Prevention of Experimental Choroidal Neovascularization and Resolution of Active Lesions by VEGF Trap in Nonhuman Primates

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Objective: To evaluate the efficacy of systemic and intravitreous administration of VEGF Trap (aflibercept) in a nonhuman primate model of choroidal neovascularization (CNV).

Methods: VEGF Trap treatment on laser-induced CNV was evaluated in 48 adult cynomolgus monkeys. In the prevention arms of the study, VEGF Trap was administered by intravenous injection (3 or 10 mg/kg weekly) or intravitreous injection (50, 250, or 500 µg/eye every 2 weeks) beginning before laser injury. In the treatment arm, a single intravitreous injection (500 µg) was given 2 weeks following laser injury. Laser-induced lesions were scored from grade 1 (no hyperfluorescence) to grade 4 (clinically relevant leakage). Representative lesions were evaluated histologically.

Results: Grade 4 leakage developed at 32.4% and 45.4% of the laser sites in animals receiving intravitreous or intravenous administration of placebo at 2 weeks following laser injury, respectively. In contrast, the development of grade 4 lesions was completely or nearly completely prevented in all groups receiving intravenous or intravitreous injections of VEGF Trap. A single intravitreous injection of VEGF Trap (500 µg) administered following the development of CNV reduced the frequency of grade 4 lesions from 44.4% to 0% within 14 days of treatment. Intravitreous VEGF Trap was well tolerated with either no or only mild ocular inflammation. Histological evaluation showed decreased scores for morphologic features of tissue proliferation in the VEGF Trap prevention groups.

Conclusions: VEGF Trap prevented the development of clinically relevant CNV leakage when administered at the lowest doses tested. Moreover, a single intravitreous injection induced inhibition of active CNV leakage.

Clinical Relevance: The animal model used in this study has an established track record as a predictor of pharmacologic efficacy of antineovascular drugs in humans having the neovascular, or wet, form of age-related macular degeneration.

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AGE-RELATED MACULAR DEGENERATION (AMD) is a leading cause of blindness whose incidence is likely to increase as the population ages.1 The great majority of individuals with AMD have the dry form, which is characterized by atrophic degeneration of the retinal pigment epithelium with secondary (and often gradual) damage to the photoreceptors. However, 80% to 90% of patients with AMD who develop severe vision loss have the wet (neovascular) form,2 which occurs when abnormal new blood vessels originating from the choroid grow through the Bruch membrane into the subretinal or intraretinal space. This choroidal neovascularization (CNV) was formerly treated with thermal laser photocoagulation according to protocols developed as part of the Macular Photocoagulation Study and related subsequent studies.3-8 Although the treatment was effective at slowing the progression of the disease, it seldom resulted in improved vision because the thermal laser also irreversibly damaged the overlying retina. The patients were often left with central scotomas from the treatment itself. Since then, drugs such as pegaptanib sodium (Macugen) and ranibizumab (Lucentis) have been developed for human use; these work by inhibiting vascular endothelial growth factor (VEGF).

VEGF Trap is a potent VEGF inhibitor comprising ligand-binding portions of human VEGF receptor 1 (VEGFR1) and VEGFR2 fused to the Fc segment of human IgG1 (Figure 1).9 VEGF Trap binds and neutralizes multiple isoforms of
VEGF-A (dissociation constant of approximately 1pM) as well as the related angiogenic factor placental growth factor (PlGF) (dissociation constant of approximately 40pM). An intravenous formulation of VEGF Trap, generically known as aflibercept, is being developed for oncology; this formulation is hyperosmotic and diluted prior to intravenous infusion. VEGF Trap-Eye, known generically as aflibercept ophthalmic solution, is an iso-osmotic, ultrapurified formulation of VEGF Trap for intravitreous injection. Phase 3 studies of VEGF Trap-Eye in patients with neovascular AMD and retinal vein occlusion are currently in progress.

The purposes of this study were to evaluate the efficacy of systemic and intravitreous administration of VEGF Trap in a primate model of CNV and to evaluate histological changes associated with the angiographic improvements observed. This study was completed prior to initiating the human clinical trial program for VEGF Trap-Eye.

