

ANATOMICAL MEASURES AS PREDICTORS OF VISUAL OUTCOMES IN RANIBIZUMAB-TREATED EYES WITH NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

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Purpose: To investigate if anatomical characteristics of eyes undergoing ranibizumab therapy were predictive of best-corrected visual acuity (BCVA) outcomes over 2 years.

Methods: Post hoc analyses of patients with age-related macular degeneration from PIER studies, defined by fundus fluorescein angiography, quantitative optical coherence tomography (OCT), and qualitative OCT, were performed to determine if associations with BCVA outcomes could be found.

Results: Ranibizumab-treated subgroups defined by baseline fundus fluorescein angiography lesion size and composition did not differ in BCVA outcomes at Month 24 ($P = 0.13$ – 1.0). Inactivity on fundus fluorescein angiography at Month 3 was associated with a 12-letter gain by Month 12 ($P < 0.01$), whereas inactivity on Month 3 qualitative OCT was not ($P > 0.05$). Qualitative OCT inactivity at Month 5 and separately at Month 8 was associated with greater BCVA gains by Month 24 (7.1 and 9.5 letters, respectively; $P \leq 0.045$) versus eyes with OCT activity.

Conclusion: When assessed separately, eyes with qualitative OCT (Months 5 and 8) or fundus fluorescein angiography (Months 3 and 5) inactivity maintained vision gain from baseline at Month 24, while those with leakage not only lost initial vision gains achieved by intraocular ranibizumab but also had net vision losses from baseline at Month 24. The PIER infrequent dosing regimen likely exaggerated and accelerated the deleterious effects of retinal fluid on BCVA, and it is not known whether these findings are applicable to treatment regimens that use more frequent monitoring and dosing of ranibizumab.

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Neovascular age-related macular degeneration (AMD) is characterized by new vessel growth and leakage in the choroidal vascular network beneath

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Bruch membrane. Although the pathologic events that precede choroidal neovascularization (CNV) are not clearly understood, disrupting activity of vascular endothelial growth factor A (VEGF-A), a diffusible cytokine that promotes angiogenesis and vascular permeability, is an effective strategy for treating CNV secondary to AMD. Ranibizumab (Lucentis; Genentech, Inc, South San Francisco, CA) is a humanized, affinity-matured, anti-VEGF antigen-binding fragment (Fab) that binds to and neutralizes all isoforms of VEGF-A and their biologically active degradation products. In two pivotal Phase III trials—*Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab In the Treatment of Neovascular Age-Related Macular Degeneration (MARINA)*¹ and *Anti-Vascular Endothelial Growth*

Factor Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-related Macular Degeneration (ANCHOR)²—monthly intravitreal injections of 0.3 mg or 0.5 mg of ranibizumab not only prevented vision loss but also improved best-corrected visual acuity (BCVA) on average. Furthermore, ranibizumab treatment was associated with significant reductions in retinal thickness, arrested growth of CNV lesions, and caused cessation of leakage from CNV, in many cases as early as 1 week after the treatment initiation. These anatomical (and BCVA) improvements were maintained with monthly dosing.

Subsequently, the Phase IIIb, Multicenter, Randomized, Double-Masked, Sham Injection-Controlled Study of the Efficacy and Safety of Ranibizumab in Subjects with Subfoveal Choroidal Neovascularization With or Without Classic CNV Secondary to Age-Related Macular Degeneration (PIER) evaluated the safety and efficacy of an alternative dosing regimen of ranibizumab (i.e., 3 monthly loading doses followed by quarterly doses). A primary analysis of PIER data³ indicated that the PIER dosing regimen was effective for treating eyes with neovascular AMD, with treated eyes losing significantly fewer BCVA letters compared with control eyes at the Month 12 evaluation. However, the magnitude of the benefit observed with quarterly dosing was disappointing compared with that observed with monthly dosing in the MARINA and ANCHOR studies.

Since U.S. Food and Drug Administration approval of ranibizumab in 2006, it has become apparent to clinicians that some patients with neovascular AMD do well with few injections, while many require monthly or near monthly dosing to maintain anatomical and visual acuity gains.^{4,5} Despite the lack of validation from Level I trials that anatomical criteria can be used as a surrogate for BCVA outcomes, most clinicians use them (e.g., optical coherence tomography [OCT] or fundus fluorescein angiography [FFA]) to help determine retreatment schedules.

Although subgroup analyses of the MARINA and ANCHOR trials^{6,7} showed that nearly all eyes did well with monthly ranibizumab therapy (both visually and anatomically), those data provided little information about which eyes would do well with less frequent treatment or if anatomical data correlated with BCVA outcomes. The PIER trial was unique in that it was a controlled clinical trial of an efficacious drug that was dosed too infrequently for the average patient with AMD; most eyes experienced recurrent retinal edema and loss of BCVA gains once monthly therapy was discontinued. In the current analysis, we tested the hypothesis that inactivity (a dry macula) on OCT and

FFA is associated with the maintenance of BCVA gains by retrospectively evaluating the degree to which anatomical features of the study eye early in ranibizumab treatment were predictive of BCVA outcomes at Months 12 and 24 in subgroups of PIER subjects.

Methods

Study Design

PIER methodology, including study design, eligibility, masking, treatment, assessments, and analyses, has been published in detail.³ The PIER protocol was approved by the appropriate institutional review board at each study site, and all subjects provided written informed consent before study participation.

Subjects

Eligible subjects were ≥ 50 years of age with a diagnosis of primary or recurrent subfoveal CNV (predominantly classic, minimally classic, or occult with no classic) secondary to AMD. Classic and occult CNV comprised $\geq 50\%$ of the total AMD lesion area, and the total lesion was ≤ 12 disk areas (DA). If a CNV lesion was minimally classic or occult with no classic component, the treated eye was required to meet protocol-defined criteria for disease progression (i.e., a 10% increase in lesion size based on FFA obtained 1 month before study initiation [i.e., Day 0] compared with FFA obtained 6 months before Day 0; a loss of ≥ 5 Early Treatment Diabetic Retinopathy Study [ETDRS] letters [1 Snellen line] of BCVA within 6 months before Day 0; or CNV-associated subretinal hemorrhage within 1 month before Day 0). Eligible subjects had baseline BCVA of 20/40 to 20/320 Snellen equivalent, measured using the ETDRS chart at a distance of 4 m.

