Suppression of Intraocular Vascular Endothelial Growth Factor During Aflibercept Treatment of Age-Related Macular Degeneration

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• PURPOSE: To determine the duration of suppression of aqueous humor concentrations of vascular endothelial growth factor (VEGF) in eyes with neovascular age-related macular degeneration (AMD) treated with aflibercept.
• DESIGN: Nonrandomized prospective clinical study.
• METHODS: Twenty-seven eyes of 27 neovascular AMD patients receiving intravitreal aflibercept injections on a pro re nata regimen driven by spectral-domain optical coherence tomography (SD OCT) were included in this study. A total of 132 aqueous humor specimens were collected before intravitreal aflibercept injections and their VEGF-A concentrations assayed by multiplex bead analysis.
• RESULTS: Mean aqueous humor VEGF concentrations before treatment initiation were 90.6 ± 37.1 pg/mL (range 23.4–190.3 pg/mL). Intravitreal injection of aflibercept suppressed the aqueous VEGF concentrations to below the lower limit of quantification (< 4 pg/mL) in all patients. The mean duration of VEGF suppression below the lower limit of quantification was > 71 ± 18 days. The earliest time after injection at which the VEGF concentration recovered to above the lower limit of quantification was 55 days in 1 patient and > 56 days, the recommended aflibercept treatment interval, in 20 patients. The aqueous VEGF recovery status of 6 patients was uncertain after 56 days.
• CONCLUSIONS: On average, VEGF concentrations in the aqueous humor were suppressed below the lower limit of quantification after intravitreal aflibercept injections for about 10 weeks. This aqueous suppression time suggests durable VEGF inhibition for most patients dosed with aflibercept every 8 weeks. (Am J Ophthalmol 2014;158:532–536. © 2014 by Elsevier Inc. All rights reserved.)

AGE-RELATED MACULAR DEGENERATION (AMD) IS a major cause of vision loss. The neovascular variant is characterized by choroidal neovascularization (CNV), in which formation of blood vessels leads to sub- and intraretinal macular edema, hemorrhage, fibrosis, and visual decay. Effective treatments have been developed recently, focusing on neutralizing vascular endothelial growth factor (VEGF) with antibodies (bevacizumab), antibody fragments (ranibizumab), or fusion proteins (aflibercept). Major clinical trials found 4-weekly injections of ranibizumab to result in best visual outcome for ranibizumab,[1] and found that injections of aflibercept every 8 weeks (following a loading phase) provided similar functional benefits.[2] As an alternative to fixed dosing intervals, pro re nata (PRN) treatments based on optical coherence tomography (OCT)-determined activity achieve similar functional results.[3,4]

Clinical trials with aflibercept suggest a longer duration of VEGF suppression than with bevacizumab or ranibizumab, which is also supported by pharmacokinetic models.[5] We have recently determined the average time for which aqueous humor VEGF concentrations are suppressed below the lower limit of quanitification of 4 pg/mL following intravitreal ranibizumab injections to be 37 days on average, with individual VEGF suppression times ranging from 26 to 69 days.[6,7] Aqueous humor concentrations appear suitable for assessing ocular VEGF levels as they correlate well with vitreous VEGF concentrations, extrapolated from retinal vascular occlusive disease and diabetic retinopathy.[8,9]

This study aimed to determine intraocular VEGF suppression duration following aflibercept treatment for neovascular AMD by sampling aqueous humor VEGF levels.

MATERIALS AND METHODS

• STUDY POPULATION: This prospective, observational study enrolled 27 eyes of 27 patients who were 60 years of age or older and had active CNV secondary to AMD. All eyes were examined and treated at the Department of Ophthalmology, University of Cologne, Germany. The study was performed in accordance with the tenets of the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the University of Cologne (reference number 11–027), and all participants gave written informed consent. The study was registered at ClinicalTrials.gov (Identifier NCT01213667).

