Change in Drusen Volume as a Novel Clinical Trial Endpoint for the Study of Complement Inhibition in Age-related Macular Degeneration

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BACKGROUND AND OBJECTIVE: To evaluate the change in drusen volume following treatment with eculizumab, a systemic inhibitor of complement component 5.

PATIENTS AND METHODS: Single-center, prospective, randomized, double-masked clinical trial. Patients were randomized 2:1 to receive intravenous eculizumab or placebo over 26 weeks. Main outcome measure: decrease in drusen volume of at least 50% at 26-week follow-up.

RESULTS: Mean drusen cube root volumes were 0.49 mm (P = .44) at baseline and 0.51 mm and 0.42 mm (P = .17) at 26 weeks in the eculizumab and placebo groups, respectively. In the placebo group, one eye had a decrease in drusen volume of at least 50% and two eyes developed neovascularization through 26 weeks.

CONCLUSION: Systemic complement inhibition with eculizumab did not significantly reduce drusen volume. Drusen growth was dependent on the number of complement at-risk alleles. Future trials should consider the use of a composite clinical trial endpoint in which efficacy is defined by the treatment’s ability to prevent drusen growth, neovascularization, and the formation of geographic atrophy over 1 year.


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influence disease progression.8–13 These trials confirmed the anecdotal observations that laser photo-coagulation to the macula caused the disappearance of drusen. Color fundus imaging confirmed these observations. However, there was no evidence that this overall loss of drusen resulted in any benefit in terms of preventing visual acuity loss or the development of choroidal neovascularization (CNV) and GA.14 Moreover, these studies relied on the en-face appearance of drusen on examination and color imaging, so the investigators could only assess the area and not the volume measurements of drusen. As a result, they based their enrollment on drusen area, which may have resulted in a diverse population of drusen at different stages of growth or regression, and many of the drusen may have already started to regress into GA. Moreover, using color fundus imaging, the investigators would not have been able to follow the incremental changes in drusen volume, outer retinal anatomy, and integrity of the retinal pigment epithelium (RPE), which are essential in assessing future visual function. However, at the time these studies were conducted, there was no reliable natural history data and no method for reproducibly measuring drusen morphology, so these trials relied on large numbers of patients to adequately power their studies to overcome all these uncertainties.15 In contrast, the use of SD-OCT imaging to assess drusen volume in the central macula has provided a unique, reliable, and reproducible method for measuring the morphology of drusen over time. This SD-OCT imaging strategy identifies a specific subset of drusen that elevate the RPE.16 As a result, the change in volume for these drusen can now be used as a novel clinical trial endpoint when studying therapies for dry AMD.5,17

While the pathogenesis of AMD is multifactorial, resulting from a combination of genetic and environmental risk factors, there is convincing evidence that the complement system plays an important role in causing AMD.18 Even before the genetic studies showed an association between AMD and complement genes, evidence of complement activation in AMD was provided by the presence of complement components 3 (C3) and 5 (C5), complement factor H, and the membrane attack complex in drusen.19–22 Genetic studies have implicated these same gene products as well as other genetic loci in the complement pathway as having a protective or risk-enhancing role for the development of AMD.23–26 In addition, the role of complement in AMD is suggested by the appearance of macular drusen in eyes of patients with complement-mediated renal disease27 and by the data from animal studies showing that complement activation has a role in CNV.28–30 Currently, there is no proven therapy that stops the progression of dry AMD, but the inhibition of complement could be a viable treatment strategy given the evidence that complement activation has a role in AMD and the encouraging unpublished results from a study investigating complement inhibition for the treatment of GA.31 Currently, the only inhibitor of terminal complement activation approved by the U.S. Food and Drug Administration is eculizumab (Soliris; Alexion Pharmaceuticals, Cheshire, CT), a humanized monoclonal antibody derived from the murine anti-human C5 antibody m5G1. Eculizumab is approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical-hemolytic uremic syndrome. Eculizumab is given intravenously and specifically binds the terminal complement protein

<table>
<thead>
<tr>
<th>Drusen Measurements</th>
<th>Study Eyes</th>
<th>Study and Fellow Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Active Treatment</td>
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<tr>
<td></td>
<td>n = 10, mm (SD)</td>
<td>n = 20, mm (SD)</td>
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<tr>
<td>Central 3 mm</td>
<td></td>
<td></td>
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<tr>
<td>Mean drusen volume, mm³</td>
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<td>0.15 (0.17)</td>
</tr>
<tr>
<td>Cube root volume, mm</td>
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<td>0.49 (0.14)</td>
</tr>
<tr>
<td>Central 5 mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean drusen volume, mm³</td>
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<td>0.17 (0.17)</td>
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<tr>
<td>Cube root volume (mm)</td>
<td>0.52 (0.15)</td>
<td>0.52 (0.14)</td>
</tr>
</tbody>
</table>

SD = standard deviation.
*Two-sided, two-sample t-test

TABLE 1
Drusen Volume Measurements at Baseline in the Central 3 and 5 mm
C5, thereby inhibiting its cleavage to C5a and C5b during complement activation and preventing the formation of the membrane attack complex.

