

Vascular Endothelial Growth Factor Is a Critical Stimulus for Diabetic Macular Edema

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- **PURPOSE:** The role of vascular endothelial growth factor (VEGF) in diabetic macular edema (DME) was tested with ranibizumab, a specific antagonist of VEGF.
- **DESIGN:** A nonrandomized clinical trial.
- **METHODS:** Ten patients with chronic DME received intraocular injections of 0.5 mg of ranibizumab at baseline and at one, two, four, and six months. The primary outcome was change in foveal thickness between baseline and seven months, and the secondary outcome measures were changes from baseline in visual acuity and macular volume.
- **RESULTS:** Mean values at baseline were 503 μm for foveal thickness, 9.22 mm^3 for macular volume, and 28.1 letters (20/80) read on an Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. At seven months (one month after the fifth injection), the mean foveal thickness was 257 μm , which was a reduction of 246 μm (85% of the excess foveal thickness present at baseline; $P = .005$ by Wilcoxon signed-rank test for likelihood that this change is due to ranibizumab rather than chance). The macular volume was 7.47 mm^3 , which was a reduction of 1.75 mm^3 (77% of the excess macular volume at baseline; $P = .009$). Mean visual acuity was 40.4 letters (20/40), which was an improvement of 12.3 letters ($P = .005$). The injections were well-tolerated with no ocular or systemic adverse events.
- **CONCLUSION:** Intraocular injections of ranibizumab significantly reduced foveal thickness and improved visual acuity in 10 patients with DME, which demonstrated that VEGF is an important therapeutic target for

DME. A randomized, controlled, double-masked trial is needed to test whether intraocular injections of ranibizumab provide long-term benefit to patients with DME. (Am J Ophthalmol 2006;142:961–969. © 2006 by Elsevier Inc. All rights reserved.)

DIABETIC RETINOPATHY IS THE MOST PREVALENT cause of vision loss in working aged individuals in developed countries.¹ Severe vision loss occurs because of traction retinal detachments that complicate retinal neovascularization, but the most common cause of moderate vision loss is macular edema. Macular edema occurs from the leakage of plasma into the central retina, which causes it to thicken because of excess interstitial fluid. The excess interstitial fluid is likely to disrupt ion fluxes and the thickening of the macula results in stretching and distortion of neurons. There is reversible reduction in visual acuity, but over time the perturbed neurons die, which results in permanent visual loss.

The leakage of plasma in patients with diabetic macular edema (DME) is visualized by fluorescein angiography and may be focal because of leakage from microaneurysms or diffuse. Microaneurysms are thought to occur because of hyperglycemia-induced pericyte death, which weakens the walls of retinal vessels and results in the small aneurysms in which endothelial cells are perturbed causing them to lose their barrier qualities and leak.² However, diffuse leakage from retinal capillaries that do not show visible structural changes (such as microaneurysms) is also a common feature of DME. This could be due to microscopic damage to retinal vessels that are not visible in images that are obtained during fluorescein angiography but could also be due the presence of excessive amounts of pro-permeability factors.

Recently, retinal hypoxia has been implicated in the pathogenesis of DME.³ Hypoxia causes increased expression of vascular endothelial growth factor (VEGF), which is a potent inducer of vascular permeability that has been shown to cause leakage from retinal vessels.^{4,5} Thus, it is reasonable to hypothesize that VEGF contributes to DME.

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Ranibizumab is a Fab fragment of an antibody that specifically binds all isoforms of VEGF-A with high affinity. Intraocular injections of ranibizumab provide benefit for patients with choroidal neovascularization because of age-related macular degeneration, which confirms studies in animal models that suggest that VEGF is an important stimulus for choroidal neovascularization (reported at the meeting of the American Society of Retina Specialists, Montreal, Canada, July 2005). In this study, we tested the hypothesis that VEGF is also an important stimulus for DME by assessing the effect of multiple intraocular injections of ranibizumab in patients with DME.

METHODS

AN OPEN-LABEL STUDY TO INVESTIGATE THE EFFECT OF intraocular injections of 0.5 mg of ranibizumab in 10 patients with DME was approved by the Federal Drug Administration and the institutional review board of the Johns Hopkins Medical Institutions. The study was designed to give patients an intraocular injection of 0.5 mg of ranibizumab at study entry and at one, two, four, and six months after entry. The dose was selected because 0.5 mg is the highest dose available and because it is reasonable to start with the highest dose and investigate other doses in future studies, if indicated. The regimen was selected to assess the effect of three monthly injections and then to determine the impact of increasing the time between injections to two months for the last two injections. The primary outcome measure was foveal thickness that was measured by optical coherence tomography (OCT)^{6,7} at seven months, compared with baseline. Secondary outcome measures were macular volume that was measured by OCT and visual acuity that was measured by the protocol of the Early Treatment Diabetic Retinopathy Study (ETDRS)⁸ at seven months, compared with baseline.

• **PATIENT ELIGIBILITY AND EXCLUSION CRITERIA:** Patients (18 or older) were eligible if they had reduction in visual acuity between 20/40 and 20/320 and met the following criteria: (1) baseline foveal thickness by OCT was 250 μ m or greater, (2) serum HbA_{1c} $\geq$ 6% for 12 months before randomization, (3) no potential contributing causes to reduced visual acuity other than DME, and (4) reasonable expectation that laser photocoagulation would not be required for the next six months. If both eyes were eligible, the eye with the greater foveal thickness was entered.

• **STUDY PROTOCOL:** Consenting patients were screened for the study with a medical history, physical examination, measurement of best-corrected visual acuity by an experienced examiner who used the ETDRS protocol,⁸ a complete eye examination, an OCT, a fluorescein angiogram, and laboratory tests on blood and urine. Eligible patients

received an intraocular injection of 0.5 mg of ranibizumab. Patients returned one week later for a repeat examination and OCT. Subsequent return visits occurred every month through seven months, which was the primary end point of the study. Additional injections of ranibizumab were performed at one, two, four, and six months. This protocol was selected to determine the effect of monthly injections for the first three months and then to try to determine whether less frequent injections would be feasible. Safety evaluations, measurement of best-corrected visual acuity, eye examinations, and OCTs were done at all study visits; fluorescein angiograms were done at three and six months. Measurements of HbA_{1c} were done at baseline and three and six months. Hematologic and blood chemistry tests were done at baseline and six months.

