Assessment of Differential Pharmacodynamic Effects Using Optical Coherence Tomography in Neovascular Age-Related Macular Degeneration

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PURPOSE. To use novel OCT parameters in assessing the differential pharmacodynamic effects of bevacizumab (Avastin; Genentech, South San Francisco, CA), pegaptanib (Macugen; OSI Pharmaceuticals, New York, NY), and verteporfin photodynamic therapy (PDT; Novartis, Basel, Switzerland) in a recently completed phase III/IV clinical trial.

METHODS. Data from 122 patients participating in the Avastin (Bevacizumab) for Choroidal Neovascularization (ABC) trial, were evaluated. OCT scans were analyzed with custom software. Changes in the volume of the neurosensory retina, amount of subretinal fluid (SRF), pigment epithelium detachment (PED), and subretinal tissue (SRT), were calculated over the 54-week trial period.

RESULTS. Reductions in retinal edema were more than twice as great from bevacizumab as from pegaptanib (-0.82 mm^3 vs. -0.31 mm^3), whereas SRF reduction was more than three times greater (-0.54 mm^3 vs. -0.15 mm^3). Both bevacizumab and pegaptanib led to rapid reductions in SRT; however, in those receiving pegaptanib, these improvements were not maintained (at week 54, -0.22 mm^3 vs. $+0.18 \text{ mm}^3$). Acute increases in SRF were seen 1 week after PDT ($+0.36 \text{ mm}^3$) and, across all treatment groups, PED volume tended to remain unchanged or to regress only slowly.

CONCLUSIONS. In clinical trials, quantitative OCT subanalysis increases the amount of clinically useful information that can be obtained from OCT images. In the emerging era of neovascular AMD therapeutics, the capacity of OCT to provide such detailed pharmacodynamic information in a noninvasive manner is likely to attain increased importance. In future comparative studies, evaluation of SRT may highlight differential effects on vascular proliferation, whereas measurement of PED volume may be useful for the estimation of retinal and subretinal pigment epithelium (RPE) therapeutic penetration. (ClinicalTrials.gov number, ISRCTN83325075.) (*Invest Ophthalmol Vis Sci.* 2012; 53:1152–1161) DOI:10.1167/iovs.11-8130

Bevacizumab (Avastin, Genentech, South San Francisco, CA), an antiangiogenic agent licensed for the management of colorectal carcinoma, has been widely adopted for the treatment of neovascular age-related macular degeneration (AMD).^{1,2} Recently, the results of the Avastin (Bevacizumab) in Choroidal Neovascularization (ABC) Trial provided the first level I evidence (i.e., evidence from a well-designed, randomized, controlled clinical trial) for the safety and efficacy of this approach.³ In the ABC trial, bevacizumab was administered as an intravitreal injection once every 6 weeks, after an initial loading phase of three intravitreal injections, further treatment was determined in large part by the assessment of disease activity using optical coherence tomography (OCT).

As in the ABC study, many clinical trials have adopted OCT-derived retreatment criteria, both for neovascular AMD and for other disorders.^{4–6} In addition, the application of OCT to clinical research has elucidated many hitherto unrecognized disease characteristics and clarified many aspects of disease pathophysiology.⁷ The advent of OCT has also allowed objective, quantitative assessment of morphologic parameters,^{8,9} with OCT-derived measurements of retinal thickness commonly used for both clinical and research purposes.^{10,11} As our knowledge of OCT image analysis has grown, however, it has become increasingly clear that even accurate measurements of retinal thickness may fail to predict visual outcomes.^{12,13} Thus, much of the focus of recent clinical imaging research has been on the identification of novel OCT-derived anatomic biomarkers.^{14–17}

For clinical trials, the discovery of novel OCT biomarkers may provide valuable information regarding therapeutic mechanisms of action, pharmacodynamics, and pharmacokinetics.^{18,19} If such biomarkers are shown to predict clinical benefit, they could also serve as surrogate endpoints in these trials, potentially leading to increased accuracy, reduced costs, and shortened duration. Similarly, in clinical practice, such parameters could extend the application of OCT imaging beyond simple diagnosis and toward prognosis.⁷ With the increased utilization of combination treatment strategies²⁰ and the likely future development of additional pathway-based therapies,²¹

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such pharmacodynamic and prognostic information may soon be crucial for the clinician in choosing the appropriate type and level of care.

