

Intravitreal Bevacizumab for Choroidal Neovascularization Caused by AMD (IBeNA Study): Results of a Phase 1 Dose-Escalation Study

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PURPOSE. To evaluate the safety of three dose regimens of intravitreal bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA) for the management of choroidal neovascularization (CNV) associated with age-related macular degeneration (AMD).

METHODS. This was a prospective, nonrandomized open-label study of 45 patients with AMD and subfoveal CNV. A standardized ophthalmic evaluation was performed at baseline and at weeks 1, 6, and 12 (± 1) after a single intravitreal injection (1.0, 1.5, or 2.0 mg) of bevacizumab. Main outcomes measures include clinical evidence of toxicity and complications. Changes in best corrected visual acuity (BCVA) and lesion characteristics-macular morphology were also evaluated.

RESULTS. The most common adverse events were conjunctival hyperemia and subconjunctival hemorrhage at the injection site. Mean BCVA improved from baseline throughout the study ($P < 0.001$; ANOVA with Geisser-Greenhouse correction). Compared with baseline, BCVA was improved at week 1 ($P = 0.001$), week 6 ($P < 0.001$), and week 12 ($P = 0.001$; Dunnett test). At week 12, the lesion area and CNV area were stable or decreased in 79.1% (34/43) and in 74.4% (32/43) of patients, respectively, with no deterioration of macular architecture observed in 83.7% (36/43). A dose-related change in BCVA (in Early Treatment Diabetic Retinopathy Study [ETDRS] lines) was observed at week 12 (1.0 mg [$+0.3$ line]; 1.5 mg [$+0.6$ line]; and 2.0 mg [$+1.0$ line]; $P = 0.02$; nonparametric test for ordered groups).

CONCLUSIONS. A single intravitreal bevacizumab injection was well tolerated and, except for minor transient local adverse events, no other adverse events were observed. In the short-term, treatment was associated with vision stabilization or improvement and no unfavorable neovascular lesion-macular changes in most patients. (*Invest Ophthalmol Vis Sci.* 2006;47:4569–4578) DOI:10.1167/iovs.06-0433

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Reports of the beneficial effect of photodynamic therapy (PDT) for choroidal neovascularization (CNV) associated with age-related macular degeneration (AMD) launched the contemporary era of management of choroidal neovascular diseases.^{1–3} Contrary to the principles arising from the Macular Photocoagulation Study (i.e., photothermal destruction of the entire neovascular lesion),^{4,5} it has been demonstrated that, by an intravenous injection of a photosensitizer that accumulates in neovascular tissue and subsequent low-irradiance specific light application, disease activity can be modulated after several PDT treatment sessions.^{6–13} Another step toward the implementation of "modulation of disease activity" rather than lesion destruction occurred at the end of 2004 with the approval of the antiangiogenic drug pegaptanib (Macugen; Eye-tech Pharmaceuticals, New York, NY), an aptamer that binds vascular endothelial growth factor (VEGF) isoform 165, for treatment of all forms of neovascular AMD.^{14,15} However, despite repeated, high-cost treatment sessions of either PDT or pegaptanib, patients generally lose vision over time because the growth of neovascular lesions is slowed, but not prevented.^{3,6–13,15}

Even if the overall benefit of pegaptanib therapy in neovascular AMD can be considered modest,¹⁵ it has been of major significance in providing proof of concept for the use of antiangiogenic therapy in neovascular AMD. Ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA) is another antiangiogenic agent currently under investigation for treatment of neovascular AMD. Unlike pegaptanib, ranibizumab binds all VEGF isoforms.^{16–21} Preliminary 2-year data from one phase III study (the MARINA Trial), which included patients with AMD who had "minimally classic CNV" and "occult CNV" neovascular lesions, demonstrated that at least 90% of patients treated with ranibizumab maintained or improved vision compared with approximately 53% of control patients.²²

Intravitreal ranibizumab is derived from the same humanized monoclonal anti-VEGF antibody of bevacizumab (Avastin; Genentech, Inc.), which is the full-length humanized monoclonal anti-VEGF neutralizing antibody designed for intravenous administration and approved for the treatment of metastatic colorectal cancer.^{23–27} Although preclinical studies demonstrated lack of retinal penetration of the full-length antibody when injected intravitreally,²⁸ rapid and favorable macular remodeling was observed in the clinical setting of neovascular AMD and macular edema due to central retinal vein occlusion after a single intravitreal injection of 1.25 mg of bevacizumab,^{29,30} thus bringing to light a new perspective of intravitreal angiogenic pharmacomodulation by using the full-length antibody.

Extensive preclinical data suggesting an important role of VEGF in choroidal neovascular diseases,^{31–54} and reported benefits of anti-VEGF therapy with pegaptanib and ranibizumab in humans,^{14,15,20,21} coupled with the encouraging initial clinical reports using intravitreal bevacizumab,^{29,30} prompted us to conduct a phase I clinical trial designed to investigate the safety

TABLE 1. Definition of Dose-Limiting Toxicity (DLT) for the Study

Ophthalmic DLTs	
Photographic evaluation	Diminished transparency of the lens (cataract induction/progression)
	Local wound-healing problems (absence of closure at the site of intravitreal injection)
Clinical examination	
	Clinically significant inflammation that is severe (obscuring visualization of the retinal vasculature) and vision threatening
	Other ocular abnormalities not usually seen in patients with age-related macular degeneration, such as retinal arterial or venous occlusion, acute retinal detachment, and diffuse retinal hemorrhage
	Best corrected visual acuity: doubling or worsening of the visual angle (loss of 3 ETDRS lines); transition to light perception or hand motion for patients whose baseline visual acuity score is less than 20/500, unless the loss of vision is due to a vitreous hemorrhage related to the injection procedure between days 1 and 7
	Tonometry: increase from baseline of intraocular pressure by 25 mmHg on two separate examinations at least 1 day apart or a sustained pressure of 30 mmHg for more than a week, despite pharmacologic intervention
Fluorescein Angiography	
	Significant retinal or choroidal vascular abnormalities not seen at baseline, such as:
	Choroidal nonperfusion (affecting one or more quadrants)
	Delay in arteriovenous transit times (>15 seconds)
	Retinal arterial or venous occlusion (any deviation from baseline)
	Diffuse retinal permeability alteration affecting retinal circulation in the absence of intraocular inflammation
Systemic DLTs	
	These include grade III (severe), such as hospitalization of the patient or an event that results in significant or persistent deficiency or inability, or grade IV (life-threatening) toxicities or any significant severe toxicity deemed related to the study drug by the investigator

and tolerability of one intravitreal injection of bevacizumab, at different dosages, in patients with neovascular AMD.

METHODS

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local Institutional Review Board. A prospective, nonrandomized, open-label trial was performed to investigate the safety and tolerability of three escalating doses of bevacizumab (1.0, 1.5, and 2.0 mg) administered as a single intravitreal injection in patients with CNV secondary to AMD. Patients were informed verbally and in writing of the potential benefits and risks of the procedure, and

all patients signed a written form stating that they understood and consented to participation. All fluorescein angiography and optical coherence tomography (OCT) evaluations were performed by a certified ophthalmic technician (DC) and interpreted by a single retinal specialist (RAC) in an unmasked fashion. If there were questions regarding interpretation of the study data, other retinal specialists (RJ, JAC, IUS) were approached in consultation.

