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# Morphologic Parameters Relevant for Visual Outcome During Anti-Angiogenic Therapy of Neovascular Age-Related Macular Degeneration

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***Purpose:*** To identify the effects of anti-angiogenic therapy in neovascular age-related macular degeneration (AMD) in respect to morphologic type and time course and to identify prognostic factors for visual outcome on the basis of standardized optical coherence tomography (OCT) analysis.

***Design:*** Subanalysis of a prospective, 12-month, multicenter, phase IIIb trial (Efficacy and Safety of Ranibizumab in Patients with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration [EXCITE]).

***Participants:*** A total of 353 treatment-naïve patients with subfoveal choroidal neovascularization (CNV) receiving quarterly or monthly ranibizumab therapy.

***Methods:*** Patients were randomized to receive 0.3 mg quarterly, 0.5 mg quarterly, or 0.3 mg monthly doses of ranibizumab. Treatment comprised a loading phase of 3 consecutive monthly injections followed by a 9-month maintenance phase of monthly or quarterly injections. Best-corrected visual acuity (BCVA) was measured using the Early Treatment Diabetic Retinopathy Study protocol, and retinal morphology was assessed by Stratus OCT (Carl Zeiss Meditec, Dublin, CA). Imaging data were evaluated by certified examiners of the Vienna Reading Center using a standardized protocol.

***Main Outcome Measures:*** The BCVA was measured using ETDRS charts and retinal morphology was assessed by OCT.

***Results:*** During the loading phase, there was a significant correlation between a reduction in central retinal thickness and an increase in BCVA ($P < 0.001$), which decreased during the maintenance phase in all treatment arms. The proportion of patients showing retinal morphologic changes, such as intraretinal cysts (IRCs), subretinal fluid (SRF), and pigment epithelial detachments (PEDs), decreased significantly in all groups ($P < 0.001$), more intensively in the 0.5 mg quarterly than in both 0.3 mg groups. Intraretinal cysts resolved most rapidly followed by SRF, whereas PED decreased at a slower rate and intensity. Patients with IRC at baseline had lower BCVA levels that remained lower over the entire study period, whereas recurrence of IRC during follow-up showed no additional negative effect on function. Baseline SRF had no effect on visual recovery; however, recurrence of SRF during follow-up showed a tendency for an additional negative effect on function ($P = 0.06$). Baseline PED showed a negative influence on visual outcome only in combination with IRC and SRF.

***Conclusions:*** There is a distinct response pattern and time course of morphologic parameters associated with anti-vascular endothelial growth factor therapy in neovascular AMD. Specific alterations, such as IRC, SRF, and PED, as baseline or follow-up features are significantly influencing the potential for visual gain. *Ophthalmology 2014;121:1237-1245 © 2014 by the American Academy of Ophthalmology.*

Vascular endothelial growth factor-A (VEGF-A) was identified as a key factor involved in the pathogenesis of neovascular age-related macular degeneration (AMD).[1–3] This insight led to the development of ranibizumab (Lucentis; Novartis Pharma AG, Basel, Switzerland, and Genentech Inc, South San Francisco, CA), a recombinant, fully humanized, affinity-matured monoclonal antigen-binding antibody fragment (Fab), which inhibits the binding of multiple biologically active forms of VEGF-A to their receptors.[4,5]

Pivotal phase III AMD trials (Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD [MARINA] and Anti-VEGF antibody for the treatment of predominantly classic choroidal neovascularization in AMD [ANCHOR]) consecutively demonstrated the efficacy of a monthly dosing regimen in improving best-corrected visual acuity (BCVA) in patients with subfoveal choroidal neovascularization (CNV) secondary to AMD.[6,7] The 1-year results showed that both 0.3 mg and 0.5 mg ranibizumab injections provide statistically significant superiority in visual acuity than sham/conventional treatment.

However, this convincing benefit was the result of a strict re-treatment regimen with 12 scheduled ophthalmic

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examinations and injections per year to be continued over the subsequent follow-up in a chronic progressive disease such as AMD. When, on completion of the controlled trials, patients were included in an Open-label Extension Trial of Ranibizumab for Choroidal Neovascularization Secondary to AMD (HORIZON) with a flexible regimen with less frequent re-treatment, an incremental decline of the initial gains was documented.[8] To reduce the socioeconomic burden in disease management, the Ranibizumab in Subjects with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration (PIER) study introduced an alternative regimen with quarterly dosing after 3 consecutive monthly injections.[9] With extension of the therapeutic intervals, the treatment benefit was lost and visual acuity returned to baseline levels. Analysis of morphologic outcomes demonstrated a correlation of recurrent leakage as seen by angiography and optical coherence tomography (OCT) and visual decline with the conclusion that the infrequent dosing regimen accelerated the detrimental effects of retinal fluid on BCVA.[10]

Fung et al were the first to suggest an individualized dosing strategy in anti-angiogenic therapy of neovascular AMD. Re-treatment based on evidence of disease activity in OCT imaging was successfully used in the Prospective Optical coherence tomography imaging of patients with Neovascular AMD Treated with intra-Ocular ranibizumab (PrONTO) trial.[11] Even during a 2-year follow-up, results proved to be excellent functionally, anatomically, and regarding re-treatment frequency.[12] This concept undoubtedly has introduced novel standards in the management of AMD, despite the small population of 40 patients in a single-center trial.