• **ADMINISTRATION OF STUDY DRUG:** Povidone iodine was used to clean the lids, and a lid speculum was inserted. Topical anesthesia was applied; in some patients, a subconjunctival injection of 2% lidocaine was given. The conjunctiva was irrigated with 5% povidone iodine. A 30-gauge needle was inserted through the pars plana, and 0.05 ml containing 0.5 mg of ranibizumab was injected into the vitreous cavity. Funduscopy examination was performed to confirm retinal perfusion, and patients were observed for one hour or until intraocular pressure returned to normal. Patients were called the day after each injection and asked whether they had decreased vision, eye pain, unusual redness, or any new symptoms.

• **OCT:** OCT scans were performed by an experienced investigator with a StratusOCT3 (Carl Zeiss Meditec, Dublin, California, USA) that used the fast macular scan protocol. This protocol consists of 6 line scans that are 6.0-mm long, centered on fixation, and spaced 30 degrees apart around the circumference of a circle. Each line consists of 128 A-scan measurements. With each A-scan, the OCT software measures the distance between the inner surface of the retina and the anterior border of the retinal pigment epithelium choriocapillaris complex on the basis of changes in reflectivity. The center point thickness, also known as the foveolar thickness, is a mean value that is generated by the StratusOCT software from the 6 central A-scan thickness values of each of the radial lines comprising the fast macular thickness map. We did not use this value generated from only 6 data points for our primary measure of central retinal thickness but instead used the foveal or central 1 mm thickness, which is an average generated value based on central 21 scans of each of the 6 lines that pass through the patient's fixation. The number of data points that are used to compute this value is $21 \times 6 = 126$, which provides a better representation of the thickness of the central retina than a value that is generated from only 6 points around fixation. Macular volume throughout the entire 6-mm zone is calculated with extrapolated values between the line scans. Excess

