ASSOCIATION BETWEEN EARLY ANATOMIC RESPONSE TO ANTI–VASCULAR ENDOTHELIAL GROWTH FACTOR THERAPY AND LONG-TERM OUTCOME IN DIABETIC MACULAR EDEMA

An Independent Analysis of Protocol i Study Data

PRAVIN U. DUGEL, MD,*† JOANNA H. CAMPBELL, PhD,‡ SZILÁRD KISS, MD,§
ANAT LOEWENSTEIN, MD,¶ VANESSA SHIH, PharmD,‡ XIAOSHU XU, MS,‡
NANCY M. HOLEKAMP, MD,** ALBERT J. AUGUSTIN, MD, PhD,†† ALLEN C. HO, MD,‡‡
VICTOR H. GONZALEZ, MD,§§ SCOTT M. WHITCUP, MD¶¶

Purpose: This post hoc analysis explores the relationship between early retinal anatomical response and long-term anatomical and visual outcomes with ranibizumab in center-involved diabetic macular edema.

Methods: Eyes randomized to the ranibizumab plus prompt laser and ranibizumab plus deferred laser treatment arms in the Protocol I study were categorized according to their proportional reduction (<20 vs. ≥20%) in central retinal thickness (CRT) after 12 weeks. Adjusted and unadjusted analyses assessed the association between early (Week 12) anatomical response and long-term (Weeks 52 and 156) anatomical and best-corrected visual acuity outcomes.

Results: Of 335 study eyes, 118 showed limited (<20%) and 217 showed strong (≥20%) CRT reduction at Week 12. In unadjusted and adjusted analyses, limited early CRT response was negatively and significantly associated with strong CRT response at Weeks 52 and 156. Sensitivity analyses indicated that this association was robust and unrelated to any "floor effect." In unadjusted analyses, a strong early CRT response was associated with greater long-term improvement in best-corrected visual acuity; after controlling for confounders, the association lost statistical significance.

Conclusion: Early CRT response to ranibizumab is a significant prognostic indicator of medium- to long-term anatomical outcome in center-involved diabetic macular edema.

RETINA 39:88–97, 2019

Diabetic macular edema (DME), a frequent microvascular complication of Type 1 and 2 diabetes,[1] is characterized by vascular leakage and accumulation of extracellular fluid in the macula because of breakdown of the blood-retinal barrier.[2] Disruption of retinal architecture (e.g., vitreoretinal interface abnormalities, loss of retinal inner layer boundaries, cell displacement by cystoid cavities, and neuroretinal detachment) associated with long-standing retinal edema[3–6] may result in neuronal cell loss and compromised retinal function.[7] Macular edema is the main cause of visual impairment in diabetic patients,[8] with symptomatic vision loss occurring when macular thickening involves or threatens the fovea.[2]

Vascular endothelial growth factor-A (VEGF-A) is a potent vasopermeability factor closely implicated in disruption of the blood-retinal barrier in DME,[9–11] making it a promising target for pharmacological intervention. Randomized clinical trials of the intravitreal anti-VEGF-A agents ranibizumab and aflibercept in eyes with center-involved DME have demonstrated their efficacy as monotherapy in comparison with laser photocoagulation in improving visual acuity[12–14] and reducing central retinal thickness (CRT).[13] Currently, intravitreal anti-VEGF-A agents are the treatment of choice for DME with central involvement.[2] Nevertheless, even with the intensive treatment schedules used in clinical trials, the morphologic and visual responses to anti-VEGF-A therapy are often incomplete, with ~20% to 65% of eyes failing to achieve resolution of retinal thickening[12,15–17] and ~30% to 70% of eyes showing <10-letter improvement in best-corrected visual acuity (BCVA)[12,13,15,18–21] after 1 year or 2 years of treatment.

From the *Retinal Consultants of Arizona, Phoenix, Arizona; †USC Roski Eye Institute, Keck School of Medicine, University of Southern California, Los Angeles, California; ‡Allergan plc, Irvine, California; §Department of Ophthalmology, Weill Cornell Medical College, New York, New York; ¶Department of Ophthalmology, Tel Aviv Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; **Pepose Vision Institute and Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri; ††Department of Ophthalmology, Staedtisches Klinikum Karlsruhe, Karlsruhe, Germany; ‡‡Mid Atlantic Retina, Plymouth Meeting, Pennsylvania; §§Valley Retina Institute, McAllen, Texas; and ¶¶Jules Stein Eye Institute, David Geffen School of Medicine at UCLA, Los Angeles, California.

Reprint requests: Pravin U. Dugel, MD, Retinal Consultants of Arizona, 1101 E. Missouri Avenue, P.O. Box 32530, Phoenix, AZ 85014-2709; e-mail: pdugel@gmail.com

Persistent and/or recurrent macular edema has been implicated as a possible contributory factor to poor visual outcome with ranibizumab therapy in DME.[22,23] Optical coherence tomography (OCT) provides an accurate and reliable measure of CRT in DME, enabling quantitative longitudinal monitoring of macular edema and its response to treatment.[24,25] In the era of anti-VEGF-A–directed treatment of center-involved DME, OCT-derived retinal thickness has become a widely used quantitative end point in major clinical studies of DME.[26] As a demonstration of the importance attached to OCT assessment in the management of DME, the Diabetic Retinopathy Clinical Research Network (DRCR.net) studies of intravitreal ranibizumab in the treatment of DME routinely use OCT-derived CRT response as a measure of treatment efficacy and the need for retreatment.[27]

Early identification of those patients for whom long-term anti-VEGF-A therapy is likely to confer that only limited visual benefit would enable more timely consideration of additional disease management strategies. To this end, the EARLY (Early Anti-VEGF Response and Long-term efficacy) program, a series of post hoc analyses of data from one of the largest studies of ranibizumab in DME—the Protocol I study[15]—was undertaken to explore the relationship between early and long-term anatomical and visual acuity outcomes of ranibizumab therapy. The primary objective of the present analysis was to assess whether retinal anatomical response after 12 weeks of ranibizumab therapy offered any indication of likely anatomical response at 1 year and 3 years. A secondary objective was to explore further the possible association between early anatomical and long-term visual acuity outcomes.

