SHORT-TERM SAFETY AND EFFICACY OF INTRAVITREAL BEVACIZUMAB (AVASTIN) FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

RYAN M. RICH, MD, PHILIP J. ROSENFELD, MD, PhD, CARMEN A. PULIAFITO, MD, MBA, SANDER R. DUBOVY, MD, JANET L. DAVIS, MD, HARRY W. FLYNN JR., MD, SERAFIN GONZALEZ, PharmD, WILLIAM J. FEUER, MS, RICHARD C. LIN, MD, PhD, GEETA A. LALWANI, MD, JACKIE K. NGUYEN, MD, GAURAV KUMAR, BA

Purpose: To evaluate the safety and efficacy of intravitreal bevacizumab (Avastin, Genentech Inc.) for the treatment of neovascular age-related macular degeneration (ARMD).

Methods: A retrospective review was performed on consented patients with neovascular ARMD receiving intravitreal bevacizumab therapy. All patients received intravitreal bevacizumab at baseline with additional monthly injections given at the discretion of the treating physician. At each visit, a routine Snellen visual acuity assessment was performed followed by an ophthalmic examination and optical coherence tomography (OCT) imaging.

Results: Fifty–three eyes of 50 patients received an intravitreal bevacizumab injection between May and August 2005. Including the month 3 visit, the average number of injections was 2.3 out of a maximum of 4 injections. No serious drug-related ocular or systemic adverse events were identified. Improvements in visual acuity and central retinal thickness measurements were evident by week 1 and continued through month 3. At month 3, the mean visual acuity improved from 20/160 to 20/125 ($P<0.001$) and the mean central retinal thickness decreased by 99.6 μm ($P<0.001$).

Conclusion: Off-label intravitreal bevacizumab therapy for neovascular ARMD was well tolerated over 3 months with improvements in visual acuity and OCT central retinal thickness measurements. While the long-term safety and efficacy of intravitreal bevacizumab remain unknown, these short-term results suggest that intravitreal bevacizumab may be the most cost effective therapy for the treatment of neovascular ARMD.

RETINA 26:495–511, 2006

From Bascom Palmer Eye Institute, Department of Ophthalmology, University of Miami School of Medicine, Florida.

Presented in part at the Retina Subspecialty Day during the American Academy of Ophthalmology meeting; Chicago, Illinois; October 14, 2005.

No financial support was received from Genentech, Inc. to perform this retrospective review. Carmen A. Puliafito, MD, MBA, is listed on a patent for optical coherence tomography and receives royalties.

Supported by the Department of Ophthalmology at the Bascom Palmer Eye Institute, Miami, Florida, and by an unrestricted grant from Research to Prevent Blindness, Inc., New York, New York.

Reprint requests: Philip J. Rosenfeld, MD, PhD, Bascom Palmer Eye Institute, University of Miami School of Medicine, 900 N.W. 17th Street, Miami, FL 33136; e-mail: prosenfeld@med.miami.edu

Vascular endothelial growth factor-A (VEGF) has been implicated as the major angiogenic stimulus responsible for neovascularization in age-related macular degeneration (ARMD).[1–6] The first anti-VEGF drug approved by the Food and Drug Administration (FDA) for the treatment of neovascular ARMD is pegaptanib sodium (MACUGEN, Eyetech/OSI Pharmaceuticals).[7] Pegaptanib binds and inhibits the extracellular isoforms of VEGF that are at least 165 amino acids in length.[8] In the phase III clinical trial using pegaptanib for the treatment of neovascular ARMD, repeated intravitreal injections of pegaptanib every 6 weeks slowed the rate of vision loss compared with a sham-injected control group after 1 year.[7] Among the patients receiving pegaptanib therapy (0.3 mg) in this study, only 6% had significant vision improvement compared with 2% in the sham-treated group.