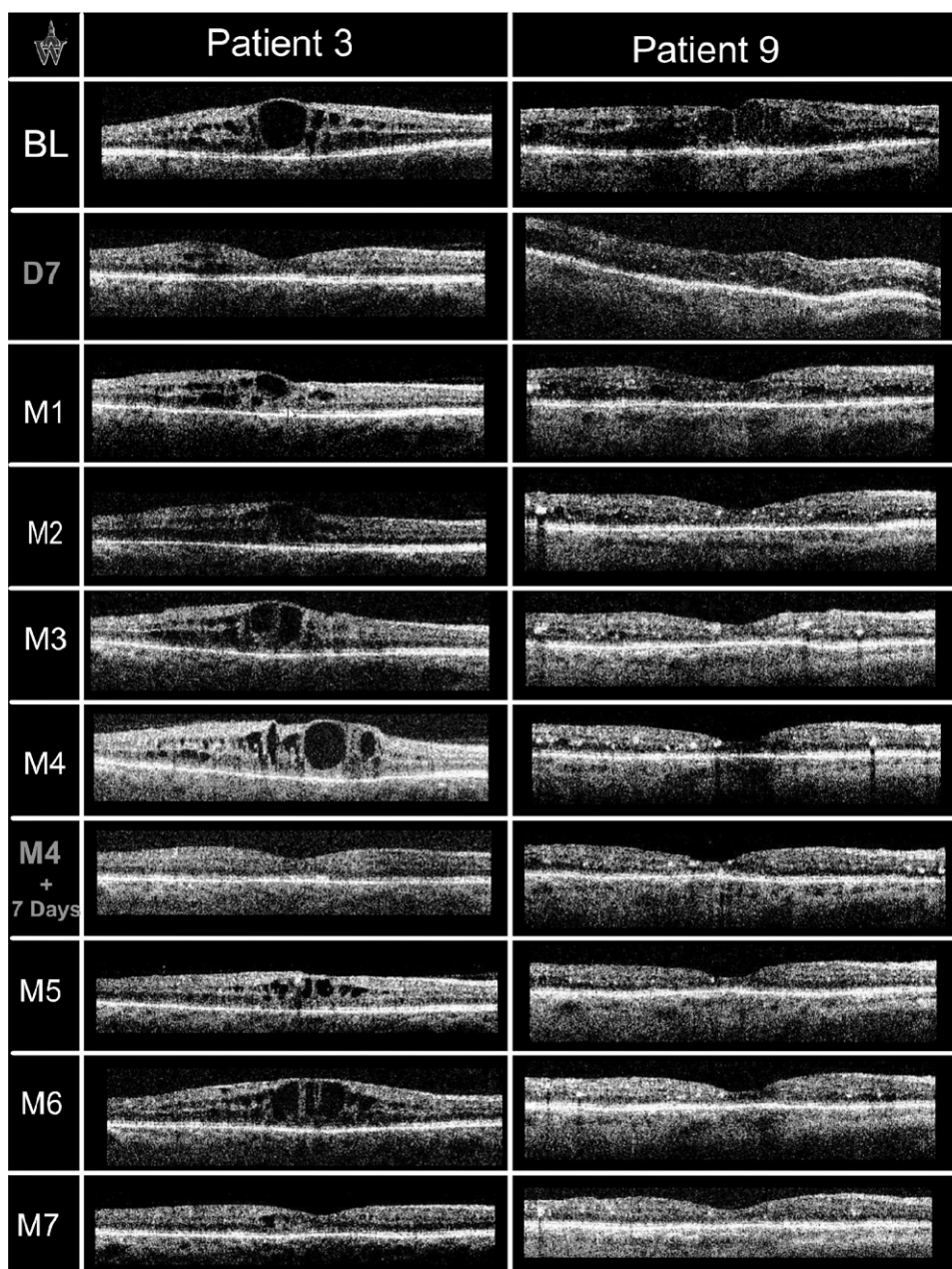


FIGURE 1. Horizontal cross sectional optical coherence tomography (OCT) scans at all time points for patients 3 and 9 with diabetic macular edema that was treated with ranibizumab to illustrate two patterns of response over time. Seven days after the first intraocular injection of 0.5 mg of ranibizumab (day seven), patient 3 showed a marked improvement in the appearance of the OCT scan with the elimination of several large cysts and the return of a normal macular contour that included a foveal depression. At month one (M1), one month after the first injection, and M2 and M3, one month after the second and third injections, respectively, the scans for patient 3 were worse than the scan at day seven, which suggests a loss of effect of ranibizumab or transient effects that are lost by one month after injection. At M4, two months after the third injection, the scan showed substantial deterioration, but seven days after the fourth injection (M4 + seven days) there was marked improvement supporting transient effect. However, there was less deterioration one month after the fourth injection (M5) than there had been one month after each of the first three injections. This was followed by deterioration at M6, two months after the fourth injection, but then at M7, the primary end point and one month after the fifth injection, there was improvement to the point that the scan looked more like the two previous scans that had been performed seven days after an injection than like those scans that had been performed one month after an injection. Like patient 3, patient 9 also showed substantial improvement at day seven compared with baseline, with resolution of several large cysts. However, unlike patient 3, patient 9 showed continued improvement and then stability at subsequent time points, regardless of the time after the injection that the scan was performed. This suggests that the beneficial effects of ranibizumab were more sustained in patient 9 than in patient 3. BL = baseline.

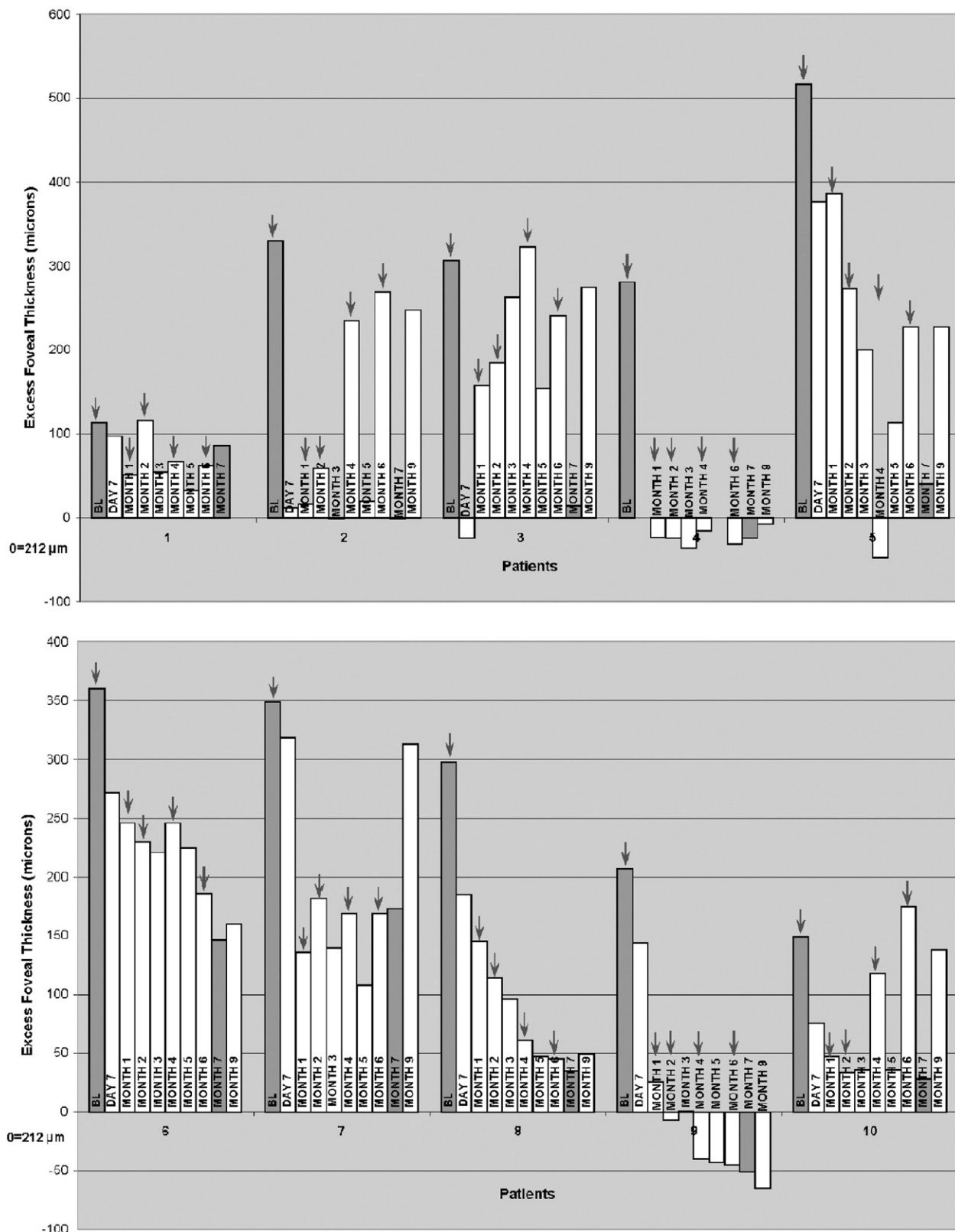


FIGURE 2. Excess foveal thickness was measured by optical coherence tomography (OCT) at each study visit in all patients with diabetic macular edema that was treated with ranibizumab. Each bar represents the foveal thickness above the normal mean value of 212 μm, which is set to zero. The arrows show intraocular injections of 0.5 mg of ranibizumab. The bars for baseline and month seven are shaded to allow quick comparison between baseline and the primary end point. The foveal thickness is less at the primary end point than at baseline for all patients.