Patients and Methods

Protocol I Study Overview

Protocol I was a prospective, multicenter Phase III study conducted by the Diabetic Retinopathy Clinical Research Network (DRCR.net) of intravitreal ranibizumab and triamcinolone in patients with center-involved DME (clinicaltrials.gov identifier NCT00445003). The methodology of this study has been detailed elsewhere.[15] In brief, study eyes (baseline BCVA of 78 to 24 Early Treatment Diabetic Retinopathy Study letters [approximate Snellen equivalent 20/32–20/320] and time-domain OCT–determined CRT ≥250 μm) were randomly assigned to treatment with 1) intravitreal ranibizumab 0.5 mg plus prompt (within 7–10 days) focal/grid photocoagulation, 2) intravitreal ranibizumab 0.5 mg plus deferred (after ≥24 weeks) focal/grid photocoagulation, 3) intravitreal triamcinolone 4 mg

plus prompt focal/grid photocoagulation, or 4) sham injection plus prompt focal/grid photocoagulation. Intravitreal injections were performed at 4-week intervals for the first 12 weeks (or for the first 20 weeks if DME persisted) and as needed thereafter; repeat use of laser was governed by the extent of central macular edema. Use of alternative DME treatments was allowed only if prespecified treatment failure or futility criteria were met. Follow-up examinations, including measurements of BCVA and OCT (Stratus, Carl Zeiss Meditec Inc, Dublin, CA)-derived CRT, were performed every 4 weeks for the first year and every 4 weeks to 16 weeks thereafter. Patient follow-up was planned for 3 years, with the primary efficacy end point being the mean change in BCVA at 1 year.[15] Review of study findings at 2 years indicated an efficacy advantage in the ranibizumab treatment arms,[28] and patients in the sham injection and intravitreal triamcinolone treatment arms were offered the option of switching to open-label ranibizumab for the third year. Follow-up findings for the subset of eyes randomized to ranibizumab plus prompt or deferred laser treatment were reported at 3 years[29] and 5 years.[30]

Anatomical and Visual Response Analysis

This analysis is based on 3-year follow-up data for those Protocol I study eyes that were randomized to ranibizumab plus prompt or deferred laser treatment and, in addition, provided an observed CRT measurement at Week 12. Eligible eyes were categorized according to their proportional change from baseline in CRT at Week 12: 1) eyes with <20% reduction ("limited early CRT response") and 2) eyes with ≥20% reduction ("strong early CRT response"). Serial (4-weekly) CRT and BCVA readings over Weeks 12 to 156 were collected for the 2 cohorts; missing readings were imputed using the last-observation-carried-forward technique. Central retinal thickness and BCVA readings obtained after initiation of add-on treatment were also included in the analysis. Anatomical response over the 3-year follow-up period was expressed as the proportion of eyes with ≥20% reduction in CRT from baseline; visual acuity response was expressed as the mean absolute change from baseline in BCVA (Early Treatment Diabetic Retinopathy Study letters). To assess the robustness of any observed association between early and long-term anatomical response (as expressed by the percent reduction in CRT), a sensitivity analysis was conducted in eyes categorized according to the change in logarithmically transformed OCT-derived CRT (logOCT) value (<1 log-step, 1–2 log-step, and >2 log-step improvement) at Week 12. For this analysis, anatomical response over the 3-year follow-up period was expressed as the

proportion of eyes with >2 log-step OCT improvement from baseline. A 1-step reduction in logOCT equates to ~20% reduction in CRT, whereas a 2-step reduction equates to ~36% reduction in CRT, irrespective of baseline CRT.[31] To address the possibility that any demonstrated association between early and late anatomical response might be attributable to the limited scope for CRT reduction in eyes with mild retinal thickening (the "floor effect"), a sensitivity analysis was performed in eyes with baseline CRT ≥350 μm.

Statistical Methods

Intercohort comparisons of baseline characteristics, as well as anatomical responses (proportion of eyes with ≥20% reduction from baseline in CRT) and visual responses (mean change from baseline in BCVA) over the study period were performed using Student's *t*-test or Kruskal–Wallis 1-way analysis of variance for continuous variables and Pearson chi-square test for categorical variables. Multiple linear and logistic regression analyses were performed on long-term (Weeks 52 and 156) anatomical responses (≥20% CRT reduction, and >2 log-step OCT improvement from baseline) and visual acuity outcome (BCVA change from baseline). In addition to early anatomical response (either <20% vs. ≥20% CRT reduction or <1 log-step and 1–2 log-step vs. >2 log-step OCT improvement at Week 12), covariates included in the regression models were age, sex, baseline BCVA and CRT, previous receipt of DME treatment, BCVA change at Week 12, and cumulative number of ranibizumab injections and laser treatments received at Week 52 or 156. *P* values were determined using Student's *t*-test (linear regression) and Wald chi-square test (logistic regression). Statistical analyses were performed with SAS versions 9.3 and 9.4 (SAS Inc, Cary, NC). All tests were 2-tailed and a *P* value of ≤0.05 was considered statistically significant.