Individualized regimens in anti-angiogenic therapy have become the leading standard of care worldwide, and OCT imaging has developed to the gold standard in diagnostic evaluation of AMD activity.[13] In early pro re nata (PRN) trials (Safety Assessment of Intravitreal Lucentis for age-related macular degeneration [SAILOR], Study of Ranibizumab in Patients With Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration [SUSTAIN]), re-treatment criteria were based on loss in visual acuity or an increase in central retinal thickness (CRT) with inferior outcomes compared with the results of the fixed monthly regimens. In novel trial designs (Comparison of Age-related Macular Degeneration Treatments Trials [CATT], The pHase III, double-masked, multicenter, randomized, Active treatment-controlled study of the efficacy and safety of 0.5 mg and 2.0 mg Ranibizumab administered monthly or on an as-needed Basis (PRN) in patients with subfoveal neOvasculaR age-related macular degeneration (HARBOR), a comprehensive approach recommending the presence of any macular fluid as a re-treatment indication still demonstrated a reduced benefit in the PRN groups compared with the monthly regimen.[13–16] Tighter OCT criteria led to a higher frequency in dosing and intensive monitoring schedules so far without consistent identification of re-treatment criteria that directly correlate with macular function.

The Efficacy and Safety of Ranibizumab in Patients with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration (EXCITE) study was the only prospective trial designed to directly compare monthly with quarterly ranibizumab dosing regimens in patients with subfoveal CNV secondary to AMD and the first study including monthly OCT monitoring by protocol.[11] This 1-year study had an active control arm of continuous monthly injections (0.3 mg) versus the less frequent schedules of 3 initial monthly injections of 0.3 mg or 0.5 mg ranibizumab followed by quarterly injections of the respective doses. The primary objective of this study was to investigate whether a maintenance strategy using a quarterly dosing regimen (0.3 mg and 0.5 mg) was noninferior to a monthly dosing regimen as determined by the mean change in BCVA from baseline to month 12.

The EXCITE design together with the complete functional and morphologic datasets offers a unique opportunity to identify structural features directly related to functional outcomes:[17] In an identical population, an optimized monthly dosing compares with continuous quarterly undertreatment allowing to monitor the effect of recurrent and persistent fluid on neurosensory function. A timely re-treatment in PRN trials eliminates fluid instantaneously and prevents a correlation of function and morphology during follow-up.

The standardized image acquisition and reading procedures in the present trial provide a detailed morphologic characterization of macular fluid accumulation regarding the occurrence of intraretinal cysts (IRCs), subretinal fluid (SRF), or pigment epithelial detachment (PED), closely reflecting the pathophysiology of neovascular AMD. These parameters have been investigated in clinical neovascular AMD trials as initial treatment and subsequent re-treatment parameters.[18–21]

The importance of different morphologic alterations as prognostic parameters is vastly controversial, as well as their value on how they can be used to optimally support re-treatment decisions. A precise understanding of the biological response of retinal and subretinal compartments within anti-VEGF treatment is essential for the identification of parameters relevant for functional improvement and mechanisms of recurrence. The extended intervals also permit the identification of the time course of recurrence/resolution of such features.

The aim of the present study was to identify the specific morphologic effects of anti-angiogenic intervention in 3 different dosing regimens of the EXCITE trial, low/high drug dose, and short/long treatment interval. Visual function was correlated with the type and time course of structural response that was documented in a reading center setting using standardized protocols.

## Methods

### Study Design

The EXCITE study was designed as a 1-year, randomized, double-masked, active-controlled, multicenter, phase IIIb study in patients with subfoveal CNV secondary to AMD, comparing the efficacy and safety of quarterly dosing regimens of ranibizumab in 2 different dosages with a monthly dosing regimen during the maintenance phase, that is, from month 3 onward.

Eligible patients were randomly assigned in a 1:1:1 ratio to any of the following 3 double-masked treatment arms (Fig 1): loading dose of 3 initial monthly intravitreal injections of 0.3 mg (arm A) or 0.5 mg (arm B) ranibizumab followed by fixed quarterly

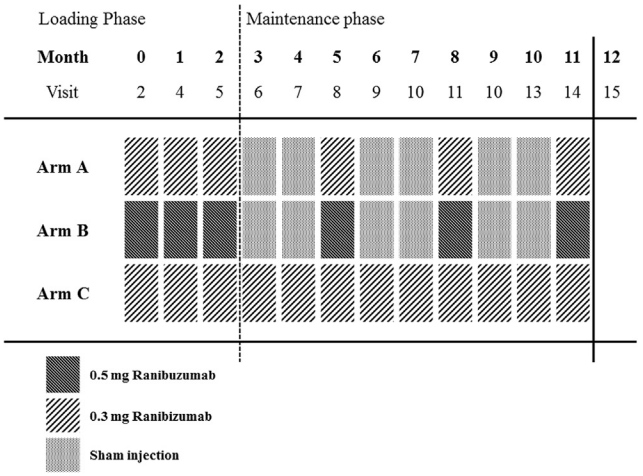


**Figure 1.** Dosing schedule of ranibizumab regimen in the Efficacy and Safety of Ranibizumab in Patients with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration (EXCITE) Study. Additional visits without treatment: V1 (screening), V3 (week 1), and V15 (month 12, end of study).

injections or 0.3 mg ranibizumab administered monthly from baseline to month 11 (arm C, active control). To maintain masking, patients in treatment arms A and B were administered a sham injection during the monthly visits, when no intravitreal injection was scheduled.

This trial was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonization Good Clinical Practice guidelines. Approval was obtained from the independent ethics committee or institutional review board at each participating study site. All patients provided signed informed consent before participating in the study. The trial is registered at clinicaltrials.gov (NCT00275821). Details regarding study sites, detailed inclusion and exclusion criteria, and safety assessment of the study have been described.[17]

### Visual Acuity Assessment

The BCVA was assessed for both eyes separately at each visit (baseline, week 1, and from month 1 to 12) using Early Treatment Diabetic Retinopathy Study–like charts at an initial testing distance of 4 m.