In the ABC trial, bevacizumab was compared to the standard therapy available at the trial's initiation: pegaptanib (Macugen; OSI Pharmaceuticals, New York, NY) or verteporfin photodynamic therapy (PDT; Novartis, Basel, Switzerland).^{3,22} Although treatment of patients in these standard therapy groups was not determined by OCT-derived criteria, OCT examinations were nonetheless performed. Thus, the ABC trial offers a unique opportunity to investigate novel OCT biomarkers, with an emphasis on differential pharmacodynamic effects, in a phase III/IV randomized clinical trial.

MATERIALS AND METHODS

Trial Design

The ABC trial was a double-masked randomized controlled trial that commenced in August 2006 and in which intravitreal bevacizumab injections were compared with standard therapy in the treatment of neovascular AMD.^{3,22} Patients enrolled in the ABC Trial were randomized to intravitreal bevacizumab or to the standard therapy available at the time of trial initiation (photodynamic therapy [PDT] with verteporfin, intravitreal pegaptanib, or sham treatment). For inclusion in the study, patients were required to have previously untreated subfoveal choroidal neovascularization secondary to AMD, with no evidence of significant ocular comorbidity. The ABC Trial was conducted according to the guidelines of the ICHGCP (International Conference on Harmonization for Good Clinical Practice in clinical research), as set out in the European Union Clinical Trials Directive (2001) and associated U.K. Regulations (2004) that comply with the principles of the Declaration of Helsinki.

Retreatment Schedule and Study Assessments

After baseline treatment, patients attended again at week 1 for a safety visit (no treatment given). Further follow-up visits with the potential for retreatment occurred at weeks 6, 12, 18, 24, 30, 36, 42, and 48. The study exit visit occurred at week 54 (~1 year), yielding a total of nine treatment visits, with the first three requiring mandatory bevacizumab treatments (baseline, week 6, and week 12) and the remaining visits dictating treatment only if standardized retreatment criteria were met (at weeks 18, 24, 30, 36, 42, and 48).

Each patient's best-refracted visual acuity was recorded at the time of enrollment using Early Treatment Diabetic Retinopathy (ETDRS) visual acuity charts at a starting distance of 4 m. Structural outcomes were assessed at every visit by using OCT measures of retinal thickness and qualitative features of choroidal neovascularization (CNV). Fundus fluorescein angiography was performed at baseline and weeks 6, 12, 24, 36, 48, and 54 (with additional fluorescein angiography at week 1 for the first 20% of patients) to allow for assessment of any change in CNV size and leak.

OCT Image Acquisition

OCT images (Stratus; Carl Zeiss Meditec, Dublin, CA), obtained with the radial lines protocol of six high-resolution B-scans (512 A-scans per 6-mm B-scan) or the fast macular scan protocol of six low-resolution B-scans (128 A-scans per 6-mm B-scan), were collected at baseline for each patient enrolled in the study. OCT imaging was again performed 1 week after the initial treatment, followed by 6-week intervals thereafter. For the purposes of this study, OCT images from baseline, week 1, and week 54, were collected and analyzed. Data for each case were exported to disk using the export feature available in the optical coherence tomograph (Stratus OCT ver. 4.0 analysis software; Carl Zeiss Meditec).