Results

The Protocol I study allocated a total of 375 eyes to treatment with ranibizumab plus either prompt or deferred laser; of these, 335 eyes provided OCT-derived CRT data (and corresponding visual acuity data) at Week 12 and were included in the present analysis.

Association Between Early and Long-Term Anatomical Response

Within the overall study population (335 eyes), 118 eyes (35.2%) showed <20% CRT reduction (mean absolute CRT change from baseline −24 μm) and 217

eyes (64.8%) showed ≥20% CRT reduction (mean absolute CRT change from baseline −183 μm) at Week 12. Comparison of the 2 cohorts over the 3-year study period indicated that of the eyes with a limited early CRT response (n = 118), 37 (31.4%) and 61 (51.7%) subsequently achieved a ≥20% reduction in CRT by Weeks 52 and 156, respectively, whereas of the eyes with a strong early CRT response (n = 217), 182 (83.9%) and 180 (82.9%) maintained the initial ≥20% reduction in CRT at Weeks 52 and 156, respectively (Figure 1). Unadjusted analysis indicated that eyes with a limited early CRT response were significantly ($P < 0.0001$) less likely to demonstrate a ≥20% reduction in CRT at Weeks 52, 104, and 156 than eyes with a strong early CRT response (Figure 1).

A limited initial anatomical response to ranibizumab did not preclude a further decrease in CRT with continued treatment. Among the cohort of study eyes with limited early CRT response (n = 118), 37 (31.4%) eyes subsequently achieved a ≥20% reduction in CRT by Week 52 ("slow responders"), whereas the remaining 81 (68.6%) eyes continued to show <20% reduction in CRT at Week 52 ("non-responders"). Subsequent response rates (proportion of eyes with ≥20% reduction in CRT) remained high in slow-responder eyes (91.9 and 86.5% at Weeks 104 and 156, respectively) but, despite a gradual improvement over time, were significantly ($P < 0.001$) lower in nonresponder eyes (23.5 and 35.8% at Weeks 104 and 156, respectively).

The significant association between early and long-term anatomical response to ranibizumab demonstrated in the unadjusted analysis persisted after controlling for potential confounding variables. Significant differences in demographic and baseline clinical characteristics and treatment intensity were noted between cohorts categorized by their proportional CRT response at Week 12 (Table 1). For example, eyes with ≥20% CRT reduction at Week 12 were younger (mean 61.5 vs. 65.0 years of age; $P = 0.002$), had lower baseline BCVA (mean 61.6 vs. 65.4 Early Treatment Diabetic Retinopathy Study letters; $P = 0.004$), and higher baseline CRT (433 vs. 345 μm; $P < 0.001$) and, as expected from their strong initial anatomical response, received fewer ranibizumab injections (mean 12.7 vs. 15.1; $P = 0.005$) and laser procedures (mean 1.7 vs. 2.4; $P = 0.005$) over the 3-year follow-up period than eyes with <20% CRT reduction at Week 12. Multiple logistic regression analysis with adjustment for differences in baseline parameters (age, sex, previous DME treatment, and baseline BCVA and CRT) indicated that <20% CRT reduction at Week 12 showed a significant negative association with ≥20% CRT reduction at Week 52 (odds ratio [OR] 0.11, 95% confidence interval [CI] 0.06–0.19; $P < 0.001$) and Week 156 (OR 0.35, 95% CI 0.20–0.61; $P < 0.001$). Of the other covariates included in this regression model, baseline BCVA and baseline CRT were significantly (positively) associated with ≥20% CRT reduction at Week 52, whereas baseline CRT remained significantly associated with ≥20% CRT reduction at Week 156. An expanded regression model that additionally adjusted for differences in treatment intensity over the 3-year study period likewise indicated that <20% CRT reduction at Week 12 was significantly (negatively) associated with ≥20% CRT reduction at Week 52 (OR 0.13, 95% CI 0.07–0.24; $P < 0.001$) and Week 156 (OR 0.45, 95% CI 0.25–0.81; $P = 0.008$) (Table 2). In this model, baseline CRT and cumulative number of ranibizumab injections were also significantly associated with ≥20% CRT reduction at Weeks 52 and 156 (Table 2).
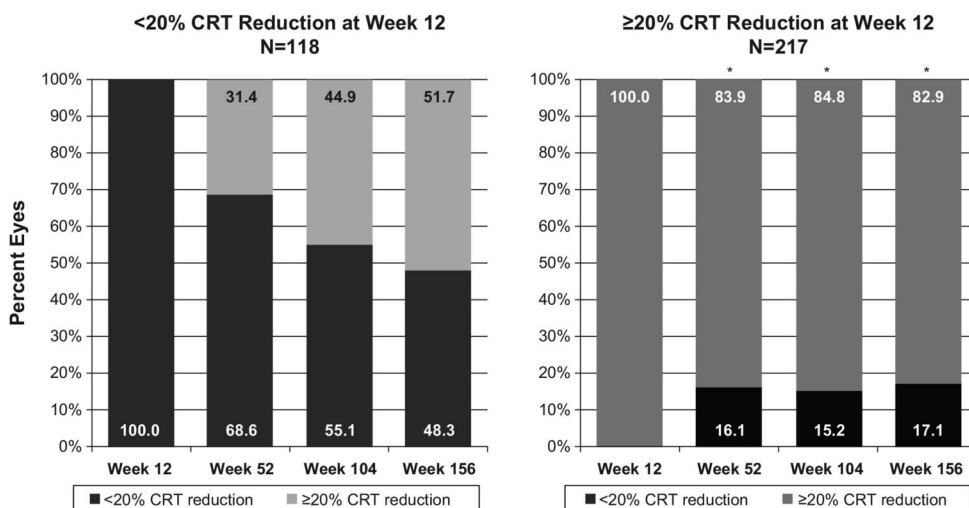

Fig. 1. Proportional CRT response over the 3-year follow-up period, categorized by proportional CRT response at Week 12. *$P < 0.0001$ at Weeks 52, 104, and 156, intercohort comparison.

