

Tolerability and Efficacy of Multiple Escalating Doses of Ranibizumab (Lucentis) for Neovascular Age-Related Macular Degeneration

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Purpose: To investigate whether multiple intravitreal doses of up to 2 mg of an antigen-binding fragment known as ranibizumab, derived from a humanized anti-vascular endothelial growth factor antibody, can be tolerated and are biologically active when injected using a dose-escalating strategy in eyes of patients with neovascular age-related macular degeneration (AMD).

Design: Open-label, 2-center, uncontrolled, randomized clinical study of 3 different dose-escalating regimens of ranibizumab.

Participants: Thirty-two patients with primary or recurrent subfoveal choroidal neovascularization secondary to AMD were enrolled. Baseline best-corrected visual acuity (VA) in the study eye was from 20/40 to 20/640 (Snellen equivalent).

Methods: Treatment regimens consisted of 5, 7, or 9 intravitreal injections of ranibizumab at 2- or 4-week intervals for 16 weeks, with escalating doses ranging from 0.3 to 2.0 mg. Patients were evaluated through day 140, 4 weeks after their last injection.

Main Outcome Measures: Safety was assessed based on ocular and nonocular adverse events, changes in VA, changes in intraocular pressure (IOP), slit-lamp ocular examination, changes in lesion characteristics based on fluorescein angiography and color fundus photography, and the presence of anti-ranibizumab antibodies.

Results: Twenty-nine patients received an injection at baseline, and 27 patients completed the study through day 140. Results were similar across the 3 treatment groups. All patients experienced ocular adverse events, most of which were mild. The most common ocular adverse events were iridocyclitis (83%) and injection-site reactions (72%). Inflammation did not increase with repeated injections, despite the increasing ranibizumab doses. Transient mild IOP elevations were common after ranibizumab injection. No serum anti-ranibizumab antibodies were detected. Overall, median and mean VAs in the study eyes improved by day 140 in all 3 groups. Only 3 of the 27 patients lost significant vision. There was no significant lesion growth, and a decrease in area of leakage from choroidal neovascularization was detected through day 140.

Conclusions: Multiple intravitreal injections of ranibizumab at escalating doses ranging from 0.3 to 2.0 mg were well tolerated and biologically active in eyes with neovascular AMD through 20 weeks. Mild transient ocular inflammation was the most common postinjection adverse event. *Ophthalmology* 2006;113:623–632 © 2006 by the American Academy of Ophthalmology.



Choroidal neovascularization is the major cause of severe vision loss in patients with age-related macular degeneration (AMD).^{1–4} In neovascular AMD, vascular endothelial growth factor (VEGF), a secreted, diffusible, pro-angiogenic

protein, is implicated as one of the major angiogenic factors responsible for neovascularization due to increased levels of expression in close proximity to choroidal neovascularization.^{5–9} Vascular endothelial growth factor has been shown

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to increase vascular permeability, induce vascular endothelial cell proliferation and promote endothelial cell survival, and serve as a chemotactic factor for leukocytes, all properties thought to be important for its role as an angiogenic factor.¹⁰ However, the most compelling evidence supporting the role of VEGF in promoting retinal and choroidal neovascularization arises from animal models in which intraocular VEGF levels are manipulated to induce or inhibit neovascularization.^{11–14} These data suggest that inhibition of VEGF could be a reasonable approach for the treatment or prevention of choroidal neovascularization in patients with AMD. This approach has now been validated in phase III clinical trials using an anti-VEGF drug known as pegaptanib sodium (Macugen, Eyetech Pharmaceuticals, New York, New York) for the treatment of neovascular AMD.¹⁵ Pegaptanib sodium is an aptamer capable of binding isoforms of VEGF that are ≥ 165 amino acids in length.

In contrast to pegaptanib sodium, ranibizumab (Lucentis, Genentech, Inc., South San Francisco, CA) is a novel antigen-binding fragment (Fab) derived from a humanized anti-VEGF antibody, and this Fab inhibits all biologically active isoforms and proteolytic fragments of VEGF.¹⁶ Ranibizumab competitively binds VEGF and inhibits its activity, based on *in vitro* vascular endothelial survival assays (Genentech, Inc., unpublished data). The Fab is derived from the same parent molecule as the full-length humanized anti-VEGF antibody bevacizumab,¹⁷ the first anti-VEGF agent with demonstrated efficacy in the treatment of cancer.¹⁸ Bevacizumab was recently approved by the Food and Drug Administration for the treatment of metastatic colorectal cancer in combination with 5-fluorouracil-based chemotherapy. The Fab molecule was developed for local ocular anti-angiogenic therapy, where a molecule smaller than a full-sized antibody is likely to have better penetration through all layers of the retina after an intravitreal injection compared with a full-sized antibody.¹⁹ In a primate model, laser-induced choroidal neovascularization was prevented in eyes pretreated with intravitreal injections of ranibizumab, and eyes with established laser-induced choroidal neovascularization showed decreased fluorescein angiographic leakage and more rapid resolution of choroidal neovascularization after intravitreal injections of ranibizumab.¹³

Three studies were designed to investigate the doses of ranibizumab that would be appropriate for the treatment of neovascular AMD in large, multicenter, randomized phase III trials. Based on these early phase I and II studies, the phase III trials were initiated, and the first positive results were presented on July 18, 2005 at the annual meeting of the American Society of Retinal Specialists. In the initial phase I dose-escalating study with neovascular AMD patients, 0.5 mg of ranibizumab was identified as the maximum tolerated single intravitreal dose, with intraocular inflammation the dose-limiting toxicity.²⁰ The second, larger, phase I/II clinical study investigated the tolerability and efficacy of multiple (up to 8) monthly intravitreal injections of ranibizumab at doses of 0.3 mg or 0.5 mg in patients with neovascular AMD. In the third trial, the phase I clinical study described in this report, doses of ranibizumab above 0.5 mg were investigated to determine if doses higher than the maximum

tolerated single dose could be tolerated when injected in an escalating stepwise fashion every 2 weeks or 4 weeks. This study was undertaken before completion of the larger phase I/II study because there was a possibility that doses of ranibizumab higher than 0.5 mg might be necessary to achieve a therapeutic benefit. This dose escalation strategy was based on primate data suggesting that dose-limiting intraocular inflammation could be avoided by increasing the dose with each sequential injection (unpublished data [Genentech studies 98-361-1757, 99-539-1757]). In these studies, cynomolgus monkeys received intravitreal injections of ranibizumab as frequently as every 2 weeks, escalating to a maximum dose of 2 mg. In these monkeys, inflammation was attenuated with higher doses, even when a 2-fold increase in the dose was given sequentially. This strategy permitted the safe administration of doses up to 2 mg. The goal of this dose-escalation study in humans was to determine if higher doses, up to 2 mg of ranibizumab, could be safely administered using a dose-escalation strategy and whether the higher doses appeared to be more efficacious than the 0.3- and 0.5-mg doses in the other study that was in progress. This report describes the tolerability and biological activity of 3 strategies used to escalate the dose of ranibizumab from 0.3 mg to a maximum of 2.0 mg (Genentech study FVF2425g).