Generation of Novel OCT-Derived Biomarkers

For each patient enrolled in the study, custom image-analysis software was used to generate novel OCT-derived anatomic biomarkers. This software (entitled OCTOR) was written by Doheny Image Reading Center software engineers to facilitate viewing and manual grading of OCT images. For any structural parameter of interest, OCTOR provides a report showing the calculated thickness/volume values for the nine ETDRS macular subfields, as well as the mean and SD of the foveal center point thickness. OCTOR is publicly accessible at www.diesel.la and has been described and validated in previous reports.^{23–25}

OCT scans were analyzed by certified OCT graders at the Doheny Image Reading Center (FMH, YO), who were masked to associated visual acuity information at the time of grading. All boundaries were drawn in accordance with the standard OCT grading protocol of the Doheny Image Reading Center.²⁴ All OCT scans included in the study met reading center criteria for sufficient image quality, including the absence of significant artifactual variations in signal intensity or generalized reductions in signal strength. No minimum value for signal strength was set, as manual grading with OCTOR often allows quantitative information to be accurately derived from images with low signal strength (image sets are only excluded when the grader cannot accurately delineate the inner and outer retinal boundaries).

After completion of grading, OCTOR was used to calculate output parameters for each OCT-derived biomarker.

Neurosensory Retina. Automated evaluation of retinal thickness/volume using vendor-provided software is often associated with segmentation errors.²⁶ Such errors are often severe in disorders with complex morphology such as neovascular AMD.²⁷ In contrast, OCTOR allows accurate quantification of the total volume of the neurosensory retina. On each OCT B-scan the neurosensory retina was defined as the area lying between the internal limiting membrane and the outer border of the photoreceptors (Fig. 1).

Subretinal Fluid. Exudation from the CNV lesion may result in the accumulation of fluid in the subretinal space.²⁴ On each OCT B-scan, subretinal fluid (SRF) was defined as the area lying between the outer border of the photoreceptors and the inner surface of the retinal pigment epithelium (RPE) or, when present, the inner surface of subretinal tissue (SRT; Fig. 1).

Subretinal Tissue. Growth of fibrovascular tissue in the subretinal space may be seen on OCT as an area of subretinal hyperreflectivity.²⁴ On each OCT B-scan, the inner and outer boundaries of this SRT, when present, were delineated by graders (Fig. 1).

Pigment Epithelium Detachment (PED). Growth of the CNV lesion in the sub-RPE space, combined with variable quantities of fluid exudation and hemorrhage, produces irregular areas of PED on clinical examination.²⁸ On OCT, PEDs appear as broad elevations of the RPE band relative to Bruch membrane.²⁴ On each OCT B-scan, PED was defined as the area lying between the inner surface of the RPE and the estimated normal position of the RPE (Fig. 1).

Statistical Methods

In each case, the total volume (subfields 1–9) of the morphologic compartment comprising each OCT-derived biomarker was calculated in cubic millimeters. The change from baseline in volume measurements was then calculated for each follow-up visit (i.e., weeks 1 and 54). A paired *t*-test or Wilcoxon signed rank test was performed to analyze these changes, depending on whether the data were normally distributed. For each paired statistical test, casewise deletion of missing data was performed, to detect whether one variable had a missing value. Because of the time necessary for manual segmentation, OCT image sets from other time points were not analyzed; changes in volume measurements between weeks 1 and 54 are therefore illustrated with dashed lines in Figures 2 to 5.

Univariate and multivariate regression was used to test for associations between visual function parameters and OCT parameters. Stepwise regression was used for selection of independent parameters

FIGURE 1. (A, C) Optical coherence tomography (OCT) B-scans of eyes demonstrating SRF, SRT, and FVPED. (B, D) The clinically relevant boundaries are graded using OCTOR (computer-assisted manual grading) software, which then computes the volumes of the spaces (retina, SRF, SRT, and FVPED) defined by these boundaries.

where improvement $\chi^2 P < 0.15$. Linearity was examined by testing for higher order polynomial terms for each continuous variable in the final multivariate model. To reduce potential collinearity, highly correlated variables ($r \geq 0.90$) were not included in the same model. Statistical analysis was performed with commercially available software (Intercooled Stata for Windows, ver. 9, Statacorp LP, College Station, TX).