Table 1. Baseline Demographics, Clinical Characteristics, and Treatment Intensity of Pooled Study Eyes, Categorized by Proportional CRT Response at Week 12 (N = 335)

Baseline Characteristics	Proportional CRT Response at Week 12		P*
	<20% CRT Reduction n = 118	≥20% CRT Reduction n = 217	
Age, mean ± SD, years	65.0 ± 9.1	61.5 ± 9.8	0.002
Male sex, n (%)	76 (64.4)	112 (51.6)	0.024
Previous DME treatment, n (%)	82 (69.5)	123 (56.7)	0.022
Baseline BCVA, mean ± SD (letters)	65.4 ± 10.9	61.6 ± 11.8	0.004
Baseline CRT, mean ± SD, μm	345 ± 83	433 ± 131	<0.001
Baseline CRT <400 μm, n (%)	89 (75.4)	101 (46.5)	<0.001
BCVA CFB at Week 12, mean ± SD (letters)	3.6 ± 6.8	9.2 ± 7.7	<0.001
CRT CFB at Week 12, mean ± SD, μm	−24.1 ± 33.5	−182.6 ± 115.7	<0.001
Cumulative no. of RAN injections at Week 52, mean ± SD	9.8 ± 3.0	7.9 ± 2.9	<0.001
Cumulative no. of RAN injections at Week 156, mean ± SD	15.1 ± 7.9	12.7 ± 7.2	0.005
Cumulative no. of laser procedures at Week 52, mean ± SD	1.6 ± 1.3	1.1 ± 1.2	<0.001
Cumulative no. of laser procedures at Week 156, mean ± SD	2.4 ± 2.1	1.7 ± 1.9	0.005

CFB, change from baseline; RAN, ranibizumab.
*Intercohort comparison, based on Student's *t*-test for continuous variables, and Pearson chi-square test for categorical variables.

Sensitivity Analyses

Sensitivity analyses indicated that the association between early and long-term anatomical response to ranibizumab was robust and was unlikely to be due to a possible "floor effect." Early-response cohorts obtained by stratification of the study eye population according to logOCT improvement at Week 12 (<1 log-step [116 eyes, 34.6%]; 1–2 log-step [110 eyes, 32.8%]; and >2 log-step [109 eyes, 32.5%]) showed significant overall differences in age, sex, baseline BCVA and CRT, proportion of eyes with baseline CRT <400 μm, change from baseline in BCVA at Week 12, and cumulative number of ranibizumab injections and laser procedures

received at Weeks 52 and 156. Multiple logistic regression with adjustment for the covariates included in the expanded regression model of the main analysis indicated that <1 log-step OCT improvement at Week 12 showed a significant negative association with >2 log-step OCT improvement at Week 52 (OR 0.06, 95% CI 0.02–0.15; P < 0.001) and at Week 156 (OR 0.36, 95% CI 0.17–0.77; P = 0.009). A statistically significant negative association was also demonstrated between 1-2 log-step OCT improvement at Week 12 and >2 log-step OCT improvement at Week 52 (OR 0.27, 95% CI 0.14–0.53; P < 0.001) and at Week 156 (OR 0.39, 95% CI 0.20–0.79; P = 0.009).

Table 2. Multiple Logistic Regression Analysis on ≥20% Reduction in CRT at Weeks 52 and 156: Pooled Study Eyes (N = 335)

Parameter	Multiple Logistic Regression on ≥20% Reduction in CRT at Weeks 52 and 156			
	Week 52, N = 335		Week 156, N = 335	
	OR (95% CI)	P*	OR (95% CI)	P*
Age	1.01 (0.98–1.04)	0.550	0.99 (0.97–1.02)	0.738
Sex (female/male)	0.95 (0.54–1.68)	0.852	1.02 (0.58–1.79)	0.950
Baseline BCVA	1.03 (1.00–1.06)	0.063	1.02 (0.99–1.05)	0.161
Baseline CRT†	2.70 (1.00–2.70)	<0.001	2.70 (2.70–2.70)	<0.001
Previous DME treatment (yes/no)	1.00 (0.56–1.81)	0.987	1.11 (0.61–2.01)	0.728
<20% CRT reduction at Week 12	0.13 (0.07–0.24)	<0.001	0.45 (0.25–0.81)	0.008
Cumulative no. of RAN injections	0.89 (0.79–0.99)	0.030	0.92 (0.87–0.96)	<0.001
Cumulative no. of laser procedures	1.11 (0.88–1.40)	0.397	1.04 (0.89–1.22)	0.598

RAN, ranibizumab.
*P value based on Wald chi-square test for OR = 1.
†Baseline CRT as expressed in 100 μm units.