Patients and Methods

This phase I, open-label, randomized study of 3 escalating multiple-dose regimens of intravitreal ranibizumab was conducted between March 14, 2002 and October 3, 2003. Institutional review boards of the 2 study sites approved the protocol, and all patients provided written informed consent before determination of their eligibility. The Health Insurance Portability and Accountability Act of 1996 was implemented during the study period, and the informed consent form was then modified to comply with the Act's requirements.

The inclusion and exclusion criteria for this study were nearly identical to the previously published criteria for the phase I study designated FVF1770g.²⁰ The only significant difference in the inclusion criteria for this study was the requirement of a best-corrected Snellen equivalent visual acuity (VA) of 20/40 to 20/400 in the study eye using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart. After a 14-day screening period to determine patients' eligibility and obtain baseline data, patients were randomized in a 1:1:1 ratio to receive 1 of 3 escalating dose regimens of ranibizumab. Each patient received injections of ranibizumab in only one eye (the study eye). The study schema is presented in Table 1. Group 1 patients received a total of 7 injections (at 2-week intervals during weeks 0–8, then at 4-week intervals during weeks 8–16) at the following doses: 0.3 mg at week 0, 0.5 mg at week 2, 0.75 mg at week 4, and 1.0 mg at weeks 6, 8, 12, and 16. Group 2 patients received a total of 9 injections at 2-week intervals: 0.3 mg at week 0, 0.5 mg at week 2, 0.75 mg at week 4, 1.0 mg at week 6, 1.25 mg at week 8, 1.5 mg at week 10, 1.75 mg at week 12, and 2.0 mg at weeks 14 and 16. Group 3 patients received a total of 5 injections at 4-week intervals: 0.3 mg at week 0, 0.5 mg at week 4, 1.0 mg at week 8, 1.5 mg at week 12, and 2.0 mg at week 16.

Ranibizumab was reconstituted from a lyophilized preparation and injected into the vitreous by a retina specialist using a 30-gauge 0.5-inch needle attached to a syringe containing 0.05

Table 1. Study Schema

		Week										
		-2	0	2	4	6	8	10	12	14	16	20
Group 1 (n = 6)	Screening period	▲	▲	▲	▲	▲	▲		▲		▲	Final study visit
		0.3	0.5	0.75	1.0	1.0			1.0		1.0	
Group 2 (n = 6)		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	
		0.3	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.0	2.0	
Group 3 (n = 6)	Screening period	▲		▲		▲		▲		▲		Final study visit
		0.3		0.5		1.0		1.5		2.0		

▲, ranibizumab administration (no. indicates dose in milligrams).

ml (50 μ l) of ranibizumab solution. The preparation of the eye before injection included the use of povidone-iodine and/or broad-spectrum antibiotic as determined by the injecting physician at the clinical site. Topical and/or subconjunctival lidocaine was applied. Ranibizumab was administered by intravitreal injection through an area 3.5 to 4.0 mm posterior to the limbus, avoiding the horizontal meridian and aiming toward the center of the globe. Injection sites were rotated from injection to injection.

The measures of the safety and tolerability of intravitreal ranibizumab injections were the incidence and severity of ocular adverse events (as identified by eye examination, including VA testing, at each study visit and by fundus photography and fluorescein angiography at study day 56 and day 140); incidence and severity of other adverse events, as identified by systemic review of systems, physical examinations (every 4 weeks), changes in vital signs (every visit), and clinical laboratory abnormalities (hematology, blood chemistries, and urinalyses every 8 weeks); and changes in fundus appearance compared with baseline (screening), as assessed by fluorescein angiography and fundus photography. Adverse event monitoring immediately after each ranibizumab injection included measurement of light perception at 5 and 30 minutes and comparison of intraocular pressure (IOP) at 30 minutes with that just before injection. If the IOP was increased by ≥ 10 mmHg, it was measured again at 60 minutes. If the IOP was increased by ≥ 10 mmHg after 60 minutes, then this was designated a grade 1 targeted adverse event. Patients returned to the clinic 1 and 3 days after each injection for safety evaluation and examination. Detailed ocular examinations, including indirect ophthalmoscopy and slitlamp examination, were performed every 2 weeks through the study.

Biological activity was assessed by evaluating the change in best-corrected VA (BCVA), as assessed by the ETDRS chart at 2 m and the change in lesion size, leakage from choroidal neovascularization, and area of subretinal fluid (SRF) as determined by fluorescein angiography. Analyses of fundus photographs and fluorescein angiography images were performed retrospectively by masked readers at the Fundus Photography Reading Center at the University of Wisconsin, Madison. Optical coherence tomography was performed as an ancillary study at one of the sites (Bascom Palmer Eye Institute) and will be discussed in a separate report. Investigators at each study site were able to view all images during the study, but all interpretations and measurements were performed by the reading center. No formal analysis was performed to compare the interpretations of the investigators with those of the reading center. Visual acuity was measured by trained personnel at the study sites, who were masked to treatment eye and study group assignment.

The pharmacokinetic parameters evaluated were serum levels of ranibizumab and plasma levels of VEGF. Measurements of serum levels of ranibizumab and anti-ranibizumab antibody and of

plasma levels of VEGF were performed using enzyme-linked immunosorbent assay methods developed at Genentech, Inc.

The analysis chosen for the data was intent-to-treat, including all patients who were randomized and who had measurements completed through day 140, without imputation for missing observations. All data were collected on case report forms and analyzed using SAS software (SAS Institute, Cary, NC). Data were statistically analyzed using the paired Student's *t* test for changes in mean measurements. The paired Wilcoxon signed rank test was used to analyze changes in median measurements.

