Influence of the Vitreomacular Interface on Outcomes of Ranibizumab Therapy in Neovascular Age-related Macular Degeneration

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Purpose: To investigate the influence of the vitreomacular interface (VMI) on the functional and anatomic efficacy of ranibizumab therapy in patients with neovascular age-related macular degeneration (AMD).

Design: Subanalysis of a prospective, 12-month, multicenter, phase IIIb trial.

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

Methods: On monthly optical coherence tomography (OCT) scan sets, the VMI configuration was graded by a certified reading center into one of the following conditions: continuous posterior vitreoretinal attachment (PVA), vitreomacular adhesion (VMA), partial vitreous detachment without vitreomacular contact, or complete posterior vitreous detachment (PVD). Best-corrected visual acuity (BCVA) and central retinal thickness (CRT) measurements were performed at monthly intervals. Analysis included patients with a minimum of 10 OCT examinations, including baseline and month 12 (n = 251). After integration of the VMI configuration over 12 months, patients were divided into one of the following categories: PVD (n = 162), release of vitreomacular contact (RELEASE; n = 48), VMA (n = 37), or PVA (n = 4). General estimation equation analyses were applied to test for noninferiority of quarterly versus monthly treatment.

Main Outcome Measures: The BCVA and CRT changes at month 12.

Results: Mean BCVA changes in letters were +4.7 (PVD), +3.2 (RELEASE), and −0.2 (VMA) in the quarterly regimen and +4.9 (PVD), +12.7 (RELEASE), and +7.5 (VMA) in the monthly regimen. No difference in therapeutic efficiency between monthly and quarterly intervention was found in eyes with PVD, and quarterly treatment was noninferior to monthly treatment ($P = 0.001$). However, monthly treatment was superior to quarterly treatment in the RELEASE ($P = 0.008$) and VMA ($P = 0.043$) groups. Mean CRT changes were −98 and −96 μm (PVD), −117 and −136 μm (RELEASE), and −93 and −87 μm (VMA) in the monthly and quarterly regimens, respectively, without statistically significant differences.

Conclusions: The configuration of the VMI seems to have an important effect on visual outcomes and need for retreatment. In patients with PVD, a lower treatment frequency may be feasible, whereas patients with RELEASE or VMA may benefit from intensive retreatment. These findings may serve as a basis for individualized treatment decisions in anti-angiogenic therapy of neovascular AMD and perhaps other indications.

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Several clinical trials have established ranibizumab (Lucentis; Novartis, Basel, Switzerland, and Genentech, South San Francisco, CA) as the gold standard in the treatment of subfoveal choroidal neovascularization (CNV) in patients with age-related macular degeneration (AMD).[1–3] This recombinant, affinity-matured monoclonal antibody fragment inhibits the binding of multiple biologically active forms of vascular endothelial growth factor (VEGF)-A to their receptors.[4–9] Pharmacokinetic studies demonstrated fast and intensive diffusion through all retinal layers, suggesting extensive inhibition of VEGF in neurosensory tissue.[10] Furthermore, a significant decrease of intraocular VEGF was measured after intravitreal injection of ranibizumab in human eyes with AMD.[11]

Controversy continues as to the optimal treatment regimen for elderly patients with AMD, who are often a multimorbid population receiving continuous treatment and monitoring and presenting with a substantial systemic cardiovascular risk. In daily practice, many physicians administer ranibizumab in a pro re nata (PRN) regimen, aiming to reduce the overall number of injections (i.e., the treatment burden) while trying to control disease activity. This strategy was supported by the 1-year results of the Comparison of AMD Treatment Trials (CATT) and

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Alternative Treatments to Inhibit VEGF in Age-related Choroidal Neovascularization (IVAN) trials, which reported equivalent effects for ranibizumab administered monthly versus PRN[12] and monthly versus loading plus PRN, respectively,[13] with only slightly less beneficial outcomes with PRN in CATT after 2 years.[14] A thorough meta-analysis of older trials recommended monthly treatment as the most effective strategy.[15] For an efficient PRN strategy, the relevant factors determining outcome, treatment, and monitoring have to be identified to individualize the PRN approach with an optimal final outcome.

Multicenter trials to date have provided solid data on overall ranibizumab efficacy but have also revealed a substantial heterogeneity in individual treatment responses. An important target of current research is therefore to identify personalized characteristics that may explain, influence, or even predict the treatment response of an individual patient. Because ranibizumab is administered by intravitreal injection, the vitreous body itself has attracted particular attention. Although it is obvious that ranibizumab is primarily delivered to the retina effectively, little is known about the pharmacokinetic mechanisms involved in the aging human eye, which may affect the efficacy and durability of intravitreal therapy.[16]

During youth, the vitreous cortex, a layer of densely packed collagen fibers, is attached to the internal limiting membrane of the retina, most firmly at the vitreous base, the optic disc, and around the foveola.[17–19] With progressing age, the central vitreous develops enlarging fluid-filled pockets that occur when the anterior-posterior oriented collagen fibrils interwoven with hygroscopic glucosaminoglycans clump together, and physiologic detachment of the vitreous starts, typically in the perifoveal region.[20–22] This process subsequently extends to include the fovea and eventually, after months to years, ends with vitreous detachment from the optic disc, leading to complete posterior vitreous detachment (PVD).[23]

Several clinical studies have addressed the incidence of vitreomacular adhesion (VMA) in patients with AMD using optical coherence tomography (OCT) to determine the configuration of the vitreomacular interface (VMI). A higher proportion of VMA has been observed in patients with AMD compared with healthy controls, suggesting abnormal vitreous detachment in the population with AMD.[24,25] Whether the rate of VMA is higher in exudative AMD has not been determined.[26–28] One group considered VMA to be relevant for disease progression in cross-sectional studies; however, results of the only prospective study so far showed similar progression rates for eyes with or without VMA.[24,27,29] A recent retrospective evaluation suggested that intravitreal anti-VEGF treatment for neovascular AMD may be less effective in eyes with VMA compared with eyes without VMA in a PRN regimen.[30]

The present study was designed to investigate the effect of defined conditions of the VMI on the functional and morphologic efficacy of intravitreal ranibizumab therapy. In a prospective randomized trial, patients classified with treatment-naïve neovascular AMD were treated with monthly or quarterly ranibizumab injections, allowing for a distinct comparison between a continuous optimized regimen and a discontinuous treatment, offering defined intervals of disease recurrence. Patients were monitored monthly using best-corrected visual acuity (BCVA) and OCT obtained by certified examiners. The VMI was evaluated according to a standardized protocol by an independent central reading center.