Among the subset of eyes with baseline CRT $\geq$350 μm (n = 199), 46 eyes (23.1%) and 153 eyes (76.9%) showed <20% and $\geq$20% CRT reduction, respectively, at Week 12. Multiple logistic regression analysis, adjusting for baseline parameters (age, baseline BCVA, baseline CRT, and previous DME treatment), and on-study treatment intensity (cumulative number of ranibizumab injections and laser procedures), indicated that $\geq$20% CRT reduction at Week 12 was significantly associated with $\geq$20% CRT reduction at Week 52 (OR 7.23, 95% CI 2.88–18.18; P < 0.001) but not at Week 156 (OR 1.24, 95% CI 0.48–3.20; P = 0.658).

Association Between Early Anatomical Response and Long-Term Visual Response

A strong early CRT response to ranibizumab was associated with greater long-term improvement in visual acuity; however, after controlling for potential confounders, the association was attenuated and statistical significance was lost (Table 3). Unadjusted analyses indicated that the mean improvement from baseline in BCVA at all time points over the 3-year study period was significantly smaller in eyes with <20% compared with $\geq$20% CRT reduction at Week 12 (mean standard deviation [SD] 4.8 [13.5] vs. 11.5 [9.5] letters at Week 52; 4.0 [15.8] vs. 10.4 [12.5] letters at Week 156; both P < 0.001). After adjustment for potential confounders (age, sex, baseline BCVA and CRT, previous DME treatment, BCVA change from baseline at Week 12, and cumulative number of ranibizumab injections and laser procedures received over the study period) using multiple linear regression, $\geq$20% CRT reduction at Week 12 was no longer significantly associated with BCVA response (change from baseline) at Week 52 (estimate [standard error, SE] 1.59 [1.25]; P = 0.205) or Week 156 (estimate [SE] 1.88 [1.70]; P = 0.268) (Table 3).

Discussion

This post hoc analysis of data from the DRCR.net Protocol I study indicates that retinal morphologic responses to ranibizumab plus prompt or deferred laser treatment generally develop rapidly in patients with center-involved DME, with two-thirds of eyes achieving a $\geq$20% reduction in CRT within the first 3 months of treatment. For eyes in this early-response category, the initial anatomical improvement was largely maintained during long-term treatment, with 83% of eyes continuing to show $\geq$20% CRT reduction at 3 years. By contrast, for the one-third of eyes that showed little or no anatomical improvement after the first 3 ranibizumab injections, prospects for future improvement with continued treatment were at best moderate, with 48% of eyes continuing to show <20% CRT reduction at 3 years, despite the intensive treatment and monitoring protocol. This would imply that resolution of macular edema after an initially limited treatment response is less readily achievable than maintenance of remission after a strong early treatment response. In those eyes that did subsequently develop a fuller anatomical response with continued treatment, improvement was generally slow: among the eyes with limited early CRT response, 31% showed $\geq$20% CRT reduction at 1 year, rising to 45% at 2 years, and 52% at 3 years. Unadjusted and adjusted analyses indicated that, at the cohort level, proportional CRT response (<20 vs. $\geq$20% reduction) at Week 12 was significantly associated with proportional CRT response over 3 years of follow-up. Using another parameter of relative anatomical change—the log-transformed

Table 3. Multiple Linear Regression Analysis on BCVA Change From Baseline at Weeks 52 and 156: Pooled Study Eyes (N = 335)

| Parameter | Multiple Linear Regression on BCVA Change from Baseline at Weeks 52 and 156 | | | |
| | Week 52, N = 335 | | Week 156, N = 335 | |
	Estimate (SE)	P*	Estimate (SE)	P*
Age	−0.13 (0.05)	0.016	−0.10 (0.07)	0.162
Sex (female/male)	0.01 (1.01)	0.991	0.66 (1.43)	0.644
Baseline BCVA	−0.22 (0.05)	<0.001	−0.25 (0.07)	<0.001
Baseline CRT	−0.01 (0.00)	0.061	−0.00 (0.01)	0.946
Previous DME treatment (yes/no)	−2.02 (1.04)	0.054	−0.16 (1.47)	0.914
BCVA CFB at Week 12	0.72 (0.07)	<0.001	0.56 (0.10)	<0.001
$\geq$20% CRT reduction at Week 12	1.59 (1.25)	0.205	1.88 (1.70)	0.268
Cumulative no. RAN injections	−0.09 (0.19)	0.658	0.04 (0.11)	0.721
Cumulative no. laser procedures	−0.66 (0.42)	0.116	−0.16 (0.39)	0.684

CFB, change from baseline; RAN, ranibizumab.
*P value based on Student's t-test.

reduction in excess retinal thickness, based on a "normal" CRT of 200 μm[32] (i.e., the change in logOCT)—we likewise demonstrated a significant association between CRT response at Week 12 and CRT response at 1 year and 3 years, which attests to the robustness of this relationship.

Our finding that a strong anatomical response ($\geq$20% CRT reduction)—whether obtained at Week 12, 52, or 156—was associated with a higher baseline CRT is consistent with reports that the greatest reductions in CRT produced by ranibizumab in DME occur in eyes with highest baseline CRT.[15,18,33] Because baseline CRT is a significant determinant of subsequent CRT response to ranibizumab therapy, it is feasible that the observed association between early and late anatomical response might be driven in part by a "floor effect" on the amount of CRT reduction that is possible in eyes with mild retinal thickening. For example, an eye with a baseline CRT of 250 μm (the threshold value required for Protocol I study eligibility) would be limited to a maximum possible CRT reduction of 50 μm (20% improvement), assuming a "normal" OCT-derived CRT value of 200 μm.[32,34,35] The demonstration of a significant association between early and late anatomical response in the subset of eyes with baseline CRT $\geq$350 μm (i.e., eyes with the potential for substantial reductions in retinal thickness) would, however, argue against a "floor effect." Moreover, findings from a previous analysis of Protocol I study data, which assessed a total of 37 baseline variables for association with 1-year anatomical and visual acuity outcomes in the ranibizumab plus prompt and deferred laser treatment arms, would suggest that the observed relationship between early and late anatomical outcome in ranibizumab-treated eyes is real.[33] To negate the possible influence of a "floor effect," the analysis chose as its parameter of anatomical response the percent reduction in excess retinal thickness, as defined by a CRT threshold of 250 μm (i.e., a value above the "normal" CRT of 200 μm). The demonstration that higher baseline CRT was associated with a greater percent reduction in excess retinal thickness, suggests that the relationship is unrelated to any "floor effect."[33]