Eyes were excluded if they had been treated for AMD with another antiangiogenic drug, photodynamic therapy, external beam radiation therapy, transpupillary thermotherapy, or subfoveal laser photocoagulation at any time or juxtafoveal or extrafoveal laser photocoagulation within 1 month before Day 0. Exclusion criteria also included structural damage to the central fovea and subretinal hemorrhage ≥ 1 DA or $\geq 50\%$ of total lesion area with foveal involvement. One eye per subject was studied.

Treatment

Eligible eyes were randomized in the ratio of 1:1:1 to sham injections, 0.3 mg of intravitreal ranibizumab, and 0.5 mg of intravitreal ranibizumab (Figure 1). Randomization was stratified by BCVA (≤ 54 ETDRS

letters, approximately 20/80 or worse Snellen equivalent vs. ≥ 55 ETDRS letters, approximately 20/80 or better Snellen equivalent) at Day 0, CNV type (minimally classic vs. occult with no classic vs. predominantly classic), and study center.

The PIER protocol mandated that eyes receive sham injections or intravitreal injections of 0.3 mg or 0.5 mg of ranibizumab every month for 3 months (Day 0, Month 1, Month 2) and every 3 months thereafter (Months 5, 8, 11, 14, 17, 20, and 23) for the remainder of the 2-year study. In addition to injection visits, clinic visits were scheduled at Months 3, 12, and 24. However, after careful review of available clinical data, including data from MARINA and ANCHOR, as they became available, it was believed to be in the best interest of sham-group subjects to receive ranibizumab. Thus, in February 2006, the protocol was amended (crossover amendment) to provide sham-group subjects who completed the Month-12 visit the opportunity to crossover to quarterly injections of 0.5 mg of ranibizumab. Subsequently, after careful review of the 12-month PIER data, it was believed to be in the best interest of all study subjects to be examined and treated more frequently than the protocol mandated. Thus, in August 2006, the protocol was again amended (rollover amendment) to provide all subjects remaining on the study the opportunity to roll over to monthly injections of 0.5 mg ranibizumab for the remainder of the study. No subjects were unmasked to

their original treatment assignment as a result of the crossover and rollover amendments.

Assessments

At each scheduled visit, subjects underwent a full ophthalmologic assessment, including BCVA testing with standardized refraction using the ETDRS chart at 4 m. Fundus photography and FFA were performed at screening and at Months 3, 5, 8, 12, and 24. Optical coherence tomography was performed at selected study sites on Day 0 and at Months 1, 2, 3, 5, 8, and 12. Fundus photography, FFA, and quantitative OCT were evaluated by a central reading center (Fundus Photograph Reading Center, University of Wisconsin, Madison, WI).

Outcome Measures

The primary efficacy outcome measure of PIER was the mean change from baseline BCVA at Month 12. We performed post hoc analyses of subgroups of ranibizumab-treated eyes (pooled 0.3-mg and 0.5-mg dose groups) defined by FFA, quantitative OCT, and qualitative OCT early in the treatment to determine how those anatomical features correlated with BCVA outcomes at Months 12 and 24, according to the masked assessment by reading center personnel.

Fundus fluorescein angiography. Fundus fluorescein angiography subgroups were composed of eyes that had active FFA lesions (i.e., total area of CNV leakage plus intense progressive retinal pigment epithelium [RPE] staining $\neq 0$) and inactive FFA lesions (i.e., total area of CNV leakage plus intense progressive RPE staining = 0). Baseline FFA characteristics assessed included total lesion area categorized as ≤ 4 and >4 DA and CNV lesion composition classified as predominantly classic, minimally classic, and occult without classic.

Quantitative optical coherence tomography. Optical coherence tomography subgroups composed of eyes with central foveal thickness (CFT) $<200 \mu\text{m}$ and CFT $\geq 200 \mu\text{m}$. Central foveal thickness corresponded to the mean point center thickness as measured by the variable labeled "center" on the fast macular thickness map.

Qualitative optical coherence tomography. As per the PIER protocol, selected study sites submitted horizontal and vertical high-resolution raster lines (512 B-scans per image) in addition to the 128-B-scans-per-image fast macular thickness maps. The 2 high-resolution raster OCT scans per eye per visit ($n = 6088$) were qualitatively evaluated by a grader (D.M.B., masked to the treatment and study eye), for evidence of CNV-associated activity, including diffuse edema, intraretinal cysts, subretinal fluid, and sub-RPE

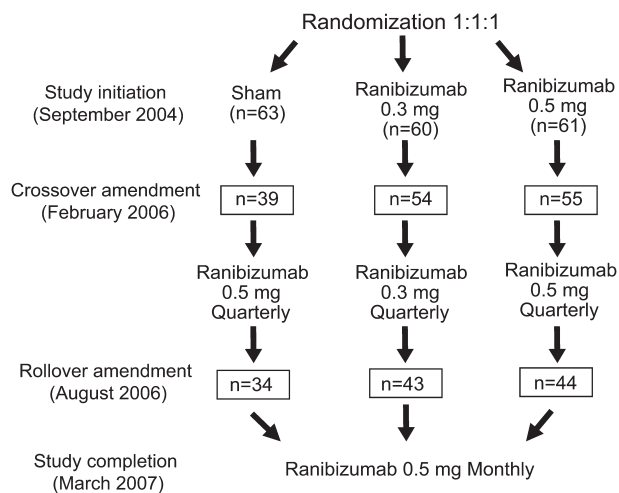


Fig. 1. Randomization, crossover, and rollover scheme. The PIER study was initiated in September 2004 and completed in March 2007. Eyes were randomized 1:1:1 to sham injection, 0.3-mg intravitreal ranibizumab, or 0.5-mg intravitreal ranibizumab. A February 2006 protocol amendment allowed sham-group subjects who remained in the study to crossover to receive 0.5-mg intravitreal ranibizumab quarterly after completing the Month-12 visit. An August 2006 amendment allowed subjects who remained in the study to rollover to receive 0.5-mg intravitreal ranibizumab monthly. Reprinted from Abraham et al⁸ with permission from Elsevier.

fluid/serous pigment epithelial detachment. Subretinal fluid was defined as a dark space between the neurosensory retina and the RPE. Sub-RPE fluid was defined as a dark space immediately under the RPE band (see Figure 2). Qualitative OCT subgroups were composed of eyes with active OCT lesions (having evidence of any type of fluid on any scan) or inactive OCT lesions (i.e., having no evidence of fluid on any scan).