Results

Baseline Characteristics

Thirty-two patients were enrolled and randomized into the study: 11 into group 1, 10 into group 2, and 11 into group 3. During the screening period, a decision was made not to proceed with the first injection of ranibizumab in 3 of the 32 patients (based on the patient's request in 1 case and on the physician's clinical judgment in 2 cases). Twenty-nine patients received treatment: 9 in group 1, 10 in group 2, and 10 in group 3 (Table 2 [available at <http://aaojournal.org>]). Patients enrolled in the study were mostly >75 years old (84%) and white (81%). Nearly two thirds of patients (63%) were female, and although the majority of patients had smoked previously (72%), few were current smokers (6.3%).

All randomized patients had a BCVA from 20/40 to 20/400 (Snellen equivalent) in the study eye at their screening visits, with 1 patient losing vision to 20/640 at baseline, the day of the first injection (Table 3 [available at <http://aaojournal.org>]). Overall, the majority of randomized patients (84%) had a VA of 20/80 or worse. Median VAs (Snellen equivalents) were 20/160 in group 1, between 20/80 and 20/100 in group 2, and 20/125 in Group 3.

Among randomized patients, the average area of choroidal neovascularization comprised at least 50% of the average total lesion size (Table 3 [available at <http://aaojournal.org>]), with the mean total area of the lesions measuring 6.5 ± 4.8 disc areas, and the mean total area of choroidal neovascularization measuring 5.2 ± 4.6 disc areas. A majority of the lesions were classified as occult only or minimally classic choroidal neovascularization, with less than a third of the lesions classified as predominantly classic. Eleven (34%) of the patients had received ≥ 1 prior therapies for AMD in the study eye, including photodynamic therapy (7 patients), laser photocoagulation (3 patients), and ranibizumab (1 patient previously enrolled in a prior phase I study 2 years before randomization into the present study).

Table 4. Patient Disposition

Status	Group 1 (n = 11)	Group 2 (n = 10)	Group 3 (n = 11)	All Patients (n = 32)
Received study drug (treated population)*	9 (81.8%)	10 (100%)	10 (90.9%)	29 (90.6%)
Completed treatment (day 112)	9 (81.8%)	9 (90.0%)	9 (81.8%)	27 (84.4%)
Completed escalating-dose regimen†	8 (72.7%)	9 (90.0%)	6 (54.5%)	23 (71.9%)
Completed main study period (day 140)	9 (81.8%)	9 (90.0%)	9 (81.8%)	27 (84.4%)
Not treated or discontinued treatment				
Total	2 (18.2%)	1 (10.0%)	2 (18.2%)	5 (15.6%)
Adverse event‡	0	1 (10.0%)	1 (9.1%)	2 (6.3%)
Patient decision to withdraw before first injection	0	0	1 (9.1%)	1 (3.1%)
Physician decision to withdraw patient before first injection	2 (18.2%)	0	0	2 (6.3%)

*Three patients who were enrolled and randomized did not receive study treatment because they elected not to undergo an injection of ranibizumab at baseline day 0.

†The patient in group 2 discontinued because of iridocyclitis and decreased visual acuity (VA). The patient in group 3 experienced an adverse event (transient ischemic attack) that indirectly led to discontinuation because the patient was treated with warfarin—a concomitant medication prohibited by the study protocol.

‡Twenty-three patients received all scheduled injections at the planned dose levels. Of the 4 patients who received dose reductions, the reasons were inflammation (2), inflammation with reduced VA (1), and reduced VA (1).

Adverse Events and Patient Disposition

Of the 29 patients enrolled, randomized, and initially injected with ranibizumab, 27 (9 in each group) completed the main study period, with their last injection given on day 112 and follow-up through day 140 (Table 4). The 2 patients who did not complete the study (1 each in groups 2 and 3) received fewer than the planned number of injections for their assigned treatment regimens. One patient was withdrawn because of an adverse event (transient ischemic attack), and the other received the first 2 injections as scheduled and a lower dose at the following injection and was finally withdrawn from treatment because of decreased

VA (>15 letters) associated with iridocyclitis. Four patients (1 in group 1 and 3 in group 3) who received all scheduled injections had their doses reduced for some injections, per the study protocol, in response to adverse events (intraocular inflammation and reduced VA). Because of iridocyclitis, the patient in group 1 received the first 4 injections at 0.3 mg and the last 3 injections at 0.5 mg. Among the patients in group 3 who received reduced doses due to iridocyclitis, one patient received 2 doses at 0.3 mg and 3 doses at 0.5 mg, whereas a second patient received the first 4 scheduled injections up to 1.5 mg, and then the fifth dose was reduced to 1.0 mg. A third patient in group 3 received the first 4

Table 5. Number (%) of Patients with Treatment-Emergent Ocular Adverse Events in Study Eye (Events Reported for ≥5% of All Patients)

Ocular Adverse Event	Group 1 (n = 9)	Group 2 (n = 10)	Group 3 (n = 10)	All Patients (n = 29)
Any adverse event	9 (100%)	10 (100%)	10 (100%)	29 (100%)
Eye disorders				
Iris and uveal tract inflammation*	8 (89%)	9 (90%)	8 (80%)	25 (86%)
Iridocyclitis	8 (89%)	9 (90%)	7 (70%)	24 (83%)
Iritis	6 (67%)	4 (40%)	2 (20%)	12 (41%)
Conjunctival hemorrhage	5 (56%)	4 (40%)	4 (40%)	13 (45%)
Visual acuity reduced	2 (22%)	2 (20%)	3 (30%)	7 (24%)
Conjunctival hyperemia	1 (11%)	0	1 (10%)	2 (7%)
Eye pain	1 (11%)	1 (10%)	0	2 (7%)
Vitreous disorder	2 (22%)	1 (10%)	1 (10%)	4 (14%)
Vitreous detachment	1 (11%)	1 (10%)	0	2 (7%)
Vitreous floaters	1 (11%)	1 (10%)	0	2 (7%)
Vitreitis	1 (11%)	1 (10%)	1 (10%)	3 (10%)
Progression of cataracts	1 (11%)	1 (10%)	0	2 (7%)
Retinal hemorrhage	0	1 (10%)	1 (10%)	2 (7%)
Injection-site reactions	5 (56%)	8 (80%)	8 (80%)	21 (72%)
Injection-site erythema	3 (33%)	8 (80%)	7 (70%)	18 (62%)
Injection-site reaction	4 (44%)	7 (70%)	7 (70%)	18 (62%)
Injection-site hemorrhage	2 (22%)	5 (50%)	5 (50%)	12 (41%)
Intraocular pressure increased‡	3 (33%)	2 (20%)	3 (30%)	8 (28%)

*Iritis refers to the presence of cell and flare in the anterior chamber (AC), whereas iridocyclitis refers to the presence of cell and flare in the AC and the anterior vitreous.