RESULTS

Baseline Characteristics

One hundred twenty-two patients, newly diagnosed with neovascular AMD and enrolled in the ABC Trial, were evaluated. Seventy-nine (65%) eyes were imaged by OCT (SAP; Stratus OCT; Carl Zeiss Meditec) with the radial lines scanning protocol, and 43 (35%) eyes were imaged with the fast macular thickness scan protocol. Of the 122 patients included in our analysis, 76 (62%) were women, and 46 (38%) were men. The mean age of patients was 80 years (SD 7.1), and the median age was 81 years (range, 58–93 years). Mean visual acuity at time of initial diagnosis was 52.15 letters (SD 11.62; range, 25–70 letters). The neovascular lesions were categorized by fluorescein angiography as classic with no occult (8 eyes, 7%), predominantly classic (20 eyes, 16%), minimally classic (37 eyes, 30%), and occult with no classic (57 eyes, 47%).

Treatments

In the bevacizumab (Avastin; Genentech) group, patients received a mean of 7.1 (range, 3–9) injections (of a possible nine). In the standard-care groups, patients received a mean of 8.9 (median, 9; range, 6–9) injections of pegaptanib (Macugen; OSI Pharmaceuticals), and active verteporfin PDT (Novartis) was administered a mean of 3.2 times (range, 2–5).

Differential Morphologic Outcomes

The mean change from baseline in each OCT-derived morphologic space was calculated for each treatment group.

Effects on the Neurosensory Retina. In patients receiving bevacizumab, total retinal volume decreased, on average, by 0.30 mm^3 at week 1 ($P = 0.0005$; Fig. 2A). By the conclusion of the study (week 54), total retinal volume had decreased by an average of 0.83 mm^3 ($P < 0.001$). In patients receiving standard therapy, total retinal volume decreased by a mean of 0.18 mm^3 at week 1 ($P = 0.0043$). By the conclusion of the study (week 54), total retinal volume had decreased, on average, by 0.37 mm^3 ($P = 0.009$).

Effects on SRF. In patients receiving bevacizumab, SRF volume decreased, on average, by 0.23 mm^3 at week 1 ($P = 0.003$; Fig. 2B). By the conclusion of the study (week 54), total SRF volume had decreased by an average of 0.44 mm^3 ($P < 0.001$). However, in patients receiving standard therapy, SRF volume increased at week 1, by mean of 0.07 mm^3 ($P = 0.6473$). By the conclusion of the study (week 54), SRF volume had decreased, on average, by 0.29 mm^3 ($P < 0.001$).

Effects on SRT. In patients receiving bevacizumab, mean SRT volume decreased by 0.24 mm^3 at week 1 ($P < 0.001$; Fig. 3A). By the conclusion of the study (week 54), SRT volume had decreased by an average of 0.30 mm^3 ($P < 0.001$). In patients receiving standard therapy, mean total SRT volume decreased by 0.19 mm^3 at week 1 ($P = 0.002$). However, by the conclusion of the study (week 54), the initial improvements had regressed, with an average increase of 0.04 mm^3 ($P = 0.6093$).

Effects on PED. In patients receiving bevacizumab, mean PED volume increased 0.06 mm^3 at week 1 ($P = 0.0669$). By the conclusion of the study (week 54), PED volume had decreased an average of 0.22 mm^3 ($P = 0.2066$). Similarly, in patients receiving standard therapy, mean PED volume increased at week 1 by 0.15 mm^3 ($P = 0.0091$), with a subsequent average decrease of 0.07 mm^3 by week 54 ($P = 0.146$).

Subgroup Analyses by Angiographic Classification

In the ABC trial, patients randomized to the standard therapy group received treatment according to their angiographic lesion classification: patients with predominantly classic/classic lesion received PDT, whereas those with minimally classic or occult lesions received pegaptanib or sham therapy. As a result, subgroup analyses according to CNV classification were also performed. Changes from baseline in total volume of the neurosensory retina and of SRF are summarized in Figure 4 and those of SRT and PED are summarized in Figure 5.