### Morphologic Analysis Using Optical Coherence Tomography

An independent, masked central Reading Center (Vienna Reading Center [VRC]) reviewed OCT images to provide a uniform evaluation of retinal morphology for each assessment (every month and at week 1) of all patients. Stratus OCT (Carl Zeiss Meditec, Dublin, CA) scans were acquired by certified operators at each study site after pupil dilatation using the fast macular thickness map (FMTM) scan mode for quantitative measures of the CRT and a 6-mm crosshair (CH) scan mode for qualitative assessment of retinal morphology. The FMTM protocol acquires six 6-mm radial cross-sectional images with a whole scan time of 1.9 seconds, each of the images consisting of 128 A-scans. The CH protocol required two 6-mm cross-sectional images (3–9 and 6–12 o'clock) with a resolution of 512 A-scans per image. Scans were performed using Stratus software version 4.0 or higher (Carl Zeiss Meditec) with all patient-identifying data being removed. Data for each measurement were sent to the VRC as digital raw data sets.

Validated computer-assisted grading software was used at the VRC. This software imports OCT scan data from the Stratus-OCT and allows the grader to evaluate various parameters in the 6-mm FMTM and CH scans following defined algorithms. The graders were trained according to the individual protocol of the VRC, and certification was awarded on the successful completion of all requirements.

All OCT scans were graded for the presence of IRC, SRF, and PED. Intraretinal cysts were defined as round, minimally reflective spaces within the neurosensory retina. Subretinal fluid was identified as a nonreflective space between the posterior boundary of the neurosensory retina and the retinal pigment epithelium (RPE) reflection. Pigment epithelial detachments were described as a focal elevation of the reflective RPE band over an optically clear or moderately reflective space. Pigment epithelial detachments were definitely graded if the radial extension of an RPE elevation exceeded 400 μm at the base or the vertical elevation from the RPE band to the surface of the choriocapillaris was >200 μm.[22]

### Quality Control and Reproducibility

All OCT images were evaluated by 4 independent VRC graders. Various levels of quality-control algorithms were performed to maintain intergrader reproducibility. Difficult cases were discussed in regular meetings with the grading supervisor. In these meetings, selected images were regarded as an exercise with the group's input, and 10% of images were randomly selected for quality control with the intent to have them re-graded by the supervisor. For comparison of results obtained by different reading centers, the reproducibility among graders of the VRC and the University of Wisconsin-Madison Reading Center was determined.[22]

### Statistical Analysis

For documentation of patient data, an SQL database was used. Statistical analyses were performed using SPSS Version 17.0 (SPSS Inc, Chicago, IL). IRC, SRF, and PED at baseline were included as potential confounders. The partial correlation coefficients are computed holding these variables constant. The BCVA was analyzed by generalized estimation equations with an unstructured covariance matrix, and normal deviates, frequency of IRC, SRF, and PED were analyzed using a logistic link function. A $P$ value $\leq 0.05$ was considered statistically significant.

## Results

### Patients

A total of 353 patients at 59 study sites were randomized for treatment with the study medication. The study was completed by 106 patients in the ranibizumab 0.3 mg quarterly group (arm A), 95 patients in the ranibizumab 0.5 mg quarterly group (arm B), and 103 patients in the ranibizumab 0.3 mg monthly group (arm C). The patients' disposition, baseline and ocular disease characteristics, treatment exposure, adverse events, BCVA, and CRT time course and contrast sensitivity analysis in the 3 different treatment arms have been described in detail.[17]

### Response and Time Course of Individual Morphologic Parameters

**Intraretinal Cysts.** Morphologic grading revealed IRC in 52 patients (51%) in the 0.3 mg quarterly group, 51 patients (52%) in the

