductions in CST, mean cube thickness, and cube volume from baseline to 24 months for eyes with SDD and eyes without SDD ($P = 0.024$, 0.003, and 0.003, and $P = 0.043$, <0.001, and 0.001, respectively) (Table 7). For comparison between groups, there was a trend of greater numerical reduction in mean CST, mean cube thickness, and cube

Table 5. Change From Baseline to 24 Months (Voxeleron, Orion)

Variable [Units]	IVR (n = 29)		Sham (n = 34)		Adjusted Difference (95% CI)[†]	P Value[†]
	Mean (SD)	P Value[*]	Mean (SD)	P Value[*]		
Total foveal subfield thickness [μm]	−5 (10)	**0.009**	−4 (17)	0.07	−2 (−9 to 6)	0.65
Total retinal thickness 1 mm circle [mm³ × 10⁻³]	−3.8 (7.6)	**0.009**	−3.0 (13.2)	0.07	−1.3 (−7.2 to 4.5)	0.65
Total retinal thickness 3 mm circle [mm³ × 10⁻³]	−49.8 (58.9)	**<0.001**	−42.7 (65.8)	**<0.001**	−9.0 (−42.4 to 24.4)	0.59
Total retinal thickness 6 mm circle [mm³ × 10⁻³]	−115.3 (215.8)	**0.002**	−100.9 (154.2)	**<0.001**	−11.5 (−106.8 to 83.8)	0.81
Inner retinal layers 1 mm circle [mm³ × 10⁻³][‡]	0.4 (7.5)	0.85	−0.7 (12.5)	0.45	0.9 (−4.6 to 6.4)	0.75
Inner retinal layers 3 mm circle [mm³ × 10⁻³][‡]	−27.3 (45.7)	**0.002**	−27.3 (52.3)	**<0.001**	−2.4 (−28.3 to 23.4)	0.85
Inner retinal layers 6 mm circle [mm³ × 10⁻³][‡]	−65.7 (166.6)	**0.008**	−63.7 (110.4)	**<0.001**	−3.5 (−74.8 to 67.7)	0.92
Outer retinal layers 1 mm circle [mm³ × 10⁻³][§]	−4.2 (5.6)	**<0.001**	−2.2 (8.5)	**0.031**	−0.3 (−3.9 to 3.3)	0.85
Outer retinal layers 3 mm circle [mm³ × 10⁻³][§]	−22.5 (22.3)	**<0.001**	−14.0 (25.8)	**0.001**	−4.4 (−17.1 to 8.2)	0.49
Outer retinal layers 6 mm circle [mm³ × 10⁻³][§]	−45.7 (67.0)	**0.002**	−37.0 (75.9)	**0.009**	0.8 (−34.1 to 35.6)	0.96

CI, confidence interval; IVR, intravitreal ranibizumab; SD, standard deviation.

[*]Within-group P value testing the null hypothesis of no change from baseline (Wilcoxon rank sum test).

[†]Between-group mean difference and P value testing the null hypothesis of no difference between groups adjusted for baseline level (analysis of covariance).

Significant P values highlighted.

[‡]Internal limiting membrane to junction of inner nuclear layer with synaptic outer plexiform layer.

[§]Synaptic outer plexiform layer + Henle fiber layer + outer nuclear layer + inner and outer segments of photoreceptors.

Table 6. Eyes With Subretinal Drusenoid Deposits Versus Eyes Without Subretinal Drusenoid Deposits at Baseline (Zeiss, Cirrus)

Variable	No SDD (n = 39)	SDD (n = 25)	P Value[*]
Central subfield thickness, mean (SD), μm	258.923 (30.912)	253.960 (35.732)	0.536
Cube thickness, mean (SD), μm	276.897 (17.578)	268.400 (16.199)	**0.024**
Cube volume, mean (SD), mm³	9.972 (0.635)	9.656 (0.586)	**0.022**
Drusen area, median (IQR), mm²	0.200 (0.000 to 0.575) [n = 38]	0.650 (0.100 to 1.650) [n = 20]	0.114
Drusen volume, median (IQR), mm³	0.010 (0.000 to 0.020) [n = 38]	0.020 (0.000 to 0.062) [n = 20]	0.184

IQR, interquartile range; SDD, subretinal drusenoid deposits; SD, standard deviation.

[*]P value of between group comparison.

volume for eyes with SDD than without SDD, given the positive point estimate of the adjusted differences between groups on all three parameters; however, they did not reach statistical significance ($P = 0.526$, 0.677, and 0.502, respectively).