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TABLE. Clinical Characteristics of the Study Population Treated With Aflibercept for Neovascular Age-Related Macular Degeneration

Study participants	27 patients
Sex, n (%)	13 (48%) male 14 (52%) female
Age at first aflibercept treatment (y), mean ± SD (range)	77.5 ± 6.4 (64–90)
Eyes, n (%)	27 eyes: 9 (33%) right, 18 (67%) left
Follow-up time per patient (mo), mean ± SD (range)	7.7 ± 2.1 (4.1–12.0)
Number of intravitreal injections per patient, mean ± SD (range)	5.5 ± 1.3 (3–8)
Aqueous VEGF concentration on day 0 (pg/mL), mean ± SD (range)	90.6 ± 37.1 (23.4–190.3)
VEGF suppression time (days), mean ± SD (range)	70.5 ± 18.0 (41–109)
Type of choroidal neovascularization, n	18 occult 4 mixed 2 classic 3 RAP
Size of choroidal neovascularization (mm^2), mean ± SD (range)	4.5 ± 3.4 (0.3–12.7)
Best-corrected visual acuity on day 0 (ETDRS letters), mean ± SD (range)	59.5 ± 17.0 (20–85)
Central retinal thickness on day 0 (μm), mean ± SD (range)	392 ± 132 (210–658)

ETDRS = Early Treatment Diabetic Retinopathy Study; SD = standard deviation; RAP = retinal angiomatous proliferation; VEGF = vascular endothelial growth factor.

• **INCLUSION AND EXCLUSION CRITERIA:** All included patients were suffering from an active sub- or juxtafoveal CNV attributable to neovascular AMD. This was confirmed by fluorescein angiography and indocyanine green angiography as well as spectral-domain optical coherence tomography (SD OCT) (HRA-2 and Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany). CNV size (mm^2) was determined from fluorescein angiograms using the HRA-2 software (Heidelberg Engineering). An additional inclusion criterion in the study eye was a best-corrected visual acuity ≥20 Early Treatment of Diabetic Retinopathy Study (ETDRS) letters. Exclusion criteria were any previous intraocular surgery (apart from cataract removal) or photodynamic therapy; any treatment with intraocular steroids; any ranibizumab/bevacizumab/pegaptanib treatment within the previous 90 days; and any previous aflibercept treatment.

• **DIAGNOSTICS AND TREATMENT:** Patients initially received 3 2-mg loading dose injections of aflibercept at intervals ranging from 4 to 6 weeks. After this first treatment phase, patients were monitored monthly by SD OCT, ETDRS best-corrected visual acuity tests, and fundus examinations. Fluorescein angiography was repeated only in unclear cases. CNV persistences or recurrences were treated by additional aflibercept injections on a PRN regimen mainly driven by morphologic findings in SD OCT. Recurrent or persistent CNV activity was detected as sub- or intraretinal fluid by SD OCT, leakage in fluorescein angiography, a loss of ETDRS letters if attributable to CNV activity, or new sub- or intraretinal macular hemorrhages. Because of variable treatment approval times of health insurances, individual disease activities, and patient appointment preferences, variable reinjection intervals occurred without any experimental study design.

• **AQUEOUS HUMOR VASCULAR ENDOTHELIAL GROWTH FACTOR MEASUREMENTS:** Samples were acquired only upon necessary treatment. Prior to each aflibercept injection, approximately 0.1 mL of aqueous humor was collected via a sterile limbal puncture with a 30 gauge needle connected to an insulin syringe. The procedure of sample collection immediately followed by aflibercept injection was randomly performed by 3 surgeons. No surgeon was assigned to specific patients, cancelling out possible dosing variabilities. Samples were immediately stored at −80 C in polypropylene tubes until they were analyzed on a Luminex xMAP microbead multiplex platform (Luminex 200; Luminex Inc, Austin, Texas, USA) following the manufacturer's assay instructions (Human Angiogenesis Panel; R&D Systems, Wiesbaden, Germany). Standard curves for VEGF were generated using the reference standard supplied with the kit. The lower limit of quantification for VEGF was 4 pg/mL.