Correlations with Visual Acuity

The associations between best corrected visual acuity and each of the OCT parameters are summarized in Table 1. At the conclusion of the study (week 54), increased volumes of SRT ($r = -0.3623$, $P < 0.001$) and neurosensory retina ($r = -0.2898$, $P = 0.002$) were associated with decreased visual acuity. Increased volumes of SRT and the neurosensory retina, at baseline, were also associated with decreased visual acuities at week 54 ($r = -0.3107$, $P < 0.001$, and $r = -0.2309$, $P =$

FIGURE 2. (A) Mean change from baseline in total volume of the neurosensory retina in those receiving bevacizumab versus those receiving standard therapy (photodynamic therapy with verteporfin, or pegaptanib). (B) Mean change from baseline in total volume of SRF in those receiving bevacizumab versus those receiving standard therapy (photodynamic therapy with verteporfin or pegaptanib).

0.010, respectively). Similarly, reductions in the total volume of SRT and the neurosensory retina, between baseline and week 54, were associated with improvements in visual acuity ($r = -0.2076$, $P < 0.026$, and $r = -0.2402$, $P = 0.010$, respectively). No statistically significant associations were found between either the total volume of SRF or the total volume of PED and visual acuity. The results of multivariate analyses are provided in Table 2. At the conclusion of the study (week 54), the total volume of neurosensory retina, in combination with the total volume of SRT, accounted for 18.34% of the variation of visual acuity at this point (Table 2, model 1).

DISCUSSION

Pharmacodynamics is the study of the biochemical and physiological effects of drugs and their mechanisms of action.^{29–31} In this report, we draw conclusions regarding differential pharmacodynamic effects through the evaluation of novel OCT-derived morphologic parameters in a recently completed phase III/IV clinical trial for neovascular AMD.

In recent years, the management of neovascular AMD has been revolutionized by the introduction of therapeutic agents that block the action of a glycoprotein, vascular endothelial growth factor (VEGF).³² VEGF plays a critical role in pathologic neovascularization through its effects both on vascular permeability and on endothelial cell proliferation.^{33,34} In the ABC trial, two such anti-VEGF therapies were evaluated: pegaptanib (Macugen; OSI Pharmaceuticals) and bevacizumab (Avastin; Genentech).³ Assessment of OCT-derived morphologic outcomes in the ABC trial thus offers an opportunity to probe the relative antipermeability and antiangiogenic efficacy of each approach.

The effects of pegaptanib and bevacizumab on vascular permeability may be chiefly seen (on OCT) through changes in total volume of the neurosensory retina and total volume of SRF. Bevacizumab led to rapid and significant reductions in CNV leakage, with reductions in retinal edema and SRF, findings consistent with those of previous reports. By comparison, the effects of pegaptanib on vascular permeability appear decidedly modest. For example, in patients with minimally classic

FIGURE 3. (A) Mean change from baseline in total volume of SRT in those receiving bevacizumab versus those receiving standard therapy (PDT with verteporfin or pegaptanib). (B) Mean change from baseline in total volume of PED in those receiving bevacizumab versus those receiving standard therapy (photodynamic therapy with verteporfin or pegaptanib).

or occult CNV lesions, reduction in SRF was more than three times greater in those receiving bevacizumab (-0.54 mm^3 vs. -0.15 mm^3 ; Fig. 4D), whereas reduction in retinal edema was more than twice as great (-0.82 mm^3 vs. -0.31 mm^3 ; Fig. 4B). These findings are consistent with the visual outcomes in the ABC trial,³ as well as with previously reported fluorescein angiographic and OCT-derived findings.^{35–37}

The effects of pegaptanib and bevacizumab on vascular proliferation may be seen chiefly on OCT through changes in the total volume of SRT (i.e., growth of fibrovascular tissue in the subretinal space).^{14,24} Both bevacizumab and pegaptanib led to rapid reductions in the total volume of SRT. In the bevacizumab group, these changes were maintained over the course of the study. However, in those receiving pegaptanib, these initial anatomic improvements were not maintained, and ultimately, the total volume of SRT exceeded baseline levels (Fig. 5B). This finding may be of particular importance given that, of all parameters investigated, SRT appeared to show the strongest correlation with visual acuity (Table 1), a finding consistent with previous studies using quantitative OCT subanalysis.^{14,15,24} Similarly, in the original clinical trials of pegap-

tanib (VISION: VEGF Inhibition in Ocular Neovascularization Study), fluorescein angiography demonstrated that pegaptanib leads only to a slowing of the growth of the CNV lesion.³⁶