‡Defined as an increase in the postinjection intraocular pressure of at least 10 mmHg recorded at 60 min after the injection.

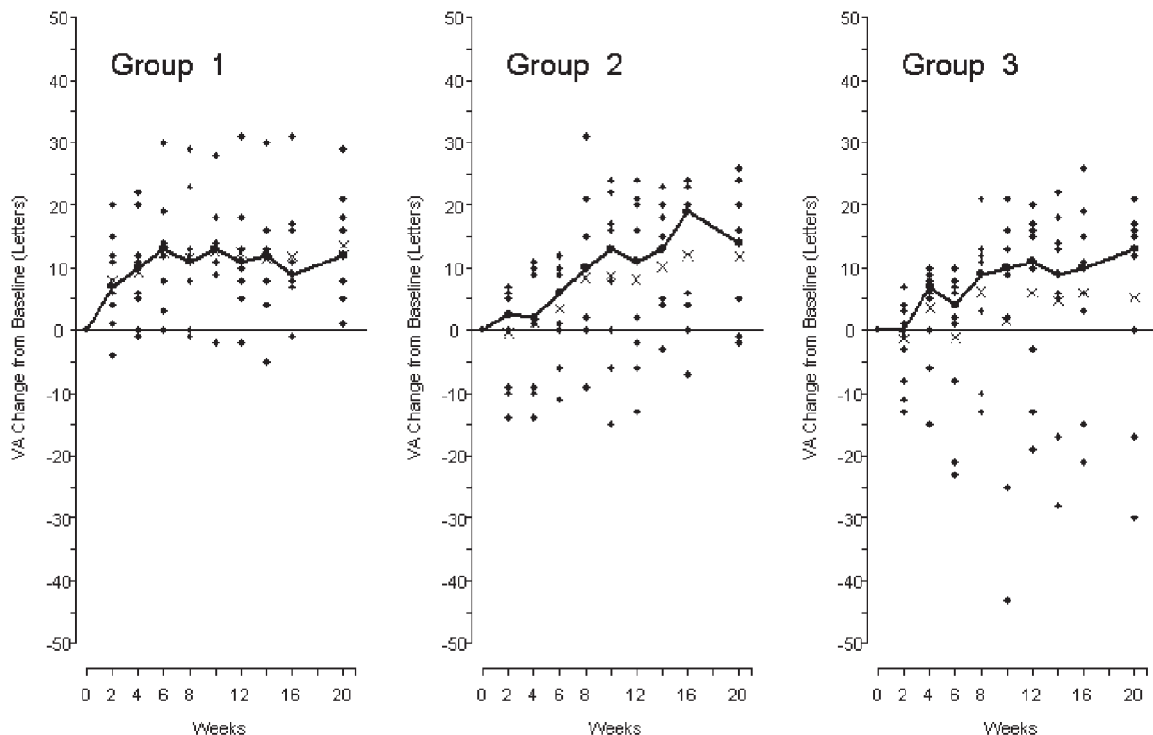


Figure 1. Change in visual acuity (VA) letter scores from baseline to day 140 for all patients in group 1 ($n = 9$), group 2 ($n = 9$), and group 3 ($n = 9$) given escalating doses of ranibizumab. Predose values are shown for each visit. $\times$ marks the mean value, and the line connects the median values. The mean increases in VA were statistically significant compared with baseline beginning at week 2 for group 1 ($P = 0.011$) and week 14 for group 2 ($P = 0.014$). Changes from baseline were not statistically significant for group 3.

injections as scheduled, then experienced a decrease in VA and received a reduced dose of 0.5 mg for the fifth injection.

All 29 treated patients experienced at least one ocular adverse event affecting the study eye during the treatment period (Table 5).

Table 6. Visual Acuity Changes from Baseline in Study Eye at Day 140 (Treated Patients)

Outcome Measure	Group 1 ($n = 9$)	Group 2 ($n = 9$)	Group 3 ($n = 9$)
No. of letters change from baseline			
n	9	9	9
Mean (SD)	13.6 (8.6)	11.9 (10.5)	5.2 (17.6)
P value	0.001	0.010	0.4
Median	12.0	14.0	13.0
P value	0.004	0.18	0.3
Range	1–29	–2 to 26	–30 to 21
Frequency distribution [n (%)]			
n	9	9	9
≥ 15 -letter increase	4 (44.4%)	4 (44.4%)	4 (44.4%)
≥ 10 -letter increase	6 (66.7%)	5 (55.6%)	6 (66.7%)
≥ 5 -letter increase	8 (88.9%)	7 (77.8%)	6 (66.7%)
± 4 letters (stable)	1 (11.1%)	2 (22.2%)	1 (11.1%)
≥ 15 -letter decrease	0 (0%)	0 (0%)	2 (22.2%)

SD = standard deviation.

Test of mean change from baseline is Student's t test. Test for median change from baseline is the paired Wilcoxon signed rank test. No pairwise comparisons (group 2 vs. group 1, group 3 vs. group 1, group 3 vs. group 2) showed a P value < 0.05 (Wilcoxon rank-sum test for pairwise comparisons).

No severe ocular adverse events were reported. Three events affecting the study eye that were categorized as moderate in severity were reported during the treatment period: retinal hemorrhage, nuclear sclerosis, and reduced VA; the latter 2 resolved by the end of the treatment period. No cases of endophthalmitis occurred during the study.