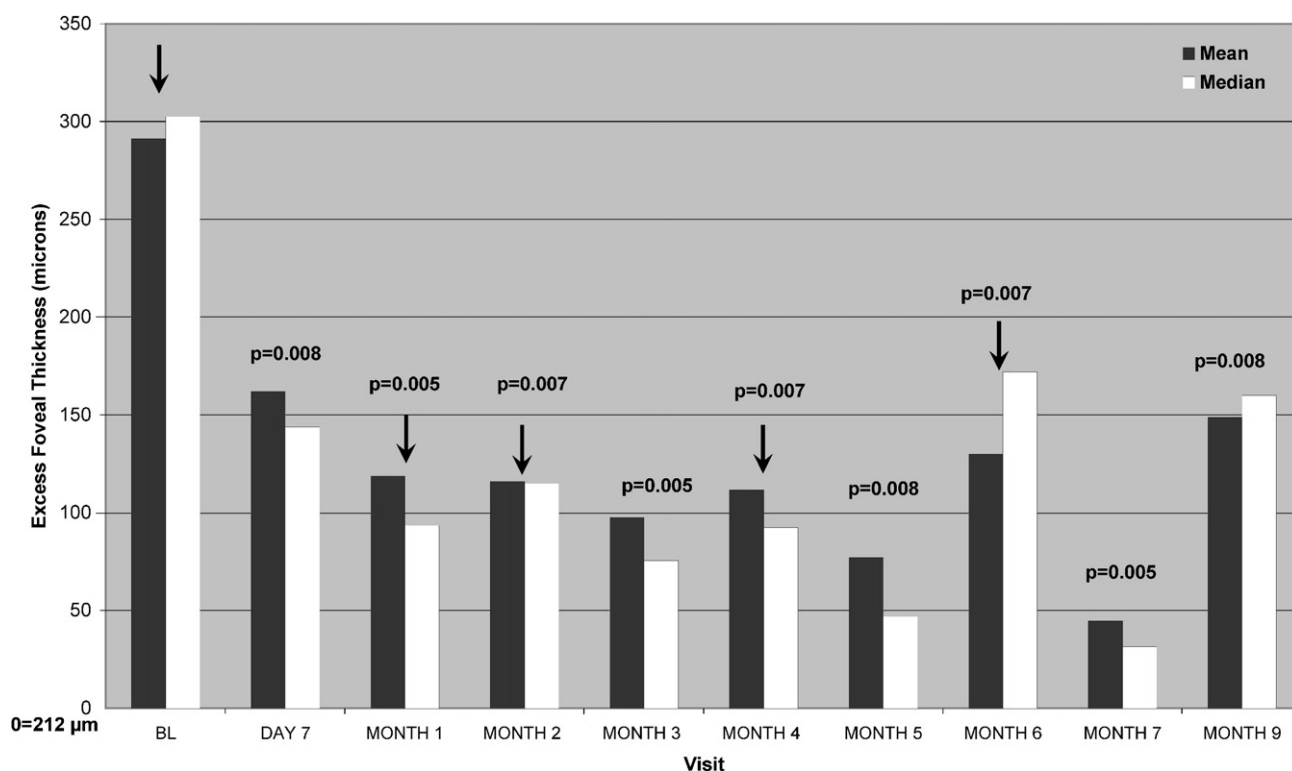


FIGURE 3. The mean excess foveal thickness at each study visit in all patients with diabetic macular edema that was treated with ranibizumab. Each bar represents the mean value for excess foveal thickness for all patients at the designated study visit (data for eight of 10 patients at month nine). The arrows show when intraocular injections of 0.5 mg of ranibizumab were administered. Compared with baseline, foveal thickness was reduced by 246 μm at the primary end point of the study, which constituted the elimination of 85% of the excess foveal thickness that had been present at baseline.

foveal thickness was calculated by subtraction of the measured foveal thickness value from the normal mean value of 212 μm that was calculated from measurements on a large population of subjects.⁹ Excess macular volume was determined by subtraction of the upper limit of the normal range of $6.94 \pm 0.37 \text{ mm}^3$ from the measured value.

• **STATISTICAL ANALYSIS:** Statistical analyses were performed with Statistical Package for the Social Sciences software (SPSS Inc, Chicago, Illinois, USA). The likelihood that the change in foveal thickness, macular volume, and visual acuity from baseline to month seven was due to ranibizumab rather than to chance was determined by the Wilcoxon signed-rank test.

RESULTS

• **CHARACTERISTICS OF THE STUDY POPULATION:** There were five men and five women in the study, with a median age of 60 years. Eight of the 10 patients were insulin-dependent diabetics. The median and mean HbA_{1C} values at enrollment were 7.50% and 7.64%, respectively, and were 7.90% and 7.91%, respectively, at month 6 ($P =$

.240). Four patients had diabetic neuropathy, and three patients had diabetic nephropathy with modest renal insufficiency that did not require dialysis. Eight patients were receiving treatment for hypertension, which was well-controlled; seven patients had hypercholesterolemia, five of whom were receiving treatment. There was no significant change in mean systolic or diastolic blood pressure during the study. The mean duration of DME was 4.75 ± 1.22 years with a median duration of 3.5 years and a range of six months to 10 years. Nine of the 10 patients with DME had received previous treatment in the study eye; eight of the patients had received at least two sessions of focal/grid laser photocoagulation not less than 5 months before study entry (range, five to 120 months), and three patients had received intraocular corticosteroids not less than 10 months before entry (range, 10 to 20 months). Despite these treatments, the mean foveal thickness at baseline was $503 \pm 115 \mu\text{m}$ (range, 326 to 729 μm). Therefore, this patient population had severe, chronic DME that was poorly responsive to standard therapies.

• **EFFECT OF RANIBIZUMAB ON FOVEAL THICKNESS:** Several patients had a large reduction in foveal thickness by seven days after the first intraocular injection of 0.5 mg of ranibizumab (median, 88 μm ; mean, 130 μm). OCT scans