Statistical Analyses

Baseline FFA characteristics (total lesion area categorized as ≤ 4 or > 4 DA and lesion composition type) were evaluated for their relationship with BCVA change from baseline at Month 24. Post hoc analyses of mean change from baseline BCVA were performed at specific time points early in the treatment for subgroups defined by FFA (active FFA lesions vs. inactive FFA lesions), quantitative OCT (CFT $< 200 \mu\text{m}$ vs. $\geq 200 \mu\text{m}$), and qualitative OCT (active OCT lesions vs. inactive OCT lesions). Because of the prespecified protocol, only specific visits had FFA and OCT performed. Month 5 and Month 8 were the only data points with both OCT and FFA data that were not immediately preceded by a mandatory injection the month prior. Data were pooled across ranibizumab dose groups, and the last-observation-carried-forward method was used for missing BCVA, FFA, and OCT data. Last observation carried forward was infrequently used, mostly after Month 12. Student *t*-test, analysis of variance, chi-square, and Pearson and polychoric correlation (observed data) were also used. Polychoric correlation relates an interval outcome with a dichotomous outcome, and its interpretation is similar to that of the Pearson correlation. Stratified tests were used to

determine changes in baseline BCVA, CNV plus intense progressive RPE staining, and CFT across time.

Results

Subject Characteristics and Treatment

Between September 2004 and March 2005, 184 subjects were enrolled at 43 U.S. investigative sites and randomly assigned to receive 0.3 mg ($n = 60$) or 0.5 mg ($n = 61$) intravitreal ranibizumab or sham injections ($n = 63$). The treatment groups were balanced on demographic and baseline ocular characteristics (Table 1). Baseline BCVA for each treatment group was 53 to 56 letters (Snellen equivalent, approximately 20/63 to 20/80). Eighty-one percent of eyes had either occult with no classic or minimally classic CNV lesions. Occult with no classic CNV was more common in the ranibizumab groups (48% in the 0.3-mg group, 49% in the 0.5-mg group) than in the sham-injection group (32%). Seventeen (27.0%) sham eyes, 7 (11.7%) 0.3-mg eyes, and 7 (11.5%) 0.5-mg eyes discontinued from study before Month 24. The primary reason for study discontinuation was the subject's decision to do so.

Forty-three (71.7%) 0.3-mg subjects and 44 (72.1%) 0.5-mg subjects who remained on study at the time of the rollover amendment rolled over to receive 0.5 mg ranibizumab monthly, beginning at Month 19. Subjects in the 0.3-mg and 0.5-mg groups received an average of 2.6 (range, 1–5) and 2.5 (range, 1–5) injections of ranibizumab, respectively, after rollover (Table 2).

At Month 12, BCVA of eyes in the 0.3-mg, 0.5-mg, and sham-treatment groups had decreased an average of 1.6, 0.2, and 16.3 ETDRS letters, respectively ($P < 0.0001$ each ranibizumab dose vs. sham). At Month 24, BCVA had decreased an average of 2.2 letters (0.3 mg), 2.3 letters (0.5 mg), and 21.4 letters

Fig. 2. Optical computed tomography images of fluid components. The images exemplify the qualitative measures of fluid, including diffuse edema, intraretinal cysts, subretinal fluid, and sub-RPE fluid.

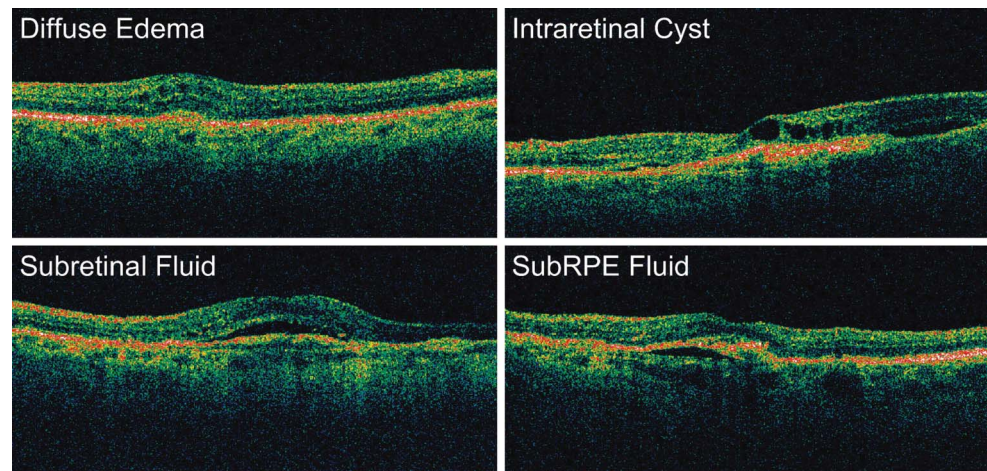


Table 1. Baseline Demographics and Ocular Characteristics

| Characteristic, n (%) | Sham (n = 63) | 0.3 mg Ranibizumab (n = 60) | 0.5 mg Ranibizumab (n = 61) |
|---|---------------|-----------------------------|-----------------------------|
| Age, years | | | |
| Mean | 77.8 | 78.7 | 78.8 |
| <75 | 16 (25) | 13 (22) | 16 (26) |
| ≥75 | 47 (75) | 47 (78) | 45 (74) |
| BCVA (with approximate Snellen equivalent)* | | | |
| Mean (ETDRS letters) | 55.1 | 55.8 | 53.7 |
| ≤54 (20/80) | 25 (40) | 29 (48) | 27 (44) |
| ≥55 (20/80) | 38 (60) | 31 (52) | 34 (56) |
| Total area of lesion | | | |
| Mean (DA) | 4.3 | 4.4 | 4.0 |
| ≤4 DA | 32 (51) | 32 (54) | 31 (51) |
| >4 DA | 31 (49) | 27 (46) | 30 (49) |
| CNV lesion subtype† | | | |
| Predominantly classic | 13 (21) | 8 (13) | 12 (20) |
| Minimally classic | 30 (48) | 22 (37) | 19 (31) |
| Occult with no classic | 20 (32) | 29 (48) | 30 (49) |

*Measured using the ETDRS chart at a starting distance of 4 m.
 †Sham = 63, 0.3 mg ranibizumab = 59, 0.5 mg ranibizumab = 61.

