

A Phase I Trial of an IV-Administered Vascular Endothelial Growth Factor Trap for Treatment in Patients with Choroidal Neovascularization due to Age-Related Macular Degeneration

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Objectives: To assess the safety, pharmacokinetics, and biological activity of IV administration of vascular endothelial growth factor trap (VEGF Trap), a recombinant protein containing the binding domains of VEGF receptors 1 and 2, in patients with neovascular age-related macular degeneration (AMD).

Design: Randomized, multicenter, placebo-controlled clinical trial.

Participants: Twenty-five patients were enrolled (11 male, 14 female); 19 received VEGF Trap (0.3 [n = 7], 1.0 [n = 7], or 3.0 mg/kg [n = 5]), and 6 received a placebo.

Methods: Patients were randomized to receive a placebo or 0.3-, 1.0-, or 3.0-mg/kg VEGF Trap—a single IV dose followed by a 4-week observation period and then 3 doses 2 weeks apart.

Main Outcome Measures: Safety and biological activity, including change in excess retinal thickness and volume assessed by optical coherence tomography and visual acuity (VA) measured by the Early Treatment Diabetic Retinopathy Study protocol.

Results: The majority of adverse events attributable to VEGF Trap were mild to moderate in severity, but 2 of 5 patients treated with 3.0 mg/kg experienced dose-limiting toxicity (1 with grade 4 hypertension and 1 with grade 2 proteinuria); therefore, all patients in the 3.0 mg/kg-dose group were withdrawn from the study. The mean percent changes in excess retinal thickness were -12%, -10%, -66%, and -60%, respectively, for the placebo and 0.3-, 1.0-, and 3.0-mg/kg groups at day 15 ($P < 0.02$ by analysis of covariance [ANCOVA]) and -5.6%, +47.1%, and -63.3% for the placebo and 0.3- and 1.0-mg/kg groups at day 71 ($P < 0.02$, ANCOVA). A significant change in VA was not noted in this small study.

Conclusions: The maximum tolerated dose of IV VEGF Trap in this study population was 1.0 mg/kg. This dose resulted in elimination of about 60% of excess retinal thickness after either single or multiple administrations. Alternative routes of delivery to increase the therapeutic window are being explored. *Ophthalmology* 2006; 113:1522-1532 © 2006 by the American Academy of Ophthalmology.



Age-related macular degeneration (AMD) is the most common cause of severe vision loss in patients over the age of 60 years in developed countries.¹ It is recognized by the presence of drusen, yellow deposits beneath the retina, and

pigmentary changes due to atrophy and proliferation of retinal pigment epithelium (RPE) cells. These changes are accompanied by gradual death of photoreceptors and moderate decreases in visual acuity (VA); this constellation of

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signs is referred to as atrophic or nonneovascular AMD. Patients with nonneovascular AMD are at risk for development of choroidal neovascularization and thereby converting to neovascular AMD. Patients with neovascular AMD account for only about 10% of patients with AMD, but account for the majority of those with severe vision loss.²

The pathogenic events underlying conversion from nonneovascular to neovascular AMD are uncertain, but studies in animal models suggest that increased expression of vascular endothelial growth factor (VEGF) is likely to play a critical role. Inhibition of VEGF receptor signaling by systemic administration of kinase inhibitors³ or a blockade of VEGF by intraocular injection of an anti-VEGF antibody fragment⁴ significantly suppresses choroidal neovascularization in animal models. These data suggest that VEGF is an important therapeutic target for treatment of choroidal neovascularization.

Several VEGF antagonists have been developed and tested in patients with neovascular AMD. Pegaptanib (Macugen, [OSI] Eyetech Pharmaceuticals, Inc., New York, New York) is an RNA molecule that binds VEGF₁₆₅ but not other isoforms of VEGF-A. Intraocular injection of pegaptanib every 6 weeks for 1 year reduced the percentage of patients with classic choroidal neovascularization due to AMD who experienced severe loss of vision (loss of ≥ 15 letters) from 45% in the sham injection group to 30%.⁵ Six percent of patients treated with pegaptanib, compared with 2% in the sham injection group, had a substantial improvement in vision (gain of ≥ 15 letters). Relative to sham treatment, the increase in size of choroidal neovascularization lesions was slowed but not stopped. These benefits are modest, but they confirmed that VEGF is a therapeutic target in neovascular AMD.

Ranibizumab (Lucentis, Genentech, Inc., South San Francisco, CA) is another VEGF antagonist; it is a Fab fragment of an antibody that binds all isoforms of VEGF-A. Monthly intraocular injections of ranibizumab in AMD patients with occult or minimally classic subfoveal choroidal neovascularization reduced the percentage of patients with severe loss of vision over the course of a year from 38% in the sham injection group to 5%, and the percentage of patients who experienced substantial improvement in vision increased from 4.6% to 34% (reported at the meeting of the American Society of Retina Specialists, July 2005, Montreal, Canada). These data suggest that antagonism of all isoforms of VEGF-A in AMD patients with choroidal neovascularization can result in stabilization of vision in the majority of patients and substantial improvement in vision in about a third of patients. Preliminary reports using ranibizumab in patients with classic subfoveal choroidal neovascularization have been equally impressive and confirm that VEGF-A is a very important target in the treatment of neovascular AMD, but suggest several questions. Are the superior results with intravitreal injection of ranibizumab compared with those with pegaptanib due to the inhibition of all VEGF-A isoforms, compared with inhibition of only VEGF₁₆₅; superior pharmacokinetics; a combination of both; or some other reason?

If the superiority of ranibizumab over pegaptanib is due to its ability to neutralize all of the VEGF-A isoforms as

opposed to just VEGF₁₆₅, then perhaps neutralizing other VEGF family members in addition to VEGF-A would provide an even greater benefit. Placental growth factor is a VEGF family member that contributes to ocular neovascularization and excessive vascular permeability, providing a rationale for targeting multiple family members and not just VEGF-A.⁶ Vascular endothelial growth factor receptors bind multiple members of the VEGF family; VEGF receptor 1 binds VEGF-A and placental growth factor, and VEGF receptor 2 binds VEGF-A, VEGF-B, and VEGF-C. A soluble form of VEGF receptor 1 consisting of just the extracellular domain—the receptor without the transmembrane or intracellular portions of the molecule—is normally produced in the body and seems to be a component of the endogenous system that modulates new vessel growth. Gene therapy resulting in elevated levels of soluble VEGF receptor 1 inhibits ocular neovascularization in animal models and has antiangiogenic activity.^{7–15}

The VEGF Trap is a recombinant soluble VEGF receptor protein in which the binding domains of VEGF receptors 1 and 2 are combined with the Fc portion of immunoglobulin G. The receptor portion of the molecule has a very high affinity for all VEGF-A isoforms ($K_d < 1$ pmol/l), placental growth factor 1 and 2, and VEGF-B, VEGF-C, and VEGF-D. So VEGF Trap is distinguished from ranibizumab by its higher potency for neutralization of all VEGF-A isoforms and its ability to inhibit other related proangiogenic and pro-permeability VEGF family members. The Fc portion slows clearance by conferring the long circulating half-life of an antibody to the molecule.¹⁶ Either systemic or intravitreal administration of VEGF Trap strongly suppressed laser-induced choroidal neovascularization in mice¹⁷ and in primates (Association for Research in Vision and Ophthalmology abstract no. 3258, 2005). The broader activity and the higher affinity of VEGF Trap are theoretical advantages over ranibizumab, but whether theoretical advantages translate into practical advantages can be determined only by clinical trials.

Although intravitreal injection of a VEGF antagonist has the benefit of limiting systemic exposure, it has the disadvantage of producing large fluctuations in intraocular levels, which cannot be easily measured, making it impossible to determine effective tissue levels. It is possible to measure plasma levels after intravascular administration, and because there is no barrier between the systemic circulation and the choroid, in this setting plasma levels approximate tissue levels, making pharmacodynamic analyses possible. Correlating plasma levels with toxicity is also extremely useful because it provides knowledge regarding plasma levels that should be avoided. This allows the rational design of alternative modes of delivery by measuring plasma levels that occur after local administration and making sure they fall below toxic levels. For this reason, probing the safety of an agent after systemic administration provides important information regardless of the ultimate mode of delivery. Systemic administration of bevacizumab (Avastin, Genentech) in combination with antimetabolites has been shown to cause hypertension and proteinuria and is associated with an increased risk of thromboembolic events.¹⁸ In an oncology trial in which patients receive

multiple subcutaneous injections of VEGF Trap, only hypertension and proteinuria have been identified as definite drug-related side effects. Based in part on this reasonable safety profile, we performed a dose-ranging, placebo-controlled, randomized trial investigating the safety, pharmacokinetics, and biological activity of IV-administered VEGF Trap in patients with neovascular AMD.