The most common category for presumed drug-related adverse events was iris and uveal tract inflammation. Inflammation occurred in 25 patients (86%) and included iridocyclitis (24 patients [83%]) and iritis (12 patients [41%]). Injection-related adverse events, which included erythema, hemorrhage, and events described simply as injection site reaction, were also common (21 patients [72%]). Thirteen patients (45%) experienced conjunctival hemorrhage, and 2 patients (7%)—1 each in groups 2 and 3—experienced worsening of a preexisting retinal hemorrhage. Reduced VA at some time during the study was reported in 7 patients (24%) (2 in group 1, 2 in group 2, and 3 in group 3) but persisted only in 2 patients in group 3 (Fig 1). Only one ocular adverse event led to permanent discontinuation of ranibizumab treatment. This was a case of mild iridocyclitis of the study eye, followed by decreased VA in that eye the following day, in a group 3 patient. The iridocyclitis developed on day 63, 1 day after the patient's third dose of ranibizumab. Adverse events affecting the nonstudy eye were reported in 6 patients (21%). The most common of these events was decreased VA (3 patients [10%]).

Postinjection mild and transient inflammatory reaction in the study eye—not necessarily related to ranibizumab—was common. Of the 29 treated patients, 25 experienced mild intraocular inflammation in the study eye, but for all but 4 of these patients, inflammation was never worse than trace to 1+. Although no formal statistical analysis was performed on the investigators' grading assessments, inflammation appeared slightly less frequent

and less severe after subsequent injections than after the first injection, despite the increasing ranibizumab doses within each treatment arm. Inflammation was rarely present 1 week after an injection. Only 1 of the 29 treated patients showed inflammation (trace) in the nonstudy eye at any visit.

Twenty of the 29 patients (69%) experienced at least one nonocular adverse event during the treatment period. The most common of these events were cardiac murmur (6 patients [21%]) and peripheral edema (6 patients [21%]). All nonocular adverse events reported during the treatment period were mild, with the exception of a moderate episode of bronchopneumonia. In addition, intraocular injection of the study drug was discontinued permanently in a patient in group 2 because she was started on systemic warfarin treatment after experiencing a mild transient ischemic attack. The only severe nonocular adverse event reported during the study—a subdural hematoma—began long after the treatment period had ended (day 388) and resolved within 2 weeks.

Intraocular pressure in the study eye was elevated from the corresponding predose pressure at 30 minutes after each dose. The mean IOP elevation typically was 4 to 5 mmHg, and in most patients, the IOP elevation was under 10 mmHg. Among those patients who had increases ≥ 10 mmHg at 30 minutes, the IOP had begun to decline by the 60-minute measurement, and measurements at 24 and 72 hours postdose were similar to predose pressures. In all but one patient, the IOP increases resolved without medication.

Posterior vitreal detachment was present in most patients (23 of 29) in both eyes before the first ranibizumab injection. Of 6 patients without posterior vitreal detachment at baseline, 2 developed it while on study. However, 1 of these 2 patients also developed posterior vitreal detachment in the nonstudy eye. No cases of vitreous hemorrhage were observed.

Fundus examinations revealed no cases of a retinal tear or

detachment in ranibizumab-treated patients, and no retinal hemorrhages in the study eye in any patient who did not have one at baseline. Two patients experienced significant worsening of a preexisting retinal hemorrhage while on treatment.

No clinically significant mean changes in clinical laboratory test values or vital signs occurred during the study. The plasma VEGF concentrations measured in the patients over the course of ranibizumab dose escalation in this study did not change significantly relative to those in pretreatment screening samples. No antibodies to ranibizumab were detected in any serum samples. Data on the pharmacokinetics of ranibizumab will be presented separately in another publication.

Visual Acuity Outcomes

Overall, mean and median VAs in the study eyes were above baseline values in all groups by day 140 (Table 6). The changes in median and mean VAs over time for each group are shown in Figure 1. During the study, the results were similar across treatment groups, with 44% of patients who completed treatment experiencing at least a 3-line (15 letters) improvement in VA measured using an ETDRS chart. The distribution of VA changes for each group is shown in Table 6.

One patient (group 3) who lost at least 3 lines of VA withdrew before day 140. Only 2 patients with ETDRS measurements at day 140, both in group 3, lost at least 3 lines of VA. The cause of vision loss in both these patients was a tear of the retinal pigment epithelium (RPE). In one patient, the RPE tear progressed through the foveal center after the first injection, and in the other patient, a significant submacular hemorrhage occurred after the first injection. Once the blood cleared in this patient, an extrafoveal tear of the RPE was observed, and this was most likely responsible for the hemorrhage. In both cases, VA remained diminished for the duration of the study.

Table 7. Lesion Characteristics at Day 140 in Study Eye (Treated Patients)

Change in Lesion Characteristics from Baseline	Group 1 (n = 9)	Group 2 (n = 10)	Group 3 (n = 10)
Total area of lesion (disc areas)			
n	9	8	8
Mean (SD)	-0.32 (1.63)	-0.19 (1.08)	0.36 (2.11)
Median	0.00	-0.13	0.47
Total area of choroidal neovascularization (disc areas)			
n	9	8	8
Mean (SD)	-1.08 (1.58)	-0.36 (1.28)	0.28 (2.98)
Median	-1.00	-0.25	-0.08
Area of classic choroidal neovascularization (disc areas)			
n	9	8	8
Mean (SD)	-0.17 (0.32)	-0.14 (0.30)	-0.69 (1.37)
Median	0.00	0.00	0.00
Total area of fluorescein leakage from choroidal neovascularization plus intense progressive RPE staining change from baseline (disc areas)			
n	9	8	8
Mean (SD)	-1.46 (1.43)	-0.59 (2.04)	0.74 (3.70)
Median	-1.25	-0.27	-0.70
Area of serous sensory retinal detachment/subretinal fluid (disc areas)			
n	7	8	9
Mean (SD)	-0.96 (3.61)	-2.75 (4.94)	-1.30 (3.66)
Median	-0.50	-1.50	-0.50

RPE = retinal pigment epithelium; SD = standard deviation.

No pairwise comparisons (group 2 vs. group 1, group 3 vs. group 1, group 3 vs. group 2) showed a *P* value < 0.05 (Kruskal-Wallis test for continuous variables, Fisher exact test for comparing proportions).