(sham group) from baseline ($P < 0.0001$ each ranibizumab dose vs. sham; Figure 3).⁸

Fundus Fluorescein Angiography

Fundus fluorescein angiographs of 63 (100%) sham-group eyes, 59 (98.3%) of 0.3 mg eyes, and 61 (100%) of 0.5-mg eyes were evaluated. For ranibizumab group eyes, the total area of CNV leakage plus intense progressive RPE staining decreased during the first 3 months, increased slightly between Months 3 and 8, and then decreased, for an average decrease from baseline of 1.4 and 1.3 DA at Month 12 and 1.5 and 1.2 DA at Month 24 in the 0.3-mg and 0.5-mg groups, respectively (Figure 4). In sham-group eyes, the total

area of CNV leakage plus intense progressive RPE staining increased across the first study year (with an average increase of 1.4 DA at Month 12) and decreased between Month 12 and Month 24 (with an average decrease of 0.8 DA at Month 24) (Month 12 $P < 0.0001$ each ranibizumab group vs. sham; Month 24 $P > 0.2$ each ranibizumab group vs. sham). The average decrease in FFA activity in the sham group during the second year of study was probably as a result of 61.9% of sham subjects crossing over to 0.5 mg ranibizumab, beginning at Month 14.

In the pooled 0.3-mg and 0.5-mg ranibizumab group, eyes with CNV lesions ≤4 DA at baseline had a mean change from baseline BCVA at Month

Table 2. Postrollover Treatment for 0.3 mg and 0.5 mg Treatment Groups

| | Ranibizumab | |
|------------------------------------|-----------------|-----------------|
| | 0.3 mg (n = 43) | 0.5 mg (n = 44) |
| Number of injections | | |
| 1 | 13 | 16 |
| 2 | 8 | 7 |
| 3 | 6 | 8 |
| 4 | 14 | 11 |
| 5 | 2 | 2 |
| First rollover injection at Month: | | |
| 19 | 3 | 3 |
| 20 | 14 | 16 |
| 21 | 6 | 4 |
| 22 | 7 | 5 |
| 23 | 13 | 16 |

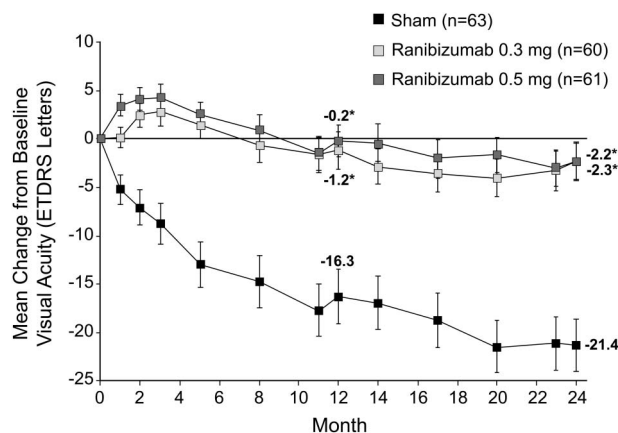
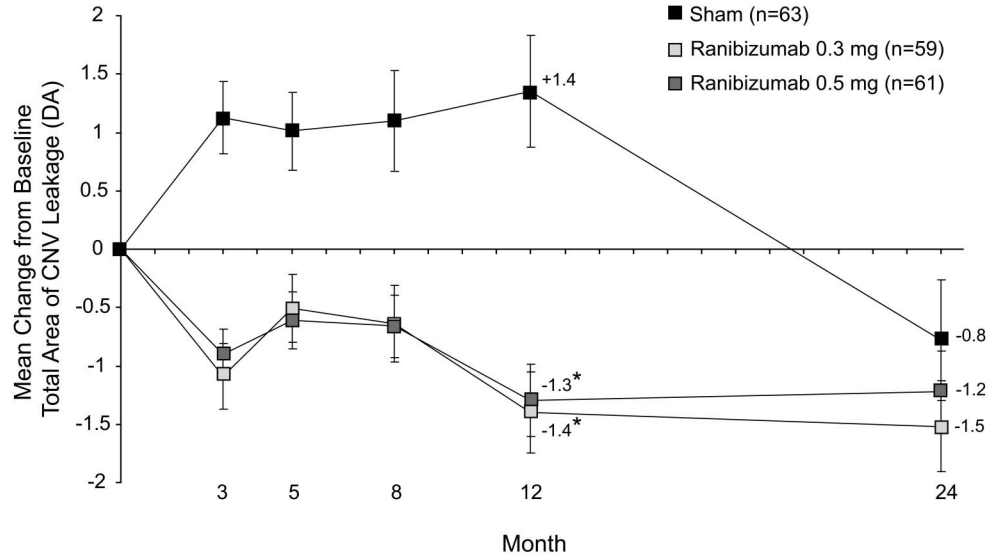


Fig. 3. Mean change from baseline visual acuity over time to Month 24. Postcrossover and postrollover data are included. All values are from the Month 24 database. The last-observation-carried-forward method was used to impute missing data. Error bars are ± 1 standard error of the mean. * $P < 0.0001$ versus sham.

Fig. 4. Mean change from baseline total area of CNV activity by FFA over time to Month 24. Fundus fluorescein angiography was performed at screening and Months 3, 5, 8, 12, and 24. Area of activity was defined as the area of choroidal neovascular leakage plus progressive retinal pigment epithelial staining. The decrease in FFA activity in the sham group may have been affected by the crossover to active drug in 61.9% of sham eyes beginning Month 14. The last-observation-carried-forward method was used to impute missing data. Error bars are ± 1 standard error of the mean. * $P < 0.0001$ versus sham.



24 of -0.10 letters compared with -4.3 letters in eyes with lesions >4 DA at baseline ($P = 0.13$). Approximately 16% of eyes with lesions ≤ 4 DA and 23% of those with lesions >4 DA lost ≥ 15 letters from baseline BCVA at Month 24 ($P = 0.34$); 14% of eyes with baseline lesions ≤ 4 DA gained ≥ 15 letters from baseline BCVA at Month 24 compared with 9% of eyes with baseline lesions >4 DA ($P = 0.35$).

In the pooled 0.3-mg and 0.5-mg ranibizumab group, eyes with minimally classic CNV at baseline had a mean change from baseline BCVA at Month 24 of -3.1 letters compared with -2.0 letters for eyes with occult with no classic CNV at baseline and -1.4 letters for eyes with predominantly classic CNV ($P = 0.90$). Approximately 20% of eyes with minimally classic, 20% with occult with no classic, and 20% with predominantly classic CNV at baseline lost ≥ 15 letters from baseline BCVA at Month 24 ($P = 1.0$), and 10% of eyes with minimally classic or occult with no classic CNV at baseline gained ≥ 15 letters from baseline BCVA at Month 24 compared with 20% of eyes with predominantly classic CNV ($P = 0.44$).