**METHODS**

**LASER INDUCTION OF CNV**

The effect of VEGF Trap treatment on laser-induced CNV was evaluated in cynomolgus monkeys (1.8-2.7 kg at initiation of dosing) using a modification of a model of CNV developed by Ryan and Ohkuma and Ryan. All of the experimental methods and techniques adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by our institutional animal care and use committee. Animals were anesthetized with ketamine hydrochloride and xylazine hydrochloride. A 532-nm diode laser (OcuLight GL; Iridex Corp, Mountain View, California) with a table-mounted slitlamp adapter was used to create small (75-µm diameter), intense laser spots except the one just temporal to the fovea, which was treated with 400 mW. If no hemorrhage occurred at a given spot, a second spot was placed adjacent to it using a laser intensity of 150 mW for all spots except the one just temporal to the fovea, which was treated with 400 mW. If no hemorrhage occurred at a given spot, a second spot was placed adjacent to it using a laser intensity of 150 mW greater than the initial burn. The development of active CNV lesions was assessed by fluorescein angiography (FA), once before injury and 15, 20, and 29 days after laser injury. The CNV lesions were graded by a masked observer (T.M.N.) using the following scale: grade 1, no hyperfluorescence; grade 2, hyperfluorescence, without leakage; grade 3, hyperfluorescence early or midtransit, and late leakage; and grade 4, bright hyperfluorescence early or midtransit, with late leakage extending beyond the borders of the laser spot.

**TREATMENT PARADIGMS**

In the prevention studies, VEGF Trap was administered by intravenous injection (3 or 10 mg/kg of body weight weekly) or intravitreous injection (50, 250, or 500 µg/eye every 2 weeks) beginning approximately 1 week before laser injury. For intravitreous injection, VEGF Trap was formulated in 10mM sodium phosphate, 135mM sodium chloride, and 0.1% polyethylene glycol 3350 (pH 6.25) and injected through a 30-gauge sterile needle in a volume of 50 µL (500 or 50 µg) using a 1-mL tuberculin syringe or 25 µL (250 µg) using a 0.3-mL syringe. VEGF Trap for intravenous injection was formulated in 5mM sodium phosphate, 5mM sodium citrate, 100mM sodium chloride, 20% sucrose, and 0.1% polysorbate 20 (pH 6.0) and infused in a volume of 4 to 5 mL/kg of body weight over 30 minutes. Control animals received weekly intravenous infusions or biweekly intravitreous injections (50 µL) of placebo comprising the appropriate vehicle solutions according to the same schedule as for corresponding VEGF Trap–treated groups.

In the treatment study, a single intravitreous injection of VEGF Trap (500 µg) was given 15 days following laser injury, at which time active CNV had already formed. Each of the experimental and control groups comprised 6 animals, including 3 males and 3 females; both eyes were treated identically (Table 1).

**INTRAVITREOUS INJECTIONS**

Animals were anesthetized with ketamine and xylazine, and the eyes were instilled with 0.5% proparacaine hydrochloride, cleaned with 2.5% povidone-iodine, and rinsed with sterile saline. Immediately following each injection, a single topical dose of tobramycin and dexamethasone (Tobradex) ointment was applied to the eye. No systemic antibiotics were used. The left and right eyes of each animal received the same dose of either VEGF Trap or placebo (as opposed to a study design that used the fellow eye as the control) to eliminate the possibility of a systemic effect on the control eye.

**OPHTHALMIC EXAMINATIONS**

Daily cage-side observations were performed on all animals to monitor for clinical signs of poor health, including any ocular abnormalities. Animals also underwent clinical ophthalmic examinations before the initiation of treatment and on postlaser days 7, 21, and 32 (intravitreous prevention groups) and days 9, 23, and 33 (intravenous prevention groups and intravitreous treatment group, excluding day 9). The anterior portion of each eye was viewed using a handheld slitlamp biomicroscope, and the ocular fundus was viewed with an indirect ophthalmoscope. Intraocular pressure was monitored. Fundus photographs were taken on the day of laser treatment (following laser injury) and approximately 4 weeks later, preceding the final FA.
deviations are based on 6 animals per group.

more than 100 cells per single field of focused beam. Scores
100 cells per single field of focused beam; a score of 4
50 cells per single field of focused beam; a score of 3
1 to 5 cells per single field of focused beam; a score of 1
0 indicating not present. The total tissue proliferation score