Methods

Treatment and Monitoring Protocol

All patients were participants of "A Randomized, Double-masked, Active-controlled, Multi-center Study Comparing the Efficacy and Safety of Ranibizumab Administered as Two Dosing Regimens in Patients With Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" (EXCITE) study (registered at clinicaltrials.gov, NCT00275821). Detailed information on study design, inclusion and exclusion criteria, and patient assessment has been reported elsewhere.[30] Briefly, the study was designed to assess the efficacy and safety of monthly versus quarterly dosing of intravitreal ranibizumab. Key inclusion criteria were age >50 years, active primary or recurrent subfoveal CNV secondary to AMD (all lesion types), and a BCVA score from 24 to 73 letters (Early Treatment Diabetic Retinopathy Study charts, testing distance 4 m). Key exclusion criteria included a wide range of pretreatments and concomitant disease entities compromising visual acuity. Patients were randomly assigned to monthly (0.3 mg) or quarterly (0.3/0.5 mg) treatment in a 1:1:1 ratio, balanced for lesion type, size, BCVA, age, and sex. All patients received 3 consecutive monthly ranibizumab injections during a common loading phase (months 0, 1, and 2). Subsequently, patients in arm A were treated at monthly intervals (12 injections per year), and patients in arm B received quarterly retreatment (6 injections per year with an 0.3- or 0.5-mg drug dose). Because no difference in any outcome parameter was noted between the 2 doses, these patients were included in a single arm.[31] All eyes underwent a complete and standardized monthly monitoring regimen according to protocol by certified examiners who were masked to the treatment. Main outcome measures were change in BCVA and central retinal thickness (CRT) from baseline to month 12 and the incidence of adverse events.

This trial was conducted in compliance with the tenets of the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice guidelines. Approval was obtained from the independent ethics committees or institutional review boards at each participating center. All patients provided written informed consent before participating in the study.

Evaluation of the Vitreomacular Interface

Eyes were examined monthly after pupil dilation and before treatment or sham injection by Stratus OCT (Carl Zeiss Meditec, Dublin, CA). Analysis was performed on raw, masked OCT datasets sent digitally to the Vienna Reading Center (VRC) from all participating sites. The scanning protocol comprised a 6-mm crosshair scan (two 6-mm sections perpendicular to each other with a resolution of 512 A-scans per section) and a fast macular thickness map scan (six 6-mm radial sections with a resolution of 128 A-scans per section) per visit, forming 1 scan set.

Certified VRC graders who were specifically trained for the VMI configuration evaluated the digital OCT images using a custom-made and validated computer-assisted grading software (Fig 1). Supervisors reviewed all scans unclear to the graders and

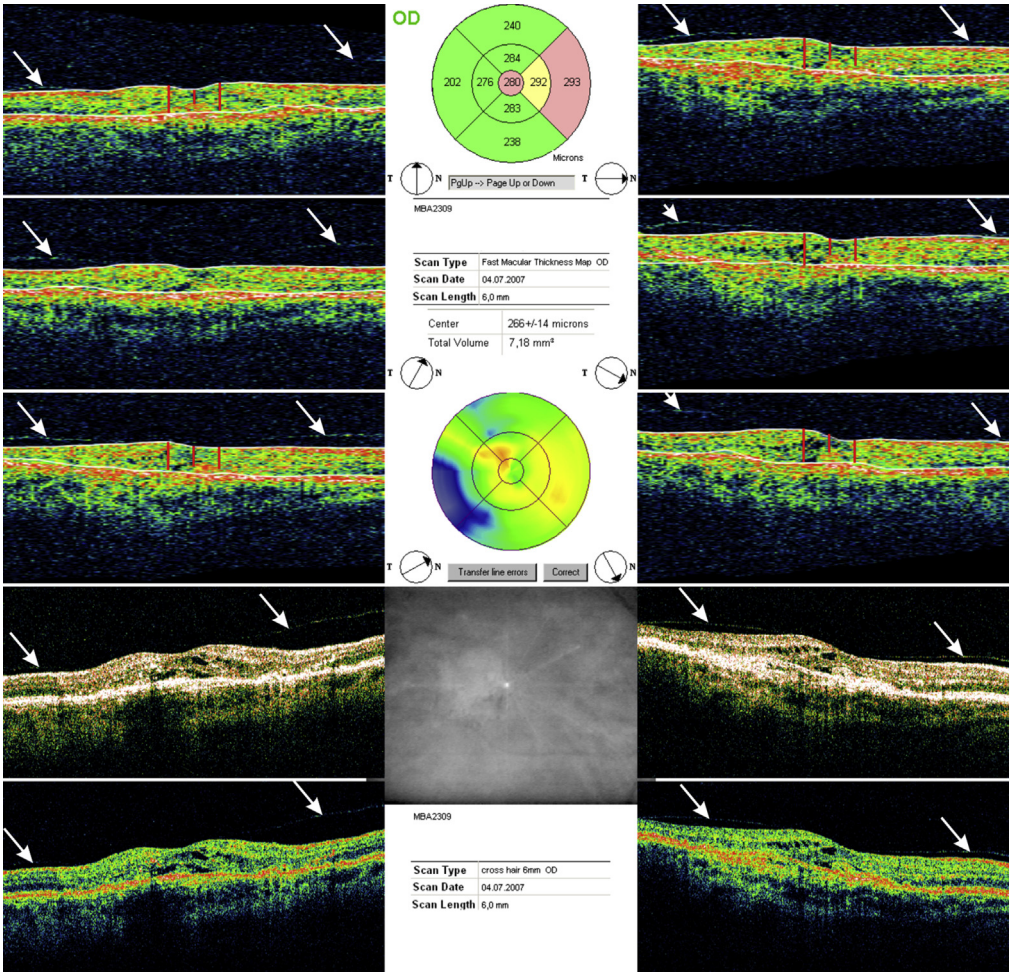


Figure 1. Each optical coherence tomography scan set was displayed to the graders on a single screen using custom-made software and consisted of 6 sections obtained with the fast macula thickness map scan pattern (upper 3 rows) and 2 sections obtained with the crosshair scan pattern (bottom 2 rows, 2 distinct false color scales). *Arrows* point to the posterior vitreous boundary visible in all sections. This particular set was graded as vitreomacular adhesion because of focal adherence of the vitreous boundary to the central macular area. OD = oculus dexter.