Materials and Methods

Ethical Considerations

The study was conducted in compliance with the Declaration of Helsinki, US Code 21 of Federal Regulations, and the Harmonized Tripartite Guidelines for Good Clinical Practice (1996). The study was reviewed and approved by the Western Institutional Review Board for some centers and by local institutional review boards for others. There were 3 participating centers: MacPherson Retina Center at Baylor College of Medicine, Houston, Texas; New York Eye and Ear Infirmary, New York, New York; and Wilmer Eye Institute of the Johns Hopkins University School of Medicine, Baltimore, Maryland. Each study subject had comprehensive discussions with one of the investigators and gave written informed consent before study entry.

Study Design

The study was a randomized, placebo-controlled, dose escalation trial in subjects with subfoveal choroidal neovascularization due to neovascular AMD. Three dose levels of VEGF Trap were investigated (0.3, 1.0, and 3.0 mg/kg). In each cohort, subjects were randomized in a 3:1 ratio (drug:placebo). After enrollment in the 0.3-mg/kg cohort was completed, there was a 2-week waiting period before the 1.0-mg/kg cohort was begun. There was also a 2-week waiting period after the 1.0-mg/kg cohort was enrolled before enrollment of the 3.0-mg/kg cohort was begun.

Study Population

The main inclusion criteria for the study were (1) male or female (any ethnicity), 50 years or older; (2) diagnosis of neovascular AMD in the study eye with leaking subfoveal choroidal neovascularization ≤ 12 optic disc areas (measured according to the protocol of the Macular Photocoagulation Study¹); (3) best-corrected VA (BCVA) score of 20/40 or worse; (4) clear ocular media and adequate pupillary dilation (able to dilate pupils to ≥ 4 mm using standard mydriatics) to permit good stereoscopic fundus photography; and (5) retinal thickness ≥ 250 μ m in the macular region as measured by optical coherence tomography (OCT).

Subjects were excluded from the study if they (1) had another disease besides neovascular AMD in the study eye that could affect vision or safety evaluation, (2) had previous laser photocoagulation to the center of the fovea in the study eye, (3) were eligible for photodynamic therapy in the study eye unless they refused it, or (4) had intraocular surgery or another treatment in the study eye within 3 months of screening. Nor were subjects eligible for the study if they had (1) symptomatic or unstable coronary artery disease, angina, congestive heart failure, or an arrhythmia requiring active medical management within the last 30 days; (2) myocardial infarction or treatment for acute congestive heart failure within the past 6 months; (3) malignancy other than basal cell carcinoma of the skin diagnosed and treated within the last 5 years; (4) a history of peripheral vascular disease; (5) blood pressure (BP), treated or untreated, $> 140/90$ mmHg on at least 3 repeated

determinations on separate days during the 6 weeks before study entry; or (6) abnormal renal function, as defined by creatinine $>$ upper limit of normal, proteinuria 1+ or greater on 2 repeat determinations, urine protein:creatinine ratio ≥ 1 , or 24-hour urinary protein excretion of ≥ 300 mg. During the treatment period of this study, the only approved treatment for subfoveal choroidal neovascularization in patients with AMD was photodynamic therapy. Because photodynamic therapy was the standard of care, all patients who were eligible for photodynamic therapy were encouraged to have photodynamic therapy and not enter the trial. Several of the patients had prior photodynamic therapy (5 subjects had multiple prior photodynamic therapy treatments, and 8 had at least 1 prior photodynamic therapy) and still showed evidence of leakage. All of these patients were eligible for photodynamic therapy and it was recommended, but they refused because they felt it was not benefiting them and they preferred to enter a trial rather than have additional photodynamic therapy. There were more of these patients (4/7 with prior photodynamic therapy) in the 0.3-mg/kg group than in the 1.0-mg/kg (2/7) and 3.0 mg/kg (2/5) groups due to random variation in a small study.

Infusions of VEGF Trap or Placebo

Patients were randomized 3:1 to receive VEGF Trap or an inactive saline infusion at each dose level. Masking of treatment assignment was the responsibility of the investigational pharmacist at each participating center. Individual patient doses were prepared by diluting the appropriate volume of VEGF Trap (25 mg/ml) with 0.9% sodium chloride to yield a final concentration of 4 mg/ml. The volume of solution to be prepared was 25 to 150 ml, depending on the patient's dose and body weight. Patients randomized to receive placebo infusions received an infusion of a volume of normal saline (0.9% sodium chloride) equivalent to the volume of active drug solution that would be administered for the patient's weight and dose level. The drug or placebo was infused over a period of no less than 1 hour by a registered nurse or physician's assistant under the guidance of one of the investigators. In addition, internists or anesthesiologists were also coinvestigators at all sites and helped to oversee the administration of the study drug and aid in the management of adverse events.

Study Activities and Assessments

Subjects were monitored closely for safety and tolerability using the following assessments and procedures: slit-lamp biomicroscopy, indirect ophthalmoscopy, tonometry, BCVA measurement, adverse event reporting, vital signs, physical examinations, serum electrolytes, creatinine, quantitative protein determination in 24-hour urine specimens, and measurement of serum neutralizing antibodies directed against VEGF Trap. Hypertension was graded based on the Common Terminology Criteria for Adverse Events. Grade 2 hypertension is defined as a recurrent or persistent (≥ 24 hours), or symptomatic increase in BP by >20 mmHg (diastolic) or to $>150/100$ if previously within normal limits; monotherapy may be indicated. Grade 3 hypertension is defined as a recurrent or persistent or symptomatic increase in BP by >20 mmHg (diastolic) or to $>150/100$ if previously within normal limits that requires treatment with more than one drug or more intensive therapy than previously. However, for purposes of this study, grade 3 hypertension that could be easily controlled with a commonly used combination of 2 agents (e.g., a diuretic, an angiotensin converting enzyme inhibitor, and/or a β -blocker) was not considered a dose-limiting toxicity (DLT). This modification of the criteria was made to reflect current standard clinical practice in the management of hypertension. Hypertension that met the criteria for a grade 3 increase and could not be controlled with a combi-

nation of commonly used antihypertensives was considered grade 3. Grade 4 or malignant hypertension is hypertension with life-threatening consequences (e.g., hypertensive crisis). A DLT was defined as any Common Terminology Criteria for Adverse Events grade 3 or 4 toxicity or grade 2 or 3 ocular toxicity. Any grade 1 or 2 toxicity that resulted in dose reduction or discontinuation of study drug was considered a potential DLT to be reviewed by the Investigator and Study Director. Other DLTs were urinary protein excretion of >2 g/24 hours or Common Terminology Criteria for Adverse Events grade 2 or greater potential immunotoxicity, including but not limited to allergic reaction/hypersensitivity (including drug fever), autoimmune reaction, vasculitis, erythema multiforme, rash/desquamation, urticaria, and/or asymptomatic bronchospasm. This includes infusion-related hypersensitivity reactions, symptoms of which may include flushing, dyspnea, tachycardia, bronchospasm (symptomatic or asymptomatic), hypotension, anxiety, myalgias, edema, and nausea.

Stereoscopic color fundus photography and fluorescein angiography (FA) were performed at baseline and days 29, 71, and 99. Optical coherence tomography was performed at each study visit. [Figure 1](http://aaojournal.org) (available at <http://aaojournal.org>) shows a flowchart of study activities. Patients in the first and second cohorts received all 4 infusions and were monitored through all study visits. All of the patients in the 3.0-mg/kg dose group were withdrawn from study after the DLT was identified. The first enrolled patient received all 4 infusions; the second patient received 2 infusions; and the third, fourth, and fifth patients each received 1 infusion.

Reading Center

The Retinal Imaging Research and Reading Center (RIRRC) at the Wilmer Eye Institute served as the reading center for fluorescein angiographic and OCT analyses. All images were evaluated by a grader who was masked with respect to treatment group. Interpretation of optical coherence tomograms is more difficult in patients with choroidal neovascularization than in patients with diabetic macular edema. In patients with choroidal neovascularization, the computer often misinterprets borders, and therefore, the computer-generated foveal thickness, on which eligibility is based, may be misleading. In some instances, baseline scans that had computer-generated values that were >250 μm , thereby allowing patients to be entered, had lower values when read later by the reading center in masked fashion. The values obtained in the reading center are more accurate because the borders selected by the computer for measurements were scrutinized and, when incorrect, were reset manually to get a more accurate reading.

Optical Coherence Tomography

Optical coherence tomography was performed using StratusOCT (Carl Zeiss Meditec, Dublin, CA). The RIRRC provided detailed instruction in the protocol for image acquisition, and a representative from the RIRRC visited each study site to certify competence and compliance. Two standard protocols (6-mm fast macular thickness map and 6×6 -mm crosshair) and 1 modified acquisition protocol (3-line 8-mm papillomacular axis scan) were used. The 3-line 8-mm papillomacular axis scans utilized the disc as a landmark to ensure reproducible placement of scan lines at each visit; 1 line was at the superior margin, 1 was at the inferior margin, and 1 passed through the center of the disc. The 6×6 -mm crosshair was a high-resolution scan used to follow morphological changes in the macula. The fast macular thickness map performed 6 linear scans 6 mm in length centered on the patient fixation at equally spaced angular orientations in 1.96 seconds. Retinal thickness at any point was defined as the distance between outer and inner reflectivity bands of the OCT cross section. Foveal thickness

(in micrometers; defined as the mean height of the neurosensory retina in a central 1-mm-diameter area) and total macular volume (in cubic millimeters) were computed automatically by the StratusOCT software (version 4.0). Due to the advanced nature of the disease and extensive change in the RPE morphology, manual scan profiling was used to correct any artifacts produced by the automated analysis algorithm.