Geographic Atrophy Area and Growth Rate

There were nine eyes with GA in this study. Six of these eyes had GA at baseline (4 in IVR and 2 in SHAM). GA growth rates over 24 months were

Table 7. Comparison of Changes in Macular Dimensions From Baseline to 24 Months for Eyes With and Without Subretinal Drusenoid Deposits

Variable	No SDD ($n = 34$)		SDD ($n = 20$)		Adjusted Difference	
	Mean (SD)	P Value[†]	Mean (SD)	P Value[†]	(95% CI)[‡]	P Value[‡]
Central subfield thickness, μm	−4.351 (−4.351)	**0.043**	−6.500 (−6.500)	**0.024**	2.148 (−4.610 to 8.905)	0.526
Mean cube thickness, μm	−4.221 (−4.221)	**<0.001**	−4.150 (−4.150)	**0.003**	0.715 (−2.717 to 4.148)	0.677
Cube volume, mm³	−0.143 (−0.143)	**0.001**	−0.155 (−0.155)	**0.003**	0.042 (−0.082 to 0.165)	0.502
Drusen area, mm*	0.054 (0.054)	0.189	0.024 (0.024) [$n = 19$]	0.66	−0.019 (−0.206 to 0.168)	0.838
Drusen volume, mm*	0.030 (0.030)	0.069	0.013 (0.013) [$n = 19$]	0.556	0.007 (−0.055 to 0.069)	0.816

CI, confidence interval; SDD, subretinal drusenoid deposits; SD, standard deviation.

*To satisfy the assumption of normality for statistical analysis, the square root transformation was applied to drusen area (original units = square mm) and the cube root transformation was applied to drusen volume (original units = cubic mm).

[†]Within-group P value testing the null hypothesis of no change from baseline (Wilcoxon rank sum test).

[‡]Between-group P value testing the null hypothesis of no difference between groups adjusted for baseline level (analysis of covariance).

Significant P values highlighted.

1.34 ± 0.79 mm²/year for ranibizumab-treated and 1.95 ± 1.73 mm²/year for sham treated eyes, with no significant difference between the 2 groups (difference = −0.62, 95% confidence interval [CI] = −4.45 to 3.21, $P = 0.61$; Figs. 4A, 4B). Square root transformation of the GA growth rate was $0.47 +/− 0.32$ mm/year for IVR, and $0.59 +/− 0.30$ mm/year for SHAM, with no statistical difference between the two groups (difference = −0.12, 95% CI = −0.71 to 0.47, $P =$ 0.61; see Fig. 4B). When the three eyes that developed GA after baseline were eliminated from assessment, there remained no significant difference in growth rates between the two groups (baseline GA area = 0.48 ± 0.12 mm² in IVR and 1.13 ± 0.80 mm² in SHAM: GA growth rates over 24 months = 1.36 ± 0.76 mm²/year for IVR and 2.74 ± 1.52 mm²/year for SHAM [difference = −1.37, 95% CI = −10.42 to 7.68, $P = 0.41$]; square root transformation: baseline GA was

A Growth Rate of in Surface Area of Geographic Atrophy (mm2) Per Year:

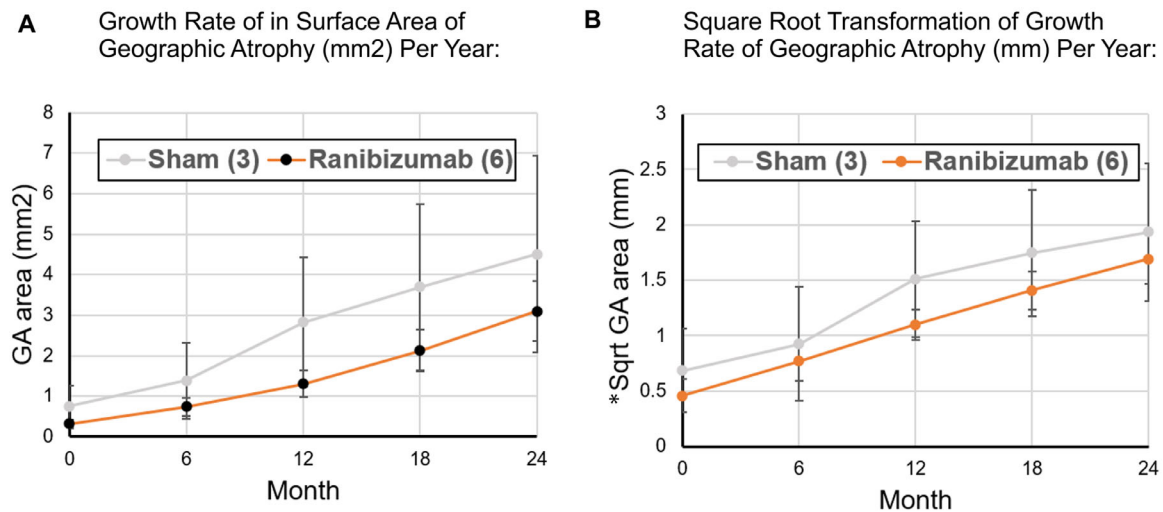**B** Square Root Transformation of Growth Rate of Geographic Atrophy (mm) Per Year:

Figure 4. (**A**) This plot depicts the growth rates of geographic atrophy in surface area from baseline to 24 months associated of eyes receiving ranibizumab (IVR) and eyes receiving sham injections (SHAM). There was no significant difference in growth rate between the two groups. (**B**) Likewise, the square root transformation of the growth rates of the two study groups shows no significant difference in GA growth rate between the two groups. *Sqrt, square root transformation.

0.69 ± 0.09 mm with IVR and 1.03 ± 0.39 mm with SHAM, and GA growth rate was 0.55 ± 0.18 mm/year for IVR, and 0.75 ± 0.18 mm/year for SHAM [difference = −0.20, 95% CI = −0.86 to 0.46, $P = 0.33$]).

Early Termination

The 18 patients who discontinued early from the study were evenly distributed between both study groups, and in no instance was their early termination related to vision loss. Eleven of them withdrew consent during the first 6 months due to personal choice unrelated to their vision status, 1 relocated to another state, 3 withdrew due to a systemic illness, and 3 died before the end of study. None of the 18 patients except one were found to have GA at baseline and at the time of termination, and the reason for early termination for that one patient was death at 21 months.

Selected Case Reports

Case 1: Ranibizumab Injection Eye

This 73-year-old man with a history of neovascular AMD in his left eye and multiple large and intermediate drusen in his right eye presented with an ETDRS BCVA of the right eye = 20/20, and the left eye = 20/80 (Figs. 5A, 5B). He was enrolled in the ranibizumab injection group in the PREVENT trial for his right eye. Figure 6 shows segmentation of his SD-OCT images at baseline (A) as well as at 24 months (B), respectively. At 24 months, the ETDRS BCVA was 20/25 in the right eye. There was a 3.9% reduction in macular volume from baseline to 24 months.

Case 2: Sham Injection Eye

An 81-year-old woman presented with neovascular AMD in her left eye, and multiple drusen and pigmentary changes consistent with intermediate age-related macular degeneration in her right eye (see Figs. 6A, 6B). Her ETDRS BCVA for the right eye was 20/50 and for the left eye: counting-fingers at 2 feet. She was randomized to the sham injection group. At 24 months, her ETDRS BCVA was 20/60 in the right eye. Figure 7 shows the segmentation of the SD-OCT images at baseline (A) and at 24 months (B), respectively. There was a 7.4% reduction in the macular volume.
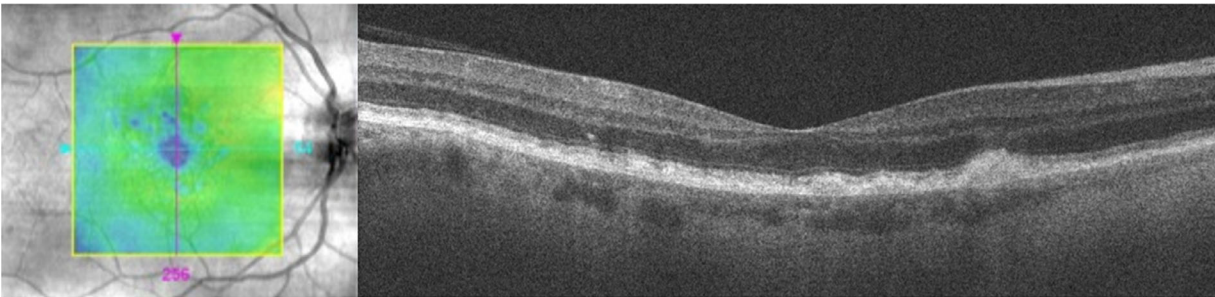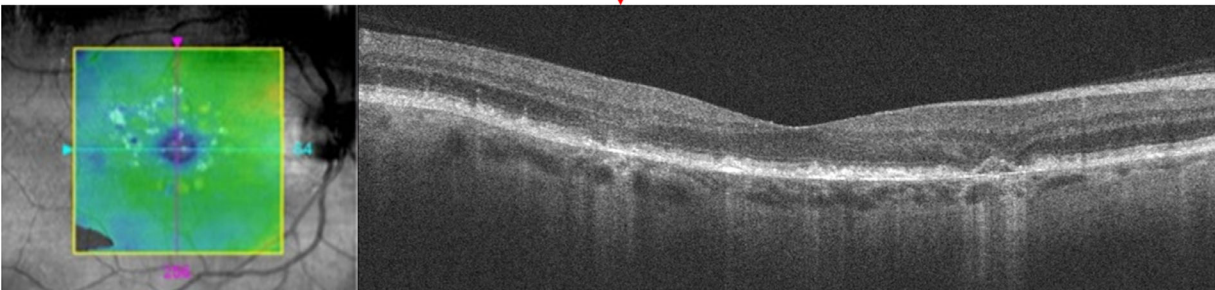