In contrast to pegaptanib therapy, injections with a different anti-VEGF drug known as ranibizumab (LUCENTIS, Genentech Inc.) resulted in vision improvement in phase III clinical trials for the treatment of neovascular ARMD. [9–11] Ranibizumab was shown to improve average visual acuity with 95% of patients having stable or improved visual acuity after receiving monthly injections for at least 1 year. In addition, 40% of patients achieved a level of visual acuity of at least 20/40. The superior results using ranibizumab compared with pegaptanib can most likely be explained by the differences in how the two drugs bind and inhibit VEGF. Unlike pegaptanib, ranibizumab binds all the biologically active forms of VEGF, including the isoforms and proteolytic breakdown products that contain fewer than 165 amino acids.[12,13]

Bevacizumab (AVASTIN, Genentech, Inc.), a full-length, humanized monoclonal antibody against VEGF, also binds and inhibits all the biologically active forms of VEGF much like ranibizumab.[14–16] The similar VEGF binding properties of bevacizumab and ranibizumab can be explained by their common molecular lineage. Both drugs are proteins that were genetically modified from the same murine monoclonal antibody against VEGF. The two proteins differ in their size and affinity for VEGF. While bevacizumab is a humanized, murine full-length antibody with two binding sites for VEGF, ranibizumab is a humanized, murine antigen binding fragment (Fab) with only a single affinity-matured binding site for VEGF.[16] The single binding site of ranibizumab has a dissociation constant (Kd) for VEGF of approximately 0.140 nM compared with an overall Kd for bevacizumab of approximately 0.5 nM to 1.0 nM.[14–16]

Ranibizumab is not yet approved by the FDA, but bevacizumab is approved for the intravenous treatment of metastatic colorectal cancer. When bevacizumab was approved by the FDA in February 2004, we initiated the Systemic Avastin for Neovascular ARMD Study or SANA Study to investigate the use of systemic bevacizumab for the treatment of neovascular ARMD. Our first cohort of 9 patients were observed for 12 weeks and had improved visual acuity with resolution of leakage from their neovascular lesions after just two or three doses of bevacizumab (5 mg/kg).[17] Except for a mild elevation in blood pressure that was easily controlled with antihypertensive medications, no other adverse events were identified. This study was expanded to include a second cohort of 9 patients and all 18 patients have been followed through 24 weeks with similar results.[18] While no additional adverse events were identified after 6 months, there was always a concern that high dose systemic therapy with bevacizumab (5 mg/kg) could result in an increased risk of thromboembolic events, the most frequent life-threatening drug-related adverse event associated with bevacizumab therapy in cancer patients and of particular concern in the older ARMD population.[19]

One way to decrease the potential risk of drug-related adverse events would be to decrease the dose of bevacizumab, and one way to decrease the dose would be to inject a small amount of drug directly into the eye. Bevacizumab was thought to be too large to penetrate the retina, a presumed requirement for any drug intended to treat neovascularization under the retina.[20] However, upon review of the literature, we found that bevacizumab was never tested to determine if it could penetrate the retina. Instead, a different antibody against an antigen known as HER-2 was used in those penetration experiments, and HER-2 is expressed in the inner retina and may have inhibited penetration from the vitreal cavity through the retina.[20] More importantly, the basic premise that retinal penetration was a requirement for the treatment of choroidal neovascularization was never tested. Intravitreal bevacizumab was never injected in any animal model of choroidal neovascularization. Even if retinal penetration was important for efficacy, it was unclear how much retinal penetration was necessary to achieve efficacy and whether penetration through a diseased human retina would be the same as the penetration through a normal animal retina. We subsequently learned that Han et al had shown that a full-length antibody was capable of penetrating a normal rabbit retina.[21]

The full-length murine precursor of bevacizumab was shown to be effective for the treatment of iris neovascularization in an animal model of neovascular glaucoma.[22] When cynomolgus monkey eyes received multiple injections of a murine anti-VEGF antibody, not only was iris neovascularization prevented but

Table 1. Changes in Central Retinal Thickness through 3 Months

	Baseline Central Retinal Thickness (μm), n = 53 Eyes	Week 1 Central Retinal Thickness (μm), n = 32 Eyes	Month 1 Central Retinal Thickness (μm), n = 51 Eyes	Month 2 Central Retinal Thickness (μm), n = 42 Eyes	Month 3 Central Retinal Thickness (μm), n = 53 Eyes	Decrease in Central Retinal Thickness (μm) from Baseline to Month 3
Median		249	242	232	228	
P value*	313	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	−85
Mean		260.7	253.5	240.2	251.4	
P value†	351	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	−99.6

*Paired Wilcoxon signed rank test.
†Paired Student *t* test.

there was no inflammation in this cross-species experiment. This study provided support for the idea that an anti-VEGF antibody could be injected into the eye without causing ocular complications even across different species.