On OCT, changes in PED volume may be reflective of both the antipermeability and antiangiogenic effects of pegaptanib and bevacizumab (i.e., fibrovascular PEDs form as a result of vascular proliferation in the sub-RPE space associated with variable quantities of exudation).³⁸ Assessment of changes in PED volume may be of particular interest from a pharmacodynamic perspective, as it may provide an indirect assessment of sub-RPE drug penetration and therapeutic effect. In patients receiving pegaptanib, PED volume showed an initial increase despite treatment ($+0.14 \text{ mm}^3$), followed by a modest reduction over the course of the study (-0.08 mm^3 ; Fig. 5D). Similarly, patients with minimally classic or occult CNV receiving bevacizumab showed little or no change in PED volume at first ($+0.02 \text{ mm}^3$); however, by the conclusion of the study, a greater reduction was seen (-0.32 mm^3). These findings are consistent with those in previous reports suggesting that PEDs regress more slowly than subretinal or intraretinal fluid in patients receiving anti-VEGF therapy for neovascular AMD.^{8,39}

FIGURE 4. (A) Mean change from baseline in total volume of the neurosensory retina in those receiving bevacizumab (Avastin; Genentech) versus those receiving PDT with verteporfin (Novartis) for classic (PC) or classic (C) lesions. (B) Mean change from baseline in total volume of the neurosensory retina in those receiving bevacizumab versus those receiving pegaptanib (Macugen; OSI Pharmaceuticals) for minimally classic (MC) or occult (O) lesions. (C) Mean change from baseline in total volume of SRF in those receiving bevacizumab versus those receiving PDT with verteporfin (PC/C lesions). (D) Mean change from baseline in total volume of SRF in those receiving bevacizumab versus those receiving pegaptanib (MC/O lesions).

Penetration throughout the retina may initially be reduced in the context of intraretinal fluid, SRF, and SRT. After treatment, reductions in these parameters may facilitate drug penetration through the outer layers of the retina and RPE and thus explain the lagging regression of the PED space.

Differences in the pharmacodynamics of pegaptanib and bevacizumab, seen in the ABC trial and described above, may result from differences in their binding of VEGF isoforms (i.e., different forms of VEGF produced by alternative gene splicing). Pegaptanib is an oligonucleotide (aptamer) that selectively binds to and thus inhibits the predominant secreted isoform of VEGF (VEGF₁₆₅).⁴⁰ Selective inhibition of a single isoform offers the theoretical advantage that normal vessels may be maintained by VEGF₁₂₁ and other isoforms, whereas pathologic neovascularization may be suppressed. Conversely, bevacizumab, a full-length monoclonal antibody, binds to and inhibits the activity of all VEGF isoforms, an approach that may provide more potent suppression of VEGF.³³ The morphologic outcomes described in this report provide further evidence for the superior antipermeability and antiangiogenic effects of bevacizumab over pegaptanib. The relative failure of pegaptanib in this regard may occur due to the activation of various proteases during the angiogenic process that cleave VEGF₁₆₅ (and longer isoforms) to generate non-heparin-binding fragments, such fragments may be sufficient to drive angiogenesis while evading pegaptanib blockade.⁴¹

The use of quantitative OCT subanalysis in the ABC trial also facilitates comparison of the mechanisms of action, and pharmacodynamic effects, of PDT with bevacizumab. Treatment with PDT begins with the intravenous injection of a photosen-

sitive compound, verteporfin.^{42–44} Activation of verteporfin by illumination with light of 689-nm wavelength induces generation of reactive oxygen species with resultant endothelial cell injury, platelet activation, and thrombus formation, a process that ultimately leads to vascular occlusion. As a result, patients in the ABC trial who received PDT (i.e., those in the standard therapy group with predominantly classic/classic CNV) demonstrated large reductions in neurosensory retinal volume as early as 1 week after treatment. In fact, the reduction in retinal volume as result of PDT was greater than in those patients with predominantly classic/classic CNV lesions who received bevacizumab. (Despite this, by the end of the study, the overall reduction in retinal volume was greater in those receiving bevacizumab; Fig. 4A.)