Patient Selection and Entry Examinations

Patients were recruited from two participating centers (Hospital de Olhos de Araraquara and Department of Ophthalmology at the Univer-

TABLE 2. Baseline Characteristics by Dose Regimen

	Dose Regimen			
	1.0 mg (n = 15)	1.5 mg (n = 15)	2.0 mg (n = 15)	Total (n = 45)
Age (y)				
Mean (±SD)	74.1 (7.01)	73.8 (4.77)	76.0 (7.25)	74.6 (6.37)
Median (Range)	73 (63–85)	72 (67–84)	77 (62–85)	74 (62–85)
Gender				
Female	6	10	8	24 (53.3%)
Male	9	5	7	21 (46.7%)
Blood pressure				
Mean ± SD				
Systolic	127.7 (10.67)	129.3 (8.21)	125.3 (9.54)	127.4 (9.45)
Diastolic	80.7 (7.29)	80.3 (6.11)	79.3 (7.04)	80.1 (6.70)
BCVA in study eye				
Mean ± SD*	1.19 (0.23)	1.11 (0.36)	1.35 (0.19)	1.22 (0.28)
Median (range)*	1.18 (0.8–1.64)	1.22 (0.36–1.6)	1.38 (0.92–1.62)	1.24 (0.36–1.64)
Median†	20/320+1	20/320–1	20/500+1	20/320–2
Range†	20/125–20/800–2	20/50+2–20/800	20/160–1–20/800–1	20/50+2–20/800–2
Lesion Size (DA)‡				
Mean ± SD	7.7 (2.47)	9.6 (3.11)	8.9 (3.38)	8.7 (2.71)
Median (range)	7 (4–11)	10 (4–15)	9 (6–13)	9 (4–15)
CNV Characteristics				
Occult	5	7	4	16 (35.5%)
Classic and Occult	8	5	8	21 (46.7%)
Classic	2	3	3	8 (17.8%)

* LogMAR.

† Snellen equivalent.

‡ The area of a circle equal to 2.54 mm².

TABLE 3. Adverse Events throughout the Study

	Dose Regimen			Total (n = 45)
	1.0 mg (n = 15)	1.5 mg (n = 15)	2.0 mg (n = 15)	
Abnormal vision	1	0	1	2
Conjunctival hyperemia	5	7	5	17
Corneal abrasion	2	1	1	4
Eye pain	0	1	0	1
Hyperemia	1	0	0	1
Ocular irritation	1	0	0	1
Ocular pruritus	0	1	0	1
Pain (at injection)	4	2	2	8
Subconjunctival hemorrhage	5	8	4	17
Vitreous floaters	0	0	1	1
Vitreous prolapse	0	1	3	4
Any adverse event	6 (40%)	8 (53.3%)	6 (40%)	19 (44.4%)

sity of São Paulo, Ribeirão Preto) from August through November 2005. All patients who met the following inclusion criteria were offered study participation: patients with neovascular AMD with logarithm of minimum angle of resolution (logMAR) Early Treatment Diabetic Retinopathy Study (ETDRS) best corrected visual acuity (BCVA) between 0.3 (Snellen equivalent, 20/40) and 1.64 (Snellen equivalent, 20/800⁻²) due to subfoveal CNV diagnosed by fluorescein angiography within the prior 96 hours. Diagnosis of neovascular (exudative) AMD was based on the criteria of the International ARM Epidemiologic Study Group.⁵⁵ For inclusion in the study, it was mandatory that the CNV caused by AMD extend under the geometric center of the foveal avascular zone and that the neovascular complex demonstrate *some* occult CNV component by fluorescein angiography. Lesions characterized angiographically by the presence of only classic CNV could also be included if logMAR ETDRS BCVA was worse than 1.0 (Snellen equivalent, 20/200). The neovascular complex could be of any size, and no restrictions existed with respect to the presence of associated serous pigment epithelial detachment and thick blood. Because of the local unavailability of pegaptanib at the time of this study, patients with the aforementioned lesion and/or BCVA criteria were not eligible for approved treatments, including photodynamic therapy with verteporfin.

Exclusion criteria included: pathologic myopia, defined as a spherical equivalent of -6 D or more, or an ocular axial length of ≥ 26.5 mm, or retinal abnormalities consistent with pathologic myopia (such as lacquer cracks)⁵⁶; angioid streaks; traumatic choroidal rupture; peripapillary changes with atrophic or pigmented "punched out" chorioretinal lesions; uveitis; or any other ophthalmic disorder, other than mild cataract, that might affect visual function. Patients were also excluded if they had (1) an allergy to fluorescein; (2) an ocular media opacity that might interfere with visual acuity, assessment of toxicity, or photographic fundus documentation of the macular area; (3) a history of vitrectomy; (4) any major surgery within the prior 6 months

or planned within the next 28 days; (5) any history of a thromboembolic event (including myocardial infarction and/or coronary disease associated with clinical symptoms or cerebral vascular accident); (6) uncontrolled systemic arterial hypertension (according to guidelines of the seventh report of the joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [JNC-7]); (7) an active infection, bleeding disorders, or peptic ulcer disease with bleeding; and (8) known coagulation abnormalities or current use of anticoagulative medication other than aspirin. Of the 51 patients offered study participation, 6 (11.8%) declined because of the estimated endophthalmitis risk of approximately 1%.

A comprehensive ophthalmic evaluation was performed at the participating center responsible for performing the intravitreal bevacizumab treatments. This evaluation included a medical history, blood pressure measurement, ETDRS BCVA testing, color fundus photography, fluorescein angiography, and third-generation OCT evaluation. Best corrected visual acuity was measured according to a standardized refraction protocol using a retroilluminated Lighthouse for the Blind distance visual acuity test chart (using modified ETDRS charts 1, 2, and R). Stereoscopic digital color fundus photography and fluorescein angiography were performed with certified fundus camera systems (UVi-60/EyeQ Pro; Canon, Tokyo, Japan, and TRC-501A/IMAGENet; Topcon, Tokyo, Japan). Third-generation OCT evaluation (Stratus Tomographer, model 3000; Carl Zeiss Ophthalmic Systems Inc., Humphrey Division, Dublin, CA) consisted of six linear 6.00-mm high-density (512 A-scans) scans oriented at intervals of 30° and centered on the foveal region.

Fifteen patients were enrolled per dose group, with enrollment in each escalating dose group only if there was no evidence of any dose-limiting toxicity at the lower dose(s) (Table 1). The protocol specified that enrollment would be stopped if three or more patients within a dose group experienced dose-limiting toxicity within 7 days after intravitreal injection of bevacizumab. No more than three patients

TABLE 4. Visual Acuity throughout the Study

Dose Regimen	Baseline	Study Visit		
		Week 1	Week 6	Week 12*
1.0 mg	1.19 (0.23)	1.15 (0.23)	1.14 (0.27)	1.17 (0.28)
1.5 mg	1.11 (0.36)	1.04 (0.38)	1.01 (0.36)	1.04 (0.38)
2.0 mg	1.35 (0.19)	1.28 (0.21)	1.24 (0.21)	1.26 (0.20)
Total	1.22 (0.28)	1.16 (0.29)	1.13 (0.30)	1.16 (0.30)
P†		=0.001	<0.001	=0.001

Data are expressed as the mean ($\pm$ SD) logMAR (n = 45).