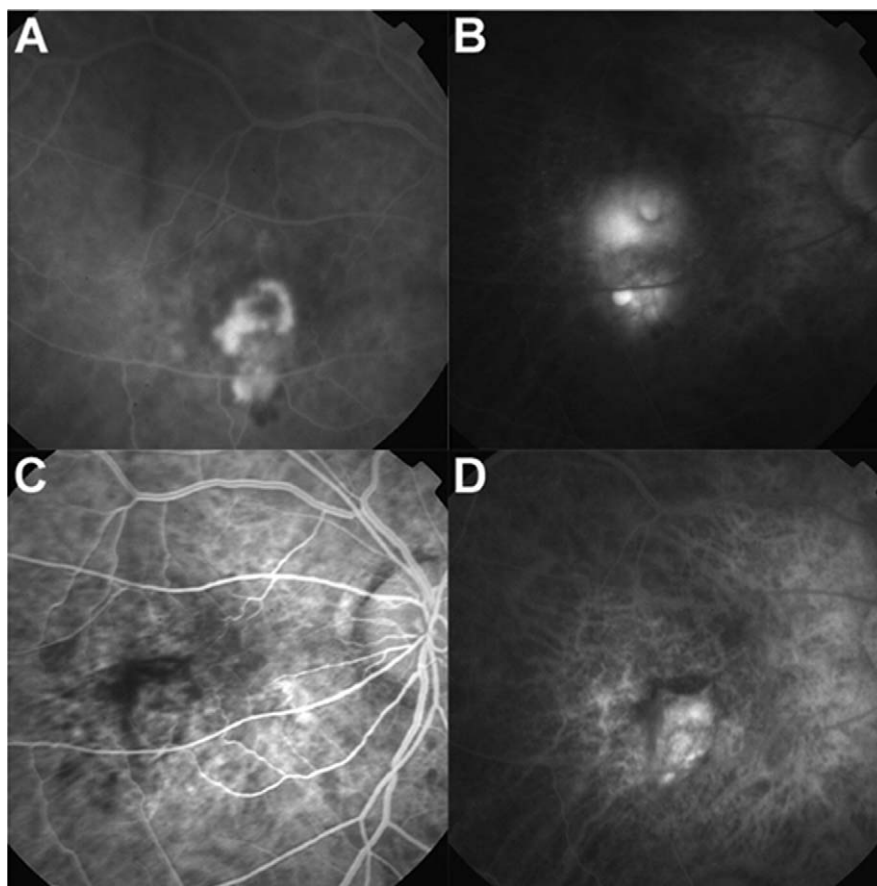


Figure 2. Early (A, C) and late (B, D) fluorescein angiographic images from a patient in group 3 with predominantly classic choroidal neovascularization arising from a prior laser scar at day 0 (A, B) and day 140 (C, D) after treatment with an escalating-dose regimen of ranibizumab. Note the absence of fluorescein leakage from the choroidal neovascularization at day 140. Vision improved from 20/63 at day 0 to 20/32 at day 140, a gain of 15 letters.

There were also times during the study when the mean VA for individual groups decreased. The mean VA declined for group 2 at day 14 (mean $\pm$ standard deviation, -0.4 ± 7.8 letters) and day 17 (-0.4 ± 9.5 letters) and for group 3 at day 14 (-1.4 ± 7.2 letters), day 29 (-0.7 ± 12.1 letters), and day 42 (-1.2 ± 12.1 letters). Of the 29 treated patients, 6 (21%) experienced a VA decline of at least 3 lines during the study, and 21 (72%) experienced an increase of at least 3 lines at some time during the treatment period.

Fluorescein Angiographic Outcomes

The total area of lesions in the study eye, as assessed by fluorescein angiography and fundus photography, showed little change from baseline, either in individual patients or overall (Table 7). In most patients, the final values for choroidal neovascularization area were near or below the baseline value. Likewise, there was a slight decrease from baseline in mean values for total area of leakage from choroidal neovascularization and the extent of SRF. No meaningful difference could be appreciated between the treatment groups. Figures 2 to 4 provide angiographic examples of patients with predominantly classic choroidal neovascularization, minimally classic choroidal neovascularization, and occult with no classic choroidal neovascularization treated with ranibizumab in this study.

Discussion

Multiple dose-escalating intravitreal injections of ranibizumab were well tolerated when administered in doses from 0.3 to as high as 2.0 mg and as frequently as every 2 weeks in patients with neovascular AMD. Although all treated patients experienced some ocular adverse events, these events were mild, except in 5 patients. One patient was withdrawn from the study because of decreased vision secondary to inflammation, and 4 patients had their doses reduced due to inflammation. As in the earlier single-injection phase I study²⁰ and the multiple-injection phase I/II study using doses of 0.3- and 0.5-mg ranibizumab every 4 weeks,²¹ the patients in this dose-escalation study experienced mild transient inflammation that typically resolved without treatment. This low-grade self-limited inflammation was very similar to the inflammation observed in the phase I study participants who received doses up to 0.5 mg.²⁰ This type of inflammation seemed to resolve spontaneously, without any long-term sequelae. In animal studies, it appeared that neither systemic nor topical steroids affected the duration or extent of the inflammation significantly (Genentech study 99-539-1757; data not