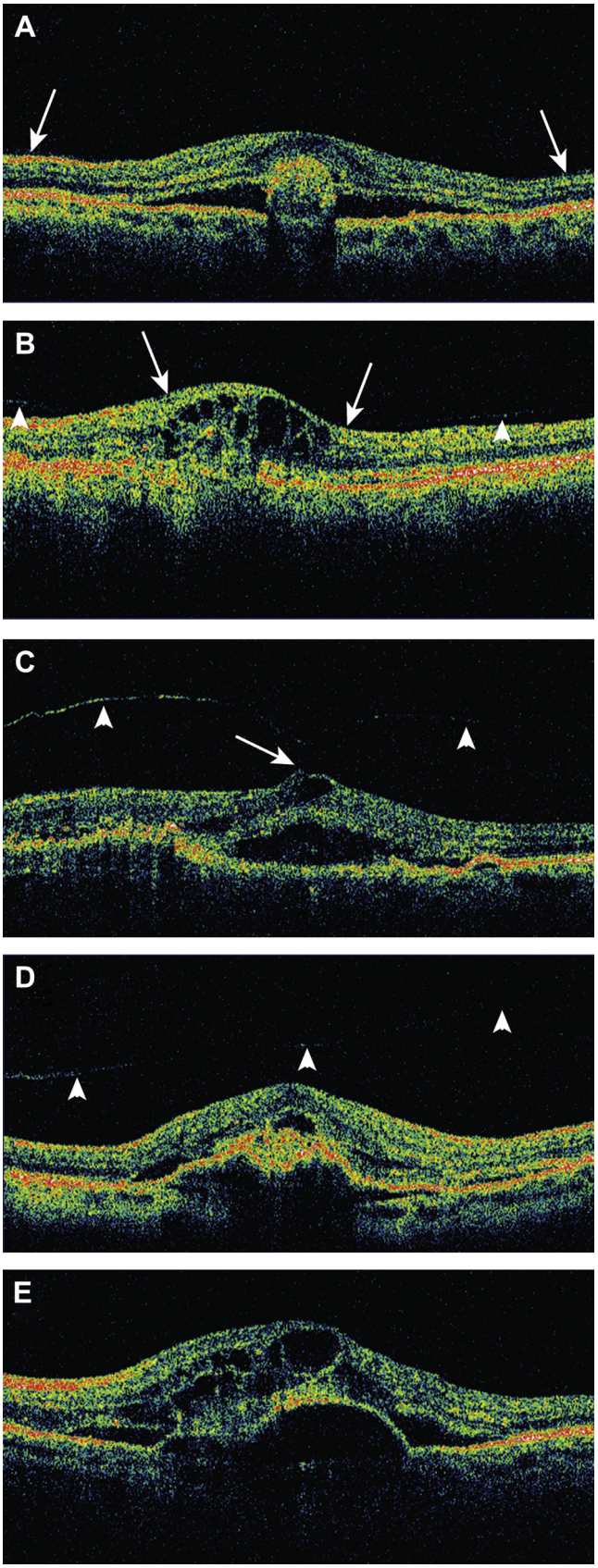
controlled the grading process by reviewing a random 10% of scans for deviations from the protocol.

According to the reading protocol for VMI configuration, a thin, continuous, reflective layer at or above the level of the internal limiting membrane of the retina was interpreted as the posterior vitreous boundary. At each visit, the scan set (i.e., crosshair and fast macular thickness map scans) was graded as posterior vitreous attachment (PVA) if the boundary layer was in continuous contact with the macula. The set was graded as VMA if a preretinal vitreous boundary in direct contact with the central macular surface could be identified. In addition, if the macular surface was focally distorted at the site of vitreomacular contact forming a distortion wedge, the set was graded as vitreomacular traction (VMT). The set was graded as vitreous border near the macula without vitreomacular contact if the vitreous boundary was visible preretinally throughout the entire scan but no contact to the retinal surface was detected (this configuration was assumed to indicate incomplete PVD, with attachment remaining at the optic nerve). Isolated sections with no vitreous border visible within a scan set with clear identification of the vitreous boundary were neglected in all of the described categories. If the posterior vitreous boundary could not be identified on any section in the entire scan set, anteposition of the boundary beyond the scanning range was assumed, and the set was graded as complete PVD. Figure 2 provides examples of the grading categories and related morphologic OCT findings.

Independent graders measured CRT according to a protocol described in detail by Lee and Koh.[30] In addition, each crosshair scan was graded for the presence of intraretinal cysts (IRCs; round hyporeflective spaces within the neurosensory retina), subretinal fluid (SRF; nonreflective space between the neurosensory retina and the retinal pigment epithelium), and pigment epithelium detachment (PED; focal elevation of the reflective retinal pigment epithelium band over an optically clear or moderately reflective space, either higher than 200 μm or wider than 400 μm). Grading examples are provided in Figure 2.

Statistical Evaluation

Data on baseline characteristics, treatment arm randomization, and BCVA were obtained from Novartis after the EXCITE study had been unmasked. Statistical analysis included patients with a minimum of 10 OCT examinations, including baseline and month 12. All patients were divided into one of the following



categories on the basis of the integrated configuration of the VMI for 12 months: (1) PVD: patients with PVD for all visits; (2) release of vitreomacular contact (RELEASE): patients with progressive vitreous detachment (from vitreous attached to the VMA or from the VMA to the vitreous border near the macula without vitreomacular contact) or with persistent grading of the vitreous border near the macula without vitreomacular contact (with vitreous detachment at the macular area but presumed attachment at the optic nerve, precluding anteposition of the posterior boundary); (3) VMA: patients with persistent grading of VMA; (4) PVA: patients with persistent grading of PVA; and (5) VMT: patients with grading of VMT during at least 1 visit.

As outlined, each of these categories reflected the integrated configuration of the VMI for each patient over the entire study duration; however, assignment to the categories was based on the individual grading of each of the 12 visits. Occasional visits with the PVD grading between 2 other visits with reliable identification of the vitreous border were considered to have been insufficiently imaged and were therefore interpolated. In case of 4 or more consecutive visits graded as PVD at the beginning of the study, these visits were considered PVA if reliable identification of the vitreous border occurred later during the trial. This was based on a cumulative percentage analysis identifying 4 consecutive visits of PVD grading as the maximum duration between 2 visits with reliable identification of the vitreous border with a probability of 95% (data on file).

Statistica 8.0 (StatSoft, Tulsa, OK) and PASW Statistics 18.0.3 (IBM/SPSS Inc., Chicago, IL) software were used for statistical testing. Analysis of variance, chi-square tests, and descriptive statistics were applied to evaluate and compare patient characteristics among different categories at baseline (age, BCVA, CRT, and presence of IRC, SRF, or PED). Pairwise comparison was performed by Tukey's honestly significant difference test in the case of a statistically significant main effect. General estimation equation (GEE) analyses were used to compare the efficacy of quarterly treatment versus monthly treatment within individual VMI categories. One-sided tests were performed to test for noninferiority of BCVA gain on the basis of the GEE parameter estimates and standard errors (margin 6.8 letters). Differences in treatment efficacy among the VMI categories within monthly and quarterly regimens were also

Figure 2. Grading examples for vitreomacular interface configurations. *Arrows* indicate vitreous attachment; *arrowheads* indicate vitreous detachment. All images are normalized horizontal "crosshair" scans. **A,** Complete vitreous attachment: The vitreous boundary membrane is in contact with the retinal surface throughout the entire section. **B,** Vitreomacular adhesion (VMA): A shallow detachment of the vitreous is seen both nasally and temporally, and the central macular area shows vitreous adherence without tractional components. **C,** Vitreomacular traction: A focal, angulated VMA is at the foveal center. The retinal surface is visibly distorted. **D,** Vitreous border near the macula without vitreomacular contact: The vitreous boundary is visible throughout the scan but not in contact with the retinal surface. This configuration was assumed to indicate macular vitreous detachment with remaining vitreous attachment at the optic nerve, precluding anteposition of the vitreous boundary. **E,** Posterior vitreous detachment (PVD): If the vitreous boundary was not visible in the entire scan set, anteposition of the boundary beyond the scanning range was assumed, and the set was graded as PVD. Grading of retinal morphology: **B–E,** The intraretinal cysts present as round hyporeflective spaces within the neurosensory retina. **A–E,** The subretinal fluid presents as a nonreflective space between the neurosensory retina and the retinal pigment epithelium. The pigment epithelial detachment (PED) was defined as a focal elevation of the retinal pigment epithelium over an optically clear or moderately reflective space, either higher than 200 μm or broader than 400 μm. **C–E,** PEDs are shown.