* n = 43.

† In comparison to baseline; all patients.

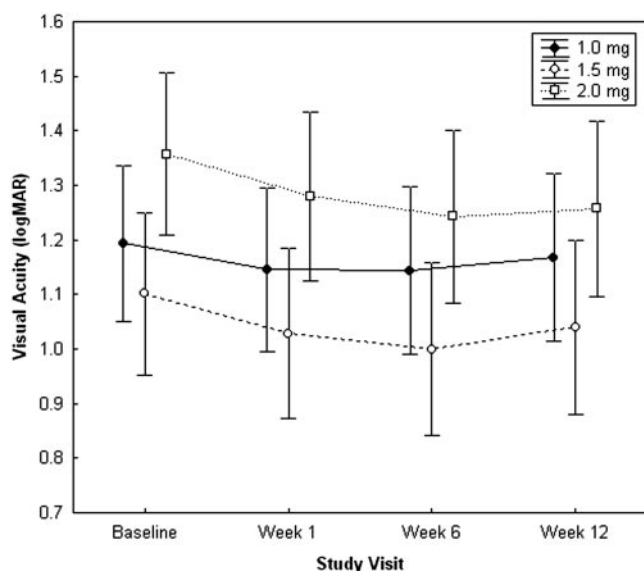


FIGURE 1. Visual acuity throughout the study. Data points: the mean; vertical bars: 95% CI.

could be enrolled into the study on any day, and no more than six patients could be enrolled into the study per week.

Treatment Procedure

Patients received one intravitreal injection of 1.0, 1.5, or 2.0 mg of bevacizumab; for these, 0.04, 0.06, and 0.08-mL aliquots of commercially available bevacizumab (25 mg/mL), respectively, were prepared for each patient and placed in insulin syringes (BD Ultra-fine; BD Biosciences, Franklin Lakes, NJ) by a compounding pharmacy using standard aseptic techniques. All treatments were performed by a single retinal specialist (RAC) using topical anesthetic (tetracaine-phenylephrine) drops under sterile conditions (eyelid speculum and povidone iodine). Patients were given 250 mg of acetazolamide orally before pupil dilation with mydriatic (tropicamide 1%) drops. Bevacizumab was injected into the vitreous cavity with a 29.5-gauge needle inserted through the inferotemporal pars plana 3.0 mm (pseudophakic) or 3.5 mm (phakic) posterior to the limbus. After the injection, central retinal artery perfusion was confirmed with indirect ophthalmoscopy and photographic documentation of the fundus and of the injection site was performed. Patients were instructed to instill one drop of 0.3% ciprofloxacin into the injected eye four times daily for 1 week after the procedure.

Follow-up Examinations and Outcome Measures

Follow-up examinations were performed at weeks 1, 6, and 12 (± 1) after injection. The same procedures performed at baseline were performed at each study visit (e.g., BCVA, complete ophthalmic examination, fundus photography, fluorescein angiography, and OCT). Local and systemic adverse events were monitored throughout the study.

These local ophthalmic and systemic adverse events were the primary end points of the study. BCVA was measured primarily as an indicator of safety, not efficacy, given the absence of a control group and the short follow-up period. However, changes in BCVA were used to compare results among the dose groups.

Secondary measures were used to evaluate the short-term effects of intravitreal bevacizumab treatment and to assist with comparisons between dose regimens: (1) total area of the neovascular complex (lesion) and characteristics of the CNV component by fluorescein angiography, and (2) macular remodeling (that is, retinal elevation) on OCT evaluation. Because of inherent OCT software flaws in the generation of reliable quantitative tomographic data in eyes with neovascular AMD,^{57,58} changes in macular architecture were qualitatively

analyzed. Based on changes in the degree of retinal elevation due to intraretinal, subretinal, and sub-RPE fluid compared with baseline, the macular architecture was classified as having undergone "partial recovery" (absence of, or decrease in, retinal elevation), "no recovery" (unchanged retinal elevation), or "deterioration" (increased retinal elevation).^{59,60}

Statistical Analysis

Repeated-measures analysis of variance (ANOVA) with the Geisser-Greenhouse correction was performed to analyze the visual acuity from baseline to the final study visit (week 12). The Dunnett test was used to compare the visual acuity after injection with the baseline measurement. A nonparametric test for trend across ordered groups was used to compare the changes in visual acuity from baseline among the three groups at weeks 1, 6, and 12.⁶¹ The significance level was set at 0.05.

RESULTS

The 45 participants included 21 (46.7%) men and 24 (53.3%) women; 43 completed all follow-up visits (one patient refused to return after the week-6 follow-up visit, and the other patient withdrew because of a death in the family). The mean ($\pm$ SD) age was 74.6 ± 6.4 years (median, 74 years; range, 62–85 years). Mean ($\pm$ SD) systolic and diastolic blood pressure was 127.4 ± 9.5 and 80.1 ± 6.7 mm Hg, respectively. The mean $\pm$ SD size of the neovascular complex was 12.2 ± 3.9 Macular Photocoagulation Study disc areas. Thirty-seven (82.2%) of 45 eyes showed some fluorescein leakage corresponding to occult CNV, and 8 (17.8%) eyes showed purely classic CNV lesions. Baseline characteristics of the patients, stratified by dose group, are summarized in Table 2.

No systemic or serious drug-related adverse events were observed. The treatment procedure was well tolerated, and no clinical evidence of inflammation, uveitis, endophthalmitis, or obvious ocular toxicity was observed. Further, there were no significant changes in blood pressure, intraocular pressure, or lens status in any of the eyes during the study follow-up period. Minor local adverse events related to the treatment procedure were observed (Table 3). Subconjunctival hemorrhage and conjunctival hyperemia were observed frequently at the intravitreal injection site and were most likely the result of the anesthesia technique rather than the injection procedure itself. In the first 15 procedures, four (26.6%) patients reported pain at the time of the injection. Seeking to minimize this pain, in the next 15 procedures we used a sterile cotton tip soaked with tetracaine-phenylephrine drops, pushed gently against the conjunctiva/sclera for 60 to 70 seconds before the injection (for the first 15 procedures the cotton tip was left in place for approximately 30 seconds). Only 2 (13.3%) patients reported pain after we implemented the latter anesthetic technique, but we did note that 8 (53.3%) patients (versus 5 [33.3%] in the first 15 patients) developed conjunctival hyperemia and subconjunctival hemorrhage within 30 minutes of the procedure.

TABLE 5. Changes in Visual Acuity Compared with Baseline

Dose Regimen	Study Visit		
	Week 1	Week 6	Week 12
1.0 mg	−0.05 (0.13)	−0.05 (0.14)	−0.03 (0.17)
1.5 mg	−0.07 (0.10)	−0.10 (0.11)	−0.06 (0.09)
2.0 mg	−0.07 (0.09)	−0.10 (0.11)	−0.10 (0.10)
P*	=0.49	=0.13	=0.02

Data are expressed as the mean ($\pm$ SD) logMAR.

* Comparison between dose regimens at each follow-up visit.