The COMPLETE (Complement inhibition with eculizumab for the treatment of non-exudative age-related macular degeneration) study was designed to evaluate the safety and efficacy of systemic eculizumab for the treatment of drusen in dry AMD. The change in drusen volume was chosen as a surrogate endpoint because it could be studied over a shorter period of time compared with other dry AMD efficacy endpoints such as the progression to advanced AMD or vision loss, which require years of follow-up. Moreover, this is the first prospective, randomized clinical trial to use complement inhibition for the treatment and assessment of drusen in dry AMD and the first study to use the volumetric assessment of drusen as a clinical trial endpoint.

PATIENTS AND METHODS

Study Design

The COMPLETE study is a 12-month investigator-sponsored, single-center, prospective, randomized, double-masked study designed to evaluate the safety and efficacy of intravenous eculizumab for the treatment of patients with drusen secondary to AMD. All subjects and study personnel other than the clinical coordinator were masked to treatment assignment. The study was performed with FDA approval (IND #104471). Before the initiation of the study, additional approval was obtained from the institutional review board at the University of Miami Miller School of Medicine. Informed consent was obtained from all patients before determination of full eligibility, and the study was performed in accordance with the Health Insurance Portability and Accountability Act. The COMPLETE study is registered at www.clinicaltrials.gov (NCT00935883).

From November 2009 to May 2011, 30 patients were enrolled. Eligibility criteria included age of 50 years or more, the presence in the study eye of high-risk drusen, and best corrected visual acuity (BCVA) of 20/63 or better (ETDRS letter score of at least 59). High-risk drusen were defined by the presence of at least one druse with a diameter of at least 250 µm observed on fundus biomicroscopy or color fundus photography and a total volume of drusen of at least 0.03 mm³ as measured by SD-OCT within a 3 mm diameter circle centered on the fovea. Exclusion criteria included visual acuity worse than 20/63, presence of any GA, or any history of macular neovascularization in the study eye. If both eyes were eligible for the study, then one eye was chosen as the study eye at the discretion of the investigator. All patients enrolled in the study received a meningococcal vaccine at least 15 days prior to the initiation of treatment as described in the package insert approved by the FDA (http://soliris.net/sites/default/files/assets/soliris_pi.pdf).32

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Changes in Drusen Volumes From Baseline to 26 and 52 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Study Eyes</td>
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<td>Placebo (n = 9)</td>
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<td>26 Weeks of Follow-up</td>
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<td>Cube root volume (mm) change in central 3 mm (SD)</td>
<td>−0.05 (0.15)</td>
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<tr>
<td>Cube root volume (mm) change in central 5 mm (SD)</td>
<td>−0.04 (0.11)</td>
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<tr>
<td>52 Weeks of Follow-up</td>
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<tr>
<td>Cube root volume (mm) change in central 3 mm (SD)</td>
<td>−0.04 (0.15)</td>
</tr>
<tr>
<td>Cube root volume (mm) change in central 5 mm (SD)</td>
<td>−0.03 (0.12)</td>
</tr>
</tbody>
</table>

SD = standard deviation.

¹ Two-sided, two-sample t-test
* Unpooled variance test
Treatment Protocol

Patients were randomized 2:1 to receive active treatment with eculizumab or placebo in a double-masked fashion. Randomization schedules were stratified with the use of a permuted-block strategy to ensure balance. During the treatment period, patients received eculizumab for a period of 24 weeks with the primary endpoint at 26 weeks. The treatment period was divided into an induction period and a maintenance period. The first 10 patients in the eculizumab group received a low dose of eculizumab (600 mg via intravenous infusion weekly for 4 weeks followed by 900 mg every 2 weeks until week 24), while the next 10 patients received a high dose (900 mg eculizumab via intravenous infusion weekly for 4 weeks followed by 1,200 mg every 2 weeks until week 24). After 26 weeks, patients were monitored without treatment every 3 months for an additional 6 months.