Anterior chamber and vitreous cell scores were deter-
m定了 for right and left eyes using a slitlamp biomicroscope as follows: a score of 0 indicates no cells observed; a score of 0.5+, 1 to 5 cells per single field of focused beam; a score of 1+, 5 to 25 cells per single field of focused beam; a score of 2+, 25 to
50 cells per single field of focused beam; a score of 3+, 50 to
100 cells per single field of focused beam; and a score of 4+, more than 100 cells per single field of focused beam. Scores
from both eyes were averaged per animal. Means and standard
deviations are based on 6 animals per group. Figure 2 shows
the timing of dosing, FA, ophthalmic examinations, and nec-
ropy relative to the day of laser treatment for each of the 3 treat-
ment arms.

STATISTICAL ANALYSIS
OF LASER LESION GRADES

For each of the 3 postlaser angiography intervals (days 15, 20,
and 29), the proportions of grade 4 counts were dichotomized
to 1 and 0 and the Cochran-Armitage trend test was applied to
the intravenous prevention and intravitreous prevention groups
separately. Fisher exact tests were also conducted for group com-
parisons between treated groups and the control group.

For the intravitreous treatment group and the intravitre-
ous prevention placebo group, data from day 15 were treated
as baseline data and were subtracted from the data on days 20
and 29. The difference was then analyzed for days 20 and 29
separately using Wilcoxon signed rank test.

All test results are exact because of the small sample sizes.
All statistical tests were conducted at the 5% level.

HISTOLOGICAL ASSESSMENT
OF CNV LESIONS

Animals were killed on postlaser day 33 (intravitreous preven-
tion groups) or day 35 (intravenous prevention groups and
intravitreous treatment group) and the upper body was per-
fused through the aorta (descending clamped) with half-
strength Karnovsky fixative. The eyes were removed, post-
fixed for 2 to 3 days in half-strength Karnovsky fixative, and
then stored in formalin until processed.

One eye from each animal in the intravitreous placebo, VEGF
Trap (500 µg) prevention, and VEGF Trap treatment groups
was selected for histopathological evaluation. The selected eyes
were representative and comprised approximately half of the
grade 4 lesions for each of the groups. Strips of tissue contain-
ing 1 or 2 lesion sites were embedded in plastic. Sections 2 µm
thick were taken at 30-µm steps through the middle of each
lesion. The sections were stained with toluidine blue, and the
sample with the most robust lesion was designated as the cen-
tral cut. This section was then evaluated by an observer (R.R.D.)
masked to the treatment condition.

A tissue proliferation score was calculated for each lesion
based on 3 criteria: the size of the spindle cell proliferative
lesion, the extent of new blood vessel proliferation in the sub-
retinal space, and the elevation of the retina above the chorio-

in capillaris (Figure 3). Each measure was graded from 0 to 3,
with 0 indicating not present. The total tissue proliferation score
comprises the sum of each of the described measures for each
laser lesion site.

RESULTS

INFLAMMATORY RESPONSE

Intravitreous administration of the VEGF Trap placebo
control article was well tolerated, with 0.5+ vitreous cells
seen in 1 of 6 animals in this group. No anterior cham-
ber cells were detected at the designated examination times in
animals receiving intravitreous injections of placebo (Table 2).
Intravitreal administration of the VEGF Trap test material at all dose levels resulted in no (0) or mild (0.5+ to 1+) inflammatory cell scores in the anterior chamber or vitreous. During the course of the study, trace (0.5+) levels of anterior chamber cells were seen in 4 of 6 animals in the mid-dose group (250 µg/eye/dose) and 3 of 6 animals in the high-dose group (500 µg/eye/dose) in the multiple (biweekly) intravitreal dose prevention experiment and in 1 of 6 animals in the single intravitreal dose treatment study (500 µg/eye following CNV formation). Vitreous cell scores were also mild (0.5+ to 1+) in all of the groups that underwent intravitreal injection of VEGF Trap, but vitreous cells were more frequent and detected in all animals in these groups at some time during the study. This finding was not unexpected, because inflammatory cells are much slower to enter and clear from the more viscous vitreous gel than the aqueous humor. These results are summarized in Table 2. At no time or dose did the mean cell inflammatory score exceed 1+ in any eye. Ocular examinations were performed approximately 2 weeks following injections in the intravitreal prevention study, so early transient inflammation may have been missed. However, the animals in the intravitreal treatment group were examined 8 days after injection and only mild inflammation was observed (on study day 23) (Table 2). No animals showed gross evidence of ocular or systemic toxic effects based on daily cage-side inspections. There were no significant effects on intraocular pressure beyond a transient elevation in all groups immediately following intravitreal injection.