Table 1. Distribution of Patients in the Vitreomacular Interface Categories for Quarterly and Monthly Treatment

	PVD (n = 162)	RELEASE (n = 48)	VMA (n = 37)	PVA (n = 4)	VMT (n = 1)	Total (n = 252)
Quarterly, % (n)	65.3 (109)	17.4 (29)	15.0 (25)	1.8 (3)	0 (0)	100 (85)
Monthly, % (n)	62.4 (53)	22.4 (19)	14.1 (12)	1.2 (1)	0.6 (1)	100 (167)

PVA = posterior vitreous attachment; PVD = posterior vitreous detachment; RELEASE = release of vitreomacular contact; VMA = vitreomacular adhesion; VMT = vitreomacular traction.

based on parameter estimates of the GEE; however, superiority tests were performed as least significant difference tests. Differences in CRT between the monthly and quarterly regimen were based on GEE analysis. No margin of noninferiority was defined in this case, and tests were applied as superiority tests. A possible influence of the number of injections on the change in VMI status was tested by comparing the study visit at which the change occurred between monthly and quarterly treatment using the Mann−Whitney test. P values ≤ 0.05 were considered statistically significant.

Results

Baseline Characteristics

The VMI configuration was evaluated on 3879 scan sets of 353 patients enrolled in the EXCITE trial. Data of 252 patients with sufficient OCT examinations according to protocol (i.e., minimum of 10 OCT examinations, including baseline and month 12) were included in the statistical analysis. A total of 167 patients received quarterly injections, and 85 patients received monthly injections.

The distribution of patients in the VMI groups was as follows: PVD was most frequent (64.3%, n = 162), followed by RELEASE (19%, n = 48) and VMA (14.7%, n = 37). Observations of PVA (1.6%, n = 4) and VMT (0.4%, n = 1) were rare because VMT was an exclusion criterion by protocol and PVA is rare in elderly patients. Because of low numbers, VMT and PVA were excluded from further analysis. The distribution of patients in the VMI groups was balanced for quarterly and monthly treatment (Table 1). Patient baseline characteristics, including morphologic parameters at the level of the retina in the different VMI categories, are presented in Table 2. Statistically significant differences were found only for baseline age: Patients with PVD were older than those with VMA (mean difference, 2.0 years; $P = 0.274$, Tukey's honestly significant difference test) and significantly older than patients with RELEASE (mean difference, 3.7 years; $P = 0.007$). Of note, despite distinct differences in the overlying vitreoretinal interface, no correlation was found among the lesion characteristics (e.g., IRC, SRF, PED) and the VMI configuration.

Table 2. Baseline Characteristics in the Vitreomacular Interface Categories

	PVD	RELEASE	VMA	P Value
Age (mean ± SD)	76.5±7.3	72.9±8.0	74.5±6.1	0.007
BCVA (mean ± SD)	56.6±12.9	57.3±13.4	55.5±11.8	0.813
CRT (mean ± SD)	326±97	312±113	320±86	0.693
IRC (%)	52.5	45.8	43.2	0.500
SRF (%)	67.9	66.7	75.7	0.613
PED (%)	75.3	81.3	73.0	0.620

BCVA = best-corrected visual acuity; CRT = central retinal thickness; IRC = intraretinal cyst; PED = pigment epithelial detachment; PVD = posterior vitreous detachment; RELEASE = release of vitreomacular contact; SD = standard deviation; SRF = subretinal fluid; VMA = vitreomacular adhesion.

Vision Outcomes in the Vitreomacular Interface Groups

After the common loading phase, mean letter gains were +5.0 for PVD, +9.7 for RELEASE, and +5.4 for VMA. Patients with RELEASE gained significantly more letters than those with PVD ($P = 0.002$) or VMA ($P = 0.030$) during this period.

From the end of the loading phase to month 12, mean letter gains were +1.1 for PVD, +0.0 for RELEASE, and −0.1 for VMA with monthly treatment, with no statistically significant differences among the configurations. With quarterly treatment, mean letter gains were −0.8 for PVD, −4.4 for RELEASE, and 4.4 for VMA from month 4 to 12, with no statistically significant differences among the configurations.

For 12 months, no significant differences among the VMI groups were detected with quarterly treatment. With monthly treatment, patients with RELEASE gained significantly more letters than those with PVD ($P = 0.014$); from baseline to month 12, comparison with VMA was not significant. Table 3 lists all pairwise differences among the VMI groups from baseline to month 12. Table 4 summarizes mean letter gains in 12 months, the loading and maintenance phase.

Quarterly Versus Monthly Treatment

In the largest group, PVD, mean letter gains after 12 months were +4.7 with quarterly treatment and +4.9 with monthly treatment. The hypothesis of the inferiority of quarterly treatment had to be clearly rejected ($P = 0.001$; margin 6.8 letters; Fig 3). Achieved letter gains during the loading phase were maintained with quarterly and monthly dosing during further follow-up (−0.8 and +1.1 letters, respectively). In the RELEASE group, mean letter gains at 12 months were +3.2 with quarterly injections and +12.7 with monthly injections ($P = 0.008$). After the loading phase, patients receiving monthly injections maintained the achieved functional gains (no letter change), whereas those receiving quarterly injections lost 4.4 letters on average. In the VMA group, mean letter changes at month 12 were −0.2 with quarterly treatment and +7.5 with monthly treatment ($P = 0.043$). Achieved gains during the loading phase were maintained with monthly treatment (−0.1 letters), whereas patients with quarterly treatment lost 4.7 letters on average during follow-up. Table 4 provides detailed comparisons of vision outcomes in the VMI groups between quarterly and monthly treatment.