Fluorescein Angiography

High-resolution digital FA was performed using an FF4 fundus camera (Carl Zeiss Meditec, Oberkochen, Germany) attached to an MRP (Boston, MA) capture station. A modified FA acquisition protocol was used for image acquisition, and compliance was monitored by a site visit. Digital images were sent to the RIRRC and analyzed using EyeRoute Proview software (version 6.1, Anka Systems Inc., McLean, VA). Two independent investigators graded each FA for markers of disease activity (progression/regression), including blood and pigment epithelial detachment. Each fluorescein angiogram was analyzed further using advanced image segmentation techniques for edge detection to determine the lesion size and maximum area and extent of leakage.

Statistical Methods

All statistical analyses were performed in SAS (version 9.0, SAS Institute, Cary, NC). Changes in continuous measures were assessed using analysis of covariance with a main effects model that included baseline excess foveal thickness as a covariate and treatment effects. Due to the small numbers of patients in this study, the assumptions of the parametric model of homoscedasticity and normality were examined, and if the parametric assumptions were unwarranted, rank analogs were to be advanced. A rank analysis of covariance was then to be performed. In fact, due to the small number of subjects in this study, parametric assumptions were not met; therefore, the primary analysis method was the rank analysis of covariance with a main effects model.

Results

Patient Population

Nine patients were enrolled in the first cohort (0.3-mg/kg VEGF Trap and placebo), 9 in the second (1.0-mg/kg and placebo), and 8 in the third (3.0-mg/kg and placebo). [Figure 1](http://aaojournal.org) (available at <http://aaojournal.org>) shows the patient disposition in the study. One patient in the second cohort who was thought initially to have occult choroidal neovascularization was determined by the reading center not to have it. Although safety data could have been obtained from this patient, because there was no possibility for the patient to receive any benefit, it was judged inappropriate to expose the patient to any possible risk; therefore, the patient was withdrawn from the study. In addition, when DLT was observed in a patient in the 3-mg/kg cohort, dosing was stopped for all patients in that cohort, and the study was terminated.

[Table 1](#) shows the demographics of the study subjects. The age and gender distributions were similar across all 4 groups. By chance, subjects who were randomized to receive placebo treatment had worse VA at baseline than patients randomized to receive VEGF Trap. Lesion sizes were similar across all groups, except for the 0.3 mg/kg, which had a smaller average lesion size. Each group had a combination of predominantly classic, occult, and minimally classic choroidal neovascularization lesions.

Table 1. Demographics and Disease History

	Placebo (n = 6)	0.3 mg/kg (n = 7)	1 mg/kg (n = 7)	3 mg/kg (n = 5)
Age (yrs) [mean (range)]	76.7 (64–86)	76.3 (58–81)	79.6 (68–88)	73.8 (69–81)
Gender (male:female)	3:3	3:4	2:5	3:2
ETDRS letters read [mean (range)]	27.8 (12–50)	47.9 (24–69)	49.9 (16–64)	47.8 (8–72)
Lesion size (DAs) (mean)	6	3	6.5	7
Lesion type (Occult:classic:minimally classic)	2:3:1	4:2:1	1:1:3	1:2:2
Foveal thickness (μm) [mean (range)]	384.4 (300–483)	299.6 (238–340)	348.8 (221–680)	414.4 (289–563)

DA = disc area; ETDRS = Early Treatment Diabetic Retinopathy Study.

Preliminary Safety Assessment

The most common adverse events were headache, hypertension, proteinuria, and hoarseness (Table 2). Hypertension, proteinuria, and hoarseness were expected adverse events, because they are class effects of systemic VEGF antagonists and had been seen in prior studies of VEGF Trap in patients with advanced malignancies (Proc ASCO abstract 3009, 2004). Adverse events were dose related and were most common in the 1.0- and 3.0-mg/kg cohorts (Table 2), but they were mild and easily managed in the 1.0-mg/kg group.

VEGF Trap administration was associated with a dose-dependent increase in mean BP. The highest readings generally were recorded 2 weeks after the first dose. Table 3 shows the change from baseline to day 15 (2 weeks after dose) in mean systolic and diastolic BPs. The increase in diastolic pressure was statistically significant at all dose levels studied. By 4 weeks after the first dose, BP generally returned to baseline (data not shown), except in one patient whose clinical course is described below. All patients with BP elevations were treated successfully for their hypertension during the repeated-dosing portion of the study. Increases in mean BP were not noted past day 29.

Severe hypertension and proteinuria occurred only in patients who received 3 mg/kg (Table 4). In one patient in the 3-mg/kg cohort, proteinuria reached a level predefined as dose limiting, and in another patient, hypertension was dose limiting (Table 5 [available at <http://aaojournal.org>]). The patient with severe hypertension was slow to respond to a change in antihypertensive regimen and developed congestive heart failure with pulmonary edema requiring hospitalization. This resolved with diuresis and more aggressive management of hypertension. Due to these DLTs, no additional VEGF Trap was administered to patients in the 3.0-mg/kg cohort, but follow-up was continued to monitor safety. All adverse events resolved after VEGF Trap was discontinued. As a result of the safety analysis, 1.0 mg/kg was determined to be the

maximum tolerated intravascular dose of VEGF Trap in this patient population.

Foveal Thickness by Optical Coherence Tomography

The prespecified primary measure of bioactivity was the effect on excess foveal thickness assessed by OCT. Normal foveal thickness is $179 \pm 17 \mu\text{m}$ ¹⁹; therefore, excess foveal thickness is measured as foveal thickness – 179 μm . Figure 2A presents sequential foveal thickness values for each patient in the 1.0-mg/kg cohort. The normal range of 145 to 213 μm , 2 standard deviations (SDs) above and below 179 μm , is shaded. Three of 5 patients who received 1.0-mg/kg VEGF Trap had reduction of foveal thickness into the normal range, and the other 2 were close to the normal range, whereas the 2 patients treated with the placebo had little or no reduction in foveal thickness. It is notable that every patient treated with 1.0-mg/kg VEGF Trap had reduction in foveal thickness 2 weeks after the first infusion and an increase over the next 2 weeks, and then most had reduction after the second, third, and fourth infusions.

Figure 2B illustrates the median percentage change in excess foveal thickness. Drug infusions are shown by arrows located at the top of the figure (days 1, 29, 43, and 57). Eight days after the initial infusion, all 3 cohorts treated with VEGF Trap showed a decrease in excess foveal thickness, but the effect with 0.3 mg/kg was marginal and lost at subsequent time points. In patients treated with 1.0-mg/kg VEGF Trap, there was a persistent effect at 2 weeks with elimination of 70% of excess foveal thickness, but by 1 month, the effect was reduced; there was a 40% decrease in excess foveal thickness. After subsequent infusions of 1.0-mg/kg VEGF Trap, there was again reduction in excess foveal thickness, and at day 71, 2 weeks after the fourth infusion, excess foveal thickness was almost eliminated (80% reduction). Although only a

Table 2. Adverse Events

	Placebo (n = 6)	0.3 mg/kg (n = 7)	1.0 mg/kg (n = 7)	3.0 mg/kg (n = 5)	All Doses (n = 19)
Headache		1 (14.3)	3 (42.9)	4 (80.0)	8 (42.1)
Hypertension			3 (42.9)	3 (60.0)	6 (31.6)
Proteinuria			3 (42.9)	3 (60.0)	6 (31.6)
Hoarseness	1 (16.7)		1 (14.3)	3 (60.0)	5 (26.3)
Arthralgia	1 (16.7)	1 (14.3)	1 (14.3)	1 (20.0)	3 (15.8)
Cough	1 (16.7)	1 (20.0)	2 (28.6)		3 (15.8)
Aggravation of arthritis pain				3 (60)	3 (15.8)

n (% of subjects at dose level).

Table 3. Mean ($\pm$ Standard Deviation) Change from Baseline in Systolic and Diastolic Blood Pressures 15 Days after Initial Dose of Vascular Endothelial Growth Factor Trap

Dose Group	Systolic (mmHg Change)	Diastolic (mmHg Change)
Placebo (n = 6)	-1.8 ± 11.2	$+3.4 \pm 6.7$
0.3 mg/kg (n = 7)	$+3.0 \pm 12.7$	$+11.0 \pm 4.0^*$
1.0 mg/kg (n = 7)	$+8.2 \pm 27.4$	$+14.7 \pm 6.4^*$
3.0 mg/kg (n = 5)	$+26.8 \pm 17^*$	$+20.4 \pm 10.1^\dagger$

* $P < 0.05$.
 $^\dagger P < 0.01$.

limited amount of data are available for patients treated with 3.0-mg/kg VEGF Trap, the effect after the first infusion appeared to be maintained for at least 1 month.