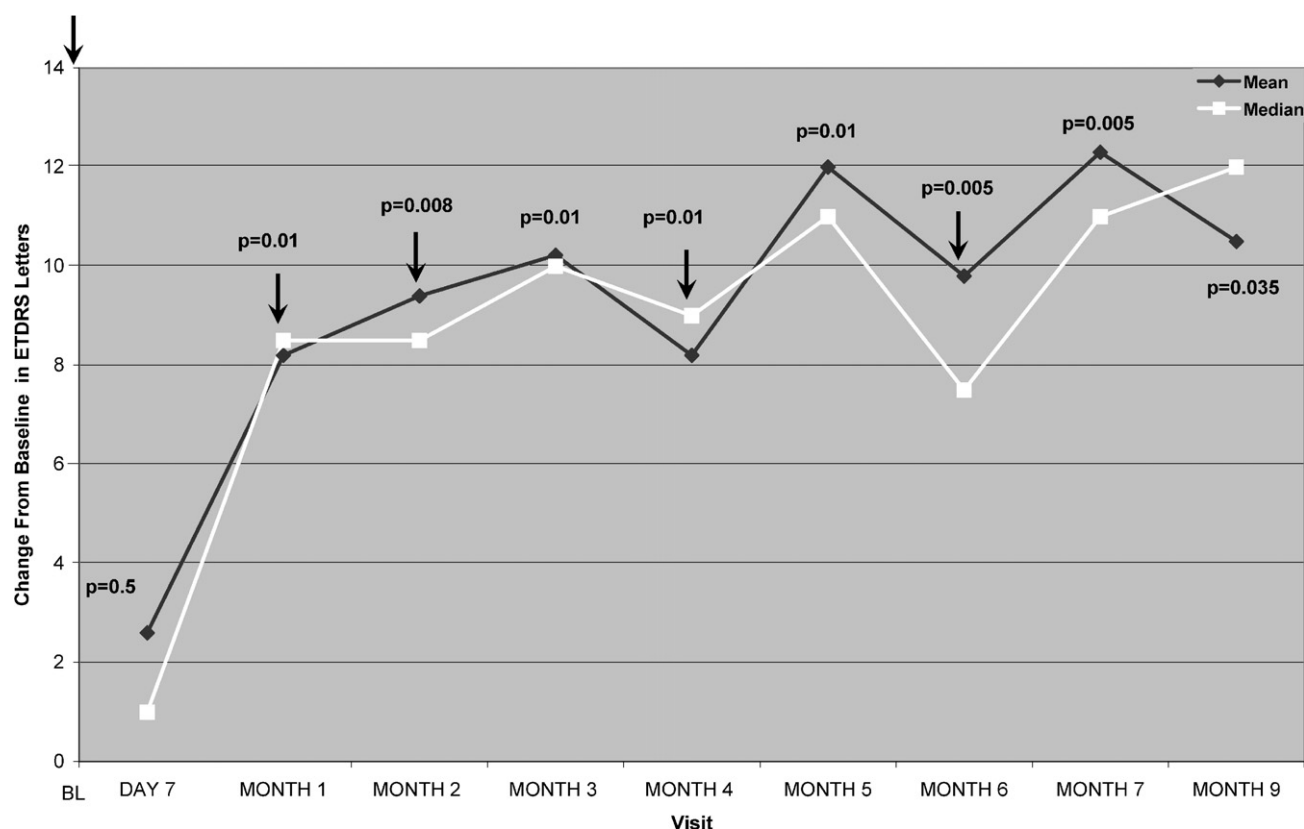


FIGURE 4. Mean and median change in visual acuity from baseline at each study visit in all patients with diabetic macular edema that was treated with ranibizumab. The black line shows the mean change in visual acuity measured in the number of letters that were read on an Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart, and the white line shows the median change in visual acuity. The arrows show times of intraocular injection of 0.5 mg of ranibizumab. At the primary end point, month seven, there was an improvement of 12.3 letters in mean visual acuity and 11 letters in median visual acuity.

from two patients whose condition showed such an immediate, dramatic response are shown in Figure 1. Patient 3 had several large cysts within the retina at baseline that resolved within a week of the first injection with return of a normal contour that included a foveal depression. Much of the improvement was lost at one month, just before the second injection. Substantial thickening and cystic changes were also seen at months two and three, which was one month after the second and third injections, respectively. To determine whether this patient had become refractory to ranibizumab, an OCT scan was done seven days after the month four injection. There was a marked improvement, with resolution of cysts and a normal foveal contour that indicated that the patient was continuing to respond well to ranibizumab, but the drug effect quickly dissipated and was not apparent by one month after each injection. The subsequent course showed that, when injections were given two months apart, there might be a longer duration of effect from a single injection. Like patient 3, patient 9 also showed substantial improvement at day seven, compared with baseline, with resolution of several large cysts. However, unlike patient 3, patient 9 showed continued improvement and then stability at subsequent time

points, regardless of the time after the injection that the scan was done. This suggests that the beneficial effects of ranibizumab were more sustained in patient 9 than in patient 3.

Excess foveal thickness is shown for each visit for all 10 patients in Figure 2. Patient 9, whose scans are shown in Figure 1, experienced the disappearance of excess foveal thickness after the first injection, with a persistent effect at each subsequent time point that included the primary end point at seven months. Patient 4 had a similar pattern. Patient 3 (Figure 1) experienced a dramatic reduction in foveal thickness at day seven, but fluctuation occurred because of the dissipation of drug effect over the course of one month and further worsening when injections were delayed for two months. This same profile is shown to some extent for patients 1, 2, 5, 7, and 10. Patients 6 and 8 had a different pattern of more gradual, steady improvement, regardless of the alteration of the injection interval.

Regardless of the different patterns that were exhibited, all patients appeared to have a beneficial response to ranibizumab. The magnitude of the beneficial response is substantial, which is shown by the change from baseline in median and mean excess foveal thickness for the entire