Ranibizumab-treated eyes with inactive FFA lesions (i.e., total area of CNV leakage plus intense progressive RPE staining = 0) at Month 3 had gained an average of 10.2 letters from baseline BCVA at Month 12. Visual acuity decreased on average in those eyes after Month 12, for a net average change from baseline BCVA of 1.7 letters at Month 24. Eyes with active FFA lesions (i.e., total area of CNV leakage plus intense progressive RPE staining $\neq 0$) at Month 3 had lost 1.8 letters at Month 12, and their visual acuity remained stable, on average, after Month 12, for a net average change from baseline BCVA of -2.5 letters at Month 24 (Figure 5A). The 12-letter

BCVA difference between the groups at Month 12 was statistically significant ($P = 0.0045$), and the 4-letter difference at Month 24 was not ($P = 0.36$). Ranibizumab-treated eyes with inactive FFA lesions at Month 5 had gained an average of 9.7 letters at Month 12 and 5.1 letters at Month 24, while those with active FFA lesions at Month 5 had lost an average of 1.6 letters at Month 12 and 2.8 letters at Month 24 (Figure 5B). Eyes with inactive FFA lesions at Month 5 had gained an average of 11.3 more letters at Month 12 ($P = 0.0096$) and 7.9 more letters at Month 24 ($P = 0.093$) compared with those with active FFA lesions at Month 5.

Quantitative Optical Coherence Tomography

Scans from 42 (66.7%) sham-group eyes, 39 (65.0%) 0.3-mg eyes, and 42 (68.9%) 0.5-mg eyes were evaluated. Central foveal thickness of ranibizumab-treated eyes decreased during the first 3 months, increased slightly from Month 3 to Month 8, and then decreased, for an average decrease of $126 \mu\text{m}$ (0.3 mg) and $123 \mu\text{m}$ (0.5 mg) by Month 12 (Figure 6). For sham-group eyes, CFT increased slightly during the first 3 months and then decreased for the remainder of the first study year, for an average decrease of $29 \mu\text{m}$ at Month 12 ($P < 0.01$ each ranibizumab group vs. sham group).

The subgroup of ranibizumab-treated eyes that had CFT $< 200 \mu\text{m}$ ($n = 53$) at Month 3 had gained an average of 0.45 letters from baseline at Month 12 and lost an average of 0.25 letters at Month 24, while those that had lesions with CFT $\geq 200 \mu\text{m}$ ($n = 24$) at Month 3 had lost an average of 2.42 letters at Month 12 and 3.33 letters at Month 24 (Figure 7A). Thus, eyes with