Macular Volume by Optical Coherence Tomography

Total macular volume is a measure of retinal thickness over a broader area than that indicated by foveal thickness. Previous studies have demonstrated that 6.47 mm^3 is the average macular volume in patients with no macular disease (Shah, Nguyen, Campochiaro, unpublished data). Three of the 5 patients treated with 1.0-mg/kg VEGF Trap had baseline total macular volume measurements more than 2 SDs above 6.47 mm^3 . All 3 patients had substantial reduction in total macular volume, and on day 71, 2 weeks after the last VEGF Trap infusion, all had total macular volume measurements very close to 6.47 mm^3 , indicating resolution of essentially all excess volume (Fig 3A [available at <http://aaojournal.org>]). By 6 weeks after the last infusion (day 99), total macular volume had begun to increase again. Two patients had normal total macular volume at baseline, although their foveal thickness was elevated; a possible explanation is that these patients had some areas of retinal thinning due to scarring and atrophy that counter areas of thickening when measurements are taken over a broad area. These patients also showed reduction in total macular volume that rebounded 6 weeks after completion of VEGF Trap infusions. The change from baseline in excess total macular volume mirrored the change from baseline in excess foveal thickness, with significant decreases in the 1.0- and 3.0-mg/kg cohorts (Fig 3B [available at <http://aaojournal.org>]).

Figure 4 shows temporal to nasal and inferior to superior transverse scans through the fovea and retinal maps at baseline and days 29, 71, and 99 for one patient who received 1.0-mg/kg VEGF

Trap. The foveal thickness at baseline was $348 \mu\text{m}$, and there was a pocket of subretinal fluid (SRF) beneath the fovea. Visual acuity was 20/80. Two weeks after the first infusion of VEGF Trap, foveal thickness was reduced to $320 \mu\text{m}$ and VA was 64 Early Treatment Diabetic Retinopathy Study letters (20/50). Two weeks later, there was an increase in foveal thickness to $420 \mu\text{m}$, although total macular volume (7.56 vs. 9.03 mm^3) and VA (20/50 vs. 20/80) remained better than baseline. At day 71, 2 weeks after the fourth infusion of VEGF Trap, the pocket of SRF had resolved, foveal thickness had improved to $232 \mu\text{m}$, and total macular volume was within the normal range at 6.69 mm^3 . Visual acuity was still 20/50. With no additional VEGF Trap, 1 month later (day 99) there was deterioration, with recurrence of the pocket of SRF, increase in foveal thickness to $248 \mu\text{m}$, and increase in total macular volume to 7.31 mm^3 . Visual acuity remained 20/50; it appears that VA lags behind OCT parameters as an indicator of bioactivity.

Pharmacokinetics

Plasma free VEGF Trap levels increased with increasing dose. However, only at the 3-mg/kg dose was free VEGF Trap present in the plasma in significant amounts beyond the day 14 time point (Fig 5). Thus, the worsening of outcomes seen 1 month after the first infusion of 1.0-mg/kg VEGF Trap is explained by the pharmacokinetics of the drug.

Fluorescein Angiography

Fluorescein angiography showed that infusions of 1.0- or 3.0-mg/kg VEGF Trap reduced leakage from choroidal neovascularization but did not cause reduction in choroidal neovascularization lesion size over the short duration of this study. A representative series of fluorescein angiograms from a patient treated with 1.0-mg/kg VEGF Trap is shown in Figure 6 (available at <http://aaojournal.org>). Compared with baseline, there was less fluorescein leakage at day 71, 2 weeks after the fourth infusion of VEGF Trap, but there was no reduction in lesion size. By day 99, there was some increase in leakage compared with day 71, but it was still less than that seen at baseline.

Visual Acuity

Patients with advanced disease that included scarring involving the fovea were included in this phase I study, so as anticipated there was not a major effect upon VA (Fig 7). Patients in the 3-mg/kg group showed an increase of 12 letters in mean VA and 2 letters in median VA 1 month after a single infusion. Patients in the 1.0-mg/kg group showed an increase in 5 letters in mean VA and 1 letter in median VA 1 month after the first infusion.

Table 4. Serious Adverse Events

Dose Level (mg/kg)	Serious Adverse Event	Doses	Related to Study Treatment	Recovered
Placebo (n = 6)	Hip pain	1	No	Yes
0.3 (n = 7)	Retinal hemorrhage	4	No	Yes*
	Squamous cell cancer (skin)	4	No	Yes*
1.0 (n = 7)	None			
3.0 (n = 5)	Hypertension [†]	1	Yes	Yes
	CHF [†]	1	No	Yes
	Pulmonary edema [†]	1	No	Yes

CHF = congestive heart failure.
 *Appeared after last dose of study drug; resolved with surgical intervention.
[†]Occurred in the same patient.

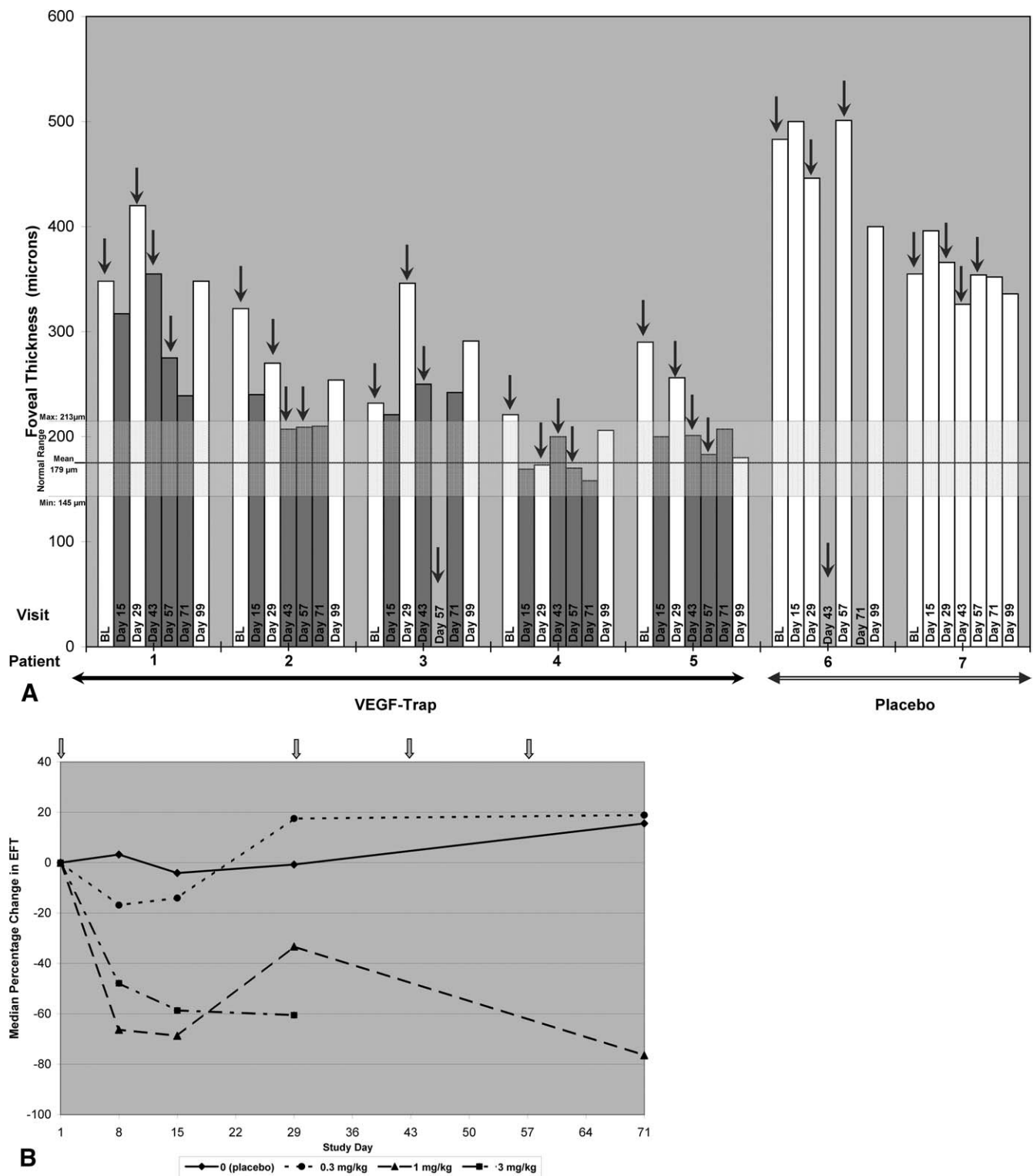


Figure 2. Reduction in excess foveal thickness from systemic infusion of 1.0-mg/kg vascular endothelial growth factor trap (VEGF Trap) in patients with neovascular age-related macular degeneration. **A**, Foveal thickness measured by optical coherence tomography is plotted at several time points for all patients in the second cohort. The measurements shown on the left and marked along the baseline (BL) as VEGF-Trap are from the 5 patients who received infusions of 1.0 mg/kg on days 0, 29, 43, and 57 (1 patient in the cohort had ungradable optical coherence tomograms). The measurements from 2 patients who received a placebo are on the right. Shaded bars are for measurements taken when based upon pharmacokinetics studies; patients were likely to have detectable plasma levels of VEGF Trap. White bars represent the beginning of the single and repeated-dosing periods of the study, when plasma VEGF Trap levels were minimal (see Fig 5). The normal range (145–213 μm), defined as the mean $\pm$ 2 standard deviations of foveal thickness determined from measurements in patients with no macular disease, is shaded. Note that for each patient the shaded bars tend to be lower than the white bars. **B**, The change from baseline in median excess foveal thickness (EFT) is plotted at several time points for patients who received a placebo or those treated with 0.3-, 1.0-, or 3.0-mg/kg VEGF Trap. There was a significant difference between the 1.0-mg/kg and placebo groups at days 15 and 71 ($P < 0.01$ by analysis of variance). The arrows at the top indicate when infusions were given.