Figure 5. (**A, B**) This 73-year-old man with neovascular age-related macular degeneration (AMD) in his left eye and intermediate AMD in his right eye was enrolled in the ranibizumab injection group for his right eye (**A**). His baseline Early Treatment of Diabetic Retinopathy best-corrected visual acuity (ETDRS BCVA) was right eye = 20/20, and left eye = 20/80 at baseline. He received ranibizumab injections in his right eye every 3 months until 24 months (**B**). There was a 3.9% reduction in macular volume from baseline to 24 months associated with ETDRS BCVA of 20/25.
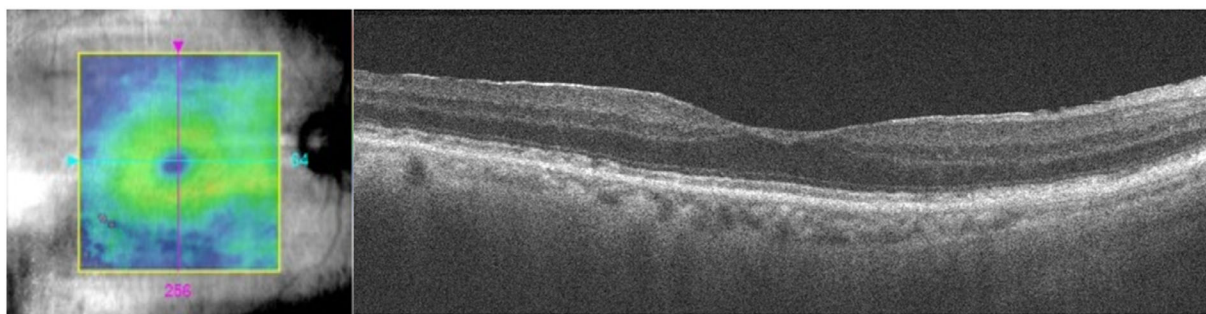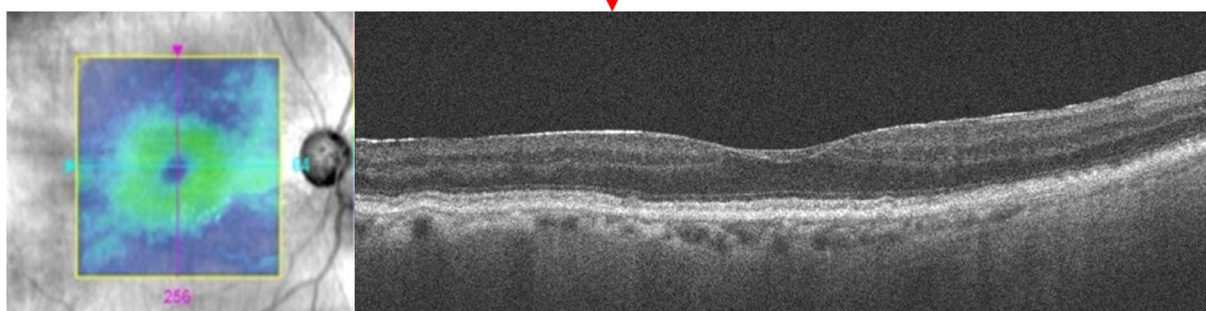
A (Baseline):

B (24 months):

Figure 6. (**A, B**) An 81-year-old woman with neovascular age-related macular degeneration (AMD) in her left eye, and intermediate AMD in her right eye was randomized to the sham injection group for her right eye. At baseline, her Early Treatment of Diabetic Retinopathy best-corrected visual acuity (ETDRS BCVA) was right eye = 20/50 and right eye (**A**). The ETDRS BCVA was 20/60 with a 7.4% reduction in macular volume at 24 months (**B**).