Subjects in the ABC trial who received PDT also demonstrated a large increase in SRF volume 1 week after their initial treatment (with a subsequent large decrease by the conclusion of the study). This finding is consistent with the known mechanism of action of PDT. Before vascular occlusion, the release of inflammatory mediators, as well as the effects of endothelial cell injury on the already hyperpermeable CNV lesion, commonly causes acute fluid extravasation.^{45,46} Transient dysfunction of the RPE, and hence the outer blood-retinal barrier, may further contribute to this temporary accumulation of fluid in the subretinal space.

Treatment with PDT also appears to cause significant early reductions in SRT volume that can be maintained over a 1-year period (although these reductions remain less marked than in patients with predominantly classic/classic lesions receiving bevacizumab). In recent years, the use of combined PDT and

FIGURE 5. (A) Mean change from baseline in total volume of SRT in those receiving bevacizumab (Avastin; Genentech) versus those receiving photodynamic therapy (PDT) with verteporfin for predominantly classic (PC) or classic (C) lesions. (B) Mean change from baseline in total volume of SRT in those receiving bevacizumab versus those receiving pegaptanib (Macugen; OSI Pharmaceuticals) for minimally classic (MC) or occult (O) lesions. (C) Mean change from baseline in total volume of PED in those receiving bevacizumab versus those receiving PDT with verteporfin for PC/C lesions. (D) Mean change from baseline in total volume of PED in those receiving bevacizumab versus those receiving pegaptanib for MC/O lesions.

anti-VEGF therapy has been advocated in neovascular AMD as a means of reducing retreatment frequency.²⁰ Evaluation of SRT volume on OCT in clinical trials may thus provide useful information to guide such an approach (i.e., the optimal blockade of pathologic vascular proliferation).

Finally, subjects in the ABC trial who received PDT demonstrated increases in PED volume at 1 week (+0.36 mm³), which remained elevated relative to baseline by the conclusion of the study (+0.19 mm³). Perhaps surprisingly, patients with predominantly classic/classic CNV who received bevacizumab

TABLE 1. Correlation of OCT-Derived Morphologic Parameters with Best Corrected ETDRS Visual Acuity

Factor	Correlation Coefficient, <i>r</i>	Model <i>R</i> ²	<i>P</i>
Correlation of Week 54 Visual Acuity with Week 54 OCT Parameters			
Neurosensory retina	-0.2898	0.0840	0.002
Subretinal fluid	0.0909	0.0083	0.334
Subretinal tissue	-0.3623	0.1313	<0.001
Pigment epithelial detachment	-0.0523	0.0027	0.579
Correlation of Week 54 Visual Acuity with Baseline OCT Parameters			
Neurosensory retina	-0.2309	0.0533	0.010
Subretinal fluid	-0.1040	0.0108	0.254
Subretinal tissue	-0.3107	0.0965	<0.001
Pigment epithelial detachment	0.0732	0.0054	0.423
Correlation of Change in Visual Acuity (Baseline to Week 54) with Change in OCT Parameters (Baseline to Week 54)			
Neurosensory retina	-0.2402	0.0577	0.010
Subretinal fluid	0.0080	0.0001	0.932
Subretinal tissue	-0.2076	0.0431	0.026
Pigment epithelial detachment	-0.0661	0.0044	0.483

Statistically significant findings are highlighted in bold.