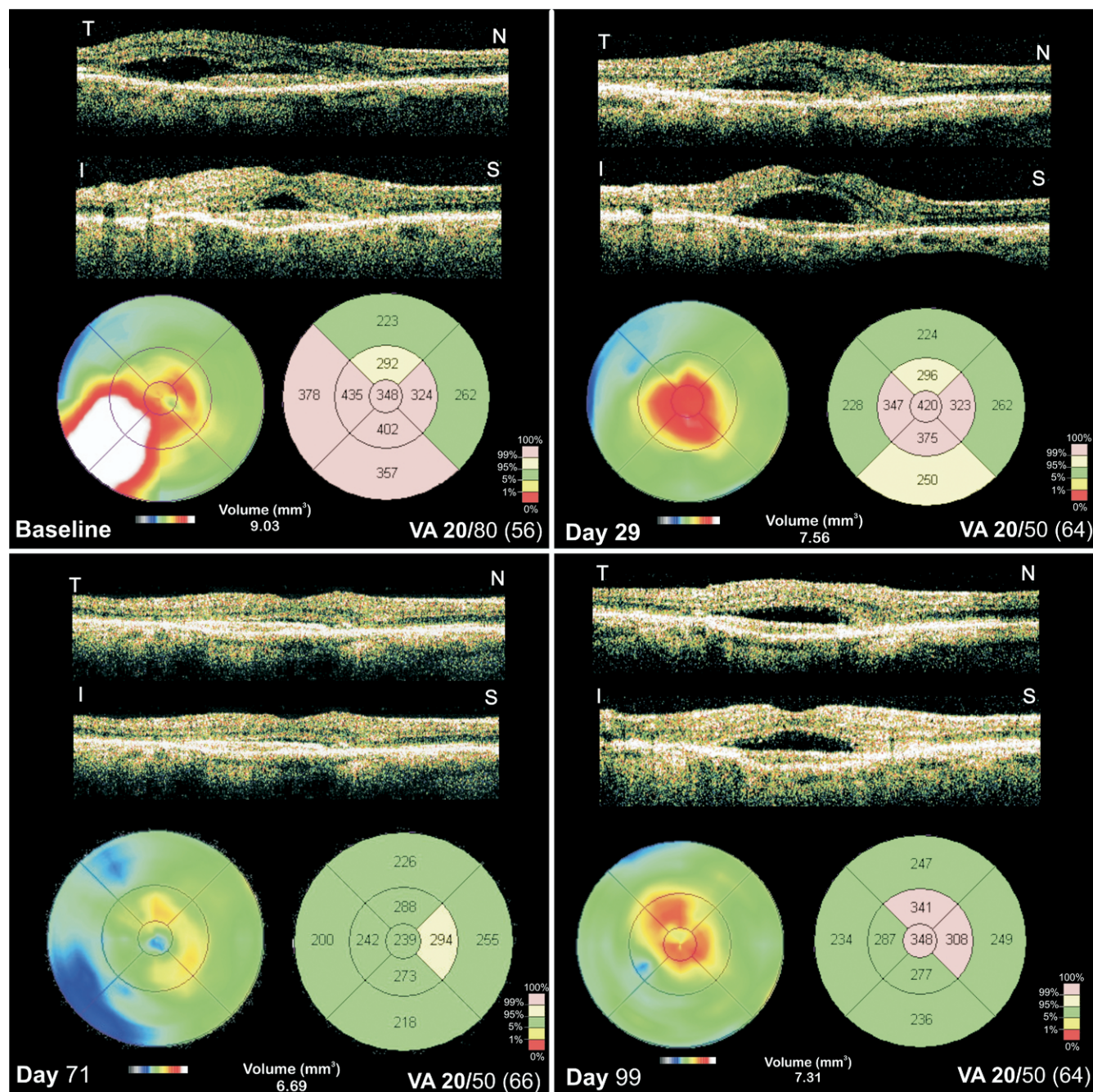


Figure 4. Temporal (T) to nasal (N) and inferior (I) to superior (S) transverse scans through the fovea and retinal maps at baseline and days 29, 71, and 99 for one of the patients who received 1.0-mg/kg vascular endothelial growth factor trap (VEGF Trap). The optical coherence tomography scans and maps are for the patient whose histograms are shown at the far left of Figures 2A and 3A. At baseline, foveal thickness was 348 μ m with a pocket of subretinal fluid (SRF) beneath the fovea. On day 29, there was little anatomic evidence of improvement, but at day 71, 2 weeks after the fourth infusion of VEGF Trap, the pocket of SRF had resolved, foveal thickness had improved to 232 μ m, and macular volume was within the normal range at 6.69 mm³. At day 99, 6 weeks after the last infusion of VEGF Trap, there was deterioration, with recurrence of the pocket of SRF, increase in foveal thickness to 248 μ m, and increase in macular volume to 7.31 mm³. VA = visual acuity.

Discussion

Preclinical studies have demonstrated that VEGF is an important stimulus for choroidal neovascularization and that systemic delivery of VEGF Trap strongly suppresses choroidal neovascularization.^{3,4,17} In this study, we report the

results of a randomized, double-masked, placebo-controlled study investigating the effect of intravascular infusion of VEGF Trap in patients with neovascular AMD. The primary objective of the study was to assess the safety of a range of doses to determine the maximum tolerated dose. This objective was met, because although 1.0-mg/kg VEGF Trap

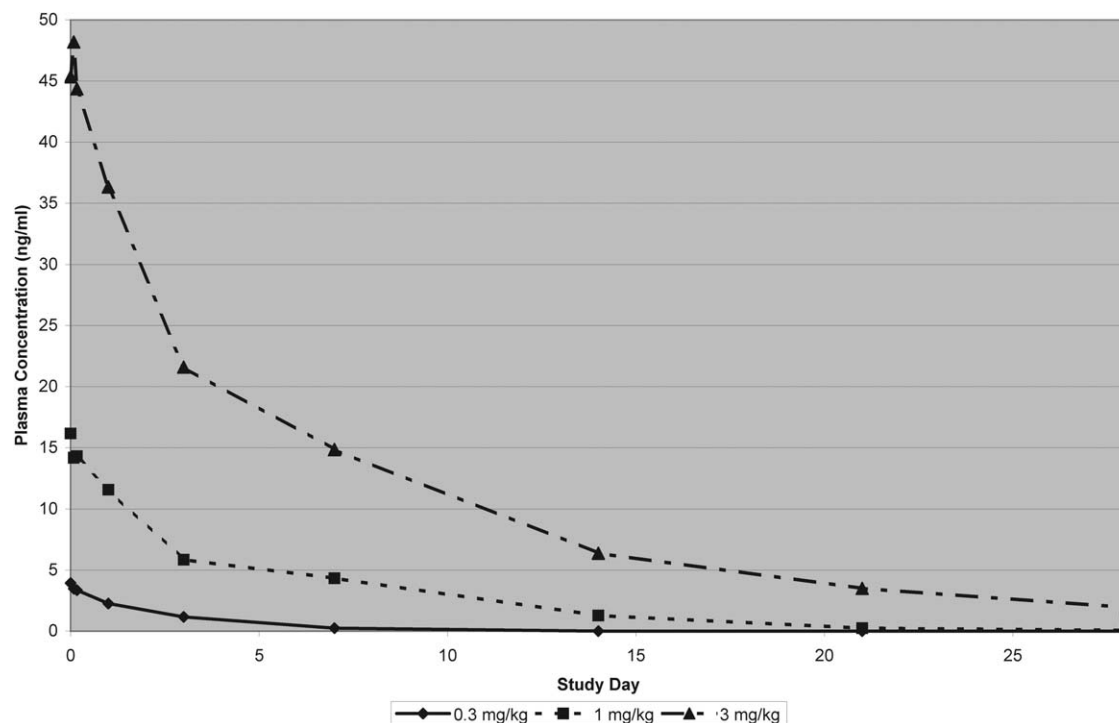


Figure 5. Pharmacokinetics after a single infusion of 0.3-, 1.0-, or 3.0-mg/kg vascular endothelial growth factor trap (VEGF Trap). Plasma levels of VEGF Trap were measured at several time points after infusion. VEGF Trap was eliminated from plasma between 3 and 7 days after infusion of 0.3 mg/kg and between 14 and 21 days after infusion of 1.0 mg/kg and was still detectable 28 days after infusion of 3.0 mg/kg.