Eyes with strong compared with limited early anatomical response to ranibizumab showed significantly greater improvement in visual acuity over the follow-up period, although intersubject variability in BCVA response was pronounced in both groups. However, eyes showing a strong early anatomical response also had significantly lower baseline BCVA and higher baseline CRT values, which are factors favoring visual acuity improvement with ranibizumab.[15,18,33] After adjusting for these and other potential confounders, the multiple regression model revealed no significant association between OCT-derived early anatomical response ($\geq$20% CRT reduction at Week 12) and BCVA improvement at either 1 year or 3 years. Previous investigations of the relationship between the anatomical and functional outcomes of anti-VEGF-A therapy in DME have yielded inconsistent results.[22,23,33,36] An independent post hoc analysis of the Protocol I study data (ranibizumab plus prompt and deferred laser treatment arms) has suggested that early and sustained CRT reduction during ranibizumab treatment is associated with a better long-term visual acuity outcome.[33] Eyes showing an early and consistent anatomical response during the first year of ranibizumab treatment ($\geq$20% CRT reduction at Weeks 16, 32, and 52) showed greater BCVA improvement at Week 52 (mean 13 letters) than eyes with an early but inconsistent response ($\geq$20% CRT reduction at Week 16 but not at Week 32 and/or 52) (mean 9 letters), eyes with a slow and variable response ($\geq$20% CRT reduction at Week 32 and/or 52) (mean 7 letters) and nonresponders (mean 4 letters).[33] A subsequent post hoc analysis of the Protocol I study data, conducted in the subset of ranibizumab plus prompt/deferred laser-treated study eyes that showed persistent macular thickening through the first 24 weeks of ranibizumab treatment (n = 117), found that long-term (3-year) visual acuity outcome was significantly worse in eyes with chronic persistent edema through the entire 3-year follow-up period than in eyes with shorter lasting edema (mean BCVA improvement from baseline to 3 years: 7 vs. 13 letters).[23] (Eyes were considered to have chronic persistent edema until they achieved a CRT <250 μm and $\geq$10% reduction relative to Week 24 at 2 consecutive study visits subsequent to Week 24.) Nevertheless, eyes with persistent macular edema through 3 years still achieved substantial reductions in CRT over this period (median CRT declined from 396 μm [baseline] to 278 μm [3 years]), consistent with the improvement in visual acuity.[23] By contrast, analysis of RISE/RIDE and READ-2 study data suggests a dissociation between early anatomical and long-term visual acuity responses to ranibizumab in DME.[22,36] An earlier DRCR.net study reported modest correlation between OCT-derived CRT and visual acuity in DME, and modest correlation between the change in CRT and change in visual acuity after focal laser photocoagulation.[37] However, the range of BCVA values observed for any given degree of retinal edema was large, and CRT was found to account for, at most, only one-quarter of the variability in visual acuity.[37] Detailed information on the spatial distribution of retinal thickness and the integrity of individual retinal layers may be required to establish a more consistent structural–functional relationship in

DME.[3,5,38,39] In addition, given the progressive course of retinal vascular leakage[40] and retinal structural damage[41,42] in DME, longitudinal assessment of CRT over time may provide a better measure of the effect of persistent macular edema on visual acuity.

Strengths of this analysis include its large sample size, randomized study design and standardized retreatment protocol, extended follow-up duration, and use of OCT methodology. Although the Protocol I study used time-domain OCT scanning, which provides poorer axial resolution and retinal layer delineation than the spectral domain OCT devices currently available in clinical practice,[25] time-domain OCT measurements are nevertheless reproducible and correlate with other morphometric parameters of macular anatomy in DME,[43] as well as with fluorescein angiographic findings.[44,45] Accordingly, time-domain OCT has been used extensively for quantification of DME severity and as a surrogate marker of treatment response.[43,46,47] Limitations of the analysis include its post hoc design, potential bias arising from use of the last-observation-carried-forward technique for imputation of missing CRT and BCVA values, and the absence of information on additional factors such as DME clinical subtype (focal vs. diffuse) and morphologic pattern (diffuse retinal thickening, cystoid macular edema, serous retinal detachment, and vitreomacular interface abnormalities) that are known to influence anatomical and visual acuity responses to anti-VEGF-A therapy.[33,48–51]

In conclusion, for physicians who primarily use OCT as a treatment guide, this analysis indicates that early (Week 12) CRT response to ranibizumab is a significant prognostic indicator of medium- to long-term anatomical outcome in DME. Although a limited early CRT response to ranibizumab does not preclude further anatomical improvement with continued treatment, consolidation of the anatomical response in these circumstances is generally protracted, and falls short of the therapeutic objective of achieving timely elimination of subretinal and intraretinal fluid.