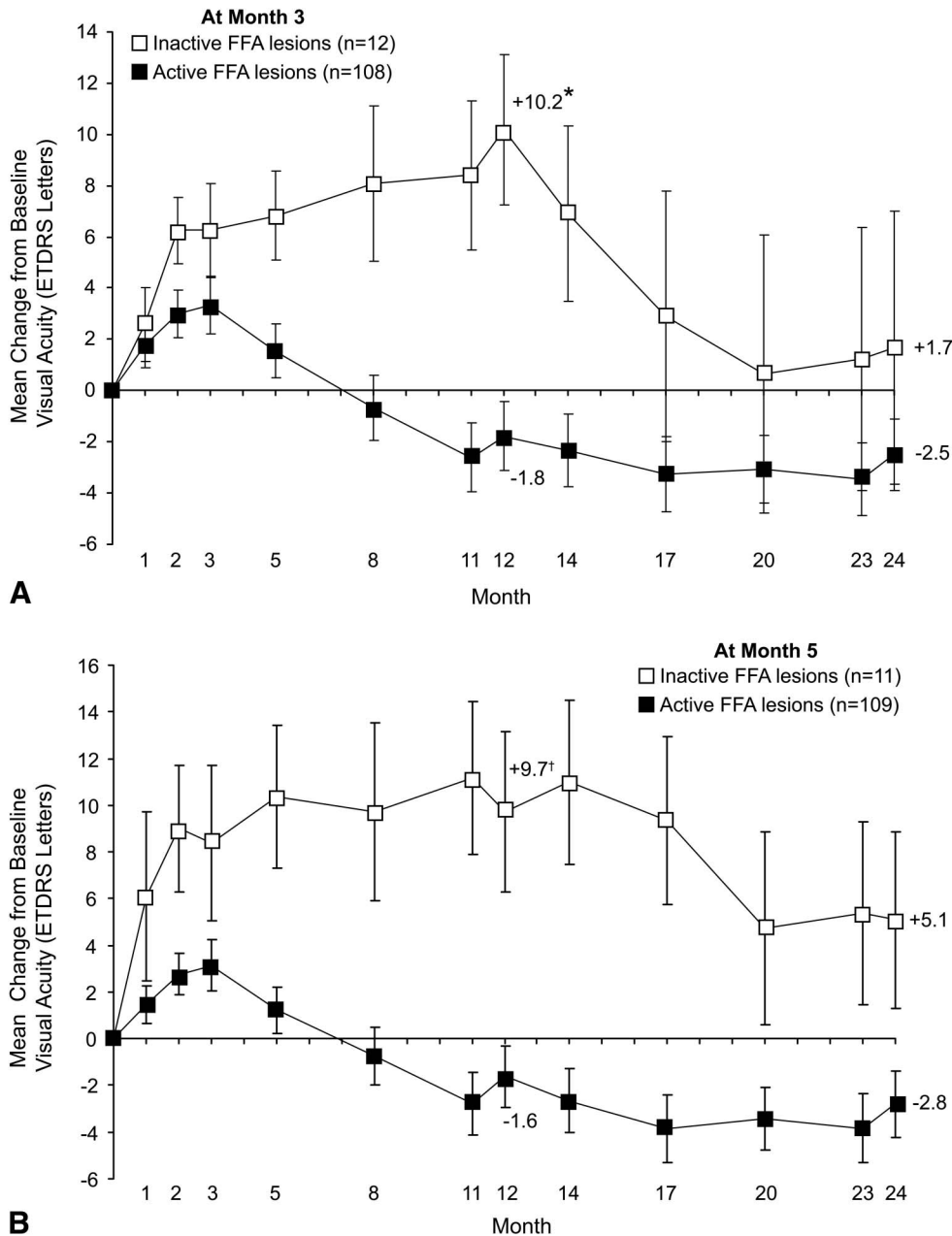


Fig. 5. Mean change from baseline visual acuity by lesion activity on FFAs for pooled ranibizumab eyes. Subgroups were composed of eyes that had active FFA lesions (i.e., total area of CNV leakage plus intense progressive RPE staining \neq 0) and inactive FFA lesions (i.e., total area of CNV leakage plus intense progressive RPE staining = 0) on FFAs at Months 3 and 5 (panels A and B, respectively). Data from 0.3-mg and 0.5-mg dose groups were pooled. Postrollover data are included. The last-observation-carried-forward method was used to impute missing data. Error bars are \pm 1 standard error of the mean. Differences in change from baseline BCVA between active FFA lesion and inactive FFA lesion subgroups were evaluated at Months 12 and 24. * $P = 0.004$ inactive FFA lesions versus active FFA lesions; † $P = 0.0096$ inactive FFA lesions versus active FFA lesions.

CFT $<200 \mu\text{m}$ at Month 3 had gained an average of 2.87 more letters at Month 12 and 3.58 more letters at Month 24 compared with those with CFT $\geq 200 \mu\text{m}$ at Month 3 (Month 12, $P = 0.45$; Month 24, $P = 0.41$).

The subgroup of ranibizumab-treated eyes with CFT $<200 \mu\text{m}$ ($n = 42$) at Month 5 had gained an average of 1.86 letters from baseline BCVA at Month 12 and 2.26 letters at Month 24, while those with CFT $>200 \mu\text{m}$ ($n = 36$) had lost an average of 2.36 letters at Month 12 and 4.61 letters at Month 24 (Figure 7B). Eyes with CFT $<200 \mu\text{m}$ at Month 5 had gained an average of 4.22 more letters at Month 12 ($P = 0.20$) and 6.87 more

letters at Month 24 ($P = 0.045$) compared with those with CFT $>200 \mu\text{m}$ at Month 5.

Qualitative Optical Coherence Tomography Analysis

Optical coherence tomography scans were qualitatively evaluated for any evidence of CNV-associated activity (Figure 2). Differences between the percentage of ranibizumab-treated eyes and sham eyes with inactive OCT lesions across the 2 years of study were statistically significant at Months 1, 2, 3, and 12 (all P values < 0.05)

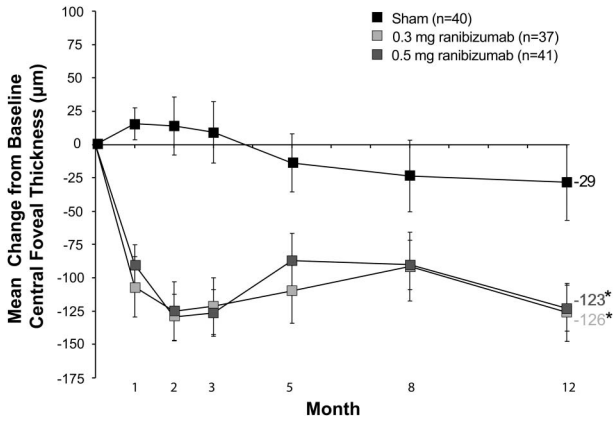


Fig. 6. Mean change from baseline CFT over time to Month 12. Optical coherence tomography was performed at selected study sites on Day 0 and at Months 1, 2, 3, 5, 8, and 12. Postcrossover and postrollover data are included. The last-observation-carried-forward method was used to impute missing data. Error bars are ± 1 standard error of the mean. * $P < 0.01$ versus sham.

(Figure 9). Ranibizumab-group eyes had received a mandatory injection 1 month before each of those time points (i.e., Day 0, Month 1, Month 2, and Month 11).

Ranibizumab-treated eyes with inactive OCT lesions at Month 2 had lost an average of 0.27 letters at Month 12 and 0.44 letters at Month 24, while eyes with active OCT lesions at Month 2 had gained an average of 0.06 letters at Month 12 and lost an average of 1.13 letters at Month 24 (Figure 8A). The mean change from baseline BCVA of eyes with inactive OCT lesions versus active OCT lesions at Month 2 was not significantly different at Month 12 ($P = 0.91$) or Month 24 ($P = 0.84$).

Ranibizumab-treated eyes with inactive OCT lesions at Month 3 had gained an average of 1.65 letters from baseline BCVA at Month 12 and 0.41 letters at Month 24, while those with active lesions at Month 3 had lost an average of 4.0 letters at Months 12 and 24 (Figure 8B). Thus, eyes that had inactive OCT lesions at Month 3 had gained an average of 5.65 more letters at Month 12 ($P = 0.091$) and 4.41 more letters at Month 24 ($P = 0.22$) compared with those that had active OCT lesions at Month 3.

Ranibizumab-treated eyes that had inactive OCT lesions at Month 5 had gained an average of 2.04 letters from baseline BCVA at Month 12 and 3.86 letters at Month 24, while those that had active OCT lesions at Month 5 had lost an average of 1.25 letters at Month 12 and 3.24 letters at Month 24 (Figure 8C). Thus, eyes that had inactive OCT lesions at Month 5 had gained an average of 3.29 more letters at Month 12 ($P = 0.33$) and 7.10 more letters at Month 24 ($P = 0.045$) compared with those that had active lesions at Month 5.

Ranibizumab-treated eyes that had inactive OCT lesions at Month 8 had gained an average of 3.88 letters