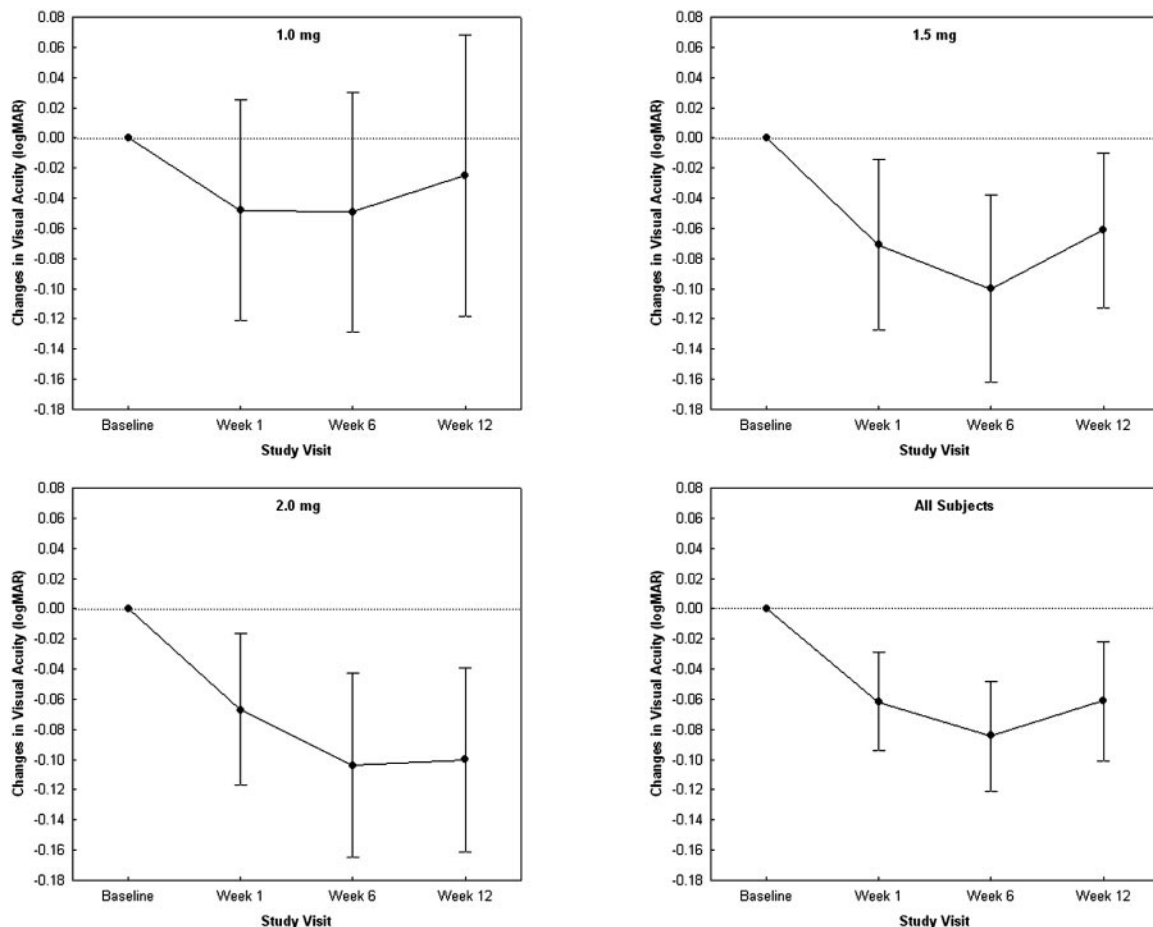


FIGURE 2. Profile of the mean visual acuity (VA) changes from baseline for each dose group after a single intravitreal injection of bevacizumab through week 12. Vertical bars, 95% CI.

Two eyes even had small subconjunctival hemorrhages before the injection. For the last 15 injections, the sterile cotton tip soaked in anesthetic (tetracaine-phenylephrine) drops was pressed against the eyewall over the injection site for only 30 to 35 seconds, with an additional 10 seconds of pressure applied just before the injection; and pain was reported by only two (13.3%) patients and subconjunctival hemorrhage developed in four (26.6%). Independent of the anesthesia technique, subconjunctival hemorrhage was resolved and absence of wound leakage was verified in all 45 patients by 1 week after injection.

The study was designed primarily as a safety trial. BCVA results are summarized in Table 4 (Fig. 1). Mean BCVA improved significantly from baseline ($P < 0.001$; ANOVA using the Geisser-Greenhouse correction). Compared with baseline, BCVA improved significantly at week 1 ($P = 0.001$), week 6 ($P < 0.001$), and week 12 ($P = 0.001$; Dunnett test; Table 4).

The mean visual acuity change (in ETDRS lines) from baseline between dose-regimens 1.0 (+0.3 line), 1.5 (+0.6 line), and 2.0 mg (+1.0 line) was significantly different at week 12, thus suggesting a dose-related response ($P = 0.02$; nonparametric test for ordered groups; Table 5; Fig. 2).

No unfavorable changes in neovascular complexes were observed during the study period by angiographic and OCT examinations. At 6 weeks, 42 (93.3%) and 40 (88.9%) patients of 45 patients exhibited stabilization (i.e., no change or decrease) of the total lesion area and CNV area, respectively, by fluorescein angiography (Fig. 3). Unchanged or decreased lesion area and CNV area was seen in 34 (79.1%) and in 32

(74.4%) patients, respectively, of 43 patients who completed the week-12 follow-up evaluation. No patients in the 2.0-mg dose group demonstrated an increase in lesion area or CNV area throughout the study period (Table 6). No patients in the 1.5-mg dose group demonstrated an increase in lesion area at the week-6 evaluation. On OCT, favorable changes in macular architecture (i.e., no deterioration) were observed in 41 (91.1%) of the 45 patients at 6 weeks, and in 36 (83.7%) of the 43 patients who completed the week-12 follow-up evaluation (Fig. 4). The most favorable macular remodeling was observed in patients in the 2.0-mg dose group at weeks 6 and 12, and at week 6 in patients in the 1.5-mg dose group (Table 6).

DISCUSSION

This phase-I clinical trial evaluated the safety profile of three different doses of a single intravitreal injection of bevacizumab, a genetically engineered humanized monoclonal antibody against all active forms of human VEGF, in patients with neovascular AMD. In short-term, bevacizumab was well tolerated when administered by intravitreal injection at doses of 1.0, 1.5, and 2.0 mg using the commercially formulation of the drug at the concentration of 25 mg/mL (volumes of 0.04, 0.06, and 0.08 mL). The most common adverse events were conjunctival hyperemia and subconjunctival hemorrhage at the injection site, events judged to be essentially the result of the injection procedure rather than the drug. No major adverse event associated with the treatment was observed during the 12-week follow-up period.