Intravenous administration of VEGF Trap placebo or VEGF Trap at a low or high dose produced no detectable anterior chamber or vitreous cells.

No evidence of a retinal inflammatory response (eg, perivascular sheathing, retinal thickening, optic nerve swelling, or retinal vascular leakage) was found on color fundus photography or FA in any of the animals.

**FLUORESCEIN ANGIOGRAMS**

Of the 4 grades assigned to the laser treatment spots, grade 4 (bright hyperfluorescence early or midtransit, with late leakage extending beyond the borders of the laser spot) corresponds to clinically significant leakage. Grade 4 lesions are thought to reflect the presence of new choroidal vessels that either have grown beyond the laser treatment spot or are leaking so intensely that the fluorescein dye has spread markedly away from the vessels. The results with respect to grade 4 leakage for all groups are shown in Table 3. The average number of grade 4 lesions in the intravitreal placebo group ranged from 26.9% to 32.4% during the times evaluated (postlaser days 15, 20, and 29), while 45.4% to 50.0% of the laser treatment areas show grade 4 leakage in the intravenous placebo group. The mean percentage of grade 4 lesions in the control groups was similar to that which has been reported by others using this animal model of CNV.10,11,13 By contrast, all of the VEGF Trap prevention groups showed marked reduction or complete absence of grade 4 lesions, irrespective of dose (Figure 4 and Figure 5). Table 4 shows the distribution of all lesion grades on day 29 for the prevention groups.

In the VEGF Trap treatment group (single intravitreal injection of VEGF Trap administered on postlaser day 15), 44.4% of laser treatment spots exhibited grade 4 leakage on day 15, similar to the percentage of grade 4 spots in the 2 placebo control groups. However, by postlaser day 20 (5 days following intravitreal administration of VEGF Trap), only 1.9% of the spots were grade
When VEGF Trap administration was begun prior to laser injury (prevention), choroidal fibroplasia and retinal elevation scores as well as CNV scores were all significantly lower in VEGF Trap–treated animals relative to placebo controls (Table 5 and Figure 9). When a single injection of VEGF Trap was given after grade 4 lesions had developed, there was also a trend toward decreased CNV, but mean scores for fibroplasia and retinal elevation were not significantly different from controls (Table 5 and Figure 10).

**Table 2. Inflammatory Response**

<table>
<thead>
<tr>
<th>Group</th>
<th>Anterior Chamber Cells</th>
<th>Vitreous Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 9</td>
<td>Day 23</td>
</tr>
<tr>
<td>Intravenous prevention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 mg/kg/dose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 mg/kg/dose</td>
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<td>0</td>
</tr>
<tr>
<td>Intravitreous prevention</td>
<td>Day 7</td>
<td>Day 21</td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
<td>0</td>
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<td>50 µg/eye/dose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250 µg/eye/dose</td>
<td>0.13 (0.2)</td>
<td>0.04 (0.1)</td>
</tr>
<tr>
<td>500 µg/eye/dose</td>
<td>0</td>
<td>0.17 (0.2)</td>
</tr>
<tr>
<td>Intravitreous treatment</td>
<td>Day 23</td>
<td>Day 33</td>
</tr>
<tr>
<td>Single dose 500 µg/eye</td>
<td>0.08 (0.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3. Mean Percentages of Grade 4 Lesions by Group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous prevention</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>45.4</td>
<td>50.0</td>
<td>45.4</td>
</tr>
<tr>
<td>3 mg/kg/dose</td>
<td>0°c</td>
<td>0°c</td>
<td>0.9°d</td>
</tr>
<tr>
<td>10 mg/kg/dose</td>
<td>0°c</td>
<td>0°c</td>
<td>0°c</td>
</tr>
<tr>
<td>Intravitreous prevention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>32.4</td>
<td>31.5</td>
<td>26.9</td>
</tr>
<tr>
<td>50 µg/eye/dose</td>
<td>0°c</td>
<td>0.9°d</td>
<td>0.9°d</td>
</tr>
<tr>
<td>250 µg/eye/dose</td>
<td>0°c</td>
<td>0°c</td>
<td>0°c</td>
</tr>
<tr>
<td>500 µg/eye/dose</td>
<td>0°c</td>
<td>0°c</td>
<td>5.8°d</td>
</tr>
<tr>
<td>Intravitreous treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single dose 500 µg/eye</td>
<td>44.4</td>
<td>1.9°f</td>
<td>0°g</td>
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</tbody>
</table>