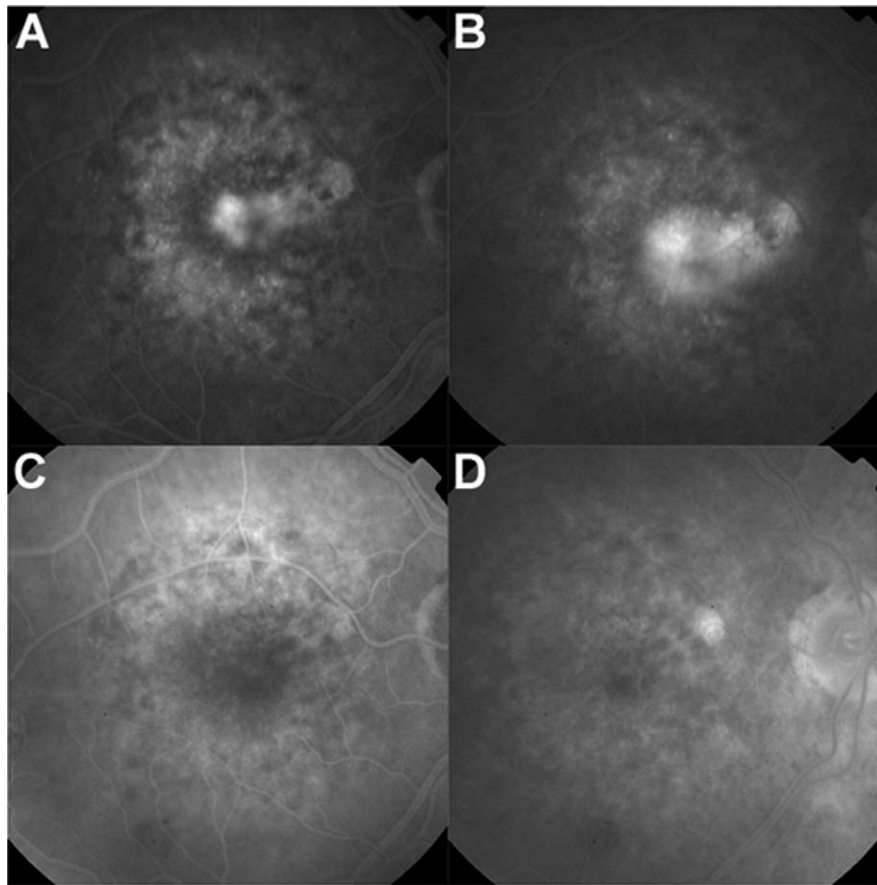


Figure 3. Early (A, C) and late (B, D) fluorescein angiographic images from a patient in group 2 with minimally classic choroidal neovascularization at day 0 (A, B) and day 140 (B, D) after treatment with an escalating-dose regimen of ranibizumab. Note the disappearance of the classic choroidal neovascularization and the absence of fluorescein leakage at day 140. Vision remained stable at 20/63 through day 140, an increase of 2 letters.

shown), and in our study population, it is unclear whether topical steroids helped to resolve the inflammation.

In the earlier single-injection dose-escalation study, the maximum tolerated dose of ranibizumab was limited to 0.5 mg due to inflammation. In the present study using a dose-escalating strategy in which the ranibizumab dose was increased incrementally every 2 weeks or 4 weeks, the maximum tolerated dose was increased to at least 2.0 mg. Despite the escalating doses, there was a tendency to observe less inflammation after each subsequent injection, with the lowest mean inflammation scores observed after the final and highest dose injected. These results are consistent with the tolerance for higher ranibizumab doses that was observed in cynomolgus monkeys exposed to a similar dose-escalating strategy (unpublished data [Genentech studies 98-361-1757, 99-539-1757]). The immunological mechanism responsible for this phenomenon remains to be elucidated. Although this study did not identify an optimal strategy for inducing such tolerance or the duration of such tolerance, the results of this study suggest that regimens employing higher doses of ranibizumab and injections as frequently as every 2 weeks may have offered viable therapeutic alternatives if lower doses and less frequent dosing schedules had proven ineffective in the ongoing phase III trials.

Visual acuity in the study eye at the end of the study period improved on average during ranibizumab treatment, with no notable differences among the treatment groups in their overall change from baseline. The changes in mean and median VA over time (shown in Fig 1) suggest that vision improved more rapidly in group 1, but there are too few patients in each group to allow any definitive conclusions. Although it is possible that one of the strategies employed in these groups is superior to the others, this study was powered insufficiently to identify small treatment benefits. Also, although all major lesion types appeared to benefit from ranibizumab therapy (as shown in Figs 2–4), the small number of lesions of any particular type in this sample of patients limits generalization beyond this study. However, similar improvement in VA across different lesion types was observed in the phase I/II study that utilized lower, multiple, fixed doses of ranibizumab (0.3 and 0.5 mg).²¹

At day 140, 2 patients in group 3 lost at least 15 letters of BCVA due to hemorrhage associated with tears of the RPE. Although it is theoretically possible that hemorrhage and a tear of the RPE might be separate consequences of anti-VEGF therapy—because we know that hemorrhage can result from systemic anti-VEGF therapy,²² and regression of choroidal neovascularization might induce contrac-

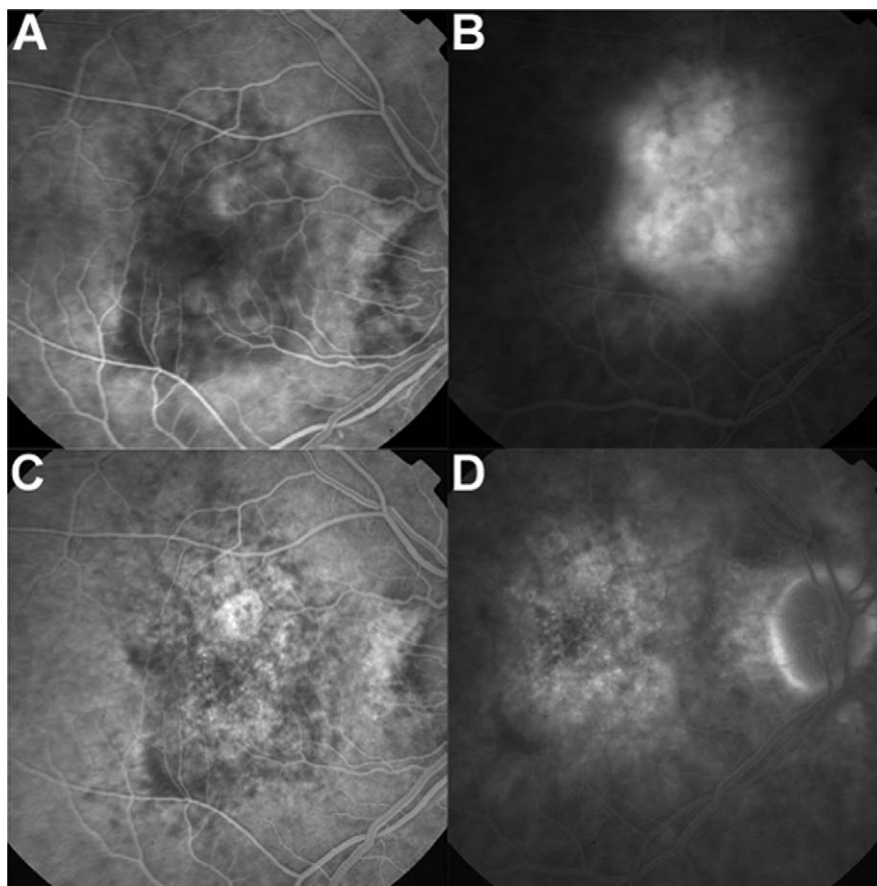


Figure 4. Early (A, C) and late (B, D) fluorescein angiographic images from a patient in group 3 with occult-only choroidal neovascularization at baseline (A, B) and day 140 (C, D) after treatment with an escalating-dose regimen of ranibizumab. Note the absence of fluorescein leakage from the choroidal neovascularization at day 140. Vision improved from 20/250 at day 0 to 20/160 at day 140, a gain of 13 letters.

tion of the RPE resulting in a tear—we consider it unlikely that ranibizumab or the specific dose-escalating regimen was responsible for these adverse events. Both patients experienced vision loss after the first injection of 0.3-mg ranibizumab—the same first dose used in the other 2 regimens. Moreover, the tears of the RPE are most likely the result of normal disease progression in neovascular AMD. However, much still needs to be learned about anti-VEGF drugs in the eye, and the larger phase III trials now underway will help determine whether these ocular adverse events were drug related.