Key words: anti-VEGF, central retinal thickness, diabetic macular edema, optical coherence tomography, ranibizumab, retinal morphology, retrospective analysis, visual acuity.

Acknowledgments

References

1. Klein R, Klein BE, Moss SE, et al. The Wisconsin epidemiologic study of diabetic retinopathy. IV. Diabetic macular edema. Ophthalmology 1984;91:1464–1474.
2. Das A, McGuire PG, Rangasamy S. Diabetic macular edema: pathophysiology and novel therapeutic targets. Ophthalmology 2015;122:1375–1394.
3. Sun JK, Lin MM, Lammer J, et al. Disorganization of the retinal inner layers as a predictor of visual acuity in eyes with center-involved diabetic macular edema. JAMA Ophthalmol 2014;132:1309–1316.
4. Murakami T, Yoshimura N. Structural changes in individual retinal layers in diabetic macular edema. J Diabetes Res 2013; 2013:920713.
5. Maheshwary AS, Oster SF, Yuson RM, et al. The association between percent disruption of the photoreceptor inner segment-outer segment junction and visual acuity in diabetic macular edema. Am J Ophthalmol 2010;150:63–67.e1.
6. Sikorski BL, Malukiewicz G, Stafiej J, et al. The diagnostic function of OCT in diabetic maculopathy. Mediators Inflamm 2013;2013:434560.
7. Pelosini L, Hull CC, Boyce JF, et al. Optical coherence tomography may be used to predict visual acuity in patients with macular edema. Invest Ophthalmol Vis Sci 2011;52:2741–2748.
8. Klein R, Klein BE, Moss SE. Visual impairment in diabetes. Ophthalmology 1984;91:1–9.
9. Qaum T, Xu Q, Joussen AM, et al. VEGF-initiated blood-retinal barrier breakdown in early diabetes. Invest Ophthalmol Vis Sci 2001;42:2408–2413.
10. El-Remessy AB, Franklin T, Ghaley N, et al. Diabetes-induced superoxide anion and breakdown of the blood-retinal barrier: role of the VEGF/uPAR pathway. PLoS One 2013;8:e71868.
11. Miller JW, Le Couter J, Strauss EC, Ferrara N. Vascular endothelial growth factor A in intraocular vascular disease. Ophthalmology 2013;120:106–114.
12. Nguyen QD, Brown DM, Marcus DM, et al. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. Ophthalmology 2012;119: 789–801.
13. Korobelnik JF, Do DV, Schmidt-Erfurth U, et al. Intravitreal aflibercept for diabetic macular edema. Ophthalmology 2014; 121:2247–2254.
14. Soheilian M, Ramezani A, Obudi A, et al. Randomized trial of intravitreal bevacizumab alone or combined with triamcinolone versus macular photocoagulation in diabetic macular edema. Ophthalmology 2009;116:1142–1150.
15. Elman MJ, Aiello LP, Beck RW, et al; Diabetic Retinopathy Clinical Research Network. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 2010;117:1064–1077.
16. Wells JA, Glassman AR, Ayala AR, et al; Diabetic Retinopathy Clinical Research Network. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. N Engl J Med 2015; 372:1193–1203.
17. Wells JA, Glassman AR, Ayala AR, et al; Diabetic Retinopathy Clinical Research Network. Alibercept, bevacizumab, or ranibizumab for diabetic macular edema: two-year results from a comparative effectiveness randomized clinical trial. Ophthalmology 2016;123:1351–1359.
18. Mitchell P, Bandello F, Schmidt-Erfurth U, et al; RESTORE Study Group. The RESTORE study: ranibizumab monotherapy

or combined with laser versus laser monotherapy for diabetic macular edema. Ophthalmology 2011;118:615–625.

19. Michaelides M, Kaines A, Hamilton RD, et al. A prospective randomized trial of intravitreal bevacizumab or laser therapy in the management of diabetic macular edema (BOLT study) 12-month data: report 2. Ophthalmology 2010;117:1078–1086.

20. Do DV, Nguyen QD, Boyer D, et al; DA VINCI Study Group. One-year outcomes of the DA VINCI study of VEGF trap-eye in eyes with diabetic macular edema. Ophthalmology 2012; 119:1658–1665.

21. Massin P, Bandello F, Garweg JG, et al. Safety and efficacy of ranibizumab in diabetic macular edema (RESOLVE Study): a 12-month, randomized, controlled, double-masked, multicenter phase II study. Diabetes Care 2010;33:2399–2405.

22. Channa R, Sophie R, Khwaja AA, et al; The READ-2 Study Group. Factors affecting visual outcomes in patients with diabetic macular edema treated with ranibizumab. Eye 2014;28: 269–278.

23. Bressler SB, Ayala AR, Bressler NM, et al; Diabetic Retinopathy Clinical Research Network. Persistent macular thickening after ranibizumab treatment for diabetic macular edema with vision impairment. JAMA Ophthalmol 2016;134:278–285.

24. Massin P, Girach A, Erginay A, Gaudric A. Optical coherence tomography: a key to the future management of patients with diabetic macular oedema. Acta Ophthalmol Scand 2006;84: 466–474.

25. Schimel AM, Fisher YL, Flynn HW Jr. Optical coherence tomography in the diagnosis and management of diabetic macular edema: time-domain versus spectral-domain. Ophthalmic Surg Lasers Imaging 2011;42:S41–S55.