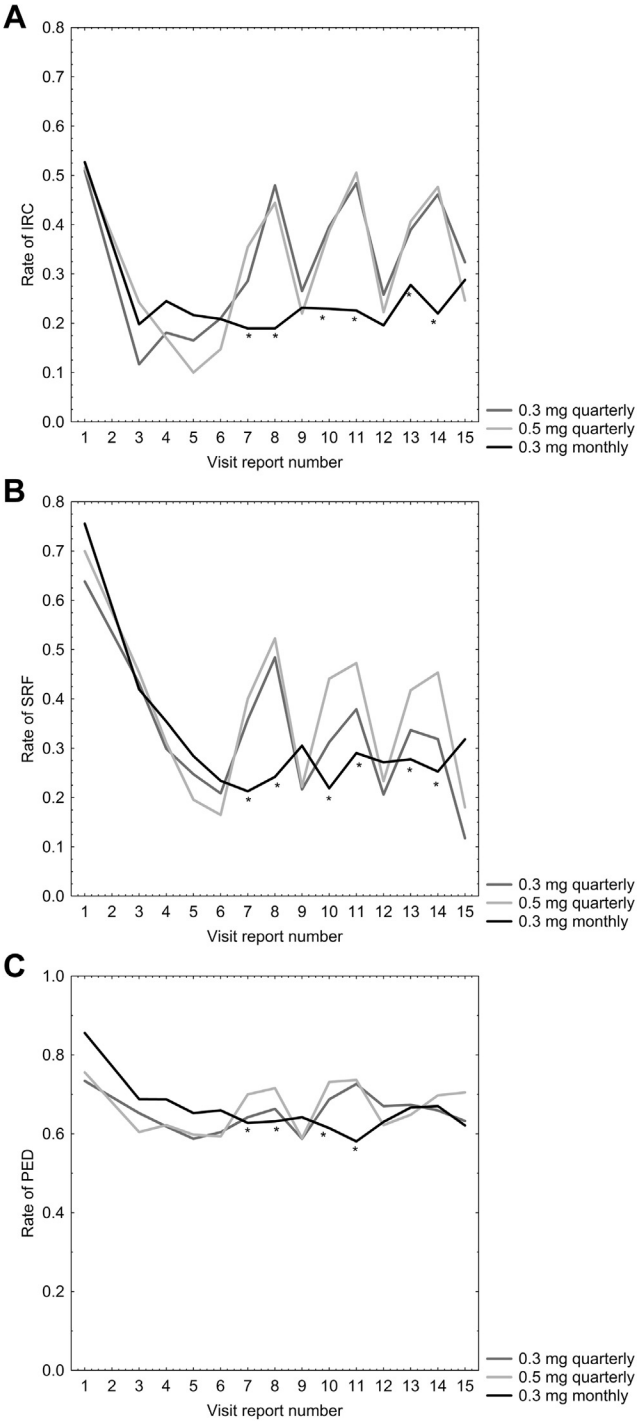


**Figure 2.** Comparison of the response of intraretinal cyst (IRC) **(A)**, subretinal fluid (SRF) **(B)**, and pigment epithelial detachment (PED) **(C)** with ranibizumab treatment in the 0.3 mg quarterly, 0.5 mg quarterly, and 0.3 mg monthly groups. After a significant reduction of IRC, SRF, and PED during the loading phase (visits 1–6), a recalcitrant rapid increase was found in each treatment-free period in the quarterly groups for IRC and SRF, whereas a slower re-increase was found for PED. Significant differences from the monthly group are indicated by an asterisk (*). V1 = screening; V2 = baseline; V3 = week 1; V4 = month 1; V5 = month 2; V6 = month 3; V15 = month 12.

0.5 mg quarterly group, and 49 patients (53%) in the 0.3 mg monthly group at baseline. A significant ($P < 0.01$) reduction of scans presenting with IRC during the loading phase (the first 3 injections) could be identified in all 3 treatment arms (Fig 2A), highlighting the consistency of this feature and the homogenous tissue response among the study arms.

Intraretinal cysts reached the lowest values at week 1 after 1 single ranibizumab injection in the 0.3 mg quarterly group and 0.3 mg monthly group (lowest rate of IRC in the 0.3 mg quarterly group) and at month 2 after 2 ranibizumab injections in the 0.5 mg quarterly group (lowest rate of IRC in the 0.5 mg quarterly group at month 1, month 2, and month 3). Thereafter, IRC did not achieve similarly low rates during the entire following study duration in all 3 treatment arms, especially in the quarterly groups (Fig 2A). The proportion of patients presenting with IRC at baseline was reduced by 80% until month 3 in the 0.5 mg group and by 68% in the 2 0.3 mg groups. The difference in IRC reduction among groups was not statistically significant during the loading phase.

During the maintenance phase, IRC showed a recurrent increase in each treatment-free period of 8 weeks in both quarterly treatment groups. This increase in the proportion of scans showing IRC was significant ($P < 0.05$) between monthly and quarterly dosing and was immediately resolved 1 month after the subsequent intravitreal injection in each quarterly dosage arm and at the end of follow-up (Fig 2A).

**Subretinal Fluid.** At baseline, 67 patients (66%) in the 0.3 mg quarterly group, 70 patients (71%) in the 0.5 mg quarterly group, and 70 patients (75%) in the 0.3 mg monthly group showed SRF. All treatment groups showed a continuous resolution of SRF during the loading phase (Fig 2B). At month 3, the proportion of scans showing absence of SRF was significantly lower in the 0.5 mg group ($P < 0.05$) when compared with the two 0.3 mg groups, indicating dose dependency.

In the 0.3 mg monthly group, the initial decrease was followed by maintenance of the improved values thereafter (Fig 2B). The 2 groups receiving quarterly treatment demonstrated a prompt and significant ($P < 0.05$) recurrence of SRF during each treatment-free phase (Fig 2B). The repetitive recurrence of SRF was similar to that of IRC, but less rapid and less intensive. Subretinal fluid reached lowest levels at the end of the study in quarterly treatment arms. In contrast to IRC, resolution of SRF after anti-angiogenic reintervention in the quarterly treatment intervals reached the same low values as after the loading phase.

**Pigment Epithelial Detachment.** A total of 75 patients (74%) in the 0.3 mg quarterly group, 77 patients (78%) in the 0.5 mg quarterly group, and 79 patients (85%) in the 0.3 mg monthly group presented with PED at baseline. All treatment groups showed a significant ($P < 0.05$) positive response on resolution during the loading phase (Fig 2C). The resolution of PED was treatment dependent, but less pronounced compared with IRC and SRF.

The 0.3 mg monthly group retained the low values of PED reached at month 3 for the entire follow-up (Fig 2C). The 2 groups receiving quarterly treatment demonstrated a significant ($P < 0.05$) recurrence of PED during each treatment-free phase except for the last phase (Fig 2C). This increase in the proportion of scans showing PED resolved again 4 weeks after the subsequent intravitreal injection; however, this "spike and wave" effect was less intensive when compared with IRC and SRF. Similar to SRF, PED resolved after anti-angiogenic intervention in the quarterly treatment intervals and reached equal values as after the loading phase. In general, PED was the least responsive feature during short- and long-term follow-ups.

Table 1. Partial Correlation Coefficients between Change of Best-Corrected Visual Acuity (Difference to Baseline) and Change of Central Retinal Thickness (All 3 Treatment Arms Corrected for the Influence of Morphologic Changes: Intraretinal Cysts, Subretinal Fluid, and Pigment Epithelial Detachment)

| Month | Quarterly (0.3 mg) | Quarterly (0.5 mg) | Monthly (0.3 mg) |
|---|---|---|---|
| 3 | −0.339* | −0.321* | −0.379* |
| 6 | −0.254* | −0.278* | −0.156 |
| 9 | −0.075 | −0.220* | −0.210* |
| 12 | −0.105 | −0.256* | −0.085 |

BCVA = best corrected visual acuity; CRT = central retinal thickness.
*$P < 0.05$.