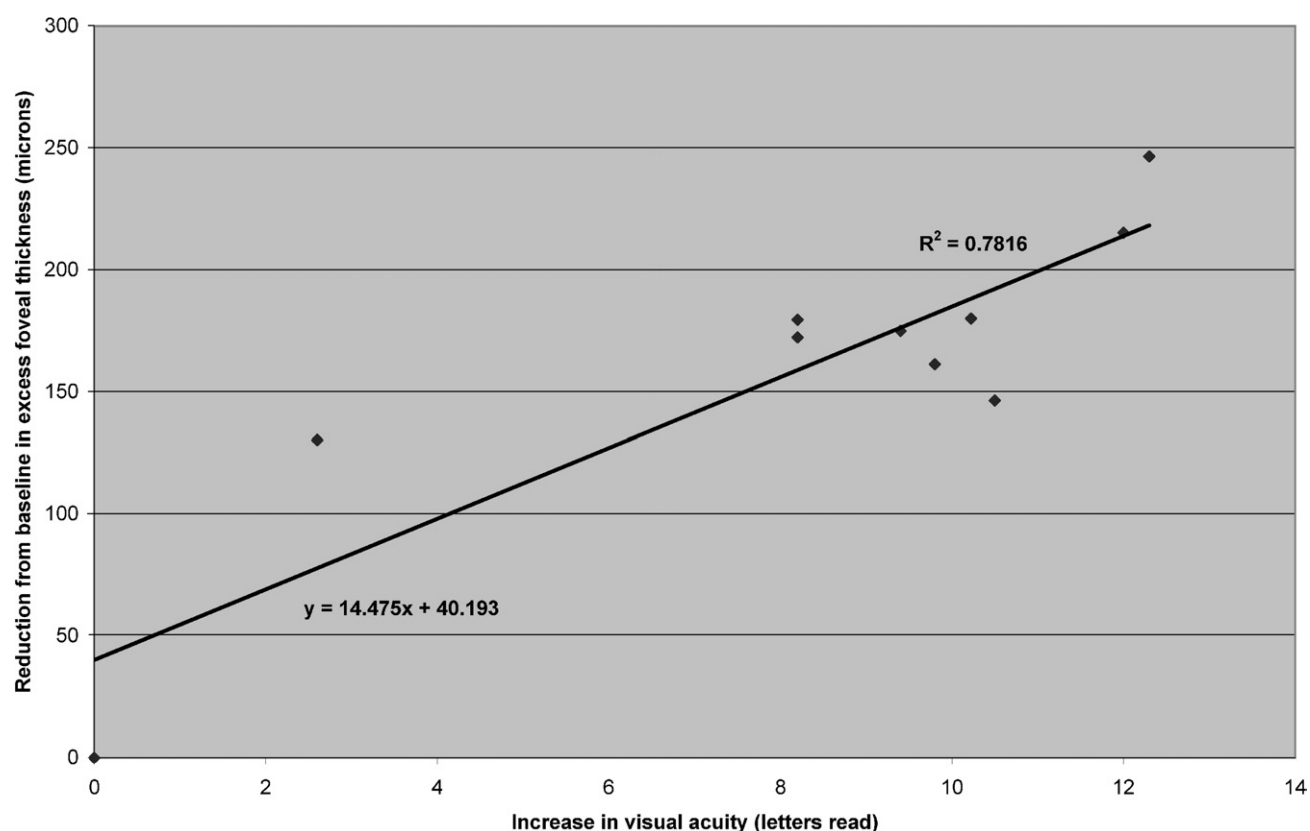


FIGURE 5. Scatter plot of reduction in foveal thickness vs gain in visual acuity at each study visit for all patients with diabetic macular edema treated with ranibizumab. The reduction in foveal thickness in micrometers on the y-axis is plotted against the improvement in visual acuity, which was measured by the numbers of letters that were read on an Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. There is a strong correlation with an R^2 value of 0.78.

group of 10 patients (Figure 3). There was a substantial drop seven days after the first injection, with further improvement to a plateau between months one and three. After an injection was skipped at month three, the foveal thickening was slightly worse at month four but, after the month four injection, improved beyond the previous plateau level. After an injection was skipped at month five, thickening was worse at month six, but after the month six injection, thickening improved to the best level of the study at the primary end point (month seven) when there were median and mean reductions in foveal thickness from baseline of 261 and 246 μm , respectively, which represented a resolution of 85% of the edema. At month nine (three months after the last injection), there was some increase in excess foveal thickness, compared with the seven-month time point, but not back to the baseline level.

• **EFFECT OF RANIBIZUMAB ON MACULAR VOLUME:** Mean macular volume was 9.22 mm^3 at baseline and was reduced to 7.47 mm^3 at seven months, which was a reduction of 1.75 mm^3 (Supplementary Figures 1S and 2S). This is a significant reduction ($P = .009$), which constitutes 77% of the excess macular volume that was present at

baseline. This large effect indicates that the reduction in thickening that occurred in the center of the macula was accompanied by global reduction in edema throughout the entire macula.

• **EFFECT OF RANIBIZUMAB ON VISUAL ACUITY:** Because of the chronicity of the DME and lack of response to other treatments, we did not expect a large improvement in visual acuity. However, mean and median visual acuities were better than the acuities at baseline at all time points and improved by 12.3 and 11 letters, respectively, at the seven-month time point (Figure 4). This is an improvement of a little more than two lines. A scatter plot of reduction in foveal thickness vs improvement in visual acuity at each visit is shown in Figure 5. There is a strong correlation with an R^2 value of 0.78. This indicates that, even for these patients with chronic macular edema, for the group as a whole improvement in foveal thickening correlates well with improvement in visual acuity at each study visit. However, the rate of change of these two outcome measures is different. A comparison of Figures 3 and 4 shows that change in visual acuity occurs more slowly than change in foveal thickness. There was rapid improvement in foveal thickness after the first injection of

ranibizumab, with more gradual improvement in visual acuity. The marked fluctuations in foveal thickness that depended on time after the last injection of the OCT scan was not accompanied by fluctuations in visual acuities, which tended to show gradual improvement (this was the first clue that patient 3 had not become refractory to ranibizumab because, despite the prominent foveal thickening seen on sequential scans that were done one month after injections, visual acuity showed progressive improvement). In view of this, it is not surprising that there was good maintenance of the improvement in visual acuity between seven and nine months, despite substantial worsening of foveal thickness, which indicates that return of thickening precedes loss of visual acuity.

• **SAFETY:** Intraocular injections of ranibizumab were tolerated well with no inflammation or other problems. The mean systolic blood pressures at baseline, months one, two, four, and six were 131.6, 139.0, 142.0, 138.4, and 135.0 mm Hg, respectively. The mean diastolic blood pressures at baseline, months one, two, four, and six were 72.3, 75.1, 78.2, 78.2, and 76.8 mm Hg, respectively. One patient received intraocular corticosteroids in the nonstudy eye for DME, and severe intractable glaucoma developed that required filtration surgery. There were no systemic adverse events, no thromboembolic events, cerebral vascular accidents, or myocardial infarctions. Capillary nonperfusion was measured by image analysis on baseline and month six fluorescein angiograms, with the investigator masked with respect to time point. The mean area of nonperfusion was 0.19812 disk areas at baseline and 0.19525 disk areas at six months. Thus, there was no significant change in capillary nonperfusion throughout the study.