The presence of any study-related adverse event that was considered to be severe in intensity by the investigators led to the discontinuation of treatment in the study, but masking was maintained. Patients were encouraged to continue with the study visits but no treatment was administered.

Ophthalmological Examination and Imaging Procedures

Ophthalmological examination included BCVA measured using the ETDRS chart at 4 meters, low luminance visual acuity testing at 4 meters using a 2.0-log unit neutral density filter (Kodak Wratten filter; Kodak, Rochester, NY), slit lamp biomicroscopy, IOP measurement, and fundus examination. All imaging studies were performed at baseline, 12 weeks, 26 weeks, 38 weeks, and 52 weeks of follow-up. These studies included color and autofluorescence imaging with a fundus camera–based flash system (TRC-50DX; Topcon Medical Systems, Oakland, NJ; AF Excitation λ: 535-585 nm, Detection λ: 605-715 nm), autofluorescence and fluorescein angiographic imaging with a confocal SLO system (Spectralis; Heidelberg Engineering, Heidelberg, Germany; AF excitation λ: 488 nm; detection λ: > 500 nm), and SD-OCT imaging with both the Cirrus (Carl Zeiss Meditec, Dublin, CA) and Spectralis instruments.

SD-OCT drusen volume maps were obtained using the Cirrus instrument and the macular 200 × 200 raster scan pattern and a proprietary algorithm, which is now available in version 6.0 of the Cirrus operating system. This algorithm calculated the difference between the elevation of the RPE caused by drusen and a virtual RPE floor free of deformations, which resulted in reproducible measurements of drusen area and volume. Only drusen that elevated the RPE would be measured using this strategy. For this study, the drusen volume was measured within a 3 mm and 5 mm diameter circle centered on the fovea. The position of the fovea was determined manually by scanning through the OCT data sets and finding the spot where the geometry of the inner retinal layers best matched the known anatomic configuration in the fovea. Drusen measurements were performed in the

Figure 1. Drusen volume changes over 26 weeks. These scatter plots depict the baseline cube root drusen volume within a 3 mm circle centered on the fovea along the x-axis and the cube root drusen volume at 26 weeks along the y-axis. The dotted lines represent the 95% test-retest confidence intervals for the variability associated with measurements. The solid diagonal line represents the cut-off for a 50% decrease in the baseline cube-root volume. (A) Study eyes. (B) Study eyes plus eligible fellow eyes.
same areas contained within these circles at baseline and at follow-up visits. A single experienced operator obtained the images, and they were actively monitored for quality and the presence of artifacts that could influence the drusen volume measurements. The same scan pattern was used to generate the OCT fundus image (OFI) and the sub-RPE slab OFIs.34,35 These images were used to assess the development of GA during the follow-up visits. Autofluorescence and fluorescein angiography images were used as well to assess the formation of GA. Fluorescein angiography and SD-OCT imaging were used to assess the formation of macular neovascularization.

Choroidal imaging was performed with the Spectralis instrument using the enhanced depth imaging (EDI) protocol. Two independent graders manually measured choroidal thickness at the foveal center, and a consensus image was used for quantitation.

Outcome Measures

The primary outcome was to determine whether the treatment with eculizumab could decrease the volume of drusen by 50% within a 3 mm circle centered on the fovea over a 6-month treatment period. The primary outcome included both the low-dose and high-dose groups. Fellow eyes that met inclusion criteria were included in the analysis of secondary outcomes. Secondary outcomes also included the change in drusen volume in the central 5 mm, the change in drusen area within the 3 mm and 5 mm diameter circles, the change in BCVA from baseline, a comparison of the low-dose and the high-dose groups, and the conversion rate from dry to wet AMD in study and fellow eyes over 12 months, which included the 6-month treatment period and the 6-month observation period.

To eliminate the influence of drusen size at baseline on the measurement of drusen growth and to eliminate the influence of drusen size on the 95% tolerance limits of the variability associated with the test-retest measurements, we performed an appropriate transformation of the data before statistical analyses were performed.4 These transformations included a square root transformation for drusen area measurements and a cube root transformation for drusen volume measurements.

Safety was assessed through the summary of ocular and nonocular adverse events, serious adverse events, ocular assessments, deaths, laboratory test results, and vital signs. Safety analyses included subjects who received at least one eculizumab infusion. Adverse events leading to discontinuation from the study were listed.