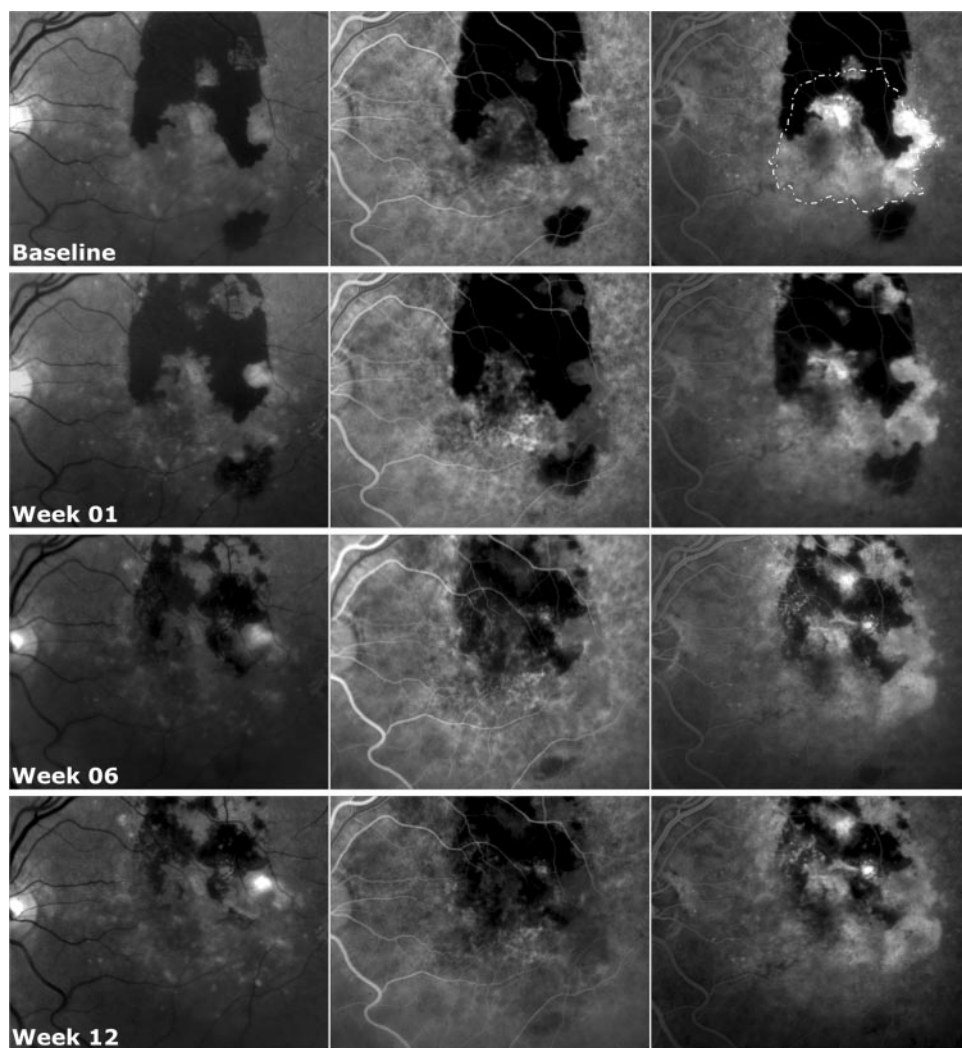


FIGURE 3. Red-free fundus photography, and early- and late-phase fluorescein angiograms of one patient in dose regimen of 1.5 mg of intravitreal bevacizumab throughout the study. *Top:* baseline evaluation performed 24 hours before intravitreal bevacizumab injection demonstrated stippled fluorescence from occult CNV component and areas of blocked fluorescence from thick blood, as well as a small retinal pigment epithelium detachment at the temporal aspect of the neovascular complex. *Top center:* 1 week after treatment fluorescence from CNV was diminished and subretinal hemorrhage has partially reabsorbed. *Bottom center:* at 6 weeks, although choroidal neovascular channels could be identified in early phases, fluorescein leakage from CNV was minimal, and subretinal hemorrhage was further diminished. *Bottom:* 12 weeks after treatment, actively leaking CNV and lesion areas were decreased in comparison with baseline.

Before study initiation we had used intravitreal bevacizumab for salvage therapy in eyes with AMD and CNV refractory to other treatments, and we observed some reflux of drug after the injection procedure. Therefore, in an effort to minimize drug reflux, 250 mg of acetazolamide was given orally 1 hour before the injection procedure. In the present study, prolapse of vitreous and/or bevacizumab at the end of the injection procedure was observed in 1 patient who received 0.06 mL and in three patients who received 0.08 mL of the drug. Unlike triamcinolone acetonide, bevacizumab is a transparent, fluid drug that may reflux when higher volumes are injected intravitreally even through a 29.5-gauge entry site.

Overall improvement in visual acuity occurred as early as 1 week after the bevacizumab injection in this study. Visual acuity continued to improve between weeks 1 and 6, and this benefit persisted up to the week 12 evaluation. The improvement observed at week 6 may reflect continued inhibition of VEGF. In conjunction with fluorescein angiographic and OCT data, which demonstrated favorable macular remodeling without documented regression of the choroidal neovascular channels in most of the patients (particularly in lesions with some classic CNV component), it seems that the beneficial effects of intravitreal bevacizumab on vision may be related to inhibition of VEGF-associated vascular permeability rather than to an effect on angiogenesis. Recently, complete retinal penetration of bevacizumab was demonstrated in rabbits after a single intravitreal injection of bevacizumab⁶²; however, no measure-

ments at the choroidal tissues were performed, and thus one may consider that low levels of the antiangiogenic drug may reach the choriocapillaris-choroid complex. This hypothesis is further supported by the remarkable beneficial effects on choroidal neovascularization characteristics of *systemic* bevacizumab in humans,^{63,64} as well as the observed effects of intravitreal bevacizumab on retinal neovascularization associated with diabetic retinopathy⁶⁵⁻⁶⁷ and choroidal neovascular lesions associated with chorioretinal anastomosis (Costa RA; unpublished data, 2006). In all, clear regression of the neovascular vessels was demonstrated, a finding not observed in the present study.

One limitation of the present study is the absence of serum testing to investigate the systemic pharmacokinetics of intravitreally administered bevacizumab. Such evaluation, however, involves high-cost procedures that are not feasible within the context of an unfunded investigator-driven study. Because hypertension is the most common systemic adverse event associated with systemic bevacizumab therapy, changes in blood pressure after treatment may roughly serve as a surrogate marker for clinically relevant systemic bevacizumab levels. Herein, as in other studies involving patients with AMD,⁶⁸⁻⁷⁰ no significant change in blood pressure was seen in the short-term after single injections of bevacizumab. Two patients were lost to the final follow-up; however, it is unlikely that this influenced the overall study results significantly, given the observed findings through 6 weeks of follow-up in both pa-

TABLE 6. Change from Baseline in Neovascular Complex Lesion Area and CNV Characteristics

	Dose Regimen							
	1.0 mg		1.5 mg		2.0 mg		Total	
	Week 6	Week 12	Week 6	Week 12	Week 6	Week 12	Week 6	Week 12
Fluorescein angiography								
Lesion area								
Decrease	1 (6.7)	1 (6.7)	2 (13.3)	2 (14.3)	3 (20)	2 (14.3)	6 (13.3)	5 (11.6)
No Change	11 (73.3)	8 (53.3)	13 (86.7)	9 (64.3)	12 (80)	12 (85.7)	36 (80)	29 (67.5)
Increase	3 (20)	6 (40)	0	3 (21.4)	0	0	3 (6.7)	9 (20.9)
CNV area								
Decrease	2 (13.3)	1 (6.7)	7 (46.7)	3 (21.4)	8 (53.3)	5 (35.7)	17 (35.6)	9 (20.9)
No Change	10 (66.7)	7 (46.6)	6 (40)	7 (50)	7 (46.7)	9 (64.3)	23 (53.3)	23 (53.5)
Increase	3 (20)	7 (46.6)	2 (13.3)	4 (28.6)	0	0	5 (11.1)	11 (25.6)
Optical coherence tomography								
Macular architecture								
Partial recovery	2 (13.3)	2 (13.3)	8 (53.3)	4 (28.6)	11 (73.3)	9 (64.3)	21 (46.7)	15 (34.9)
No recovery	10 (66.7)	8 (53.3)	6 (40)	8 (57.1)	4 (26.7)	5 (35.7)	20 (44.4)	21 (48.8)
Deterioration	3 (20)	5 (33.3)	1 (6.7)	2 (14.3)	0	0	4 (8.9)	7 (16.3)

Data are expressed as the number of cases with the percentage of the total in parentheses.