26. Baskin DE. Optical coherence tomography in diabetic macular edema. Curr Opin Ophthalmol 2010;21:172–177.

27. Aiello LP, Beck RW, Bressler NM, et al; Diabetic Retinopathy Clinical Research Network. Rationale for the diabetic retinopathy clinical research network treatment protocol for center-involved diabetic macular edema. Ophthalmology 2011;118: e5–14.

28. Elman MJ, Bressler NM, Qin H, et al: Diabetic Retinopathy Clinical Research Network Writing Committee. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 2011;118:609–614.

29. Elman MJ, Qin H, Aiello LP, et al; Diabetic Retinopathy Clinical Research Network. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. Ophthalmology 2012;119: 2312–2318.

30. Elman MJ, Ayala A, Bressler NM, et al; Diabetic Retinopathy Clinical Research Network. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: 5-year randomized trial results. Ophthalmology 2015; 122:375–381.

31. Ferris FL III, Miller KM, Glassman AR, Beck RW; Diabetic Retinopathy Clinical Research Network. A proposed method of logarithmic transformation of optical coherence tomography data for use in clinical research. Ophthalmology 2010;117: 1512–1516.

32. Browning DJ, McOwen MD, Bowen RM Jr, O'Marah TL. Comparison of the clinical diagnosis of diabetic macular edema with diagnosis by optical coherence tomography. Ophthalmology 2004;111:712–715.

33. Bressler SB, Qin H, Beck RW, et al. Factors associated with changes in visual acuity and central subfield thickness at 1 year after treatment for diabetic macular edema with ranibizumab. Arch Ophthalmol 2012;130:1153–1161.

34. Bressler NM, Edwards AR, Antoszyk AN, et al: Diabetic Retinopathy Clinical Research Network. Retinal thickness on Stratus optical coherence tomography in people with diabetes and minimal or no diabetic retinopathy. Am J Ophthalmol 2008; 145:894–901.

35. Kiernan DF, Hariprasad SM, Chin EK, et al. Prospective comparison of Cirrus and Stratus optical coherence tomography for quantifying retinal thickness. Am J Ophthalmol 2009;147:267–275.

36. Pieramici DJ, Wang PW, Ding B, Gune S. Visual and anatomic outcomes in patients with diabetic macular edema with limited initial anatomic response to ranibizumab in RIDE and RISE. Ophthalmology 2016;123:1345–1350.

37. Browning DJ, Glassman AR, Aiello LP, et al; Diabetic Retinopathy Clinical Research Network. Relationship between optical coherence tomography-measured central retinal thickness and visual acuity in diabetic macular edema. Ophthalmology 2007;114:525–536.

38. Kardon RH. Role of the macular optical coherence tomography scan in neuro-ophthalmology. J Neuroophthalmol 2011;31: 353–361.

39. Ebneter A, Wolf S, Abhishek J, Zinkernagel MS. Retinal layer response to ranibizumab during treatment of diabetic macular edema: thinner is not always better. Retina 2016;36:1314–1323.

40. Sander B, Larsen M, Engler C, et al. Diabetic macular oedema: a comparison of vitreous fluorometry, angiography, and retinopathy. Br J Ophthalmol 2002;86:316–320.

41. Soliman W, Sander B, Jørgensen TM. Enhanced optical coherence patterns of diabetic macular oedema and their correlation with the pathophysiology. Acta Ophthalmol Scand 2007;85: 613–617.

42. Deák GG, Bolz M, Ritter M, et al. A systematic correlation between morphology and functional alterations in diabetic macular edema. Invest Ophthalmol Vis Sci 2010;51:6710–6714.

43. Browning DJ, Glassman AR, Aiello LP, et al; Diabetic Retinopathy Clinical Research Network. Optical coherence tomography measurements and analysis methods in optical coherence tomography studies of diabetic macular edema. Ophthalmology 2008;115:1366–1371.

44. Kang SW, Park CY, Ham DI. The correlation between fluorescein angiographic and optical coherence tomographic features in clinically significant diabetic macular edema. Am J Ophthalmol 2004;137:313–322.

45. Özdek ŞC, Erdinç MA, Gürelik G, et al. Optical coherence tomographic assessment of diabetic macular edema: comparison with fluorescein angiographic and clinical findings. Ophthalmologica 2005;219:86–92.

46. Hussain A, Hussain N, Nutheti R. Comparison of mean macular thickness using optical coherence tomography and visual acuity in diabetic retinopathy. Clin Exp Ophthalmol 2005; 33: 240–245.

47. Krzystolik MG, Strauber SF, Aiello LP, et al; Diabetic Retinopathy Clinical Research Network. Reproducibility of macular thickness and volume using Zeiss optical coherence tomography in patients with diabetic macular edema. Ophthalmology 2007;114:1520–1525.

48. Kim M, Lee P, Kim Y, et al. Effect of intravitreal bevacizumab based on optical coherence tomography patterns of diabetic macular edema. Ophthalmologica 2011;226:138–144.

49. Roh MI, Kim JH, Kwon OW. Features of optical coherence tomography are predictive of visual outcomes after intravitreal bevacizumab injection for diabetic macular edema. Ophthalmologica 2010;224:374–380.

50. Wu PC, Lai CH, Chen CL, Kuo CN. Optical coherence tomographic patterns in diabetic macula edema can predict the effects of intravitreal bevacizumab injection as primary treatment. J Ocul Pharmacol Ther 2012;28:59–64.

51. Shimura M, Yasuda K, Yasuda M, Nakazawa T. Visual outcome after intravitreal bevacizumab depends on the optical coherence tomographic patterns of patients with diffuse diabetic macular edema. Retina 2013;33:740–747.