Statistical Analysis

The principal treatment efficacy outcome was the success rate at 6 months of follow-up. Success was defined as a decrease in the drusen cube root volume of at least 50% compared with the baseline cube root volume without evidence of progression to GA or
neovascular disease.\textsuperscript{5} Success rates were compared between study eyes randomized to eculizumab and those randomized to placebo with the Fisher exact test. Volume and area reductions were compared with the two-sample \( t \)-test. Average change in drusen volume and area over time constituted a secondary outcome variable and were compared between groups with the two-sample \( t \)-test. Analysis of variance was used to examine the effect of genotype on the change in lesion size.

The rationale for sample size determination is presented in a previously published natural history study.\textsuperscript{5} Assuming a 5% rate of success in placebo-treated patients, this randomized trial was designed with 80% power, at an alpha error level of 0.05, to detect a 65% or greater success rate in patients randomized to eculizumab.

**Pharmacokinetic and Complement Factors Analysis**

During the study, blood samples for pharmacokinetic and complement factor screening were drawn and analyzed in a masked fashion at baseline and at every planned visit at weeks 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 38, and 52. Alexion Pharmaceuticals laboratory measured soluble C5b-9 and performed a hemolytic assay for C5 activity. Levels of factor H and C3 were also measured.

**Genetic Analysis**

Genetic testing for seven single-nucleotide polymorphisms (SNPs) was performed in all patients as previously described,\textsuperscript{36} and the prevalence of the following alleles was assessed: CFH-rs1061170 [C], C3-rs2230199 [G], CFH-rs2274700 [C], HTRA1-rs10490924 [T], CFB-rs641153-R32Q [G] C2-rs9332739-E318D [G]. Blood samples from each patient were sent to the University of California San Diego for the testing. Genomic DNA was extracted from peripheral blood leukocytes according to established protocols. All SNPs were genotyped using the SNaPshot method according to the manufacturer’s recommendations.\textsuperscript{36}
Baseline Characteristics

Thirty eyes of 30 patients were enrolled in the study and randomized 2:1 to active eculizumab treatment and placebo. Patients in the eculizumab group were randomized to receive the low-dose or high-dose treatment regimen. The first 10 patients on eculizumab received the low-dose regimen and the next 10 patients received the high-dose regimen. Twelve fellow eyes met inclusion criteria and were analyzed as a secondary endpoint.

The mean ages (standard deviation) of patients in the placebo and active treatment groups were 70.7 (7.8) and 70.7 (6.8), respectively. Mean ages in the low-dose and high-dose regimen groups were also similar: 72.4 (6.5) and 69.0 (7.0), respectively. Mean ETDRS visual acuity was 78.0 (10.0) letters in the placebo group and 80.9 (5.9) in the active treatment group \( (P = .33) \). Low-dose and high-dose groups had similar ETDRS visual acuity letter scores of 81.1 (3.8) and 80.7 (7.7) letters, respectively \( (P = .88) \).

The drusen volumes at baseline are shown in Table 1 (page 19). At baseline, the mean cube root drusen volume (SD) in the central 3 mm was 0.49 mm \(^3\) (0.14) and 0.47 mm \(^3\) (0.10) in the eculizumab and placebo groups, respectively \( (P = .64) \). Volumes on the untransformed scale were 0.15 (0.17) mm \(^3\) and 0.12 (0.08) mm \(^3\) in the eculizumab and placebo groups. Mean square root drusen area at baseline in the central 3 mm was 1.40 (0.33) mm \(^2\) and 1.37 (0.24) mm \(^2\) for the eculizumab and placebo groups, respectively \( (P = .71) \). Areas on the untransformed scale were 2.07 (1.00) mm \(^2\) and 1.93 (0.71) mm \(^2\) in the eculizumab and placebo groups. The baseline measurements of placebo and eculizumab groups were similar by all volume and area measurements.

Patient Retention, Treatment Compliance, and Safety

Twenty-eight patients completed 52 weeks of follow-up. Two of the patients in the high-dose group refused further participation after 3 weeks of follow-up because of personal conflicts with the treatment schedule and exited the study. No adverse events were identified in these patients. One placebo patient failed to complete a full course of infusions because of chest discomfort during one of the infusions, which required a hospitalization. However, she remained masked, treatment was halted, and she continued to be monitored in the study without further infusion. After unmasking at the end of the study, she was found to have received placebo. Systemic therapy with eculizumab was well tolerated through 6 months, and no drug-related adverse events were identified in the patients included in the study.