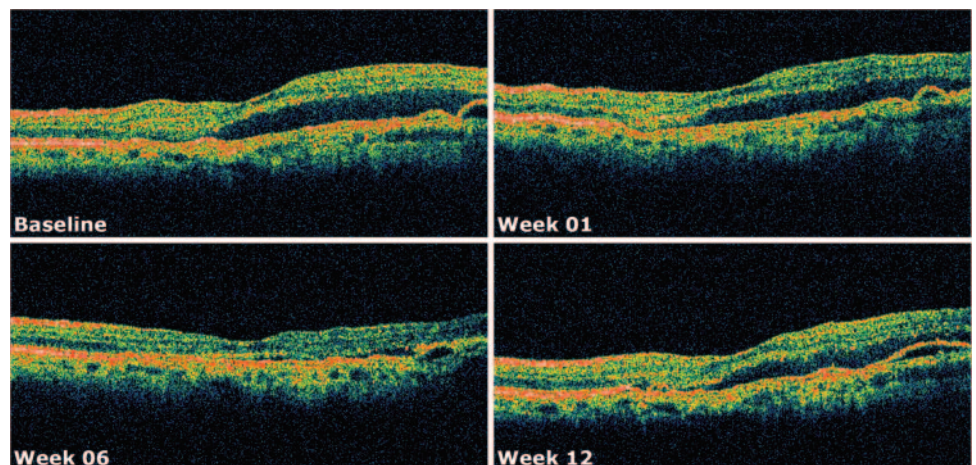
tients. Additional limitations of the present study include the small number of subjects and short follow-up duration. Finally, the variability of the injection volume within each dose regimen, which could also have interfered in the study results, might have been minimized in this study by using preloaded syringes prepared by a compounding pharmacy.

As mentioned earlier, limitations inherent in the study's design preclude extrapolation of our results. Conclusions regarding efficacy cannot be made in the absence of a control group, but differences among the dose groups may provide clues regarding drug effects. Patients who received 1.5 or 2.0 mg of bevacizumab were more likely to demonstrate no change or a reduction in lesion area as well as in CNV area at weeks 6 and 12 than were patients who received 1.0 mg. Overall, and keeping in mind the short follow-up of the study and the variable course of the disease, at week 6 more eyes demonstrated a smaller area of leakage from CNV with associated favorable macular remodeling on angiographic and OCT evaluations, thus suggesting the effects of bevacizumab may be maximal up to week 6 under the strict conditions of this study. Overall visual acuity improved throughout the study, and no differences existed among dose regimens at weeks 1 and 6. However, the change in visual acuity observed in treated pa-

tients at week 12 was related to the dose regimen, thus suggesting a possible longer duration of action (i.e., drug persistence in the eye) of the higher dosage (2.0 mg) of bevacizumab, but not necessarily a dose dependence related to better initial efficacy. In light of this and the fact that beneficial effects of bevacizumab have just been reported in neovascular AMD after intravitreal injections of 2.5 mg (0.1 mL),⁷⁰ future studies probably should include higher dose regimens; nevertheless, as discussed earlier, issues related to drug-fluid vitreous reflux should be cautiously monitored. One may also argue that even lower doses should have been tested since for pegaptanib therapy it was the lowest dose of the drug that appeared the most effective.¹⁵ However, in contrast to pegaptanib, in our study the highest dose worked best, suggesting that low-dose inhibition is not an issue. Furthermore, dose regimens lower than 1.0 mg are difficult to measure and require dilution of the commercially available drug so that reproducible volumes can be injected. This would require reformulation of the drug with stability testing, which is outside the scope of clinical practice.

In 1971, Folkman⁷¹ hypothesized that the growth of tumors is angiogenesis dependent and that antiangiogenic therapy may represent an option for the treatment of solid tumors.⁷² Since

FIGURE 4. Third-generation optical coherence tomography evaluation (high-density [512 A-scans] 6 mm in length B-scans, oriented obliquely at 330°) of one patient in dose regimen of 1.5 mg of intravitreal bevacizumab throughout the study (same patient illustrated in Fig. 3). *Top left:* focal regions of fusiform thickening of as well as fragmentation and irregular elevation of the outer highly reflective layer corresponding to the retinal pigment epithelium-choriocapillaris hyperreflective complex associated with intraretinal and subretinal fluid thus characterizing a fibrovascular detachment of the RPE was seen at baseline. *Top right:* 1 week after treatment, decrease in retinal elevation was noted. *Bottom left:* 6 weeks after the procedure, marked decrease in retinal elevation was seen. *Bottom right:* by week 12, subretinal and sub-RPE fluid recurred; in comparison to baseline, no deterioration of the macular architecture was verified.



then, numerous reports have supported the crucial role of neovascularization in malignancy and various non-neoplastic diseases, including neovascular AMD,⁷² which is the leading cause of blindness in elderly persons in developed nations. Unfortunately, currently approved treatments are characterized by both limited clinical significance and high-cost that restricts access to treatment, particularly in underdeveloped nations. Characterization of the various processes involved in the pathogenesis of any disease is a prerequisite to developing effective therapies. Vascular endothelial growth factor is a protein involved in the onset and progression of AMD.³¹⁻⁵⁴ Thus, VEGF and its signaling receptors are potential targets for pharmaceutical intervention. Results from phase III clinical trials evaluating intravitreal anti-VEGF agents have led to the approval of pegaptanib for the treatment of neovascular AMD and indicate favorable results associated with ranibizumab. In this era of pharmacomodulation for AMD management, bevacizumab may have a crucial role not only because of the promising results reported herein and additional safety data and clinical evidence of its beneficial effects reported recently for a variety of diseases,^{65-67,73-77} but also because intravitreal bevacizumab therapy, if proved safe and efficacious, may represent a treatment option for neovascular AMD that could be accessible to all patients, independent of their socioeconomic condition.