Even though there were limited data available, we offered intravitreal bevacizumab, a humanized murine monoclonal antibody, to a patient with neovascular ARMD who had failed verteporfin photodynamic therapy (PDT) and pegaptanib therapy and was continuing to lose vision.[23] One week after the bevacizumab injection, OCT revealed dramatic improvement in the central retinal thickness of the macula, and 1 month after the injection, fluorescein angiography showed no evidence of leakage from the neovascular lesion. The response to intravitreal bevacizumab was very similar to the responses previously seen with intravenous bevacizumab and with intravitreal ranibizumab in the early phase I/II studies.[24,25] Based on our preliminary experience with intravitreal bevacizumab, we began to offer off-label intravitreal injections of bevacizumab primarily to patients with neovascular ARMD who were losing vision despite receiving FDA-approved therapies such as PDT and pegaptanib therapy. This retrospective report describes our initial 3-month experience using intravitreal injections of bevacizumab for the treatment of neovascular ARMD at the Bascom Palmer Eye Institute.

Patients and Methods

Approval for this retrospective review was obtained from the Institutional Review Board (IRB)/Ethics Committee at the University of Miami School of Medicine. All patients signed an informed consent to participate in this retrospective review. To be eligible for this retrospective review, patients had received an intravitreal injection of bevacizumab as part of their routine clinical care for the treatment of neovascular ARMD at the Bascom Palmer Eye Institute. Intravitreal bevacizumab was primarily offered to patients who were losing vision while undergoing treatment with FDA-approved therapies for neovascular ARMD or as primary therapy only after a thorough discussion of all their therapeutic options. All patients had evidence of increased 1 mm central retinal thickness as determined by optical coherence tomography (Stratus OCT, Version 4.0.2, Carl Zeiss Meditec, Dublin, CA). This increased central retinal thickness consisted of subretinal fluid and/or cystic changes within the retina. Before each injection of bevacizumab, patients signed a standard institutional consent describing the potential risks and benefits of treatment. Although there were no formal exclusion criteria, patients with a history of uncontrolled hypertension and recent thromboembolic events were not usually injected with bevacizumab, but this decision was at the discretion of the treating physician.

At each visit, patients underwent Snellen visual acuity measurements according to the procedures followed by individual physicians within their practice. An attempt was made to obtain best-corrected visual acuity at each visit; however, visual acuity measurements were not standardized and were performed as part of routine clinical care. At each visit, an ophthalmic examination was performed consisting of a slit-lamp evaluation and a biomicroscopic fundus examination. Ocular imaging consisted of fluorescein angiography and/or OCT at the time of the first bevacizumab injection and at each follow-up visit. Most patients did undergo OCT imaging at each visit (Table 1). OCT imaging consisted of 6-diagonal fast, low-density (low resolution, 128 a-scans per diagonal) 6 mm scans and 6-diagonal slow, high-density (high resolution, 512 a-scans per diagonal) 6 mm scans performed at 30 degree intervals. The 1 mm central retinal thickness measurements were determined from the fast macular thickness maps calculated from the 6 low resolution diagonal scans. The 6 high-density,

high resolution radial diagonal scans were used to qualitatively evaluate the macula and to determine if retreatment was needed.

In May 2005, when the off-label use of intravitreal bevacizumab was initiated, a patient's blood pressure was not routinely measured before an intravitreal injection at the Bascom Palmer Eye Institute. This policy was subsequently changed so that all patients receiving an intravitreal injection of any drug underwent blood pressure monitoring. However, no standard protocol for measuring blood pressure was implemented. As a result, blood pressure was measured manually or by using an automated blood pressure monitor. Only one measurement was routinely taken just before injection. Patients with systolic blood pressures above 150 mmHg and diastolic blood pressures above 90 mmHg were routinely referred to their internists for further evaluation and management.