Figure 4. Increase in drusen volume over 52 weeks. This left eye of a 72-year-old man treated with placebo demonstrated an increase in drusen volume over 52 weeks. (A, F, K) Color fundus images with white box representing the spectral-domain OCT (SD-OCT) scan area (200 × 200) and a line centered on the fovea representing the location of the SD-OCT B-scan at baseline, 26 weeks, and 52 weeks. (B, G, L) Heidelberg autofluorescence images at baseline, 26 weeks, and 52 weeks. (C, H, M) SD-OCT B-scan centered on the fovea at baseline, 26 weeks, and 52 weeks. (D, I, N) Retinal pigment epithelium segmentation maps at baseline, 26 weeks, and 52 weeks. (E, J, O) Drusen volume maps with a 3 mm circle centered on the fovea. The drusen volume in the central 3 mm was calculated to be 0.11 mm \(^3\) at baseline (E), 0.15 mm \(^3\) at 26 weeks (J), and 0.18 mm \(^3\) at 52 weeks (O).
Primary Outcome

Figure 1 (page 21) shows the change in drusen volumes in the study eyes from baseline to 26 weeks. At 6 months of follow-up, none of the 18 eculizumab-treated study eyes demonstrated a 50% reduction in drusen volume, whereas one eye (10%) in the placebo group showed a 50% reduction in drusen volume ($P = .36$, Fisher exact test; Figure 3, page 23). One additional placebo-treated eye experienced a decrease in drusen volume that was less than 50% of the baseline volume. In the eculizumab group, no study eyes decreased in volume beyond the 95% tolerance interval for test-retest variability limits shown by the dotted lines in Figure 1. The remainder of the eyes either showed an increase in drusen volume beyond the test-retest variability limits (Figure 4, page 7) or remained stable (Figure 5). The 95% confidence interval around the difference between the treatment and placebo groups with respect to the proportions with a successful outcome ranged from $-0.55$ to $0.21$, which effectively ruled out a treatment-related positive outcome of a 50% drusen reduction in 22% or more of the eyes treated with eculizumab.

Secondary Outcomes

There was no difference in drusen growth between the placebo- and eculizumab-treated study eyes (Table 2, page 20). At 26 weeks of follow-up, the placebo eyes ($n = 9$, excluding the eye that progressed to neovascular disease) averaged a $-0.05$ mm (SD = .15) change in cube root drusen volume within the central 3 mm, whereas the eculizumab-treated group ($n = 18$) averaged a $0.02$ (SD = .01) increase in cube root drusen volume ($P = .17$, unpoled variance $t$-test). The 95% confidence interval for the difference in the mean change in drusen volume between the eculizumab and placebo groups ranged from $-0.04$ to $+0.19$. Thus, a reduction in drusen volume growth due to eculizumab was not detected (Table 2). Between weeks 26 and 52 (Table 2; Figure 2, page 22), no additional eyes experienced a 50% reduction in drusen volume.

A subset of 12 fellow eyes met the study inclusion criteria. One eye was excluded from the final analysis because the patient exited the study. Figures 1 and 2 (pages 21 and 22, respectively) show the changes in drusen volumes from baseline to 26 weeks and from baseline to 52 weeks for both the study and the fellow eyes. There were no differences between the eculizumab- and placebo-treated groups when the fellow eyes were included in the analyses (Tables 1 and 2, pages 2 and 3, respectively). In addition, there were no detectable differences when comparing the effects of low-dose with high-dose eculizumab regimens on the change in drusen volume over 26 weeks ($P = .63$) or 52 weeks ($P = .59$).
By 26 weeks of follow-up, a single placebo study eye progressed to neovascular disease with the development of a vascularized retinal pigment epithelial detachment (PED) and the appearance of intraretinal fluid (Figure 6). A single non-study but eligible fellow eye, also in the placebo group, progressed to neovascularization at 20 weeks of follow-up. No additional eyes developed neovascularization between weeks 26 and 52. None of the eyes developed GA over 52 weeks.

The BCVA of both study and fellow eyes remained stable over 26 weeks of follow-up with small increases in the number of ETDRS letters read in both groups: +2.4 (3.9) in the active eculizumab group and +4.0 (7.0) in the placebo group (\(P = .36\)). The maximum number of letters lost by any eye meeting inclusion criteria was five letters (one eculizumab eye). There was a deficit in the low luminance visual acuity compared to standard visual acuity test at baseline of 14.4 (5.1) letters and 15.5 (5.4) letters in the eculizumab and placebo groups, respectively (\(P = .60\)). After 26 week of follow-up, the deficit increased to 16.5 (4.8) and 16.3 (5.8) letters in the eculizumab and placebo groups, respectively (\(P = .92\)). The difference between baseline and 26 weeks of follow-up was not statistically significant (\(P = .37\)).