### Correlation Between Visual Acuity and Central Retinal Thickness

Treatment effects on CRT and BCVA were moderately although significantly correlated (−0.3 to −0.4) at the end of the loading phase. During the maintenance phase, the correlation decreased. This decrease was more pronounced in the 0.3 mg treatment arms. The correlation in the 0.5 mg quarterly regimen was still significant at month 12 (Table 1). In contrast to treatment effects on BCVA and CRT as defined by difference to baseline values, BCVA and CRT values themselves were correlated only at baseline (Fig 3). No significant correlation could be found at month 3, 6, 9, or 12.

### Correlation of Morphologic Parameters with Visual Function

Visual function measured in Early Treatment Diabetic Retinopathy Study letters was correlated to morphologic changes as documented by OCT analysis. The time courses of BCVA change in patients showing different individual combinations of morphologic parameters (±IRC, ±SRF, ±PED) at baseline were analyzed for treatment arms individually (Fig 4). Absolute and relative numbers of patients showing different combinations of morphologic parameters at baseline are summarized in Table 2. Approximately 24% of all patients initially presented with a combination of all 3 alterations simultaneously, and 27% of patients exhibited SRF and PED without intraretinal fluid.

Overall, combinations including IRC at baseline (Fig 4, curves indicated in red) showed a significantly lower mean BCVA when compared with alterations without IRC (Fig 4, curves indicated in blue). During treatment, BCVA improved significantly in all groups; however, the mean BCVA of patients with initial IRC remained significantly lower than mean BCVA of patients without IRC during the entire follow-up and in all treatment regimens (Fig 4). However, reoccurrence of IRC during follow-up did not have an additional negative effect on visual function (Table 3).

In regard to the prognostic value of SRF, there was no significant difference in BCVA outcome between patients presenting with or without SRF at baseline, as well as during follow-up in any of the 3 regimens. However, recurrence of SRF during the maintenance phase showed a tendency ($P = 0.06$) for an additional negative impact on visual function. Pigment epithelial detachments showed a significant negative effect on BCVA levels at baseline and during follow-up only in combination with IRC and SRF at baseline.

## Discussion

In the present investigation, change in overall CRT and individual morphologic parameters (IRC, SRF, PED) were documented in a quantitative and qualitative manner and correlated with functional outcomes during a 12-month follow-up. Datasets from a prospective multicenter trial offering a direct comparison between a continuous immediate re-treatment strategy and a discontinuous delayed regimen allowing exacerbation of morphologic alteration were analyzed. Certified graders of an independent reading center interpreted retinal morphology according to standardized protocols providing the currently most reliable method to handle OCT data.

Consistent with the findings of other large studies evaluating the efficacy of intravitreal ranibizumab in neovascular AMD, all 3 treatment arms achieved a similarly effective increase in BCVA and decrease in CRT during the loading phase.[8–10,17] However, CRT measurements include a composite of multiple individual alterations between the internal limiting membrane and the RPE layer. A detailed qualitative analysis of retinal and subretinal compartments, namely, IRC, SRF, and PED, may reveal functionally more relevant changes in retinal microstructure that might be neglected in comprehensive measurements of total CRT, thereby leading to inaccurate correlations between functional and morphologic parameters in neovascular AMD and other exudative macular diseases treated with anti-angiogenic strategies.

Intraretinal cysts at baseline revealed a significant ($P < 0.05$) and rapid resolution during the loading phase, reaching the lowest values in 2 of 3 treatment arms 1 week after the first single ranibizumab injection. Subretinal fluid and PED also resolved significantly during the loading phase; however, optimal responses were reached mostly after the third injection at month 3. In the absence of further continuous treatment in the 2 quarterly regimens, all morphologic parameters showed a recurrent increase in each treatment-free period of 12 weeks. From all measured parameters, IRC recurred most rapidly, followed by SRF and immediately resolved after the subsequent intravitreal injection. This suggests IRC to be the morphologic parameter responding most sensitively to anti-VEGF therapy in neovascular AMD. Explanations might be that ranibizumab rapidly diminishes active neovascular leakage into the retina, that the intraretinal bioavailability of the drug is rapid and efficient, and that the plasticity of the neurosensory retina for structural repair is high. In contrast, resolution of SRF and particularly sub-RPE fluid proceeds more slowly, because these compartments may show a reduced accessibility and prolonged absorption of exudate.

In addition, this study showed a dose dependency of the effect of ranibizumab on the graded morphologic parameters. The proportion of scans showing absence of IRC, SRF, and PED at the end of the loading phase was higher in the 0.5 mg arm when compared with the two 0.3 mg dosing arms. Previous studies investigating multiple escalating doses of ranibizumab concluded that the change in mean BCVA occurred similarly in the 0.3 mg and the 0.5 mg groups, with no clear dose-response relationship.[23,24] In terms of morphologic