The systemic safety of the higher intravitreal doses was reassuring. Only one nonocular adverse event, a subdural hematoma during the long-term follow-up period, required hospitalization and was designated as severe. During the study, cardiovascular events predominated. Although no serious adverse events were thought to be drug related, the possibility that the transient ischemic attack suffered by one patient was related to intravitreal anti-VEGF therapy cannot be excluded, because systemic anti-VEGF therapy has been shown to increase the risk of thromboembolic events.²³ However, this seems very unlikely in our case because the one episode of a transient ischemic attack occurred after a low dose of ranibizumab, and very small amounts of the intravitreal drug would be expected to reach the systemic

circulation. In addition, systemic VEGF concentrations did not change significantly from baseline during the study.

This study was intended to test whether ranibizumab could be tolerated at doses of up to 2.0 mg when given as an escalating dose regimen, and it was not powered to detect rare but potentially serious adverse events that might result from this treatment. Events that occurred with an incidence of 10% would have a 95% probability of being seen at least once, and an 80% probability of being seen at least twice among the 29 treated subjects in this study. Events that occurred with an incidence of $\leq 5\%$ would have a $<50\%$ probability of being seen. Even if an event was seen only once in a study with such an elderly patient population, it is generally difficult to distinguish whether the event was due to treatment or to concomitant conditions. The larger ongoing phase III trials will help to establish if any causal relationship exists between the study drug and systemic adverse events.

In summary, ranibizumab was well tolerated when administered as repeated intravitreal injections using 3 different regimens of escalating doses. Dose escalation up to 2.0 mg in this small uncontrolled clinical study appeared to result in significant vision improvement in over 40% of patients, but these results did not seem to add any benefit beyond that seen with repeated injections at lower doses in

the larger phase I/II study. Although these results are promising, and the VA improvements are consistent with previous results, the study is limited by the small number of patients and the uncontrolled study design. However, these results do provide further support for the continued investigation of ranibizumab for the treatment of neovascular AMD.

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Table 2. Demographic and Nonocular Baseline Characteristics of Randomized Patients

Characteristic	Group 1 (n = 11)	Group 2 (n = 10)	Group 3 (n = 11)	All Patients (n = 32)
Age (yrs) [Mean (SD)]	79.2 (4.3)	81.4 (3.5)	78.6 (6.7)	79.7 (5.0)
Gender (female)	9 (81.8%)	6 (60.0%)	5 (45.5%)	20 (62.5%)
Race/ethnicity				
White	9 (81.8%)	9 (90.0%)	8 (72.7%)	26 (81.3%)
Hispanic	1 (9.1%)	1 (10.0%)	3 (27.3%)	5 (15.6%)
Black	1 (9.1%)	0	0	1 (3.1%)
Tobacco use				
Never	4 (36.4%)	2 (20.0%)	1 (9.1%)	7 (21.9%)
Previous	7 (63.6%)	8 (80.0%)	8 (72.7%)	23 (71.9%)
Current	0	0	2 (18.2%)	2 (6.3%)

SD = standard deviation.

Table 3. Ocular Baseline Characteristics of Randomized Patients

Characteristic	Group 1 (n = 11)	Group 2 (n = 10)	Group 3 (n = 11)	All Patients (n = 32)
Median visual acuity*	20/160	20/80–20/100	20/125	20/125
≤20/40 but >20/80	0	3 (30%)	2 (18%)	5 (16%)
≤20/80 but >20/160	5 (45%)	3 (30%)	4 (36%)	12 (38%)
≤20/160 but >20/320	4 (36%)	3 (30%)	2 (18%)	9 (28%)
≤20/320 but ≥20/400	2 (18%)	1 (10%)	2 (18%)	5 (16%)
<20/400	0	0	1 (9%) [†]	1 (3%)
Mean letter score (SD)	42.4 (11.1)	48.9 (13.0)	41.4 (18.0)	44.1 (14.3)
Median letter score	41.0	52.0	49.0	46.5
Total area of lesion (disc areas)				
n	11	10	10	31
Mean (SD)	7.49 (5.91)	4.94 (4.01)	7.08 (4.28)	6.53 (4.82)
Median	5.70	4.43	5.08	5.15
Total area of choroidal neovascularization (disc areas)				
n	11	10	10	31
Mean (SD)	5.73 (5.50)	3.92 (4.02)	5.93 (4.26)	5.21 (4.60)
Median	3.00	2.05	4.45	3.15
Area of classic choroidal neovascularization (disc areas)				
n	11	10	10	31
Mean (SD)	2.11 (4.86)	0.88 (1.22)	0.99 (1.42)	1.35 (3.04)
Median	0	0.58	0.08	0.15
Area of classic choroidal neovascularization (% of total lesion area)				
n	11	10	10	31
Mean (SD)	26.7% (35.5%)	27.1% (34.6%)	25.9% (37.3%)	26.6% (34.6%)
Median	0	11.5%	0.8%	2.0%
Lesion type distribution				
n	11	10	11	32
100% occult	6 (54.5%)	3 (30.0%)	5 (45.5%)	14 (43.8%)
>0–<50% classic	1 (9.1%)	4 (40.0%)	2 (18.2%)	7 (21.9%)
≥50% classic	4 (36.4%)	3 (30.0%)	3 (27.3%)	10 (31.3%)
Cannot grade or missing	0	0	1 (9.1%)	1 (3.1%)

SD = standard deviation.

*Approximate Snellen equivalent.

[†]One patient had vision better than 20/400 when screened, but it dropped to 20/640 on the day of injection, day 0.