All intravitreal injections were performed using a standard protocol at the Bascom Palmer Eye Institute. Preinjection antibiotic drops were not routinely used. The eye was topically anesthetized and a povidone-iodine (10%) scrub was performed on the lids and lashes. A sterile speculum was placed between the lids. Povidone-iodine (5%) drops were then applied over the ocular surface three times several minutes apart. Additional topical anesthesia was achieved by applying a sterile cotton swab soaked in sterile 4% lidocaine to the area designated for injection in the inferotemporal quadrant. Bevacizumab (0.05 mL; 1.25 mg) in a tuberculin syringe with a 30-gauge needle was then injected through the pars plana into the vitreous cavity inserted through the sclera 3 to 4 mm posterior to the limbus. Postinjection light perception was assessed and the intraocular pressure (IOP) was monitored until the IOP was below 30 mmHg. After the injection, the patient was instructed to apply topical antibiotics to the injected eye 4 times a day for 3 days. All patients received a call within 24 hours to assess their status and remind them to take their antibiotic drops.

The timing of the postinjection follow-up visits and the need for retreatment with intravitreal bevacizumab were at the discretion of the treating physician; however, most patients were seen monthly and injected if there was evidence of cystic maculopathy and/or subretinal fluid as determined by OCT imaging. Some patients also underwent a safety visit 1 week after the first injection.

Preparation of Bevacizumab for Intravitreal Injection

Bevacizumab is commercially available as a solution (100 mg; 25 mg/mL) and was not diluted, reconstituted, or altered in any way. All manipulations of bevacizumab were performed using proper aseptic technique under a laminar flow hood (IS0 Class 5) by a licensed and registered pharmacy in compliance with United States Pharmacopeia (USP) guidelines in Chapter 797. The vial of bevacizumab was punctured with a device called a Mini-Spike Dispensing Pin with Security Clips (B-Braun, catalog # DP-1000SC). The use of this or a similar device is recommended because bevacizumab is available in a single-use vial so entering the vial multiple times is not recommended. While this device contains a bacterial retentive air-venting filter, it does not actually filter the drug itself, and filtering of the drug was not performed due to the possibility that the protein could stick to the filter. Approximately 0.12 mL of bevacizumab was drawn up into multiple 1 mL polypropylene tuberculin syringes (Becton Dickinson & Co., Franklin, NJ, Ref # 309602, NDC # 08290309602). A sterile cap was then placed on each syringe. The syringes were then labeled, placed in light-resistant brown bags to protect bevacizumab from light, and stored in a refrigerator at 2 to 8 ˚C until used for injection. Approximately 25 syringes were prepared from each 4 mL vial of bevacizumab. At least two syringes were submitted to the Microbiology Department for sterility and endotoxin testing. A 14-day expiration was assigned to each syringe based on USP Chapter 797 for a low-risk, refrigerated preparation. A 14-day expiration date was considered to be a conservative estimate of stability because the drug was known to be stable in its original glass vial for 18 months. If all testing results were negative, then the syringe was relabeled to have a 90-day expiration date; however, this practice was abandoned because the long-term stability of bevacizumab in syringes was unknown and the syringes were routinely used within the original 14-day expiration date. The syringe containing bevacizumab was removed from the refrigerator before injection and did not remain at room temperature for longer than 30 minutes. Before injection, a sterile standard 30-gauge needle (5/8 inch) was placed on the syringe, and the plunger was advanced to 0.05 mL (50 μL) so that all the dead space was removed. Stability information for the drug in the syringes is not currently available.