was well tolerated, 2 serious adverse events occurred in patients receiving a dose of 3.0 mg/kg. The adverse events were severe hypertension and proteinuria, which are both class effects of VEGF antagonists. Systemic hypertension is felt to occur because inhibition of VEGF in arterial endothelial cells reduces release of nitric oxide, which acts on arterial smooth muscle cells to cause vasodilation²⁰; therefore, systemic inhibition of VEGF increases vascular tone and results in hypertension. Hypertension is a potentially manageable side effect, because drugs that promote relaxation of vascular smooth muscle could be used to counter it, but intervention must be timely and aggressive to avoid consequences of acute hypertension, which can be serious. Proteinuria is also a class effect of VEGF antagonists, but its mechanism is not as well understood as that of hypertension. Proteinuria is reversible by stopping the VEGF antagonist, and although preliminary indications are that chronic VEGF blockade-related proteinuria can be tolerated without leading to deterioration of the glomerular filtration rate, it is still unknown whether prolonged proteinuria during continuing administration of VEGF-blocking agents would result in permanent structural damage. Therefore, the maximum tolerated dose for IV administration of VEGF Trap in this patient population was 1.0 mg/kg. Higher dose levels may be tolerated with expectant management of BP in hypertensive patients, or in younger patient populations.

A second goal of the study was to determine if the maximum tolerated dose of VEGF Trap has bioactivity. The primary measure of bioactivity was excess foveal thickness assessed by OCT. Every patient treated with 1.0-mg/kg

VEGF Trap had reduction in foveal thickness 2 weeks after the first infusion. At day 71, 2 weeks after the fourth infusion, the median reduction in excess foveal thickness was 80%, a dramatic difference from placebo-treated patients, who had no reduction in excess foveal thickness. Similar dramatic reductions in excess total macular volume were seen after infusions of 1.0-mg/kg VEGF Trap. These effects were sustained for at least 2 weeks, but not 1 month, after a single infusion of 1.0 mg/kg, whereas the limited data available for patients treated with 3.0-mg/kg VEGF Trap suggest a sustained effect for at least 1 month. This difference in duration of effect is explainable by the pharmacokinetics of VEGF Trap, because active VEGF Trap was still detectable in patient plasma 1 month after infusion of 3.0 mg/kg, but not 1.0 mg/kg. Six weeks after a fourth 1.0-mg/kg infusion, the median excess foveal thickness was 40%, suggesting loss of about half of the maximal treatment effect; therefore, repeated infusions of 1.0-mg/kg VEGF Trap may be needed to maintain benefits.

The rapid reduction of excess foveal thickness after infusion of 1.0- or 3.0-mg/kg VEGF Trap suggests that blockade of VEGF results in reduced leakage from choroidal neovascularization. Similar rapid effects were observed in a case series of patients with neovascular AMD treated with systemic infusions of bevacizumab,²¹ a full-length antibody that binds VEGF. Reduction in excessive permeability also was suggested by comparison of posttreatment and baseline fluorescein angiograms, but reduction in lesion size was not seen. This suggests that antagonism of VEGF for at least 2 months does not cause regression of choroidal

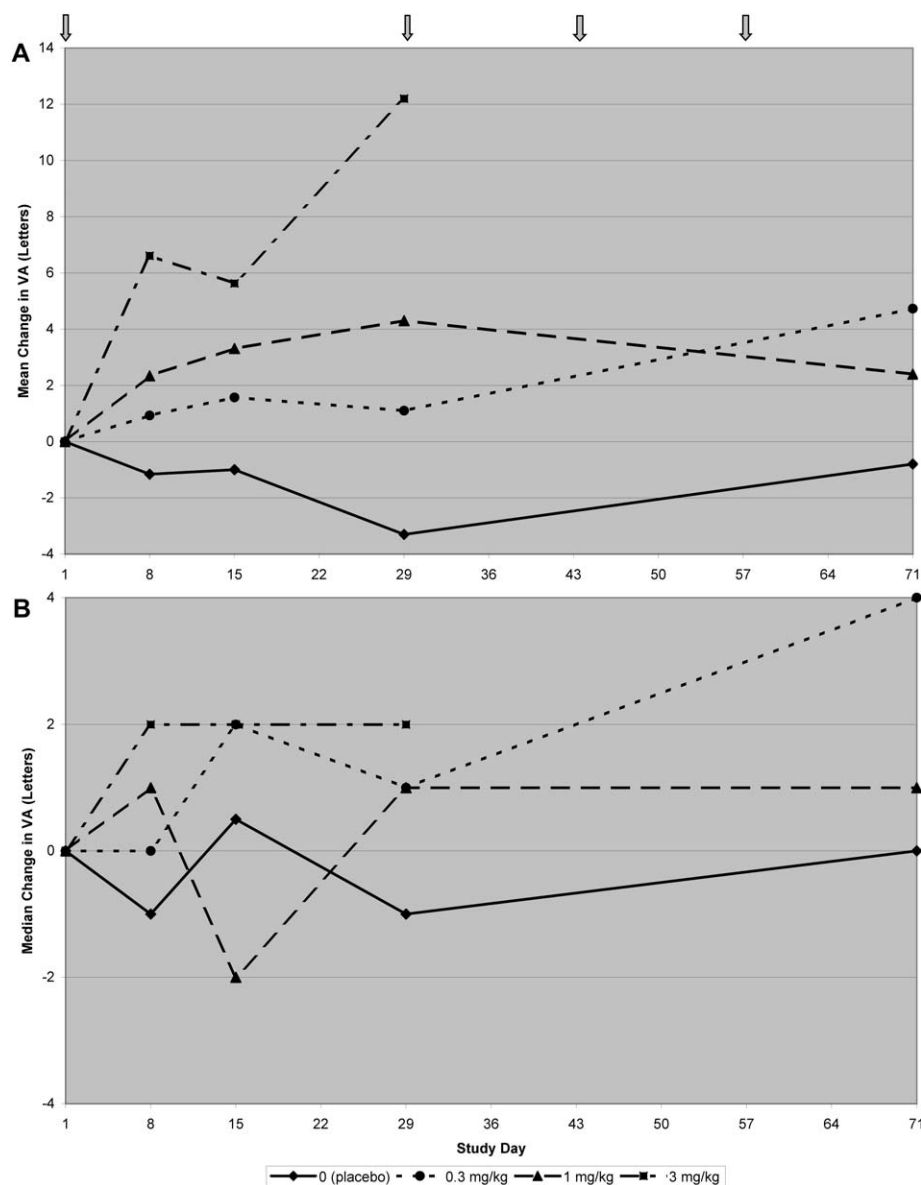


Figure 7. Effect of systemic infusion of vascular endothelial growth factor trap (VEGF Trap) on visual acuity (VA). The graphs show the mean (A) and median (B) change in letters read on the Early Treatment Diabetic Retinopathy Study VA chart from baseline at several time points in each of the 4 cohorts (placebo and 0.3-, 1.0-, and 3.0-mg/kg VEGF Trap). Arrows, when infusions were given.

neovascularization. This is consistent with results seen 1 year after intravitreal injections of pegaptanib, an aptamer that binds VEGF; there was continued growth of choroidal neovascularization, although at a slower rate than that seen in patients receiving sham injections.⁵

This study is a small phase I study but was randomized and placebo controlled. Whenever randomized studies are small, there is a high likelihood that groups will be unbalanced with respect to one or more relevant characteristics. This was the case in our study. Compared with the placebo group, lesions were smaller in the 0.3-mg/kg group but larger in the 1.0- and 3.0-mg/kg groups. Likewise, foveal thickness was less in the 0.3-mg/kg group than in the placebo group, but it did not significantly differ from placebo in the other 2 groups. Whether or not there are a few

more classic lesions in the placebo group has little relevance because other VEGF antagonists have shown equal effectiveness for all lesion types, and the study is very short in duration. The study was not powered to detect a difference in VA, nor was the patient population ideal because many of the patients had multiple previous photodynamic therapies and substantial subfoveal fibrosis. However, despite relatively advanced disease many of the patients had a component of reversible vision loss and showed some improvement in vision, with reduction in retinal thickness and SRF. This correlation of visual improvement and reduction in foveal thickness and SRF suggests that with an adequately powered study a treatment benefit in VA should be detectable. However, more data are needed before the effect of VEGF Trap can be compared with that of other treatments.

Our data support previous studies suggesting that antagonism of VEGF is a good treatment strategy for patients with neovascular AMD.^{5,21} They also suggest that up to 4 systemic infusions of 1.0-mg/kg VEGF Trap are well tolerated. Future studies will explore other modes of delivery of VEGF Trap to try to identify the best way to utilize this promising therapeutic agent.