**Comment**

**BACKGROUND**

Important advances were made in the treatment of AMD by the application of drugs that act to destroy and/or prevent formation of the new blood vessels. The first of these to be approved for human use was photodynamic therapy using the photosensitizing dye verteporfin (Visudyne; Novartis, Basel, Switzerland) administered intravenously followed by exposure of the CNV to 689-nm low-energy laser. Photodynamic therapy greatly reduced direct retinal damage from prior thermal laser therapy. However, there were problems with recurrence, and patients continued to have a decline in vision over time.14

Following the development of photodynamic therapy, a new family of drugs that act to inhibit the cytokine VEGF-A was developed. VEGF-A has been implicated as a causal factor in the development of the wet form of AMD as well as other ocular vascular diseases characterized by pathological neovascularization and vascular leak and/or edema. A number of strategies are being developed to inhibit VEGF-A signaling in these conditions, including application of antibodies to VEGF-A or the VEGF receptors, VEGF-binding aptamers, and small interfering RNAs and treatment with kinase inhibitors. The first of these

Consonant with the FA findings, histological evaluation revealed that intravitreous administration of VEGF-Trap reduced proliferative responses of the retina to laser injury, particularly neovascular proliferation.

When VEGF Trap administration was begun prior to laser injury (prevention), choroidal fibroplasia and retinal elevation scores as well as CNV scores were all significantly lower in VEGF Trap–treated animals relative to placebo controls (Table 5 and Figure 9). When a single injection of VEGF Trap was given after grade 4 lesions had developed, there was also a trend toward decreased CNV, but mean scores for fibroplasia and retinal elevation were not significantly different from controls (Table 5 and Figure 10).
to be approved for human use was pegaptanib (Macugen), an RNA aptamer directed against the VEGF-A 165 isoform.\textsuperscript{15,16} Inhibition of VEGF-A 165 was shown to slow the progression of vision loss in wet AMD but did little to reverse vision loss. More recently, intravitreous administration of ranibizumab (Lucentis) has been approved for the treatment of AMD. Ranibizumab is a humanized monoclonal antibody Fab fragment that is directed against all isoforms of VEGF-A. It has largely replaced pegaptanib in clinical practice following 2 large, clinical, phase 3 trials (Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD [MARINA]\textsuperscript{17} and Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD [ANCHOR]\textsuperscript{18,19}) showing that 94% to 96% of patients receiving 0.5 mg of ranibizumab monthly lost fewer than 15 letters of visual acuity and 34% to 40% actually gained 15 letters. The related drug bevacizumab (Avastin), a humanized whole IgG1 antibody approved for oncology, is also used off-label by clinicians.\textsuperscript{20} Despite these advances, the current treatment of choice for AMD (either ranibizumab or bevacizumab) requires repeated intravitreous injections on a monthly basis for an indeterminate period—possibly years—to maintain improvements in visual acuity.

VEGF, PlGF, AND CNV

Extensive literature demonstrates that VEGF-A is a critical factor contributing to the development of ocular neovascularization (for a review, see the article by Witmer et al\textsuperscript{21}). In contrast to other agents that bind and neutralize only VEGF-A, VEGF Trap also binds and neutralizes PlGF.\textsuperscript{9} Placental growth factor is a member of the VEGF family of cytokines that is expressed prominently in the placenta, the tissue from which it was first isolated.\textsuperscript{22} It can promote angiogenesis directly or by enhancing VEGF-A activity.\textsuperscript{23,24} In contrast to VEGF-A, which also plays an indispensable role in normal vascular development, PlGF has been specifically implicated
in promoting pathological neovascularization. While genetic deletion of even a single allele of VEGF-A results in profound impairments in vascular development, normal vascular development and function are not appreciably impaired in PlGF-null mice. However, genetic deletion or pharmacological inhibition of PlGF significantly reduces pathological neovascularization as well as the associated vascular leakage in numerous disease settings. Like VEGF-A, PlGF appears to be involved in promoting ocular vascular disease in both humans and animals. For example, PlGF is present in CNV membranes excised from human eyes, and experimental CNV is decreased in PlGF-null mice and mice treated with PlGF neutralizing antibodies relative to controls.