Statistical Analysis

For purposes of statistical analysis, all Snellen visual acuity data were converted to an equivalent letter score from a standard 2-m protocol using an Early Treatment Diabetic Retinopathy chart.[26] Data were statistically analyzed using the paired Student *t* test for changes in mean blood pressure measurements, visual

Table 2. Change in Visual Acuity through 3 Months

	Baseline Visual Acuity Letters/ Snellen Equivalent, n = 53 Eyes	Week 1 Visual Acuity Letters/ Snellen Equivalent, n = 32 Eyes	Month 1 Visual Acuity Letters/ Snellen Equivalent, n = 51 Eyes	Month 2 Visual Acuity Letters/ Snellen Equivalent, n = 42 Eyes	Month 3 Visual Acuity Letters/ Snellen Equivalent, n = 53 Eyes	Change in Visual Acuity from Baseline to Month 3 in Letters
Median	35	35	35	55	50	+15
P value*	20/200	20/200 $P = 0.003$	20/200 $P < 0.001$	20/80 $P = 0.001$	20/100 $P < 0.001$	
Mean	38.1	43.8	44.1	46.9	46	+7.9
P value†	$20/160^{-1}$	$20/125^{-1}$ $P = 0.005$	$20/125^{-1}$ $P < 0.001$	$20/125^{+2}$ $P = 0.001$	$20/125^{+1}$ $P < 0.001$	

*Paired Wilcoxon signed rank test.
†Paired Student *t* test.

acuity letter scores, and central retinal thickness measurements at week 1 through month 3 compared with mean baseline values. Median measurements at week 1 through month 3 were compared with median baseline values using the paired Wilcoxon signed rank test. Systolic blood pressure values were analyzed separately from the diastolic values. Statistical significance was defined as $P < 0.05$.

Results

Baseline Characteristics

A total of 53 eyes from 50 consecutively consented patients received an initial intravitreal injection of bevacizumab during the period from May 2005 to August 2005. Three patients had bevacizumab injected into both eyes. These 50 patients had a mean age of 78 years and a median age of 80 years (range: 62 to 91 years). There were 28 women (56%). Of the 53 eyes, 40 eyes (75%) had received some prior therapy before receiving an intravitreal injection of bevacizumab. Prior therapy consisted of PDT in 23 eyes, pegaptanib therapy in 33 eyes, and PDT progressing to pegaptanib therapy in 16 eyes. Thirteen eyes (25%) received intravitreal bevacizumab as primary therapy. The baseline median and mean visual acuity and OCT central retinal thickness measurements are shown in Tables 1 and 2. At baseline, median and mean visual acuity measurements were 20/200 and 20/160, respectively. Baseline median and mean 1 mm central retinal thickness measurements were 313 μm and 351 μm, respectively. Blood pressure measurements were performed on only 20 patients at baseline. The median and mean systolic/diastolic blood pressure values were the same at 130/80.

Safety

Table 3 summarizes the number of injections performed during the first 3 months. A total of 123 injections of bevacizumab were performed. On average, an eye received 2.3 injections out of the maximum of 4 injections if a patient had received an injection at baseline, month 1, month 2, and month 3. There were no episodes of inflammation or severe vision decrease immediately after an injection. There were no cases of endophthalmitis, retinal detachment, or lens damage. During the 3 months, there were no thromboembolic events which included cerebrovascular accidents, transient ischemic attacks, myocardial infarctions, or peripheral vascular disease.

Based on the 1 year data from the Phase III pegaptanib trial, an annual thromboembolic rate of 6% and an annual death rate of 2% would be expected.[7] In this retrospective study with a sample size of 50 patients over 3 months, the probability of detecting at least one thromboembolic event given a true 3 month

Table 3. Distribution of Injections through Month 3

Follow-up Visit	Number of eyes injected at this visit (%)	Total of one injection, n (%)	Total of two injections, n (%)	Total of three injections, n (%)	Total of four injections, n (%)	Average number of injections per eye ± SD
Month 1 (n = 51)	28 (55)	23 (45)	28 (55)	0	0	1.5 ± 0.5
Month 2 (n = 42)	21 (50)	12 (29)	19 (45)	11 (26)	0	2.0 ± 0.7
Month 3 (n = 53)	21 (40)	11 (21)	19 (36)	18 (34)	5 (9)	2.3 ± 0.9

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.