Morphologic Outcomes in the Vitreomacular Interface Groups

During the loading phase, CRT was progressively reduced in all VMI categories (−101.8 μm PVD, −117.5 μm RELEASE, −83.7 μm VMA). This reduction was maintained during further follow-up with monthly injections (change from month 3 to 12: +3.5 μm PVD, −0.1 μm RELEASE, −9.3 μm VMA). Quarterly injections resulted in

Table 3. Pairwise Differences Between Mean Change in Best-Corrected Visual Acuity (95% Confidence Interval) and Mean Letter Gains for Quarterly (Upper Triangle) and Monthly (Lower Triangle) Treatment

	Mean (SD) monthly	PVD	VMA	RELEASE
Mean (SD) quarterly		4.7 (13.84)	−0.3 (13.48)	3.2 (14.15)
PVD	4.9 (11.77)		**5.0 (−1.0 to 11.1)**	**1.5 (−4.2 to 7.3)**
VMA	7.5 (10.69)	**2.6 (−4.8 to 10.0)**		**−3.5 (−11.1 to 4.1)**
RELEASE	12.7 (11.03)	**7.8 (1.6–14.0)**	**5.2 (−3.0 to 13.4)**	

Mean letter gains = normal letters; pairwise differences = bold letters
PVD = posterior vitreous detachment; RELEASE = release of vitreomacular contact; SD = standard deviation; VMA = vitreomacular adhesion.

a periodic increase and decrease of CRT in all categories (Fig 4), although, as with monthly injections, CRT at 12 months was similar to that after loading (change from month 3 to 12: +6.0 μm PVD, −18.9 μm RELEASE, −3.2 μm VMA). No influence of the VMI configuration on CRT could be detected at baseline or after treatment.

With quarterly treatment, SRF rates were reduced in all categories ($P < 0.001$), whereas the frequency of IRC and PED remained similar ($P = 0.381$, $P = 0.263$, respectively; Table 5). With monthly treatment, IRC, SRF, and PED were statistically significantly reduced in patients with PVD ($P < 0.001$, $P < 0.001$, $P = 0.006$, respectively). In patients with VMA or RELEASE, the frequency of SRF was statistically significantly reduced ($P = 0.008$), whereas the proportions of IRC and PED remained constant.

Discussion

This standardized subanalysis of a controlled, randomized trial of neovascular AMD identified the vitreous configuration as a relevant factor for the efficacy of ranibizumab treatment. Of note, outcomes were linked to the VMI configuration per se by so far unknown mechanisms, but outcomes within certain configurations were substantially different depending on the treatment regimen used. Whether the role of the VMI is causative or related to other conditions remains to be determined.

Posterior vitreous detachment was the most frequent VMI configuration in this AMD-specific elderly population and was observed in 64.3% of study eyes, a percentage in line with other studies.[26] Patients with PVD gained approximately 1 line of vision on Early Treatment Diabetic Retinopathy Study charts after 12 months. Quarterly treatment and monthly treatment resulted in nearly identical effects, and the inferiority of quarterly treatment at month 12 could be clearly rejected in this specific subgroup ($P = 0.001$, margin 6.8 letters; also confirmed for margin 5.0 letters; Table 4; lower border of 97.5 confidence interval, >−5). Particular strengths of the current study are that the 2 treatment regimens were compared directly and that all patients underwent a rigorous monthly, standardized, prospective, functional, and morphologic follow-up, even within therapy-free intervals. Moreover, because the quarterly regimen represented undertreatment, this comparison allowed for enhanced rates of recurrence due to sham injections, procedures that are not available in current trials for obvious ethical reasons. We could clearly observe similar BCVA gains in monthly and quarterly treated patients with PVD at each visit during the entire 12-month trial (Fig 3), with halved retreatment rates in the latter arm. We believe this finding is highly relevant to clinical practice because it demonstrates for the first time that a large subgroup of patients—identifiable by OCT at baseline—could be treated effectively with quarterly injections of ranibizumab, significantly reducing treatment and monitoring burden.

Table 4. Change in Best-Corrected Visual Acuity in the Study Eye

	PVD		RELEASE		VMA	
	Quarterly (n = 109)	*Monthly (n = 53)*	*Quarterly (n = 29)*	*Monthly (n = 19)*	*Quarterly (n = 25)*	*Monthly (n = 12)*
Change from baseline to month 12, mean (SD)	4.7 (13.84)	4.9 (11.77)	3.2 (14.15)	12.7 (11.03)	−0.3 (13.48)	7.5 (10.69)
Change from baseline to month 4, mean (SD)	5.6 (9.15)	3.8 (8.84)	7.7 (11.21)	12.7 (8.03)	4.4 (5.18)	7.6 (9.64)
Change from month 4 to month 12, mean (SD)	−0.8 (11.50)	1.1 (10.30)	−4.4 (12.68)	0.0 (9.53)	−4.7 (9.33)	−0.1 (10.16)
Comparison of change from baseline to month 12 vs. monthly dosing						
Mean difference (SE)	−0.1 (2.21)		−9.5 (3.84)		−7.8 (4.45)	
95% CI	−3.8 to 3.5		−15.9 to −3.0		−15.3 to −0.3	
97.5% CI	−4.5 to 4.2		−17.2 to −1.7		−16.8 to 1.2	
P value against −6.8	0.001		0.756		0.588	
P value against 0	0.473		0.008		0.043	

CI = confidence interval; PVD = posterior vitreous detachment; RELEASE = release of vitreomacular contact; SD = standard deviation; SE = standard error; VMA = vitreomacular adhesion.