TABLE 2. Multivariate Models of Visual Acuity and Optical Coherence Tomography Predictive Factors

Factor	Model R ² (Adjusted)	Improvement χ^2 P*
Model 1 (week 54 VA; week 54 OCT)	0.1834	
Total volume of neurosensory retina (mm ³)		0.003
Total volume of subretinal tissue (mm ³)		<0.0001
Model 2 (week 54 VA; baseline OCT)	0.0965	
Total volume of subretinal tissue (mm ³), no other variables significant		<0.0001
Model 3 (change in VA; change in OCT (baseline to week 54))	0.0663	
Total volume of neurosensory retina (mm ³)		0.030
Total volume of subretinal tissue (mm ³)		0.083

* Stepwise regression was used to select the variables that were independently associated with visual acuity.

also demonstrated increased PED volumes at week 1 (+0.19 mm³) which remained elevated at week 54 (+0.14 mm³). Previous studies using quantitative OCT subanalysis have shown that predominantly classic CNV lesions are associated with the smallest volumes of PED (classic patterns of fluorescein leakage have traditionally been thought to correspond to type 2 [subretinal], CNV on histology).^{14,47} The disparity between the effects of bevacizumab on PED in minimally classic and occult lesions versus the effects on PED in predominantly classic/classic lesions suggests that the presence of large quantities of SRT hinders sub-RPE penetration of this agent.

Our study has several strengths—in particular, the utilization of manual image grading, performed at a dedicated OCT image reading center, to quantify any morphologic space of interest in an objective, reproducible, manner. Furthermore, the OCT images sets were evaluated in the context of a phase III/IV clinical trial, with standardized follow-up and retreatment protocols, obtained in conjunction with ETDRS visual acuities after protocol refraction by trained personnel. Our study was performed with custom image-analysis software (OCTOR), similar analyses are possible with the Layer Editing Tool in current Stratus OCT software (ver. 5.0 and above). OCTOR, retitled 3D-OCTOR, has recently been updated to allow for analysis of spectral-domain OCT image sets, from multiple OCT vendors.²³ Although 3D-OCTOR is not yet publically available, many spectral-domain OCT systems (e.g., Cirrus HD-OCT, Carl Zeiss Meditec; and Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) now allow for some form of manual segmentation and thus for quantitative subanalysis of novel parameters similar to those described in this study.⁴⁸

Our study also has limitations, including the use of Stratus OCT rather than newer OCT systems based on spectral-domain OCT technology. As with several recently completed clinical trials on neovascular AMD (e.g., the recently published Comparison of AMD Treatment Trial [CATT]),⁴⁹ spectral-domain OCT was not yet widely available at the conception and initial stages of the ABC trial. The high speed of spectral-domain OCT allows dense raster scanning of the macula, reducing the need for interpolation algorithms and thus increasing the accuracy of any quantitative information.⁵⁰ The improved sensitivity and resolution of spectral-domain OCT systems may also allow identification and quantification of other novel morphologic parameters as yet unknown. Our study was also limited by the time necessary for manual grading of OCT images. As a result, we did not analyze OCT images from every available study visit. Such exhaustive analyses may be useful for assessing the fluctuations in each OCT parameter that are likely to occur in as-required, OCT-derived retreatment regimens.^{51,52}

In conclusion, commercially available OCT image analysis software is restricted, for the most part, to the quantification of retinal thickness and volume.^{53,54} OCT subanalysis, either through man-

ual grading or improved automated algorithms, allows quantification of other morphologic compartments (e.g., SRF, SRT, and PED).⁸ In the emerging era of neovascular AMD therapeutics,^{21,55} the capacity of OCT to provide such detailed pharmacodynamic assessments, in a noninvasive manner, is likely to attain increased importance. For example, in the recent CATT study, the effects of ranibizumab were compared to those of bevacizumab via the assessment of OCT-derived retinal thickness.⁴⁹ The findings from CATT suggest that ranibizumab leads to greater reductions in retinal thickness than bevacizumab (i.e., ranibizumab may have a stronger effect on vascular permeability than bevacizumab); however, no statistically significant difference was found between their visual outcomes. Thus, in future comparative studies, differences in other pharmacodynamic parameters may be of greater clinical significance. For example, evaluation of SRT may elucidate different effects on vascular proliferation, whereas measurement of PED volume may be useful for the estimation of retinal and sub-RPE therapeutic penetration. Finally, with the rapid evolution of OCT technology, other potentially significant morphologic parameters are likely to emerge, particularly for the evaluation of co-existing atrophic (dry) age-related macular changes.

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