No correlation was found between choroidal thickness and the area or volume of drusen at baseline either with or without adjusting for age and axial length (all \(P > .12\)), and no correlation was found between choroidal thickness and the progression of drusen area or volume over 26 and 52 weeks of follow-up (all \(P > .53\)).

**Genetic Analysis**

The distribution of patients according to the number of at-risk alleles they carry is shown in Table 3 (page 27). This table also shows the association between these genotypes and the volume of drusen at baseline and the change in volume of drusen over 26 and 52 weeks. The drusen volume at baseline in the central 3 mm measured by SD-OCT was not associated with
the number of at-risk alleles in each patient \((P = .87)\) or whether a particular locus was homozygous or heterozygous (all \(P > .05\)). However, the growth of drusen volume over 26 and 52 weeks was associated with the number of at-risk alleles for the SNP CFH-rs1061170 carried by a patient, and this association was statistically significant \((P < .001)\). This SNP identifies the H1 at-risk haplotype. For the SNP CFH-rs2274700, there was a statistically significant difference in the growth of drusen volume at 26 weeks if the patients carried one or both copies of the at-risk allele \((P = .03)\). However, the statistical significance observed at 26 weeks was lost at 52 weeks. This SNP identifies both the H1 and the H3 CFH haplotypes. No other at-risk allele tested showed a correlation between the allelic burden and the growth of drusen volume through 52 weeks (all \(P > .05\)). A secondary analysis was performed to determine whether the genotype had any influence on the treatment outcome. There was no correlation between any of the SNPs and the potential treatment effect of eculizumab (all \(P > .05\)).

### Pharmacokinetics

During the study, blood samples were collected to assay for eculizumab and C5 activity. Among patients receiving eculizumab, all samples collected between the onset of treatment and week 26 showed measurable levels of drug. Eculizumab levels rose rapidly during treatment weeks 1 to 4, with the low-dose cohort averaging 91 to 285 mg/mL and the high-dose cohort averaging 84 to 239 \(\mu g/mL\). Differences between levels in the low- and high-dose groups were not statistically significant (all \(P > .2\)). Between weeks 6 and 26, eculizumab levels continued to increase by only 1.5 \(\mu g/mL/week\) in actively treated patients. On average, C5 activity decreased to less than 8% of normal levels by 1 week after treatment and less than 0.5% by week 26. Among the patients

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**TABLE 3**

<table>
<thead>
<tr>
<th>Single-Nucleotide Polymorphism [At-Risk Allele]</th>
<th>Number of At-Risk Alleles (Number of Patients With Genotype)</th>
<th>Cube Root of Drusen Volume at Baseline (SD)</th>
<th>(P) Value</th>
<th>Change in Cube Root of Drusen Volume at 26 Weeks (SD)</th>
<th>(P) Value</th>
<th>Change in Cube Root of Drusen Volume at 52 Weeks (SD)</th>
<th>(P) Value</th>
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<td>CFH-rs1061170 [C]</td>
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<td>.69</td>
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\(SD =\) standard deviation.

\(^1\)One-way analysis of variance.

*One patient converted to wet AMD and was not included in the drusen change over 26 weeks. All patients carried both at-risk alleles for SNP C2_rs9332739-E318D (data not shown).
receiving placebo injections, levels of eculizumab were undetectable.