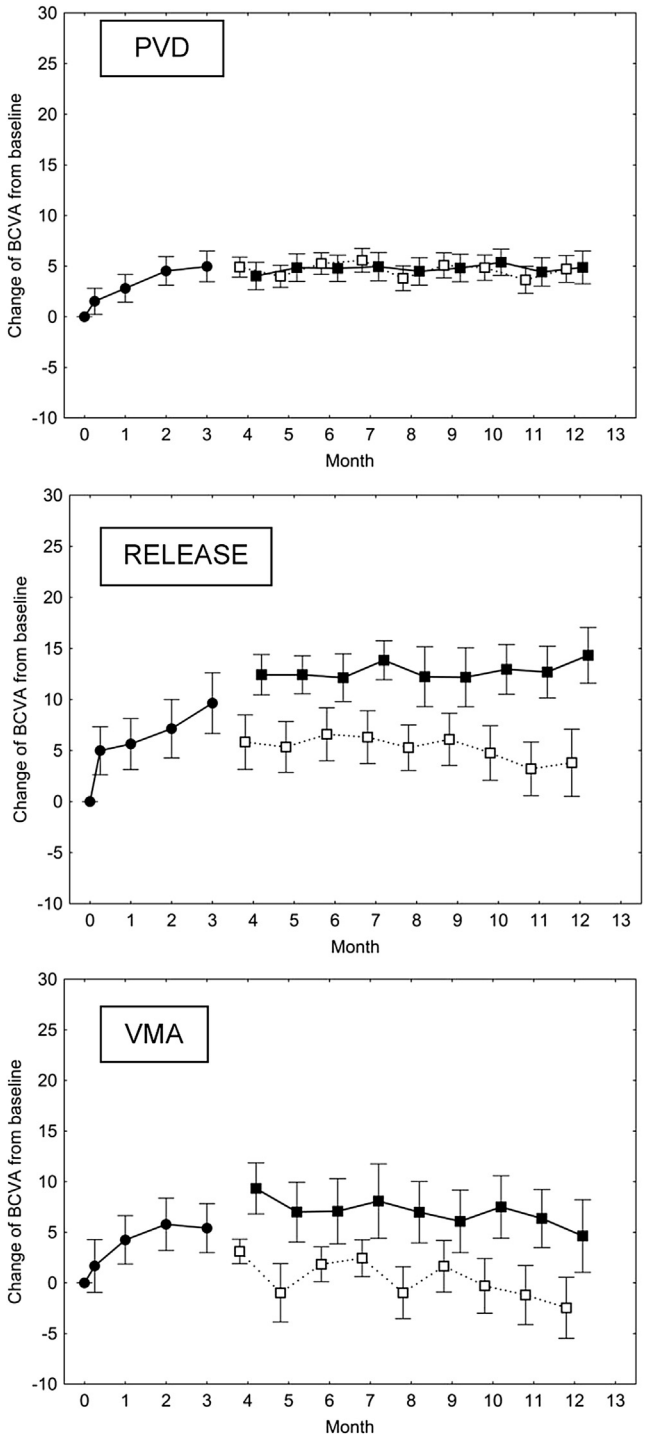


Figure 3. Mean change and 95% confidence interval of best-corrected visual acuity (BCVA) measurements from baseline to month 12 for the posterior vitreous detachment (PVD), release of vitreomacular contact (RELEASE), and vitreomacular adhesion (VMA) categories. Loading phase for all patients (*black circles*), quarterly treatment (*white squares*), and monthly treatment (*black squares*).

With reference to 5.0 letters, quarterly treatment was found to be inferior to monthly treatment for the overall EXCITE population, despite the high frequency of PVD.[31] Indeed, the outcomes of the RELEASE and VMA groups contrasted strongly with the outcomes of the PVD group, with monthly treatment being clearly superior to quarterly treatment ($P = 0.008$, $P = 0.043$, respectively). Vision gains with monthly injections were excellent and exceeded those of the PVD group. On the other hand, in both the RELEASE and VMA categories, function was continuously lost after the loading phase with quarterly retreatment, and outcomes were inferior to monthly treatment at all visits after month 4.

To date, there are few reports of the influence of VMI configuration on anti-VEGF treatment. All studies are retrospective and include small patient populations or retreatment regimens without a standardized protocol.[30] To our knowledge, the current large-scale prospective trial performed by our group provides the most compelling evidence. Another recent retrospective study described results of a loading plus PRN regimen with ranibizumab or bevacizumab (OCT-based retreatment; 3.87±1.77 injections in 12 months), resulting in inferior BCVA outcomes of eyes with VMA compared with eyes without VMA.[30] The authors postulated a negative association between VMA and the visual outcome of anti-VEGF treatment in neovascular AMD. This hypothesis and the average number of injections in the study, which would to date be considered undertreatment, correspond to the results of our VMA group treated quarterly. However, on the basis of the results of monthly treatment, we suggest that VMAs per se, whether stable (VMA) or dynamic (RELEASE), may not represent a disadvantage for anti-VEGF therapy because these patients can achieve favorable outcomes with the appropriate choice of regimen.

In our study, patients with RELEASE generally gained more letters than patients with VMA. It is interesting that BCVA gains were already different during the loading phase, although patients in the VMA and RELEASE groups had similar baseline findings (i.e., focal VMAs for all patients with VMA and most patients with RELEASE). Progression of detachment usually occurred later during the study year in the RELEASE group and was more frequent with monthly than with quarterly dosing but did not cause sudden BCVA changes (data on file). We may explain the difference between vision outcomes by hypothesizing that the RELEASE group probably contained more patients with physiologic adhesions than the VMA group because the average age was lower and PVD was eventually triggered by repeated intravitreal injections. Some authors have proposed that inflammatory processes at the retinal level may lead to an abnormal fixation of physiologic vitreous adhesions in patients with AMD, which may also explain why VMA is more frequent in patients with AMD compared with healthy controls.[24,27] It is conceivable that fixation of adhesions during the disease is linked to a smaller potential for recovery of retinal function during anti-VEGF treatment or to more advanced disease state.

Because the individual outcomes in the VMI groups were strongly linked to the treatment regimen, pharmacokinetic mechanisms may be responsible for our findings. Eyes with RELEASE and VMA configurations exhibit a confined fluid compartment between the vitreous and the retina. Given the excellent treatment outcomes in these groups with monthly treatment, we may postulate that high drug concentrations in

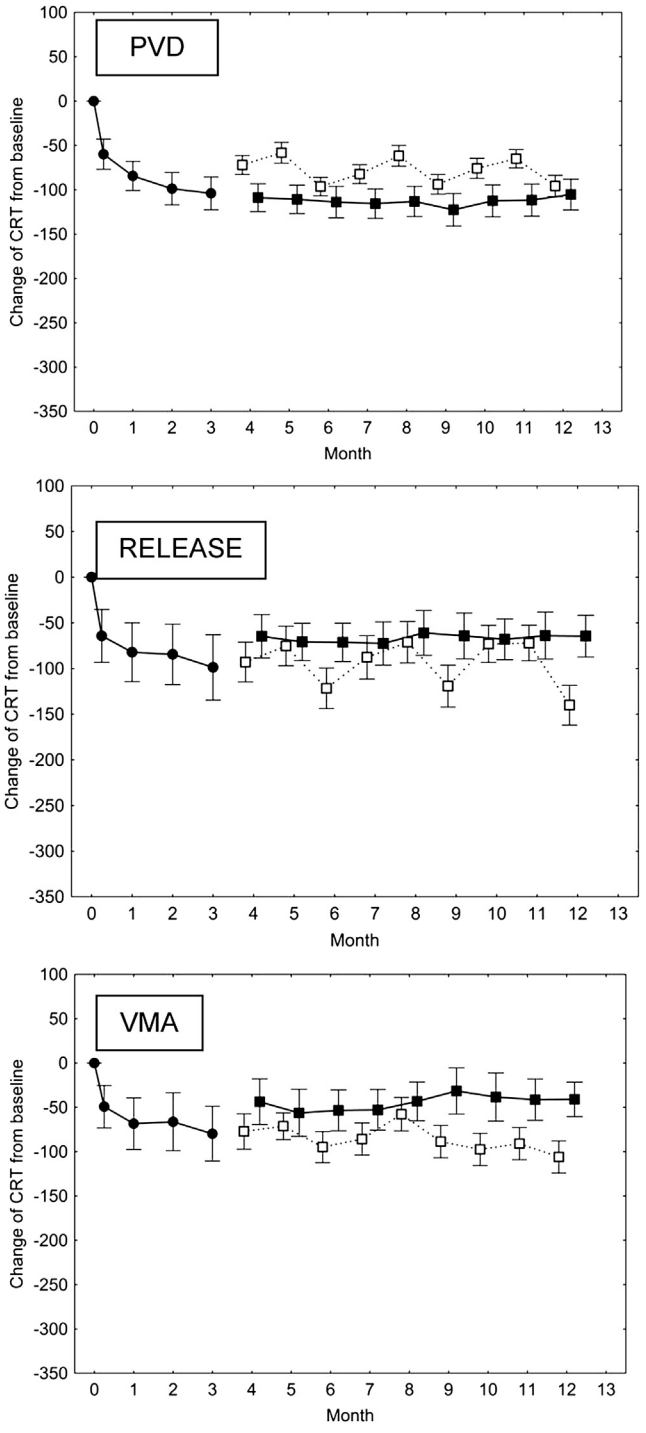


Figure 4. Mean change and 95% confidence interval of central retinal thickness (CRT) measurements from baseline to month 12 for the posterior vitreous detachment (PVD), release of vitreomacular contact (RELEASE), and vitreomacular adhesion (VMA) categories. Loading phase for all patients (*black circles*), quarterly treatment (*white squares*), and monthly treatment (*black squares*).