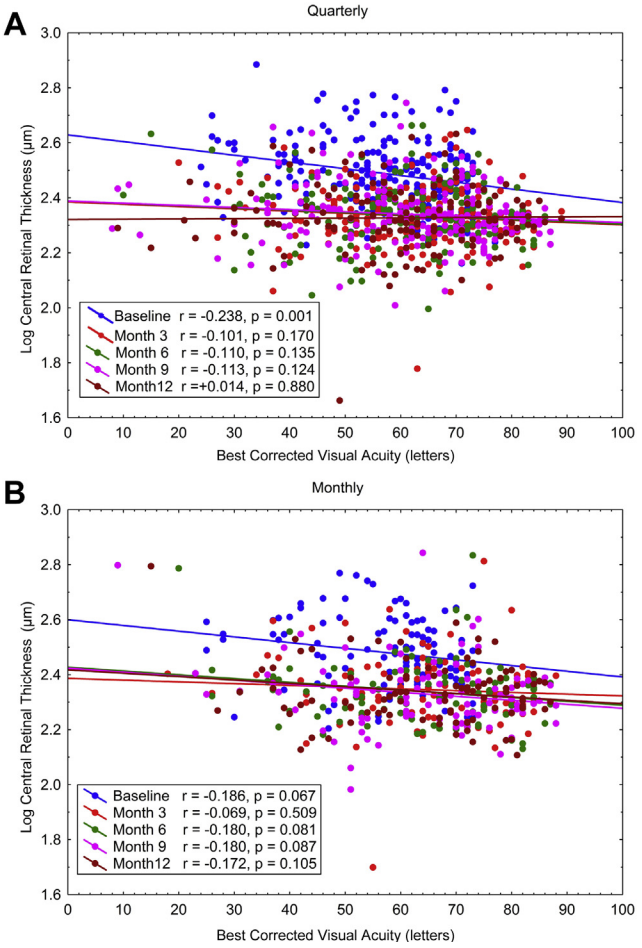


**Figure 3.** Scatter plots showing the correlation of best-corrected visual acuity (BCVA) and CRT in the quarterly (0.3/0.5 mg) groups **(A)** and the 0.3 mg monthly group **(B)** at baseline and months 3, 6, 9, and 12.

efficacy, a difference in favor of the higher dose was noted. In the PIER Study, the differences between the 0.3 mg and the 0.5 mg ranibizumab dose groups in mean change in BCVA, CRT, and area of leakage from CNV were not statistically significant.[9] However, although the 0.5 mg group showed a more intensive reduction of all morphologic alterations, it exhibited no clinically meaningful benefit relative to the 0.3 mg group. There was no difference in the rate of recurrence of morphologic changes in each treatment-free period in the 2 quarterly treatment groups or with regard to BCVA outcomes; rather, recurrence of morphologic changes appeared more rapid in the higher dose group.[17] In the HARBOR trial, a higher proportion of patients gaining ≥15 letters was noted in the higher dose group unrelated to the change in total CRT. Further OCT analysis may be worthwhile to identify the underlying morphologic mechanisms.[16]

An analysis of function and morphology during the maintenance phase is of particular interest, because OCT data are mainly used to guide the long-term management of neovascular AMD. Our investigation reveals that morphologic

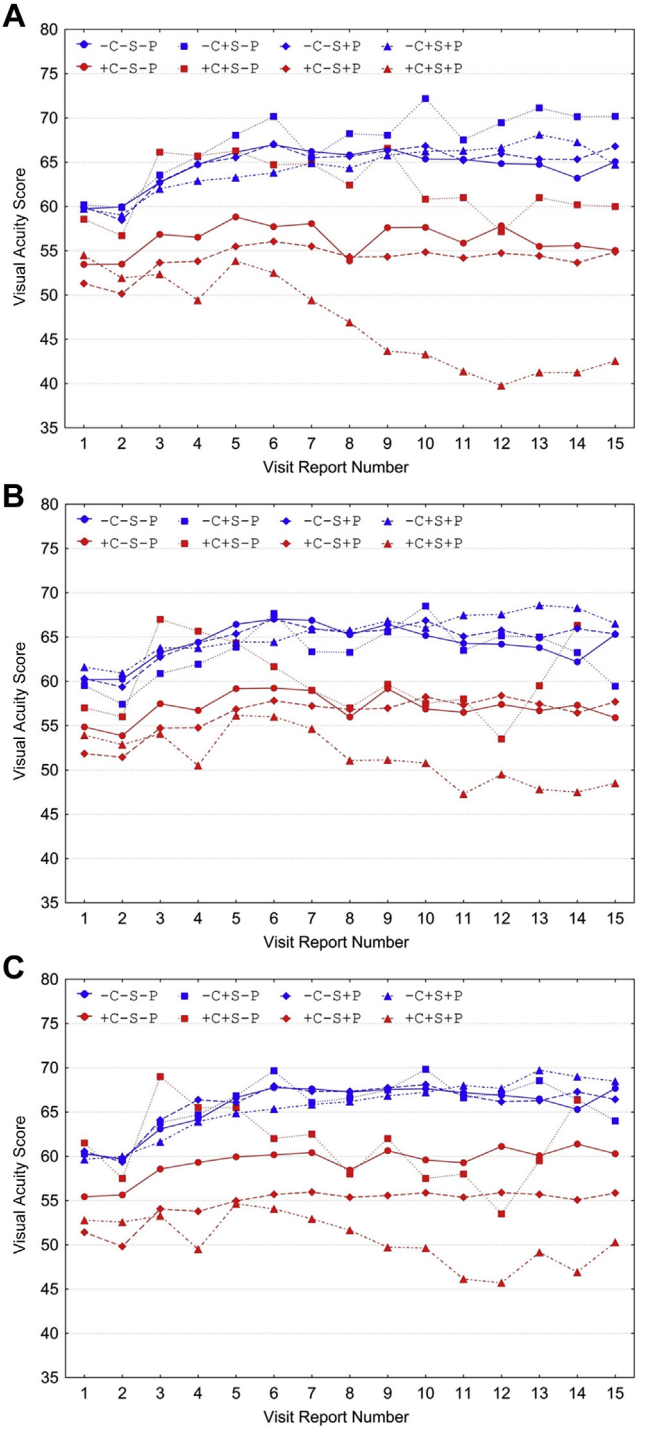


**Figure 4.** Time course of best-corrected visual acuity (BCVA) change in Early Treatment Diabetic Retinopathy Study letters after 0.3 mg quarterly **(A)**, 0.5 mg quarterly **(B)**, and 0.3 mg monthly **(C)** ranibizumab treatments in patients with different combinations of morphologic parameters at baseline. +/− C = intraretinal cysts present/absent; +/− P = pigment epithelial detachment present/absent; +/− S = subretinal fluid present/absent; V1 = screening; V2 = baseline; V3 = week 1; V4 = month 1; V5 = month 2; V6 = month 3; V15 = month 12. *Blue lines*: patients without cysts at baseline. *Red lines*: patients with cysts at baseline.