References

- Macular Photocoagulation Study Research Group. Argon laser photocoagulation for neovascular maculopathy: five year results from randomized clinical trials. *Arch Ophthalmol* 1991; 109:1109–14.
- Klein R, Klein BEK, Linton KP. The Beaver Dam Eye Study: the relation of age-related maculopathy to smoking. *Am J Epidemiol* 1993;137:190–200.
- Kwak N, Okamoto N, Wood JM, Campochiaro PA. VEGF is an important stimulator in a model of choroidal neovascularization. *Invest Ophthalmol Vis Sci* 2000;41:3158–64.
- Kryzstolik MG, Afshari MA, Adamis AP, et al. Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. *Arch Ophthalmol* 2002;120:338–46.
- Gragoudas ES, Adamis AP, Cunningham ET Jr, et al. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med* 2004;351:2805–16.
- Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* 2001;7:575–83.
- Goldman CK, Kendall RL, Cabrera G, et al. Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis, and mortality rate. *Proc Natl Acad Sci U S A* 1998;95:8795–800.
- Takayama K, Ueno H, Nakanishi Y, et al. Suppression of tumor angiogenesis and growth by gene transfer of a soluble form of vascular endothelial growth factor receptor into a remote organ. *Cancer Res* 2000;60:2168–77.
- Mahasreshi PJ, Navarro JG, Kataram M, et al. Adenovirus-mediated soluble FLT-1 gene therapy for ovarian carcinoma. *Clin Cancer Res* 2001;7:2057–66.
- Shiose S, Sakamoto T, Yoshikawa H, et al. Gene transfer of a soluble receptor of VEGF inhibits the growth of experimental eyelid malignant melanoma. *Invest Ophthalmol Vis Sci* 2000; 41:2395–403.
- Lai C-M, Brankov M, Zaknich T, et al. Inhibition of angiogenesis by adenovirus-mediated sFlt-1 expression in a rat model of corneal neovascularization. *Hum Gene Ther* 2001; 12:1299–310.
- Honda M, Sakamoto T, Ishibashi T, et al. Experimental subretinal neovascularization is inhibited by adenovirus-mediated soluble VEGF.flt-1 receptor gene transfection: a role of VEGF and possible treatment for SRN in age-related macular degeneration. *Gene Ther* 2000;7:978–85.
- Kong H-L, Hecht D, Song W, et al. Regional suppression of tumor growth by *in vivo* transfer of a cDNA encoding a secreted form of the extracellular domain of the *flt-1* vascular endothelial growth factor receptor. *Hum Gene Ther* 1998;9: 823–33.
- Bainbridge J, Mistry A, Alwis MD, et al. Inhibition of retinal neovascularization by gene transfer of soluble VEGF receptor sFlt-1. *Gene Ther* 2002;9:320–6.
- Lai YK, Shen WY, Brankov M, et al. Potential long-term inhibition of ocular neovascularization by recombinant adeno-associated virus-mediated secretion gene therapy. *Gene Ther* 2002;9:804–13.
- Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci U S A* 2002;99:11393–8.
- Saishin Y, Saishin Y, Takahashi K, et al. VEGF-TRAP_{R1R2} suppresses choroidal neovascularization and VEGF-induced breakdown of the blood-retinal barrier. *J Cell Physiol* 2003; 195:241–8.
- Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335–42.
- Hee MR, Puliafito CA, Duker JS, et al. Topography of diabetic macular edema with optical coherence tomography. *Ophthalmology* 1998;105:360–70.
- Tang JR, Markham NE, Lin YJ, et al. Inhaled nitric oxide attenuates pulmonary hypertension and improves lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor. *Am J Physiol Lung Cell Mol Physiol* 2004;287: L344–51.
- Michels S, Rosenfeld PJ, Puliafito CA, et al. Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration. *Ophthalmology* 2005;112:1035–47.

Appendix: CLEAR-AMD 1 Study Group

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Table 5. Dose-Limiting Toxicities

Dose Level (mg/kg)	n	Dose-Limiting Toxicity	Doses	Serious	Recovered
0.3	7	None	None		
1.0	7	None	None		
3.0	5	Grade 2 hypertension*	2	No	Yes
		Grade 2 proteinuria*	2	No	Yes
		Grade 4 hypertension†	1	Yes	Yes
		Grade 3 CHF†	1	Yes	Yes
		Grade 3 pulmonary edema†	1	Yes	Yes

CHF = congestive heart failure.

*Both encountered by subject no. 001–306.

†All encountered by subject no. 001–365.

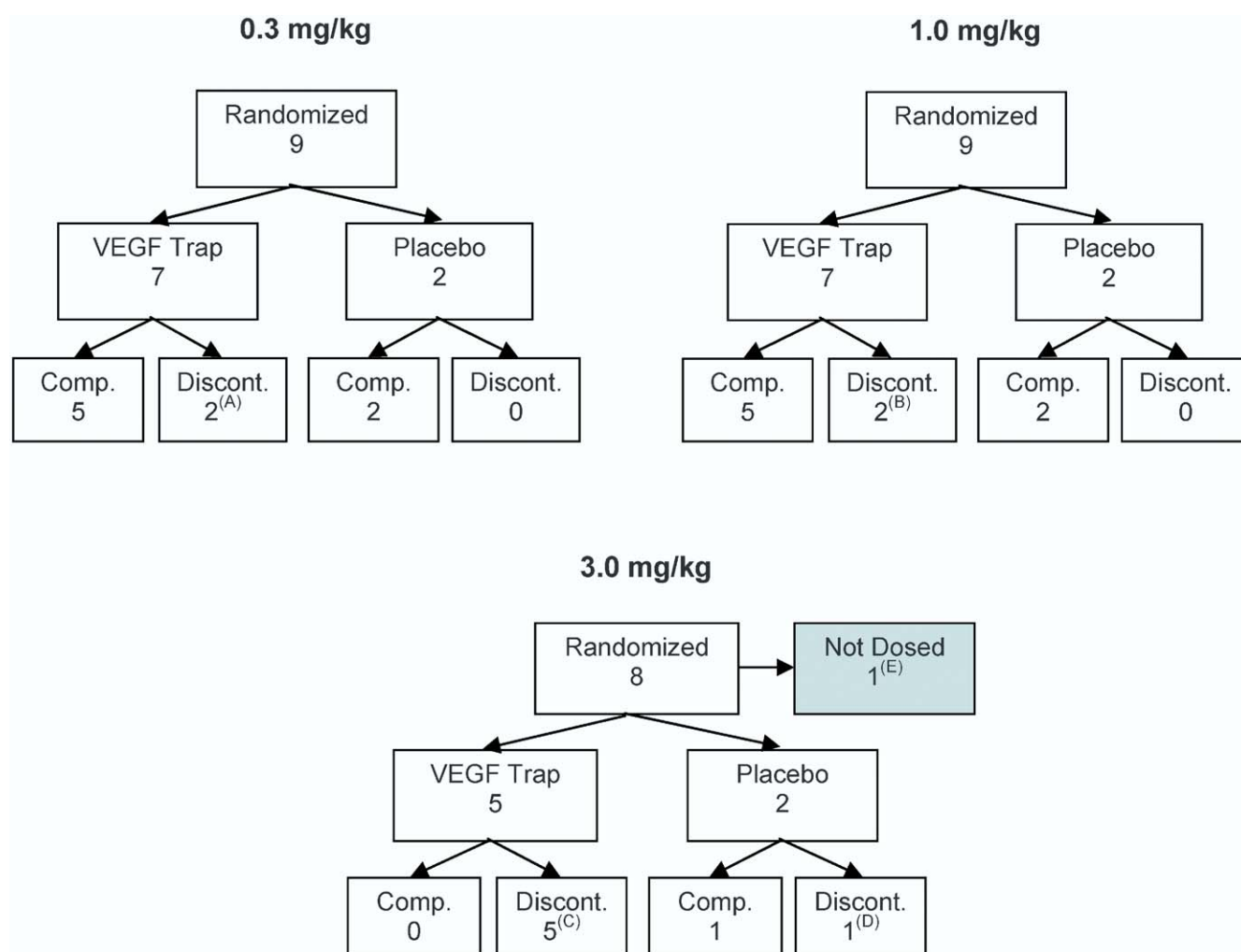


Figure 1. Patient disposition. This schematic shows the disposition of patients in the study. The reasons for discontinuation of patients in each of the groups were (A) withdrawal of consent in 2 patients; (B) an adverse event (1 patient) and failure to meet eligibility criteria (1 patient); (C) an adverse event (2 patients), need for excluded medication (2 patients), and sponsor request due to safety concerns (1 patient); (D) loss to follow-up (1 patient); and (E) randomization to the study at the same time a decision was made to discontinue dosing for all patients and, therefore, no dosing. Comp. = completed; VEGF Trap = vascular endothelial growth factor trap.