DISCUSSION

The COMPLETE study is the first randomized, phase 2 clinical trial performed to evaluate the clinical efficacy and safety of complement inhibition for the treatment of drusen in dry AMD. A number of strategies for modulating the complement system are currently being used in dry AMD patients, and these approaches include the inhibition of complement proteins such as C3, C5, and factor D. In this study, eculizumab, an anti-complement drug that blocks the activation of C5, was intravenously administered in a population of patients with drusen secondary to AMD. The effects of eculizumab on drusen volume and area were assessed and compared against placebo. As described above, the square root transformation of drusen area measurements and the cube root transformation of drusen volume measurements were performed to eliminate the influence of drusen size on their 95% test-retest reproducibility limits and on their overall growth rates. Overall, there was no statistically significant effect of eculizumab on drusen volume and area. The majority of patients in the active treatment and placebo groups maintained a stable drusen volume over 52 weeks with some dynamic changes around the test-retest 95% confidence intervals shown as the dotted lines in Figures 1 and 2 (pages 21 and 22 respectively). Figure 1 shows the scatter plots of the baseline and follow-up cube root drusen volumes at 26 weeks for the placebo and eculizumab patients. Two eyes included in the placebo group had a significant decrease in the drusen volume over 26 weeks, and these two eyes can be observed below the test-retest dotted line. Only one eye lost at least 50% of its baseline volume, and this eye is shown below the solid diagonal line. Neither eye progressed to GA or CNV. Figure 3 (page 23) shows the color fundus image, autofluorescence image, OCT B-scans, and drusen volume maps of an eye in the placebo group that experienced a significant reduction in drusen volume over 26 weeks. In this example, some drusen that would be classified as hard or calcific drusen in the color image appeared to resolve during the follow-up period. One study eye in the placebo group progressed to CNV during follow-up (Figure 6, page 26). This patient presented with an increase in drusen volume over the first 20 weeks of follow-up before developing subretinal fluid and a vascularized RPE detachment at week 24. In addition, one fellow eye of a patient included in the placebo group presented with neovascularization at week 20. Overall, there were no differences in the drusen volumes between treatment groups through 52 weeks.

In this study, we were not able to identify any effect of systemic eculizumab treatment on drusen volume measured with SD-OCT. These results raise questions of whether complement inhibition is a viable treatment strategy, whether our study was too small and too short to detect a treatment effect, whether C5 is the appropriate target to prevent complement-mediated disease progression, whether systemic therapy is appropriate for the treatment of dry AMD, whether an adequate dose of eculizumab was used, whether intravitreal injections would have been a better route for drug delivery, or whether complement inhibition may only be effective in a genetic subgroup of AMD patients who carry the at-risk alleles of complement genes. While eculizumab inhibits C5 and prevents terminal complement activation, it is entirely possible that more proximally activated complement proteins such as C3a and C3b need to be suppressed to affect drusen volume and eculizumab is unable to suppress these anaphylotoxins. While the doses used in this study were based on the effective doses of eculizumab used to treat paroxysmal

<table>
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<th>30% Failure Rate</th>
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<tr>
<td>90%</td>
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*Based on an estimated failure rate of 60% in the placebo group and 1:1 randomization. Failure was defined as growth of drusen volume and formation of any neovascularization or geographic atrophy at 1 year.
nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, and these doses completely inhibit systemic complement C5 in these diseases.\textsuperscript{38, 39} It is possible that an even higher dose is needed to penetrate the back of the eye to reduce drusen volume. Of course, this assumes that C5 activation in the retina has a role in the growth of drusen. However, if C5 inhibition is needed in the choroid to induce drusen regression, then the doses used in this study should have been adequate to show a treatment effect. While it’s possible that complement activation may not have a role in the reduction of drusen volume, our genetic data suggest that complement activation clearly has a role in drusen growth.

Although the number of patients in this study is small for a genetic association study, it is striking that there was a highly statistically significant correlation between the growth of drusen volume and the number of at-risk CFH alleles carried by the patients. Of the seven SNPs evaluated in this study (Table 3, page 27), only the SNP associated with the high-risk H1 haplotype, the CFH allele (CFH-rs1061170), showed an association with the growth of drusen volume at 26 and 52 weeks ($P < .001$). This highly significant association suggests that complement may play an important role in the growth of drusen volume, and it is unlikely due to alpha error inflation as a result of multiple statistical comparisons.\textsuperscript{40, 41} Perhaps a more appropriate endpoint might have been the inhibition of drusen growth rather than the reduction of drusen volume. However, our study was not powered to study the inhibition of drusen growth. It is also interesting that two placebo eyes converted from dry to wet AMD. While it is possible that complement inhibition might be an effective treatment to prevent the conversion of dry to wet AMD, no conclusion is possible due to the small size of our study. However, such a prevention strategy could be used as a clinical trial endpoint in the study of complement inhibition for dry AMD. Previously, this prevention endpoint was used unsuccessfully to study anecortave acetate for the treatment of dry AMD (www.clinicaltrial.gov; NCT00333216).