this small compartment resulted from monthly injections, whereas inadequate drug concentrations and poor vision outcomes resulted from quarterly injections. In eyes with PVD, in which this fluid compartment is larger, drug concentrations may have been lower, resulting in minor therapeutic efficiency of either regimen. Otherwise, equal vision outcomes for the PVD monthly and quarterly groups may also imply that optimum effects may have already been reached with quarterly treatment, making better outcomes with monthly treatment impossible. However, the pathophysiologic and pharmacokinetic mechanisms behind the clinical differences among the VMI groups cannot be explained by the current trial, and further studies are needed to investigate these phenomena.

Several treatment regimens for ranibizumab have been tested in clinical trials, and the results have been extensively reviewed. Mitchell et al,[15] taking into account the results of the Anti-VEGF Anti-body for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-related Macular Degeneration (ANCHOR), Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-related macular Degeneration (MARINA), EXCITE, Phase IIIb, multicenter, randomized, double-masked, sham injection-controlled study of the efficacy and safety of Ranibizumab in subjects with subfoveal choroidal neovascularization with or without classic CNV secondary to age-related macular degeneration (PIER), Safety Assessment of Intravitreal Lucentis for Age-Related Macular Degeneration (SAILOR), prospective OCT imaging of Patients with Neovascular AMD Treated with Intraocular Ranibizumab (PRONTO), and a 12-month, phase III, multicenter, single-arm, open-label trial conducted in 10 European countries and Australia to evaluate the safety and efficacy of ranibizumab in treating subfoveal CNV secondary to AMD (SUSTAIN) studies, suggested that after an initial loading phase of 3 injections, monthly dosing of ranibizumab was generally superior to less frequent dosing. By contrast, the large CATT trial, which tested a monthly and a PRN regimen of ranibizumab and bevacizumab based on retreatment criteria including vision loss or any fluid on OCT, showed equivalent results after 12 months for both dosing regimens, with a small advantage of monthly treatment after 2 years.[12,14] One-year results of the IVAN trial report equivalent effects for monthly treatment and loading plus PRN regimen, supporting the CATT results.[13] These studies were balanced for factors such as baseline age, BCVA, lesion type, and lesion size, variables that are known to influence treatment outcomes. The current subanalysis points to the importance of the VMI configuration in the context of treatment regimens. Distribution of the VMI configurations within the trials listed earlier may have influenced their outcomes, and a potential imbalance may help to explain how incongruent outcomes could result from similar trial designs.

Retinal morphology was evaluated for all patients in the current subanalysis. All VMI categories had a similar CRT and comparable rates of morphologic findings at baseline. However, in strong contrast to vision outcomes, no influence of the VMI configuration on CRT could be detected throughout the entire study period.

Study Limitations

The EXCITE study was not designed to evaluate differences between VMI configurations, and the current evaluation of

Table 5. Proportions of Patients within the Vitreous Categories Presenting with Intraretinal Cysts, Subretinal Fluid, or Pigment Epithelial Detachment at Baseline and Month 12

	Quarterly			Monthly		
	PVD	*RELEASE*	*VMA*	*PVD*	*RELEASE*	*VMA*
Baseline						
IRC	53.2	48.3	44.0	50.9	42.1	41.7
SRF	63.3	72.4	68.0	77.4	57.9	91.7
PED	69.7	82.8	72.0	86.8	78.9	75.0
Month 12						
IRC	46.8	44.8	48.0	15.1	36.8	33.3
SRF	33.0	37.9	40.0	15.1	31.6	58.3
PED	66.1	75.9	60.0	64.2	78.9	75.0

IRC = intraretinal cyst; PED = pigment epithelial detachment; PVD = posterior vitreous detachment; RELEASE = release of vitreomacular contact; SRF = subretinal fluid; VMA = vitreomacular adhesion.

treatment response in the VMI groups was not part of the original study protocol. Nevertheless, we believe that this subanalysis still bears the strength of a prospective study because it was planned and conducted while the EXCITE trial was fully masked to the VRC, with independent readers evaluating raw OCT data sets. Although patients in the quarterly treatment arm received slightly different doses of ranibizumab, no difference in treatment outcomes related to dose was seen in any analysis of this or similar trials between 0.3/0.5 mg or even 0.5/2.0 mg.[31,32] In contrast, the extended intervals of the quarterly regimen allowed for higher recurrence rates and accentuated differences among the VMI groups. Naturally, the relatively small numbers of patients in the VMA and RELEASE groups represent a limitation, although this is a consequence of the per se low incidences of these VMI configurations in elderly patients with AMD. However, it is important to keep in mind that the PVD group represents a large proportion of patients within each treatment arm, and our results in this particular patient group have higher statistical power.

Grading of the VMI undoubtedly is challenging, and the imaging technology used (Stratus OCT) may be regarded as a further limitation of this analysis. Several approaches were united to gain reproducible grading in this study. First, grading was based on 8 single scans (forming a scan set) per visit and then categories reflected the vitreous status over 12 months, taking into account at least 10 of 12 possible visits, including baseline and month 12. Further interpolation of visits, when the vitreous border could not be identified, was conducted on the basis of a cumulative percentage analysis with a probability of 95% (this is covered in the Methods section). All of this has been applied to reduce the drawbacks from time domain technologies offering only 6 radial scans.

Furthermore, a study including a comparison of VMI imaging with time domain OCT and spectral domain OCT was conducted, finding comparable sensitivity for adhesions with repetitive imaging.[29] However, the outcome of this study should not be affected by the sensitivity of the VMI imaging because the same procedures and deficiencies apply equally to the analysis of both treatment arms, and nevertheless significant differences were observed.

Whether the restriction to patients with large scan sets imposed inadvertent bias in the study was investigated by comparing baseline data of included and excluded eyes. Values for mean BCVA (56.7 letters/56.5 letters, $P = 0.84$) and mean CRT (324.8/304.5 μm, $P = 0.15$) at baseline were comparable, and proportions of eyes presenting with IRC ($P = 0.89$), SRF ($P = 0.66$), or PED (0.96) were similar. We therefore conclude that limitation to patients with at least 10 complete OCT examinations did not cause significant bias in this analysis.