References

1. Miller JW, Schmidt-Erfurth U, Sickenberg M, et al. Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration: results of a single treatment in a phase 1 and 2 study. *Arch Ophthalmol*. 1999;117:1161-1173.
2. Schmidt-Erfurth U, Miller JW, Sickenberg M, et al. Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration: results of retreatments in a phase 1 and 2 study. *Arch Ophthalmol*. 1999;117:1177-1187.
3. Treatment of age-related macular degeneration with photodynamic therapy (TAP) Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: one-year results of 2 randomized clinical trials. TAP report (published correction in *Arch Ophthalmol* 2000; 118:488). *Arch Ophthalmol*. 1999;117:1329-1345.
4. Macular Photocoagulation Study Group. Subfoveal neovascular lesions in age-related macular degeneration. Guidelines for evaluation and treatment in the macular photocoagulation study. *Arch Ophthalmol*. 1991;109:1242-1257.
5. Macular Photocoagulation Study Group. Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration: results of a randomized clinical trial. *Arch Ophthalmol*. 1991;109:1220-1231.
6. Bressler NM; Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials. TAP report no. 2. *Arch Ophthalmol*. 2001;119:198-207.
7. Verteporfin In Photodynamic Therapy Study Group. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization. VIP report no. 2. *Am J Ophthalmol*. 2001;131:541-560.
8. Bressler NM, Arnold J, Benchaboune M, et al. Verteporfin therapy of subfoveal choroidal neovascularization in patients with age-related macular degeneration: additional information regarding baseline lesion composition's impact on vision outcomes. TAP report no. 3. *Arch Ophthalmol*. 2002;120:1443-1454.
9. Rubin GS, Bressler NM. Treatment of Age-Related Macular Degeneration with Photodynamic therapy (TAP) study group. Effects of verteporfin therapy on contrast on sensitivity: results from the Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) investigation. TAP report no. 4. *Retina*. 2002;22:536-544.
10. Blumenkranz MS, Bressler NM, Bressler SB, et al. Verteporfin therapy for subfoveal choroidal neovascularization in age-related macular degeneration: three-year results of an open-label extension of 2 randomized clinical trials. TAP report no. 5. *Arch Ophthalmol*. 2002;120:1307-1314.
11. Bessler NM and the VAM Study Writing Committee. Verteporfin therapy in age-related macular degeneration (VAM): an open-label multicenter photodynamic therapy study of 4,435 patients (published correction in *Retina*. 2004;24:990). *Retina*. 2004;24:512-520.
12. Azab M, Benchaboune M, Blinder KJ, et al. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: meta-analysis of 2-year safety results in three randomized clinical trials: Treatment of Age-Related Macular Degeneration with Photodynamic Therapy and Verteporfin in Photodynamic Therapy Study Report no. 4. *Retina*. 2004;24:1-12.
13. Kaiser PK; Treatment of Age-related Macular Degeneration with Photodynamic Therapy (TAP) Study Group. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: 5-year results of two randomized clinical trials with an open-label extension. TAP Report no. 8. *Graefes Arch Clin Exp Ophthalmol*. Published online March 15, 2006.
14. The Eyetech Study Group. Preclinical and phase 1A clinical evaluation of an anti-VEGF pegylated aptamer (EYE001) for the treatment of exudative age-related macular degeneration. *Retina*. 2002; 22:143-152.
15. Gragoudas ES, Adamis AP, Cunningham ET Jr, et al. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med*. 2004; 351:2805-2816.
16. Presta LG, Chen H, O'Connor SJ, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res*. 1997;57: 4593-4599.
17. Muller YA, Chen Y, Christinger HW, et al. VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 Å—a resolution and mutational analysis of the interface. *Structure*. 1998;6:1153-1167.
18. Chen Y, Wiesmann C, Fuh G, et al. Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity-matured Fab in complex with antigen. *J Mol Biol*. 1999;293:865-881.
19. Krzystolik MG, Afshari MA, Adamis AP, et al. Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. *Arch Ophthalmol*. 2002;120:338-346.
20. Rosenfeld PJ, Schwartz SD, Blumenkranz MS, et al. Maximum tolerated dose of a humanized anti-vascular endothelial growth factor antibody fragment for treating neovascular age-related macular degeneration. *Ophthalmology*. 2005;112:1048-1053.
21. Heier JS, Antoszyk AN, Pavan PR, et al. Ranibizumab for treatment of neovascular age-related macular degeneration: a phase I/II multicenter, controlled, multidose study. *Ophthalmology*. Published online February 13, 2006.
22. Genentech: Press Releases—News Release February 28, 2006. Available at: <http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=9427>. Accessed March, 2006.
23. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med*. 2003;349:427-434.
24. No author listed. Adding a humanized antibody to vascular endothelial growth factor (bevacizumab, Avastin) to chemotherapy improves survival in metastatic colorectal cancer. *Clin Colorectal Cancer*. 2003;8:85-88.
25. Kabbinnavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol*. 2003;21:60-65.
26. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004;350:2335-2342.
27. Yang JC. Bevacizumab for patients with metastatic renal cancer: an update. *Clin Cancer Res*. 2004;10:6367S-70S.