Discussion

In this post hoc analysis of the PREVENT trial, we found no statistical differences on drusen area and volume between the 2 study groups, despite borderline increase in these parameters over 24 months for PR-treated eyes ($P = 0.046$ and $P = 0.052$, respectively).

Regarding macular thickness and volume, prior studies have shown progressive reduction in these parameters in eyes with intermediate AMD in the passage of time. Lamin et al. reported longitudinal reduction in total retinal volume (TRV) from baseline to year 2. In the same study, retinal sublayer analysis showed progressive decrease in volume of the inner retinal layers (ganglion cell layer, inner plexiform layer, and inner nuclear layer), as well as the outer nuclear layer, outer plexiform layer, and drusen volume ($P = 0.033$ to 0.000, respectively).[13] In a longitudinal prospective study by Chiang et al. with 143 participants, 27 had unilateral intermediate AMD (group 1), 61 had either bilateral intermediate AMD or unilateral intermediate AMD with contralateral late AMD (group 2), and 21 eyes had SDD (group SDD).[14] Over a period of 4 years, they found progressive decreased total retinal thickness in all 3 groups of study eyes in the following order of ranking: group 1 < group 2 < group SDD in the amount of retinal thinning. In our study, both groups of study eyes are analogous to Chiang et al.'s group 2 cohort, and a subset of both groups of our study with SDD are comparable with their group SDD cohort. Similar to their group 2 cohort, both IVR and SHAM eyes in our study developed significant reductions in TRT (mean CST [$P = 0.006$ and 0.018, respectively] and mean cube thickness [$P < 0.001$ and $P = 0.001$, respectively]) over the study course. Also consistent with Lamin et al.'s study, both IVR and SHAM eyes in our study developed significant reduction in macular volume (mean cube volume [$P < 0.001$ and $P = 0.003$, respectively]) over the course of the study.

In addition, both our SDD and non-SDD cohorts developed significant reductions in CST, mean cube thickness and mean cube volume (P values from 0.043 to < 0.001). However, between group comparison in our study did not show significantly more reductions in macular dimensions over time in eyes with SDD than in eyes without SDD, although there appeared to be such a trend given the positive point estimate of the adjusted differences between them. It is possible

but unknown whether comparison of SDD with non-SDD cohorts with a much larger sample size or longer duration of follow-up would have led to significant differences between these groups.

Concerning retinal sublayer analysis, multiple authors have reported progressive reductions in outer retinal layers over time for eyes with intermediate AMD. Schuman et al., Sadigh et al., and Rogala et al. reported consistent outer retinal layer thinning primarily driven by outer ONL degeneration and particularly overlying areas of prominent drusen.[9,11,12] However, the layer reported to be ONL in these OCT studies has been shown to include a substantial contribution from HFL, and not reflect the true ONL thickness.[27] Consistent with these reports, our study has shown significant increased ONL + HFL thinning from baseline to 24 months for both study groups. As scans were not acquired to disambiguate these layers, they are grouped together for precise assessment. Future studies should acquire images in such a way as to differentiate these structures (ONL and HFL) more accurately, in order to provide a more precise measure of photoreceptor loss.[27] In agreement with the study by Lamin et al.,[13] our study also found inner retinal layer reductions in both study groups from baseline to 24 months.