The findings of this article have potentially important implications for the design of future therapeutic trials of interventions for the treatment of neovascular AMD in terms of the stratification of this variable between treatment groups. It is tempting to conclude that these findings may justify less intensive treatment regimens in patients with PVD and more intensive treatment regimens in patients with RELEASE or VMA; however, the practical implications of this for the treating physician may be limited. Novel imaging devices, such as higher-resolution fast raster scanning OCT using swept source technology, will resolve this diagnostic issue.

In conclusion, the configuration of the VMI has a significant effect on the efficacy of ranibizumab therapy in neovascular AMD. Patients with PVD seem to be less sensitive to a less intensive treatment schedule and may require less frequent dosing. Patients with RELEASE and VMA may derive the best benefit from intensive monthly treatment. Our study may serve as a pivotal point for future trials evaluating intravitreal anti-VEGF compounds and as a foundation for individualized treatment decisions in patients with neovascular AMD and perhaps other indications.

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References

1. Rosenfeld PJ, Brown DM, Heier JS, et al; MARINA Study Group. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 2006;355:1419–31.
2. Brown DM, Kaiser PK, Michels M, et al; ANCHOR Study Group. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med 2006;355:1432–44.
3. Brown DM, Michels M, Kaiser PK, et al; ANCHOR Study Group. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: two-year results of the ANCHOR Study. Ophthalmology 2009;116:57–65.
4. Keyt BA, Berleau LT, Nguyen HV, et al. The carboxyl-terminal domain (111-165) of vascular endothelial growth factor is critical for its mitogenic potency. J Biol Chem 1996;271:7788–95.
5. Ferrara N, Mass RD, Campa C, Kim R. Targeting VEGF-A to treat cancer and age-related macular degeneration. Annu Rev Med 2007;58:491–504.
6. Penn JS, Madan A, Caldwell RB, et al. Vascular endothelial growth factor in eye disease. Prog Retin Eye Res 2008;27:331–71.

7. Grisanti S, Tatar O. The role of vascular endothelial growth factor and other endogenous interplayers in age-related macular degeneration. Prog Retin Eye Res 2008;27:372–90.
8. Lowe J, Araujo J, Yang J, et al. Ranibizumab inhibits multiple forms of biologically active vascular endothelial growth factor in vitro and in vivo. Exp Eye Res 2007;85:425–30.
9. Ferrara N, Damico L, Shams N, et al. Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. Retina 2006;26:859–70.
10. Meyer CH, Holz FG. Preclinical aspects of anti-VEGF agents for the treatment of wet AMD: ranibizumab and bevacizumab. Eye (Lond) 2011;25:661–72.
11. Funk M, Karl D, Georgopoulos M, et al. Neovascular age-related macular degeneration: intraocular cytokines and growth factors and the influence of therapy with ranibizumab. Ophthalmology 2009;116:2393–9.
12. CATT Research Group; Martin DF, Maguire MG, Ying GS, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med 2011;364:1897–908.
13. IVAN Study Investigators, Chakravarthy U, Harding SP, Rogers CA, et al. Ranibizumab versus bevacizumab to treat neovascular age-related macular degeneration: one-year findings from the IVAN randomized trial. Ophthalmology 2012;119:1399–411.
14. Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group, Martin DF, Maguire MG, Fine SL, et al. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. Ophthalmology 2012;119:1388–98.
15. Mitchell P, Korobelnik JF, Lanzetta P, et al. Ranibizumab (Lucentis) in neovascular age-related macular degeneration: evidence from clinical trials. Br J Ophthalmol 2010;94:2–13.
16. El Sanharawi M, Kowalczuk L, Touchard E, et al. Protein delivery for retinal diseases: from basic considerations to clinical applications. Prog Retin Eye Res 2010;29:443–65.
17. Foos RY. Vitreoretinal juncture; topographical variations. Invest Ophthalmol 1972;11:801–8.
18. Sebag J, Balazs EA. Morphology and ultrastructure of human vitreous fibers. Invest Ophthalmol Vis Sci 1989;30:1867–71.
19. Le Goff MM, Bishop PN. Adult vitreous structure and postnatal changes. Eye (Lond) 2008;22:1214–22.
20. Sebag J. Age-related changes in human vitreous structure. Graefes Arch Clin Exp Ophthalmol 1987;225:89–93.
21. Sebag J. Age-related differences in the human vitreoretinal interface. Arch Ophthalmol 1991;109:966–71.
22. Johnson MW. Perifoveal vitreous detachment and its macular complications. Trans Am Ophthalmol Soc 2005;103:537–67.
23. Uchino E, Uemura A, Ohba N. Initial stages of posterior vitreous detachment in healthy eyes of older persons evaluated by optical coherence tomography. Arch Ophthalmol 2001;119:1475–9.
24. Krebs I, Brannath W, Glittenberg C, et al. Posterior vitreomacular adhesion: a potential risk factor for exudative age-related macular degeneration? Am J Ophthalmol 2007;144:741–6.
25. Nomura Y, Ueta T, Iriyama A, et al. Vitreomacular interface in typical exudative age-related macular degeneration and polypoidal choroidal vasculopathy. Ophthalmology 2011;118:853–9.
26. Mojana F, Cheng L, Bartsch DU, et al. The role of abnormal vitreomacular adhesion in age-related macular degeneration: spectral optical coherence tomography and surgical results. Am J Ophthalmol 2008;146:218–27.
27. Robison CD, Krebs I, Binder S, et al. Vitreomacular adhesion in active and end-stage age-related macular degeneration. Am J Ophthalmol 2009;148:79–82.
28. Lee SJ, Lee CS, Koh HJ. Posterior vitreomacular adhesion and risk of exudative age-related macular degeneration: paired eye study. Am J Ophthalmol 2009;147:621–6.
29. Waldstein SM, Sponer U, Simader C, et al. Influence of vitreomacular adhesion on the development of exudative age-related macular degeneration: 4-year results of a longitudinal study. Retina 2012;32:424–33.
30. Lee SJ, Koh HJ. Effects of vitreomacular adhesion on anti-vascular endothelial growth factor treatment for exudative age-related macular degeneration. Ophthalmology 2011;118:101–10.
31. Schmidt-Erfurth U, Eldem B, Guymer R, et al; EXCITE Study Group. Efficacy and safety of monthly versus quarterly ranibizumab treatment in neovascular age-related macular degeneration: the EXCITE Study. Ophthalmology 2011;118:831–9.
32. Singer MA, Awh CC, Sadda S, et al. HORIZON: an open-label extension trial of ranibizumab for choroidal neovascularization secondary to age-related macular degeneration. Ophthalmology 2012;119:1175–83.

Footnotes and Financial Disclosures

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