28. Mordenti J, Cuthbertson RA, Ferrara N, et al. Comparisons of the intraocular tissue distribution, pharmacokinetics, and safety of 125I-labeled full-length and Fab antibodies in rhesus monkeys following intravitreal administration. *Toxicol Pathol.* 1999;27:536-544.
29. Rosenfeld PJ, Moshfeghi AA, Puliafito CA. Optical coherence tomography findings after an intravitreal injection of bevacizumab (Avastin) for neovascular age-related macular degeneration. *Ophthalmic Surg Lasers Imaging.* 2005;36:331-335.
30. Rosenfeld PJ, Fung AE, Puliafito CA. Optical coherence tomography findings after an intravitreal injection of bevacizumab (Avastin) for macular edema from central retinal vein occlusion. *Ophthalmic Surg Lasers Imaging.* 2005;36:336-339.
31. Amin R, Puklin JE, Frank RN. Growth factor localization in choroidal neovascular membranes of age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 1994;35:3178-3188.
32. D'Amore PA. Mechanisms of retinal and choroidal neovascularization. *Invest Ophthalmol Vis Sci.* 1994;35:3974-3979.
33. Frank RN, Amin RH, Elliott D, Puklin JE, Abrams GW. Basic fibroblast growth factor and vascular endothelial growth factor are present in epiretinal and choroidal neovascular membranes. *Am J Ophthalmol.* 1996;122:393-403.
34. Aiello LP. Vascular endothelial growth factor and the eye. Past, present and future (editorial). *Arch Ophthalmol.* 1996;114:1252-1254.
35. Lopez PF, Sippy BD, Lambert HM, et al. Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. *Invest Ophthalmol Vis Sci.* 1996;37:855-868.
36. Kvanta A, Algvere PV, Berglin L, Seregard S. Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. *Invest Ophthalmol Vis Sci.* 1996;37:1929-1934.
37. Wells JA, Murthy R, Chibber R, et al. Levels of vascular endothelial growth factor are elevated in the vitreous of patients with subretinal neovascularisation. *Br J Ophthalmol.* 1996;80:363-366.
38. Schlingemann RO, van Hinsbergh VWM. Role of vascular permeability factor/vascular endothelial growth factor in eye disease (review). *Br J Ophthalmol.* 1997;81:501-512.
39. Yi X, Ogata N, Komada M, et al. Vascular endothelial growth factor expression in choroidal neovascularization in rats. *Graefes Arch Clin Exp Ophthalmol.* 1997;235:313-319.
40. Kliffen M, Sharma HS, Mooy CM, et al. Increased expression of angiogenic growth factors in age-related maculopathy. *Br J Ophthalmol.* 1997;81:154-162.
41. Mousa SA, Lorelli W, Campochiaro PA. Role of hypoxia and extracellular matrix-integrin binding in the modulation of angiogenic growth factors secretion by retinal pigmented epithelial cells. *J Cell Biochem.* 1999;74:135-143.
42. Frank RN. Growth factors in age-related macular degeneration: pathogenic and therapeutic implications. *Ophthalmic Res.* 1997;29:341-353. Comment in: *Ophthalmic Res.* 1999;31:243-244.
43. Campochiaro PA, Soloway P, Ryan SJ, Miller JW. The pathogenesis of choroidal neovascularization in patients with age related macular degeneration. *Mol Vis.* 1999;5:34-39.
44. Campochiaro PA. Retinal and choroidal neovascularization. *J Cell Physiol.* 2000;184:301-310.
45. Spilsbury K, Garrett KL, Shen WY, Constable IJ, Rakoczy PE. Overexpression of vascular endothelial growth factor (VEGF) in the retinal pigment epithelium leads to the development of choroidal neovascularization. *Am J Pathol.* 2000;157:135-144.
46. Lip PL, Belgore F, Blann AD, et al. Plasma VEGF and soluble VEGF receptor FLT-1 in proliferative retinopathy: relationship to endothelial dysfunction and laser treatment. *Invest Ophthalmol Vis Sci.* 2000;41:2115-2119.
47. Ohno-Matsui K, Morita I, Tombran-Tink J, et al. Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. *J Cell Physiol.* 2001;189:323-333.
48. Schwesinger C, Yee C, Rohan RM, et al. Intrachoroidal neovascularization in transgenic mice overexpressing vascular endothelial growth factor in the retinal pigment epithelium. *Am J Pathol.* 2001;158:1161-1172.
49. Mori K, Gehlbach P, Yamamura S, et al. AAV-mediated gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization. *Invest Ophthalmol Vis Sci.* 2002;43:1994-2000.
50. Ogata N, Wada M, Otsuji T, et al. Expression of pigment epithelium-derived factor in normal adult rat eye and experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci.* 2002;43:1168-1175.
51. Renno RZ, Youssri AI, Michaud N, Gragoudas ES, Miller JW. Expression of pigment epithelium-derived factor in experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci.* 2002;43:1574-1580.
52. Hera R, Keramidas M, Peoc'h M, et al. Expression of VEGF and angiopoietins in subfoveal membranes from patients with age-related macular degeneration. *Am J Ophthalmol.* 2005;139:589-596. Comment in: *Am J Ophthalmol.* 2005;139:710-711.
53. Tong JP, Chan WM, Liu DT, et al. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. *Am J Ophthalmol.* 2006;141:456-462.
54. Bhutto IA, McLeod DS, Hasegawa T, et al. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. *Exp Eye Res.* 2006;82:99-110.
55. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol.* 1995;39:367-374.
56. Verteporfin in Photodynamic Therapy Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in pathologic myopia with verteporfin. 1-year results of a randomized clinical trial. VIP report no. 1. *Ophthalmology.* 2001;108:841-852.
57. Costa RA, Calucci D, Skaf M, et al. Optical coherence tomography 3: automatic delineation of the outer neural retinal boundary and its influence on retinal thickness measurements. *Invest Ophthalmol Vis Sci.* 2004;45:2399-2406.
58. Costa RA, Skaf M, Melo Jr LA, et al. Retinal assessment using optical coherence tomography. *Prog Retin Eye Res.* 2006;25:325-353.
59. Costa RA, Calucci D, Cardillo JA, et al. Selective occlusion of subfoveal choroidal neovascularization in angioid streaks using a new technique of ingrowth site treatment. *Ophthalmology.* 2003;110:1192-1203.
60. Costa RA, Teixeira LF, Calucci D, et al. Selective occlusion of subfoveal choroidal neovascularization in pathologic myopia using a new technique of ingrowth site treatment. *Am J Ophthalmol.* 2003;135:857-866.
61. Cuzick J. A Wilcoxon-type test for trend. *Stat Med.* 1985;4:87-90.
62. Shahar J, Avery RL, Heilweil G, et al. Electrophysiologic and retinal penetration studies following intravitreal injection of bevacizumab (Avastin). *Retina.* 2006;26:262-269.
63. Michels S, Rosenfeld PJ, Puliafito CA, Marcus EN, Venkatraman AS. Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration: twelve-week results of an uncontrolled open-label clinical study. *Ophthalmology.* 2005;112:1035-1047.
64. Nguyen QD, Shah S, Tatlipinar S, et al. Bevacizumab suppresses choroidal neovascularisation caused by pathological myopia. *Br J Ophthalmol.* 2005;89:1368-1370.
65. Spaide RF, Fisher YL. Intravitreal bevacizumab (Avastin) treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. *Retina.* 2006;26:275-278.
66. Avery RL. Regression of retinal and iris neovascularization after intravitreal bevacizumab (Avastin) treatment. *Retina.* 2006;26:352-354.
67. Jorge R, Costa RA, Calucci D, Cintra L, Scott IU. Intravitreal bevacizumab (Avastin) for persistent new vessels in diabetic retinopathy (IBEPe Study). *Retina.* In press.

68. Avery RL, Pieramici DJ, Rabena MD, et al. Intravitreal bevacizumab(Avastin) for neovascular age-related macular degeneration. *Ophthalmology*. 2006;113:363-372.
69. Spaide RF, Laud K, Fine H, et al. Intravitreal bevacizumab treatment of choroidal neovascularization secondary to age-related macular degeneration. *Retina*. 2006;26:383-390.
70. Bashshur ZF, Bazarbachi A, Schakal A, et al. Intravitreal bevacizumab for the management of choroidal neovascularization in age-related macular degeneration. *Am J Ophthalmol*. Published online April 16, 2006.
71. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285:1182-1186.
72. Witmer AN, Vrensen GFJM, Van Noorden CJF, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. *Prog Retin Eye Res*. 2003;22:1-29.
73. Iturralde D, Spaide RF, Meyerle CB, et al. Intravitreal bevacizumab (Avastin) treatment of macular edema in central retinal vein occlusion: a short-term study. *Retina*. 2006;26:279-284.
74. Manzano RP, Peyman GA, Khan P, Kivilcim M. Testing intravitreal toxicity of bevacizumab (Avastin). *Retina*. 2006;26:257-261.
75. Maturi RK, Bleau LA, Wilson DL. Electrophysiologic findings after intravitreal bevacizumab (Avastin) treatment. *Retina*. 2006;26:270-274.
76. Oshima Y, Sakaguchi H, Gomi F, Tano Y. Regression of iris neovascularization after intravitreal injection of bevacizumab in patients with proliferative diabetic retinopathy. *Am J Ophthalmol*. Published online March 20, 2006.
77. Paula JS, Jorge R, Costa RA, Rodrigues MLV, Scott IU. Short-term results of intravitreal bevacizumab (Avastin) on anterior segment neovascularization in neovascular glaucoma. *Acta Ophthalmol Scand*. In press.