Regarding geographic atrophy, multiple previous studies, including subanalyses of prior pivotal clinical trials have shown formation or progression of GA in eyes that underwent anti-VEGF therapy for nAMD over time.[19–23] For instance, the SEVEN-UP study, including patients in the ANCHOR, MARINA, and HORIZON clinical trials, revealed macular atrophy in 98% of study eyes with a growth rate of 0.28 mm^2 per year correlating with visual decline over 5 years ($P < 0.001$), and final macular atrophic lesion size was related significantly to final vision.[19] In 2014, Grunwald et al. reported approximately one fifth of patients in the Comparison of Age-related Treatment Trials (CATT) developed GA within 2 years of treatment with anti-VEGF therapy.[20] A subsequent report indicated 41% of gradable eyes with GA in CATT had subfoveal location in 17% of these eyes.[21] A 5-year follow-up study of CATT also reported the GA incidence to be 12% at 1 year, 17% at 2 years, and 38% at 5 years with the GA growth rate of 0.33 mm per year.[22] In another study involving treatment of eyes with polypoidal choroidal vasculopathy, Kawaii et al. reported the development of macular atrophy correlated with the number of anti-VEGF injections.[18] These reports provide evidence suggestive of a contributing role of anti-VEGF therapy to formation and progression of GA in eyes with AMD. However, all these studies regarding GA progression pertained to eyes receiving anti-VEGF therapy that have already developed nAMD. To our knowledge, we are not aware of any studies on potential contribution of anti-VEGF therapy to GA formation or progression in eyes with intermediate AMD. In the PREVENT trial, 50% of the study eyes with nonexudative AMD in the intermediate stage received ranibizumab injections, whereas the other 50% received sham injections. Our analysis showed no significant difference in the GA growth rate between the 2 study groups over 24 months. Therefore, our study provides no evidence of increased magnitude or rate of growth in GA due to ranibizumab injections in eyes with intermediate AMD.

In summary, despite the substantial longitudinal thickness or volume reductions from baseline to 24 months for both TRT as well as retinal sublayer analyses, our study has shown no differences between IVR and SHAM in these changes. Given the lack of differences between groups for thickness and volume reductions as well as GA growth, this means that quarterly ranibizumab injections over 24 months for eyes with intermediate AMD have not aggravated or accelerated the process of retinal degeneration in comparison to the sham eyes over the study time course.

Although OCT segmentations were not performed manually, the use of automated segmentation algorithms provided consistency and its accuracy was manually confirmed. In addition, color fundus and FAF imaging assessment for AREDS severity score and GA at the UC Davis Reading Center were performed by two independent graders with adjudication for any disagreements by a third senior grader.

This study has relevant clinical implications. First, for eyes with nAMD that have reverted to the nonexudative state after resolution of neovascularization with repeated anti-VEGF therapy, this study provides no evidence of harmful effects with continuation of periodic anti-VEGF therapy to prevent reactivation of neovascularization. Moreover, given the lack of such evidence associated with conventional doses of anti-VEGF drugs, further investigation with more potent anti-VEGF drugs to prevent neovascularization in nonexudative eyes at high risk for neovascular conversion remains a viable option from a safety standpoint.

There are a number of limitations associated with this study. First, this is a post hoc analysis of a prospective clinical trial and therefore these secondary end points were not prespecified at the onset of the clinical trial. In addition, the sample size was limited in this study. There were a small number of eyes with GA at baseline. The elimination of patients that developed neovascular conversion as well as those who terminated early from the analyses to avoid confounding

effects further reduced the sample size. The sample size issue likely had the greatest impact on the GA and SDD analysis. This study was not originally powered to investigate the effect of anti-VEGF therapy on GA growth and macular thinning. Nevertheless, this effect has practical utility in determining whether there is harm in administering anti-VEGF therapy to these eyes. Despite a number of patients who terminated early, there was no data imputation or last-observation-carried-forward (LOCF) with the GA data. None of these patients dropped out of the study due to development or progression of GA. Given the small sample size, the results of the GA analysis in this study should be considered preliminary, pending confirmation in a future study with a more robust sample size. The study course was also limited to 24 months. It is unknown whether a more robust sample size or a longer follow-up time would have altered the results of this study. Nevertheless, the prospective design with a control group, incorporation of the service of third-party imaging experts, and a reading center, as well as adherence to a careful follow-up schedule and prespecified imaging protocols are the strengths of this study, allowing the generation of pertinent results useful to the ophthalmic community.

In conclusion, our post hoc analysis of the PREVENT trial has confirmed progressive increased retinal thinning and GA in eyes with intermediate AMD on a longitudinal basis, consistent with previous publications. More importantly for the purpose of this study, our results have shown that this progressive retinal degenerative process was not worsened by quarterly dosing of ranibizumab in these eyes at risk for vision loss. Future studies with a more robust sample size and longer follow-up time may provide confirmation of the outcomes shown in this study and may elucidate additional relevant information on this topic.

Acknowledgments

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