Our data suggest that a novel composite clinical trial endpoint should be used when designing future phase 2 clinical trials to test potential therapies for nonexudative AMD (Table 4). This composite endpoint uses the three likely outcomes when following the normal progression of drusen, and the power calculations in Table 4 are derived from the natural history study previously published by Yehoshua et al\textsuperscript{5} and confirmed in the current study. We found that when eyes with a baseline drusen volume of 0.03 mm$^3$ or greater in the absence of any GA are followed up for 1 year, the proportion of eyes at follow-up with decreasing, stable, and increasing drusen volumes are 20%, 26%, 54%, respectively. If we then assume, based on our previous research, a cumulative incidence of CNV at 1 year of 4.5% and a cumulative incidence of GA at 1 year of 5.0%, then we can design a composite clinical trial endpoint in which the goal of treatment is to prevent normal disease progression. If normal disease progression occurs, then this would be considered a failure, and failure would be defined as the growth of drusen volume beyond test-retest limits, the appearance of any neovascularization, or the appearance of GA at 1 year. If we assume an expected failure rate of 60% in the placebo group, then Table 4 shows the number of subjects in each group required to perform a study with 80% or 90% power to detect a decrease in the defined failure rate.

The current study had an 80% power to detect a 50% decrease in the drusen volume in 65% of subjects receiving drug compared with 5% of subjects receiving placebo. After 6 months, we not only failed to detect this predetermined treatment effect, but based on our outcomes, we are confident that we could have detected a treatment effect that decreased the drusen volume in as few as 30% of the subjects receiving drug. If we now apply this novel composite clinical trial endpoint that focuses on drusen growth rather than a decrease in drusen volume to the outcomes from the current study, then eculizumab once again failed to show a positive treatment effect. However, we never would have expected to see a positive treatment effect using the composite clinical trial endpoint given the small number of patients and the short follow-up in this current study. In fact, the current study design would have only been suitable if we had hypothesized a reduction in the composite failure rate of 90% or more following treatment with eculizumab, which would have been an overly optimistic prediction. Table 4 shows the actual number of subjects that would be needed to design a study with 80% power to detect a 50% decrease in the failure rate from 60% to 30% at 1 year using this composite endpoint. The study would require 48 subjects in both the placebo and the eculizumab arms. While this number is greater than the number of patients enrolled in our current study, it still represents fewer subjects than the number enrolled in previous and ongoing phase 2 clinical trials designed to test novel therapies for nonexudative AMD.

The use of composite endpoints has been controversial especially when used to increase the statistical power of a trial by combining several outcomes that occur individually at a low incidence.\textsuperscript{42, 43} This was not the motivation behind our suggested compos-
ite endpoint. Rather, the principal outcome of interest and the one with the highest incidence in historical controls is the growth of drusen volume. The analytical issue then becomes how to handle progression to either neovascularization or GA. One option is to label them as adverse events and include them in a safety evaluation. This would involve censoring the enrolled eyes of patients at the time of progression in the final analysis of efficacy. Because these are the worst possible outcomes for patients, we feel their proper role in the efficacy analysis is to be considered failures; however, trials employing this composite outcome should examine the component outcomes separately as well.

In summary, systemic complement inhibition with eculizumab was well tolerated through 6 months, and no drug-related adverse events were identified, but complement inhibition with eculizumab did not reduce the volume of drusen in patients with dry AMD. While patients in the placebo group did have a higher incidence of neovascularization, suggesting an effect of complement inhibition in preventing the conversion of dry to wet AMD, the study was too small to draw any definitive conclusions about eculizumab in preventing this conversion. The study showed an association between the number of at-risk CFH alleles and drusen growth, suggesting that complement activation has a role in the formation of drusen, but the study was not powered adequately to look for a decrease in drusen growth. Whether C5 inhibition is the appropriate target or intravitreal injections might be a better way to deliver the drug remains to be determined. Although it failed to meet the primary outcome measure, the study demonstrated the utility of using SD-OCT to measure drusen volume as a clinical trial endpoint. The results from this first trial investigating the effects of C5 inhibition on drusen volume are sufficiently intriguing to suggest that future investigations of complement inhibition for dry AMD should be pursued using a composite clinical trial endpoint, which would include the prevention of drusen growth and the prevention of neovascularization and GA.

REFERENCES

16. Yehoshua Z, Gregori G, Sadda SR, et al. Comparison of drusen area and the one with the highest incidence in historical controls is the growth of drusen volume. The analytical issue then becomes how to handle progression to either neovascularization or GA. One option is to label them as adverse events and include them in a safety evaluation. This would involve censoring the enrolled eyes of patients at the time of progression in the final analysis of efficacy. Because these are the worst possible outcomes for patients, we feel their proper role in the efficacy analysis is to be considered failures; however, trials employing this composite outcome should examine the component outcomes separately as well.

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