Table 2. Absolute and Relative Numbers (%) of Patients Showing Different Combinations of Morphologic Parameters at Baseline Presentation

| Morphology | Quarterly (0.3 mg) | Quarterly (0.5 mg) | Monthly (0.3 mg) |
|---|---|---|---|
| Not gradable | 11 | 11 | 13 |
| | 9.17% | 9.32% | 11.40% |
| −C−S−P | 10 | 9 | 4 |
| | 8.33% | 7.63% | 3.51% |
| +C−S−P | 7 | 4 | 7 |
| | 5.83% | 3.39% | 6.14% |
| −C+S−P | 9 | 7 | 4 |
| | 7.50% | 5.93% | 3.51% |
| +C+S−P | 6 | 4 | 3 |
| | 5.00% | 3.39% | 2.63% |
| −C−S+P | 7 | 8 | 8 |
| | 5.83% | 6.78% | 7.02% |
| +C−S+P | 16 | 12 | 11 |
| | 13.33% | 10.17% | 9.65% |
| −C+S+P | 31 | 29 | 35 |
| | 25.83% | 24.58% | 30.70% |
| +C+S+P | 23 | 34 | 29 |
| | 19.17% | 28.81% | 25.44% |
| Total | 120 | 118 | 114 |

+/− C = intraretinal cysts present/absent; +/− S = subretinal fluid present/absent; +/− P = pigment epithelial detachment present/absent.

changes are not translated in an identical pattern into BCVA outcomes. Although the initial decrease of CRT during the loading dose showed a highly significant correlation with an improvement in BCVA, this association becomes less and less evident during follow-up because damage in the retinal architecture may become irreversible. The correlation between a reduction in CRT and gain in BCVA decreased in all treatment arms during the maintenance phase, particularly in the 0.3 mg regimens. This finding explains the lack in efficiency of time-domain OCT-guided PRN trials when treatment indications were based on a specific threshold of CRT increase, mostly $\Delta = 100\ \mu m$ or $\Delta = 50\ \mu m$.[13,14] Accordingly, current expert opinions recommend a "no mercy" regimen with re-treatment indicated by any evidence of fluid on OCT imaging.[15] Of note, CRT decreases to an identical low final value in the EXCITE trial in all monthly/quarterly regimens because of irreversible damage to the neurosensory structures during the increase in CRT at untreated intervals, but BCVA does not recover accordingly. An analysis of SUSTAIN and PIER data confirmed the strong influence of adequate timing of reintervention because lost function is not recovered with delayed discovery.[10,14] In an interventional PRN study, vision decline was anticipated by an increase in CRT in the preceding 1 to 2 months. If >4 letters had been lost, eyes failed to recover vision despite later treatment.[25] Qualitative data, such as a cystoid intraretinal pattern, may offer superior insight into the condition of the neurosensory retina, because IRCs may be the result of active vascular leakage, and readily disappear on treatment, or reflect intraretinal neurodegeneration referred to as "cystic degeneration." Accordingly, insufficient quarterly treatment leads to a pronounced increase in IRCs together with inferior and irreversible visual loss in our study. This morphologic finding may highlight why a simple quarterly ranibizumab treatment regimen results in inferior functional outcomes compared with monthly, PRN, or treat and extent schedules and can therefore not be recommended in any way.

Table 3. Change in Best-Corrected Visual Acuity from Month at First Reappearance of Cysts during Maintenance Phase to Month 12 (Last Observation Carried Forward)

| | 0.3 mg Quarterly | | | 0.5 mg Quarterly | | | 0.3 mg Monthly | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Mean (SEM)* | *n* | *Significance* | *Mean (SEM)* | *n* | *Significance* | *Mean (SEM)* | *n* | *Significance* |
| No cysts | −0.7 (1.24) | 40 | ns | −2.2 (1.79) | 41 | ns | 1.4 (0.92) | 62 | ns |
| Month 4 | −2.8 (2.93) | 20 | | 4.7 (2.51) | 14 | | −2.4 (1.72) | 19 | |
| Later | −2.7 (1.49) | 51 | ns | −5.4 (1.39) | 53 | <0.05 | 1.2 (1.20) | 30 | ns |
| No cysts | 0.2 (1.23) | 41 | ns | −2.5 (1.65) | 41 | ns | 0.6 (0.79) | 62 | ns |
| Month 5 | −5.4 (3.52) | 14 | | −3.8 (2.40) | 20 | | 5.5 (2.02) | 4 | |
| Later | 0.9 (1.95) | 37 | ns | −5.3 (1.69) | 32 | ns | 0.7 (1.46) | 26 | ns |
| No cysts | 0.3 (1.21) | 40 | ns | −0.7 (1.60) | 39 | ns | −0.3 (0.70) | 62 | ns |
| Month 6 | −0.3 (2.39) | 16 | | −2.1 (2.62) | 13 | | −0.8 (1.02) | 5 | |
| Later | −0.6 (1.79) | 21 | ns | −4.7 (1.94) | 20 | ns | 0.9 (1.93) | 20 | ns |
| No cysts | 0.0 (0.99) | 41 | ns | −1.1 (1.53) | 41 | ns | 0.5 (0.66) | 62 | ns |
| Month 7 | 3.5 (4.25) | 4 | | −1.7 (0.88) | 3 | | −3.8 (3.39) | 6 | |
| Later | −2.4 (1.95) | 17 | ns | −7.9 (2.41) | 16 | ns | 3.3 (2.05) | 14 | ns |
| No cysts | 0.7 (0.90) | 41 | ns | −1.3 (1.65) | 38 | ns | −0.4 (0.72) | 61 | ns |
| Month 8 | 0.6 (3.25) | 5 | | −4.2 (2.29) | 6 | | 3.3 (1.75) | 4 | |
| Later | −3.8 (2.38) | 12 | ns | −11.6 (2.82) | 11 | ns | 4.4 (2.32) | 11 | ns |
| No cysts | −0.1 (1.02) | 41 | ns | −0.4 (1.08) | 39 | ns | 0.2 (0.85) | 58 | ns |
| Month 9 | −3.6 (2.44) | 8 | | −7.0 (2.02) | 5 | | 0.0 (1.08) | 4 | |
| Later | 0.8 (2.66) | 4 | ns | −12.4 (5.15) | 5 | ns | 3.4 (3.50) | 7 | ns |
| No cysts | 0.4 (0.93) | 40 | ns | −0.6 (1.02) | 38 | ns | 0.2 (0.80) | 58 | ns |
| Month 10+ | 3.8 (6.02) | 4 | | −7.5 (4.15) | 6 | | 2.0 (2.28) | 7 | |