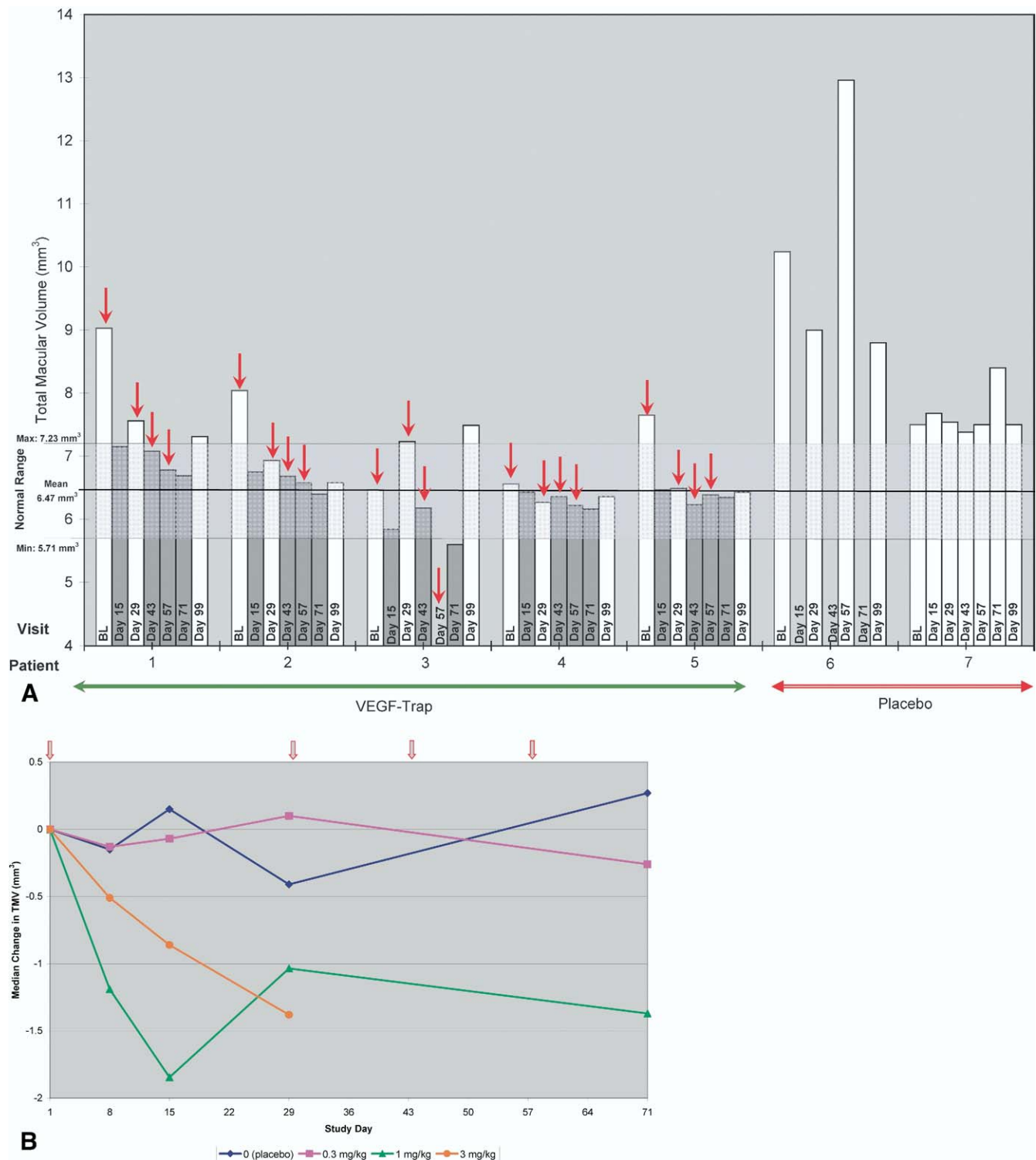


Figure 3. Reduction in excess total macular volume (TMV) from systemic infusion of 1.0-mg/kg vascular endothelial growth factor trap (VEGF Trap) in patients with neovascular age-related macular degeneration. **A**, Excess macular volume measured by optical coherence tomography is plotted at several time points for all patients in the second cohort. The measurements shown on the left and marked along the baseline (BL) as VEGF-Trap are from the 5 patients who received infusions of 1.0 mg/kg on days 0, 29, 43, and 57. The measurements from 2 patients who received a placebo are on the right. Shaded bars are for measurements taken when based upon pharmacokinetics studies; patients were likely to have detectable plasma levels of VEGF Trap. White bars are for measurements taken when patients were likely to have undetectable levels of VEGF Trap. The normal range (5.71–7.23 mm³), defined as the mean $\pm$ 2 standard deviations of macular volume determined from measurements in patients with no macular disease, is shaded. Note that for each patient, the shaded bars tend to be lower than the white bars. **B**, The change from baseline in TMV is plotted at several time points for patients who received a placebo or those treated with 0.3-, 1.0-, or 3.0-mg/kg, VEGF Trap. The arrows at the top indicate when infusions were given.

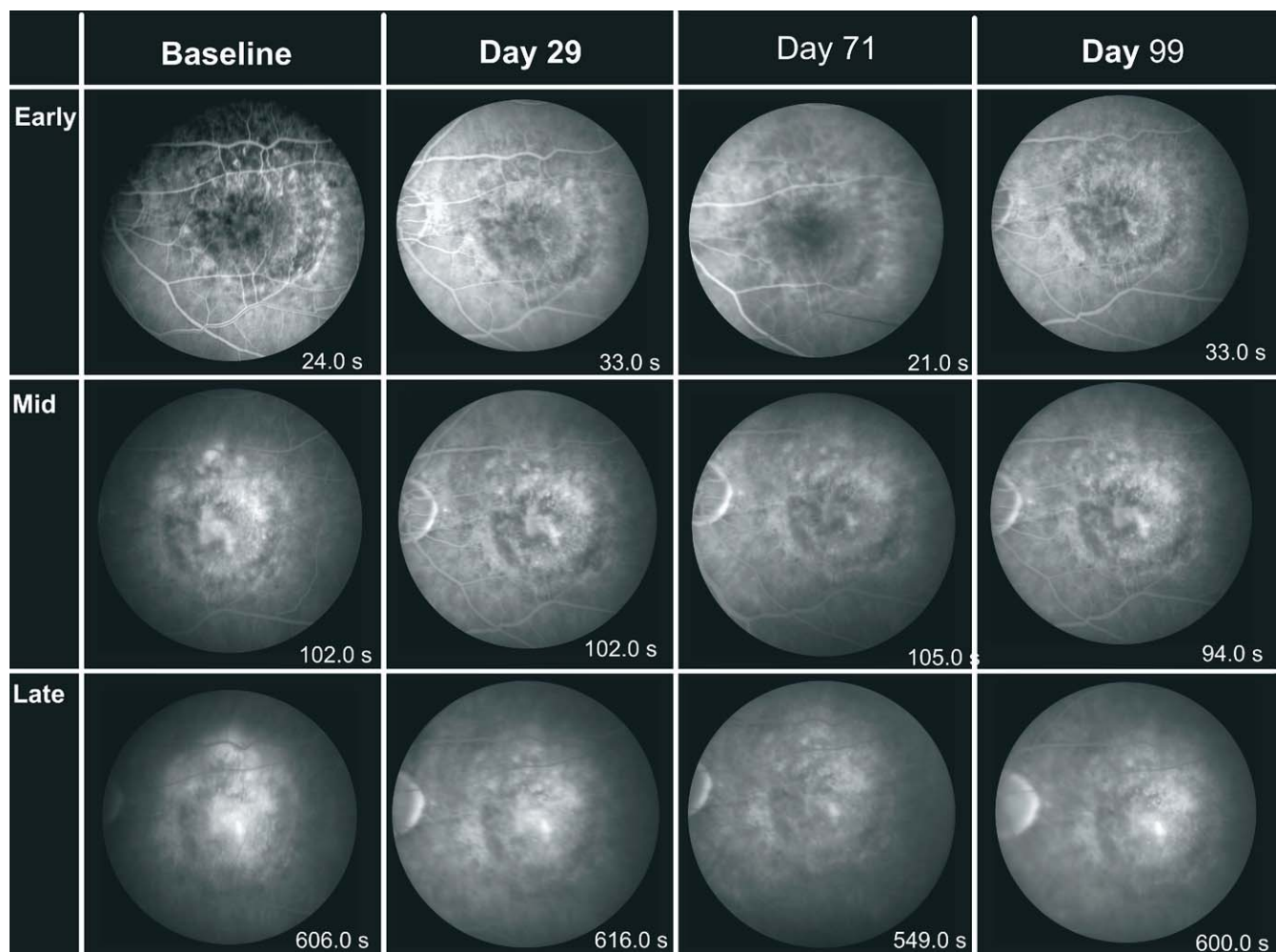


Figure 6. Effect of treatment with 1.0-mg/kg vascular endothelial growth factor trap (VEGF Trap) on fluorescein leakage from subfoveal choroidal neovascularization. Digital fluorescein angiography was done at baseline and 3 time points after initiation of treatment with 1.0-mg/kg VEGF Trap. At baseline, there is a large area of occult subfoveal choroidal neovascularization that leaks during the midphase and late phase of the angiogram, so that there is white dye pooled beneath the entire macula. At day 29, there is mildly reduced leakage, but by day 71, there is a substantial reduction in leakage and pooling of dye. At day 99, there is some increase in leakage compared with day 71, but it is still less than that seen at baseline. s = seconds.