The proangiogenic and propermeability effects of VEGF-A are thought to be mediated primarily through VEGFR2 expressed on vascular endothelial cells. A structurally related receptor, VEGFR1, binds both VEGF-A and PlGF. In addition to being present on endothelial cells, where receptor ligation is also thought to promote angiogenesis and vascular permeability, albeit more weakly, VEGFR1 is expressed by many other cell types including leukocytes, pericytes, smooth muscle cells, and endothelial progenitor cells. Thus, in addition to promoting angiogenesis and vascular permeability by acting directly on endothelial cells, VEGF and PlGF can also act via VEGFR1 on a variety of other cell types involved in blood vessel formation and stabilization. Moreover, VEGF and PlGF serve as potent chemoattractants and activators of leukocytes, particularly monocytes, in a variety of pathological conditions.

EFFECTS OF VEGF TRAP IN RODENT MODELS OF OCULAR NEOVASCULARIZATION

VEGF Trap, administered either as serial subcutaneous injections or as a single intravitreous injection, has been shown to suppress laser-induced CNV in mice. Moreover, VEGF Trap given subcutaneously inhibits retinal neovascularization in transgenic mice that overexpress VEGF in photoreceptors. Furthermore, VEGF Trap was found to reduce breakdown of the blood-retinal barrier following intravitreous injection of VEGF and in transgenic mice that overproduce VEGF in the retina. Systemic administration of VEGF Trap also has been shown to suppress neovascularization and the associated inflammatory cell infiltrate following corneal injury and to delay corneal allograft rejection in mice. More recently, VEGF Trap has been reported to prevent the development and promote the regression of recently formed CNV following subretinal injection of matrigel in rats. Interestingly, VEGF Trap treatment also reduced CNV-associated fibrosis and inflammation in this model.

THIS STUDY

Although CNV can be induced in other species, only nonhuman primates have maculae similar to the human macula. Thus, the model by Ryan of inducing CNV using intense, small laser spots applied to the macular retina to break the Bruch membrane has become a standard means of assessing the preclinical efficacy of pharmacological treatments for wet AMD (ie, CNV). For example, this model was used for preclinical evaluations of photodynamic therapy and Lucentis. Even so, the model has its limitations. The young nonhuman primates have otherwise healthy retinas (including retinal pigment epithelia) and the induced CNV, unlike CNV in elderly humans with AMD, is self-limiting, resolving in 6 to 8 weeks without treatment. Also, the model has considerable variability. Only about 40% of the treatment spots go on to develop grade 4 lesions and 20% of the animals are nonresponders, with no CNV developing in either eye (T.M.N. and B.J.C., unpublished data, May 2008). Therefore, it is important to have an adequate number of subjects in each group.
In this animal model of CNV, VEGF Trap was highly effective at preventing the development of grade 4 leakage on FA regardless of dose or whether it was administered intravenously on a weekly schedule or intravitreally every 2 weeks (Table 3, Figure 4, and Figure 5). Histological assessment confirmed that choroidal new vessel formation, fibrotic changes, and retinal thickness also were markedly less in the treated eyes (Table 5).

Moreover, when a single intravitreous injection of VEGF Trap was given after grade 4 CNV had developed, leakage was stopped within 5 days in approximately 93% of previously active grade 4 lesions and within 14 days following treatment in 100% of the lesions (postlaser days 20 and 29, respectively) (Table 3, Figure 6, and Figure 7). Although the effect of a single intravitreous injection of placebo was not evaluated, grade 4 lesions persisted for the duration of the study in all animals receiving multiple intravitreous or intravenous injections of placebo (Table 3). Histological examination revealed a trend toward decreased CNV and fibrosis relative to controls, which was not statistically significant. VEGF is a powerful mediator of vascular permeability in addition to new vessel formation, so VEGF Trap may have blocked VEGF-induced leakage from choroidal neovessels. Alterna-
tively, VEGF Trap may have reduced or stopped blood flow through the new vessels.