DISCUSSION

THE DEVELOPMENT OF OCT HAS PROVIDED AN EXTREMELY useful tool for the study and management of DME. It allows noninvasive cross-sectional imaging of the retina that provides reproducible measurements of retinal thickness with a resolution of 10 μm .⁶ OCT provides an objective assessment of treatment response that is not influenced by observer or patient bias. Because reproducibility is high and sudden changes in DME are unusual, spontaneous short-term changes of more than 30 μm are rarely seen.⁹ One week after a single injection of 0.5 mg of ranibizumab (a specific antagonist of VEGF) in 10 patients with chronic DME, there were median and mean reductions in foveal thickness of 88 and 130 μm , respectively. This strongly suggests that VEGF is a stimulus for retinal thickening, which is a conclusion that is supported by the added improvement in foveal thickness that is achieved with four additional injections of ranibizumab that result in median and mean reductions in foveal thickness of 261 and 246 μm , respectively, at seven months, which was the primary end point

of the study. This was an 85% reduction of the excess foveal thickening that was present at baseline. There was a strong correlation between the change in foveal thickness over time and the change in macular volume over time, which provided added confidence that the results are reliable and meaningful. Visual acuity measurements are most reliable when both patients and examiners are masked to whether a treatment was actually administered, but the anatomic evidence of improvement and the strong correlation between reduction in foveal thickening and improvement in vision support the reliability of the measured gains in median and mean visual acuity of 11 and 12.3 letters, respectively.

Previous studies have also suggested that VEGF may play a role in DME. An orally administered kinase inhibitor that blocks VEGF receptor signaling caused a dose-dependent reduction in foveal thickness in patients with DME, but because of other activities of this drug, the improvement in DME could not be attributed solely to inhibition of VEGF receptors.¹⁰ Recently, intraocular injections of pegaptanib, an aptamer that selectively binds VEGF₁₆₅, combined with focal laser photocoagulation when considered needed, was found to cause a possible small beneficial effect in patients with DME.¹¹ Patients who were randomly assigned to receive intraocular injections of 0.3 mg of pegaptanib had a median visual acuity of 20/50 at the primary end point, compared with 20/63 at baseline, which was an improvement of approximately one line. This was no different from the sham injection group, which showed improvement of approximately one line from 20/80 at baseline to 20/63. Change in foveal thickness from baseline was -68.0 , -22.7 , and -5.3 μm in the 0.3-, 1-, and 3-mg pegaptanib treatment groups compared with $+3.7$ μm in the sham injection group. These results are confounded by the concomitant use of focal laser photocoagulation in this study; fewer patients in the 0.3-mg treatment group (11/44 patients) compared with the sham injection group (20/42 patients) were treated with focal laser photocoagulation. The small benefit in the 0.3-mg treatment group cannot be attributed solely to the inhibition of VEGF₁₆₅, but rather to the combination of VEGF₁₆₅ blockade and focal laser compared with focal laser alone in the sham injection group. Also, the relatively small effect of this combination therapy on foveal thickness in patients with DME suggests that VEGF₁₆₅ plays a relatively small role in DME and/or that pegaptanib is an inefficient inhibitor. Although the current study included only 10 patients, it is not confounded by any concomitant treatments and demonstrates that a specific VEGF antagonist that is given over seven months causes reductions in median and mean retinal thickening of 261 and 246 μm , respectively which results in the resolution of most excess thickening (85%). This supports the conclusion that VEGF-A (probably multiple isoforms) plays a major role in DME.

This study raises several questions. Are the different patterns of response to intraocular injections of ranibizumab in different patients because of different levels of VEGF production in these patients? What is the optimal timing for injections? There appeared to be a plateau in the amount of reduction of foveal thickening during the first three months of the study when monthly injections of ranibizumab were given, with additional benefit achieved by switching to injections every other month. It is important to determine whether this is a valid observation that can be confirmed or simply random variation. If it is a valid observation, it suggests that there may be some compensatory response during the monthly injection phase, such as increased expression of VEGF that is circumvented by less frequent injections. The most important question raised by the study is whether intraocular injections of ranibizumab can provide long-term benefit in patients with DME. The mean improvement of 12.3 letters of visual acuity over seven months is suggestive, but a larger double-masked, randomized, controlled trial that will span several years is needed to determine the ultimate value of ranibizumab for patients with DME. Such a trial is being planned.