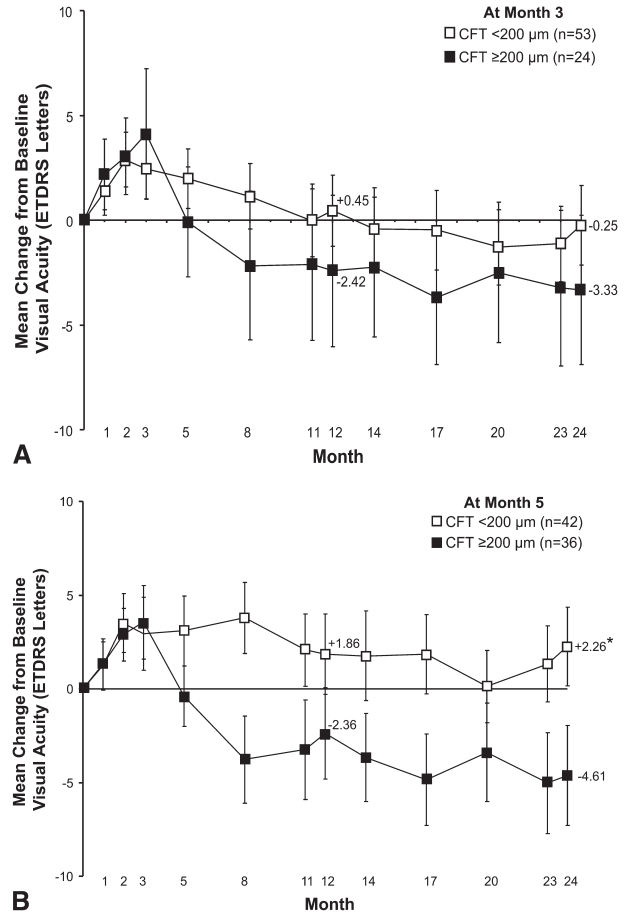


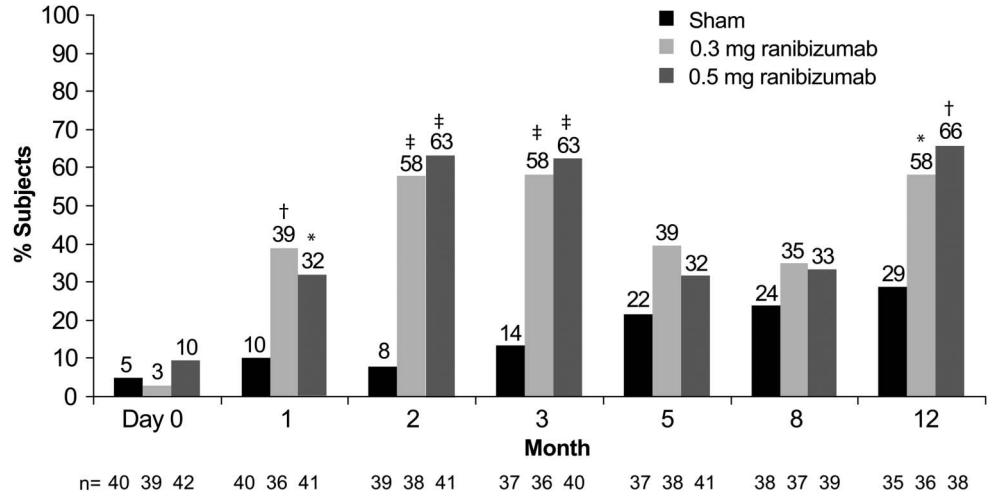
Fig. 7. Mean change from baseline visual acuity by CFT for pooled ranibizumab eyes. Subgroups were defined by CFT $< 200 \mu\text{m}$ and CFT $\geq 200 \mu\text{m}$ at Months 3 and 5 (panels A and B, respectively). Data from the 0.3-mg and 0.5-mg dose groups were pooled. Postrollover data are included. The last-observation-carried-forward method was used to impute missing data. Error bars are ± 1 standard error of the mean. Evaluated at Months 12 and 24, there were no statistically significant differences in change from baseline BCVA between CFT $< 200 \mu\text{m}$ and CFT $\geq 200 \mu\text{m}$ subgroups (Panel A). * $P = 0.045$, CFT $< 200 \mu\text{m}$ versus CFT $\geq 200 \mu\text{m}$.

from baseline BCVA at Month 12 and 5.5 letters at Month 24, while those that had active OCT lesions at Month 8 had lost an average of 2.0 letters at Month 12 and 4.04 letters at Month 24 (Figure 8D). Thus, eyes that had inactive OCT lesions at Month 8 had gained an average of 5.88 more letters at Month 12 ($P = 0.09$) and 9.54 more letters at Month 24 ($P = 0.009$) compared with those with active lesions at Month 8.

Correlation of Anatomical Criteria and Best-Corrected Visual Acuity Outcomes

Pearson correlation coefficients of change from baseline leakage plus RPE staining (leakage) at any visit with change from baseline BCVA at the same or later visits ranged from -0.18 (Month 5 FFA and Month

Fig. 9. Percentage of eyes with inactive lesions by qualitative OCT during study Year 1. A larger percentage of ranibizumab-treated eyes than sham-injection eyes had inactive lesions (i.e., remained fluid free) during study Year 1. ‡*P* < 0.0001 versus sham; †*P* < 0.01 versus sham; **P* < 0.05 versus sham.



The largest negative correlations (showing an association between CFT decreases and BCVA increases) were typically observed at the same visits or visits that were close in time. For both FFA and OCT, correlations of early anatomical change with later BCVA change were slightly negative or positive, indicating no linear relationship between early anatomical improvement and later BCVA improvement.

Correlations were also assessed for change from baseline BCVA at the same or later visits and dichotomized FFA (i.e., active FFA lesions vs. inactive FFA lesions), and separately, with dichotomized qualitative OCT (i.e., active OCT lesions vs. inactive OCT lesions). Polychoric correlations supported the inference that improved BCVA was associated with anatomical dryness (a positive correlation given the dichotomous coding). For example, for over 24 months, polychoric correlation coefficients for FFA and OCT ranged from +0.21 to +0.48 and +0.13 to +0.32, respectively.

Discussion

This study evaluated the degree to which the anatomical characteristics of the study eye early in treatment affected BCVA outcomes in subgroups of ranibizumab-treated PIER study eyes. The findings demonstrate concepts that may help guide clinicians in the use of anti-VEGF agents in neovascular AMD. First and foremost, an initial anatomical (according to FFA and/or OCT analyses) or visual improvement after three monthly ranibizumab injections does not guarantee long-term success. For eyes with FFA lesion activity at Month 3, CFT ≥200 μm at Month 3, and qualitative OCT activity at Months 2 and Month 3 (Figures 5A, 7A, and 8A and B, respectively), the

average BCVA gain from 3 monthly loading doses of ranibizumab was lost after switching to quarterly dosing, and eyes lost vision compared with baseline at Months 12 and 24. This demonstrates that eyes that experience improvements after three monthly loading doses still need to be closely monitored because many will need frequent retreatment with anti-VEGF therapy. Second, it appears that the longer anatomical improvements were maintained (according to FFA or OCT), the more likely it was that the BCVA benefits of ranibizumab persisted on a quarterly dosing regimen. Eyes with inactive FFA lesions at Month 5 (Figure 5B) or inactive OCT lesions at Month 5 (Figure 8C) or Month 8 (Figure 8D) were much more likely to maintain their BCVA gains (analogous to the entire treated cohorts in MARINA and ANCHOR that had continuous monthly dosing). It is not our belief that this indicates eyes with an anatomical improvement should be observed less frequently or treated less frequently. However, the data imply that analogous to the successful repair of rhegmatogenous retinal detachments, maintained anatomical success (absence of disease activity on OCT or FFA) seems important for BCVA to be gained and maintained.