ns = not significant; SEM = standard error of the mean.
Comparison for same time interval with patients without cysts during maintenance phase and with patients with later occurrence of cysts. Significance assessed Bonferroni corrected for multiple time intervals.

On the other hand, a comparison of BCVA with IRC recurrence after the loading dose showed no correlation during follow-up in the 0.3 mg monthly and the 0.3/0.5 mg quarterly treatment arms identifying reversible cysts as a transient sign of vascular leakage responding rapidly to re-treatment as opposed to irreversible cystic degeneration. However, there was a trend for correlation between recurrence of SRF and decrease in BCVA during the maintenance phase. This is consistent with previous studies in which a reduction of SRF correlated well with an improvement in BCVA during the initial injections of the loading phase and after re-treatment during the maintenance phase.[20] However, a direct comparison is not possible because those data are based on spectral-domain OCT compartment analysis using SRF volume measurements rather than Stratus OCT grading for the presence or absence of SRF. Three-dimensional, raster scanning provided by spectral-domain OCT offers far superior qualitative and quantitative imaging for a refined analysis. So far, the majority of prospective multicenter trials have used time-domain OCT data, and few have undertaken an analysis of qualitative parameters. The CATT group provided reproducibility in grading IRC, SRF, and sub-RPE fluid with a kappa between 0.48 and 0.8 based on the same OCT instrumentation used in our study.[26] A greater foveal thickness and presence of RPE elevation on OCT were identified as negative predictors for improvement.[27] Spectral-domain OCT may allow for further analysis of other function-related parameters, such as photoreceptor integrity, in future studies.[28]

Finally, our analysis demonstrates that patients with IRC at baseline showed a lower level of initial visual acuity that remained lower over the entire study period. Subretinal fluid at baseline had no negative predictive value for visual recovery, and PED showed a significant negative predictive value for visual outcome in combination with IRC and SRF. The latter fact is complementary to the CATT subanalysis, but highlights the association with retinal alteration.[27]

These findings suggest that IRC at baseline particularly indicates preexisting and irreversible retinal damage, decreasing the potential for visual gain throughout therapy. This is in accordance with findings of a recent study by Pelosini et al[29] showing that the cross-sectional area of retinal tissue between the plexiform layers in cystoid macular edema, as imaged by OCT, can be used as an indicator for visual acuity.

Most important, the RPE plays an important role in the pathophysiology of exudative and atrophic AMD. Advanced RPE alterations strongly affect the overlying neurosensory retina by multiple mechanisms, such as cystic degeneration or inflammatory activation.[30,31] In advanced AMD, cystic degenerative changes in the retina regularly occur over areas of RPE loss and sub-RPE fibrosis as shown by selective imaging of the RPE by advanced OCT imaging technology.[32] The integrity of the RPE could not be assessed in this trial.

In conclusion, this study demonstrates the importance of distinct qualitative morphologic parameters with respect to visual prognosis and a guidance in future patient-individualized disease management of anti-VEGF treatment in neovascular AMD to optimize functional outcomes and socioeconomic value.

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## Footnotes and Financial Disclosures

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Abbreviations/Acronyms:
**AMD** = age-related macular degeneration; **BCVA** = best-corrected visual acuity; **CATT** = Comparison of Age-Related Macular Degeneration Treatments Trials; **CH** = crosshair; **CNV** = choroidal neovascularization; **CRT** = central retinal thickness; **EXCITE** = Efficacy and Safety of Ranibizumab in Patients with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration; **Fab** = fragment antigen-binding; **FMTM** = fast macular thickness map; **HARBOR** = The pHase III, double-masked, multicenter, randomized, Active treatment-controlled study of the efficacy and safety of 0.5 mg and 2.0 mg Ranibizumab administered monthly or on an as-needed Basis (PRN) in patients with subfoveal neOvasculaR age-related macular degeneration; **IRC** = intraretinal cyst; **OCT** = optical coherence tomography; **PED** = pigment epithelial detachment; **PIER** = Ranibizumab in Subjects with Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration; **PRN** = pro re nata; **RPE** = retinal pigment epithelium; **SRF** = subretinal fluid; **SAILOR** = Safety Assessment of Intravitreal Lucentis for age-related macular degeneration; **SUSTAIN** = Study of Ranibizumab in Patients With Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration; **VEGF-A** = vascular endothelial growth factor-A; **VRC** = Vienna Reading Center.

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