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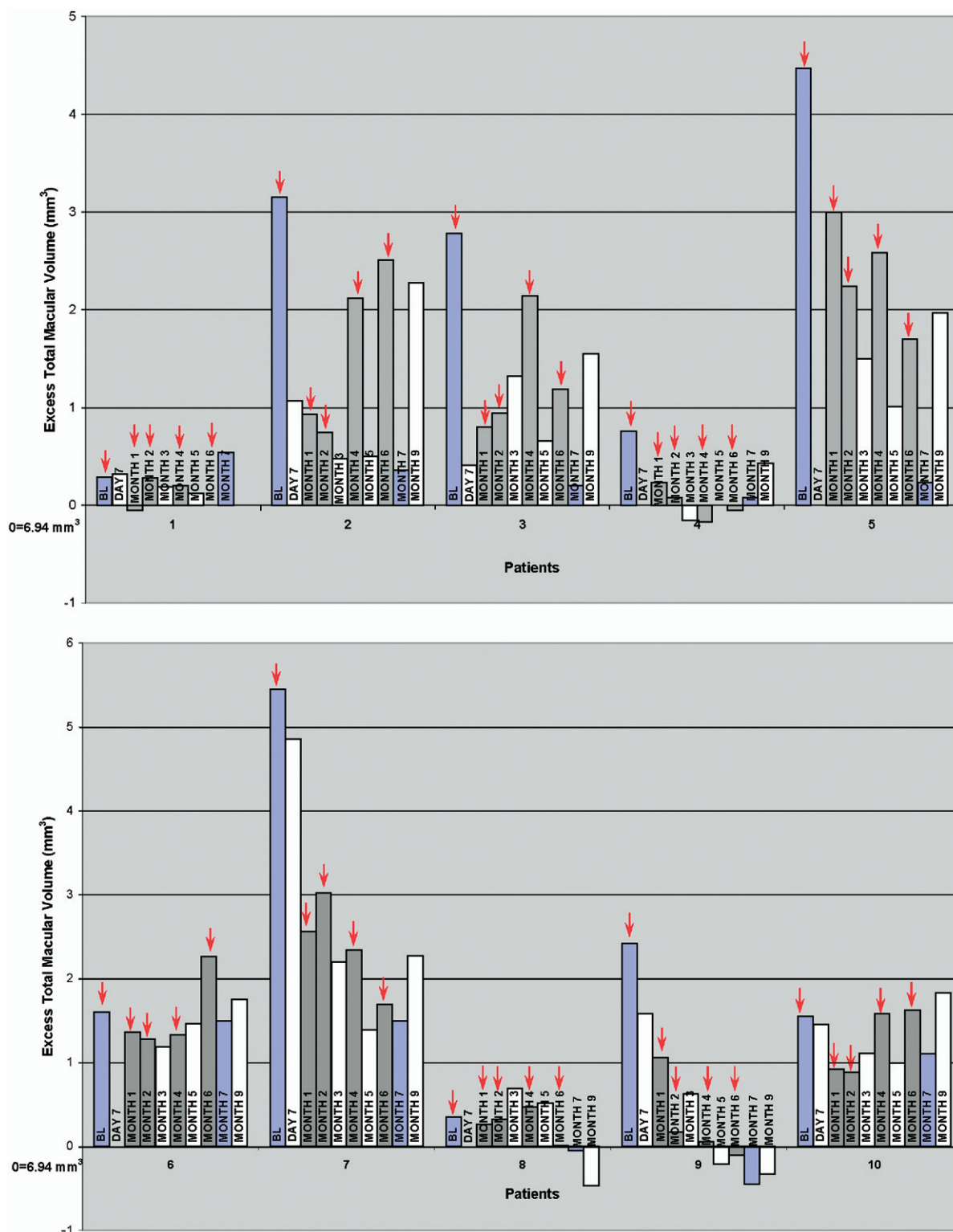
Biosketch

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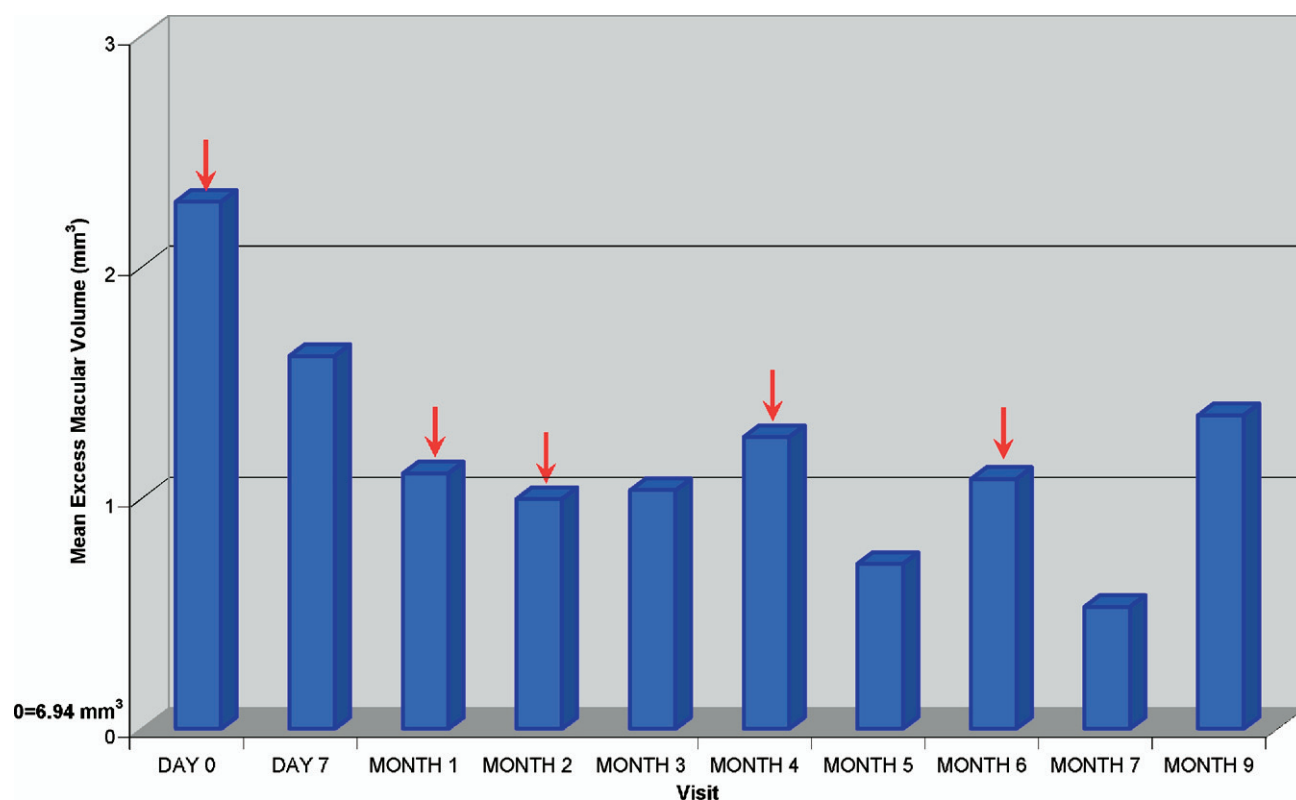


Biosketch

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SUPPLEMENTARY FIGURE 1S. Excess macular volume measured by optical coherence tomography (OCT) in all patients at each study visit. Each bar represents the macular volume above the normal mean value of 6.94 mm³, which is set to 0. The arrows show intraocular injections of 0.5 mg of ranibizumab.



SUPPLEMENTARY FIGURE 2S. The mean excess macular volume for all 10 patients at each study visit. Each bar represents the mean value for excess macular volume for all patients at the designated study visit. The arrows show when intraocular injections of 0.5 mg of ranibizumab were administered. Compared to baseline, macular volume was reduced by 1.75 mm³ at the primary endpoint of the study, constituting elimination of 77% of the excess macular volume that had been present at baseline.