Intravitreous administration of VEGF Trap was well tolerated, with only a mild inflammatory response noted in the eyes that underwent intravitreous VEGF Trap treatment. Except for 1/1100 or fewer anterior chamber and vitreous cells in some eyes, no other ophthalmoscopic signs of inflammation were seen.

HUMAN TRIALS OF VEGF TRAP-EYE

VEGF Trap is now in clinical trials (for a recent review, see the article by Dixon et al42). A phase 1 trial of 25 patients with exudative AMD evaluated the tolerability and efficacy of intravenous administration of VEGF Trap at 3 different dose levels. Subjects had a significant decrease in retinal thickness as determined by optical coherence to-

tography,43 although visual acuity was not significantly improved in this small safety study. However, 1 subject experienced grade 4 hypertension and 1 subject developed grade 2 proteinuria. Hypertension and proteinuria are now well-established class effects of systemic VEGF inhibition, and both patients exhibiting these adverse events in the study by Nguyen et al43 had received the highest intravenous dose of VEGF Trap (3 mg/kg).

Another phase 1 study (Clinical Evaluation of Anti-angiogenesis in the Retina, CLEAR-IT 1) used intravitreous administration of VEGF Trap-Eye (aflibercept ophthalmic solution).44 The first part of this study was a sequential cohort dose escalation (from 0.05 to 4.0 mg/eye) in 21 patients with exudative AMD. No serious systemic or ocular toxic effects were observed. However, a marked decrease in retinal thickness44 and improvement in visual acuity44 were noted. VEGF Trap-Eye also

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### Table 5. Histological Scores

<table>
<thead>
<tr>
<th>Histological Finding</th>
<th>CNV Prevention&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CNV Treatment&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo VEGF Trap</td>
<td>Placebo VEGF Trap</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Fibroplasia</td>
<td>1.74</td>
<td>1.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Retinal elevation</td>
<td>1.31</td>
<td>0.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>0.69</td>
<td>0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>3.74</td>
<td>1.79&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>

Abbreviation: CNV, choroidal neovascularization.

<sup>a</sup> Mean scores for all lesions in all eyes (n = 6 per group).

<sup>b</sup> Mean scores for lesions that were grade 4 at postlaser day 15 (prior to the single VEGF Trap injection).

<sup>c</sup> P < .05, Mann-Whitney U test.

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![Figure 9. Intravitreous prevention study, showing late-phase fluorescein angiograms at postlaser day 29 and histological sections at postlaser day 33 (glycol methacrylate sections stained with toluidine blue; scale bar=250 µm) for 2 animals that received 3 intravitreous doses of either placebo or VEGF Trap (500 µg/eye/dose). The representative histological sections correspond to the numbered laser treatment areas in the fluorescein angiograms. The placebo-treated sections are thicker and more vascular compared with the VEGF Trap–treated eyes. Note the presence of subretinal fluid in lesion 3 on day 33.](https://example.com)
has been used in a small open-label safety study for treatment of diabetic macular edema. A single dose of 4 mg was administered intravitreally to 5 patients who had undergone multiple prior treatments for diabetic macular edema. There was a median decrease in central macular thickness of 79 µm as well as some improvement in vision. A phase 2 trial in diabetic macular edema is in progress.

In a double-masked phase 2 trial (CLEAR-IT 2), VEGF Trap-Eye was evaluated in 157 patients with exudative AMD randomized to either monthly or quarterly intravitreal injections for 12 weeks at doses of 0.5 or 2 mg. Another study arm used 3 initial monthly doses of 2 mg followed by 2-mg doses given at 8-week intervals. The active control arm comprised subjects receiving ranibizumab (0.5 mg) at 4-week intervals. The 1-year outcomes from these studies are pending publication.

Using an established primate model of CNV, administration of VEGF Trap in a prevention protocol markedly reduced vasoproliferative responses of the macaque retina to laser injury, substantially preventing the development of all components of CNV lesions as well as vascular leakage. When a single intravitreal VEGF Trap injection was given after grade 4 lesions had developed, there was resolution of vascular leakage. This also resulted in a trend toward lower histological scores for the neovascular components of the lesions, suggesting partial regression of newly formed vessels.

CONCLUSIONS

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