Qualitative OCT grading revealed that approximately 60% of eyes showed no lesion activity after 2 loading doses of ranibizumab, and this was maintained with a third dose (Figure 9). Thus, it may be that the majority of “anatomical responders” or “incomplete anatomical responders” can be determined after 2 doses of ranibizumab. Subsequent to Month 3, when subjects were mandated to switch to quarterly dosing, more than one third of the initial responders demonstrated recurrent activity on OCT at Month 5 and/or Month 8. While the response at Month 12 (after a mandatory injection at Month 11) showed an anatomical success rate similar to that seen after the initial 2 or 3

doses, this did not result in a concomitant rebound in mean BCVA. This implies that the relative undertreatment and resulting anatomical activity resulted in retinal injury and irreversible loss of BCVA gains. Further evidence of this irreversibility is that even though approximately 72% of patients initially randomized to ranibizumab “rolled over” to monthly therapy beginning at Month 19 (and had on average 2.7 injections), there was minimal improvement in mean BCVA (Figure 3), and despite the best intentions of the rollover amendment, there was a net loss in mean BCVA from baseline to Month 24.

While a surprisingly low number of eyes demonstrated inactive FFA lesions after 3 loading doses of ranibizumab (i.e., 10% at Month 3), eyes with a dry FFA showed the strongest association with BCVA outcomes at Months 12 and 24. At the same 3-month time point, 60% of evaluated eyes were dry on qualitative OCT grading. This disparity may result from the sampling error introduced by having only two scans available for grading (rather than all 6 radial line scans available from a Stratus macular thickness map or the greatly increased sample size of currently available spectral-domain OCT devices). It is also known that an effective RPE pump sometimes keeps the retina dry and gives a “dry” OCT reading, despite active CNV leakage. This may have been the case for approximately 5% of eyes at Day 0, for which no fluid was seen with qualitative OCT analysis by a masked grader.

Comparison of the 12-month and 24-month BCVA outcomes and correlations between anatomical factors raise important implications for the design of clinical trials for neovascular AMD because it appears that the deleterious effects of undertreatment may take longer than 12 months to become evident. While eyes that had inactive FFA lesions at Month 3 had statistically greater gains from baseline BCVA at Month 12, the advantage was lost by Month 24. In contrast, according to qualitative OCT grading at Months 5 and 8, Month 12 BCVA outcomes of the subgroups of eyes with consistently dry OCTs were not statistically different than those with fluid at Months 5 and 8 ($P = 0.33$ and 0.090 , respectively). However, their BCVA outcomes were significantly different at Month 24 ($P = 0.045$ and 0.0096 , respectively). This implies that the resilience of the retina is finite and that it may take time to demonstrate a difference in treatment strategies. This not only justifies the 2-year end point required in U.S. Food and Drug Administration registration trials for AMD therapies but also emphasizes that the 2-year end point of the Comparison of Age-related Macular Degeneration Treatments Trials (CATT): Lucentis-Avastin Trial⁴ trial may be critical to see the ultimate effects of a pro re nata (PRN) treatment regimen on BCVA.

The CATT study monthly treatment arms demonstrated that 53% of eyes with monthly ranibizumab and 71% of monthly bevacizumab had fluid at Month 12 on reading center qualitative OCT analysis of all 6 Stratus radial line scans, but overall, the vision gains were excellent in both the groups,⁴ indicating that small amounts of fluid may not be deleterious to 12 month BCVA outcomes with monthly therapy. A higher percentage of eyes had fluid on OCT at Month 12 in the CATT PRN arms (71% ranibizumab and 79% bevacizumab),⁴ and the overall reduction in BCVA gains at 12 months in these cohorts may be indicative of the deleterious effects of more fluid with PRN therapy. In the PIER study, anatomical characteristics at time points not immediately after monthly doses were the most predictive of BCVA at Months 12 and 24. Analysis of OCT and FFA data available at the time of PRN therapy visits in the CATT study through 24 months (and the ongoing HARBOR 2.0-mg ranibizumab study PRN arms) will help clarify the correlation of OCT fluid and BCVA outcomes because both of these studies have more complete OCT data sets and larger sample sizes.

Some limitations were inherent to our study. The fixed 3-month dosing in PIER is obviously way too long for most patients and allowed more recurrence and persistence of retinal fluid for much longer than a monthly “tight PRN” CATT approach or even a “treat-and-extend” regimen. The PIER dosing regimen probably exaggerated the deleterious effects of fluid in the retina, and as such, it may not be applicable to smaller excursions of retinal thickness seen with tight PRN or treat-and-extend regimens. Also, anatomical subgroups were limited because OCT data were available for only about two thirds of eyes during the first year of study. Additionally, qualitative OCT evaluation might have been confounded by the quality of the scans. The original scans had been printed out at PIER study sites and were subsequently scanned into portable document format for grading on a computer monitor. Moreover, only two upper-resolution scans were available per subject visit, rather than the six that are standard with a macular thickness map protocol on the Stratus OCT. High-resolution imaging with spectral-domain OCT will probably prove to be a more sensitive method for detecting recurrence of CNV leakage.

Our results demonstrate that subgroups of eyes that had no recurrent CNV leakage on FFA at Month 5 or OCT at Months 5 and 8 (visits 3 months after the last injection) maintained an overall net vision gain compared with baseline BCVA at the 24-month end point. In contrast, those with FFA and OCT activity at Months 3 (after 3 consecutive monthly doses), 5, or 8 not only

lost the initial BCVA gains achieved but finished with net vision losses compared with baseline at the Month 24 end point. This association between specific anatomical markers and ultimate BCVA will need to be validated with the anatomical data sets available with the ongoing CATT and HARBOR PRN arms.

Key words: neovascular AMD, ocular coherence tomography, fundus fluorescein angiography, anatomic predictors, ranibizumab, anti-VEGF therapy.

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