RESULTS

INTRAOCULAR VEGF CONCENTRATIONS WERE ASSAYED IN samples of aqueous humor from 27 patients undergoing PRN aflibercept treatment for neovascular AMD. The clinical characteristics of the study population are listed in the Table. We analyzed 132 aqueous humor samples of 149 intravitreal aflibercept injections administered during the study. VEGF levels (y-axis) were plotted in relation to the interval from the previous aflibercept injection (x-axis) for each patient; the very first aflibercept injection was defined as day 0. Representative examples are depicted in Figure 1. Complete aqueous humor VEGF suppression was assumed when VEGF levels were below the lower limit of quantification of the analytical method (4 pg/mL).

Aflibercept led to complete suppression of aqueous VEGF in all patients at early times after injection. As

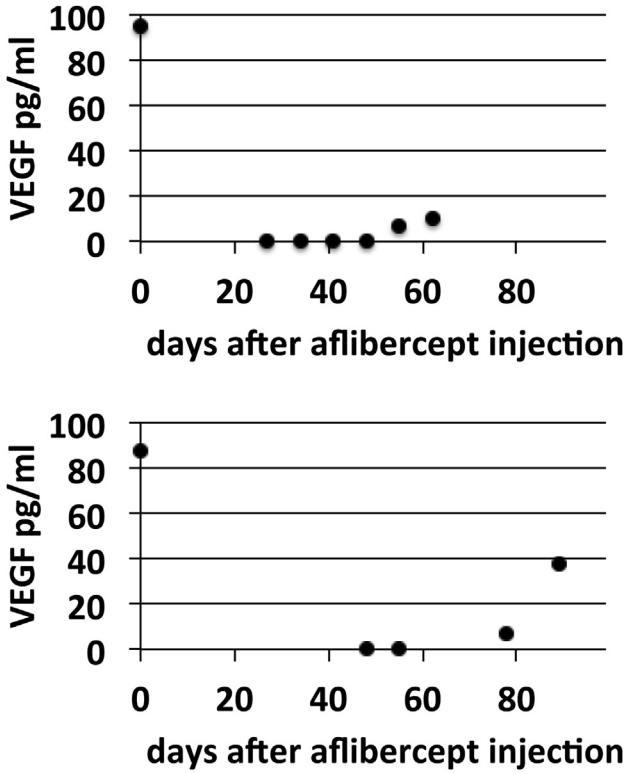

FIGURE 1. Scatter diagrams of 2 patients with neovascular age-related macular degeneration, showing the time interval since the previous intravitreal aflibercept injection and the corresponding vascular endothelial growth factor concentrations in aqueous humor.

patients were seen monthly and only injected with aflibercept PRN, and subsequently were only assayed for aqueous humor VEGF levels at that time, it is difficult to measure the precise moment at which VEGF suppression is lost. Therefore, the latest sampling time of complete aqueous VEGF suppression and the earliest sampling time of aqueous VEGF suppression loss was determined for each patient; the true duration of aqueous VEGF suppression lies between these 2 times. The earliest time at which complete aqueous VEGF suppression was lost could be determined for only 7 of the patients (Figure 2), but not for the remaining 20 patients, as the latest available sample still showed complete suppression.

After the recommended dosing interval of 56 days, aqueous VEGF levels were still completely suppressed in 20 patients; 1 patient was definitely no longer suppressed; and the suppression status of the remaining 6 patients was uncertain owing to nonavailability of samples for this time point.

The mean VEGF suppression time was greater than 71 ± 18 days. All VEGF suppression time data are shown in Figure 2. Individual VEGF suppression times were apparently stable because suppression was never lost at a time at which other assays of the same individual showed it to be present (data not shown).

When excluding the upload phase, and excluding patients with persistent activity, only 6 injections were dictated before 8 weeks based on signs of recurrent activity on SD OCT. The mean recurrence interval from the previous injection in these 6 cases was 49 ± 5 days.

DISCUSSION

IN THIS STUDY WE MEASURED VEGF CONCENTRATIONS IN the aqueous humor of neovascular AMD patients undergoing aflibercept treatment. At early times after injection, aflibercept completely suppressed aqueous VEGF below the lower limit of quantification of our analytical method in all 27 patients. The mean duration of complete aqueous VEGF suppression was at least 71 days, 2 weeks longer than the recommended dosing interval.

The aflibercept VEGF suppression time of at least 71 days is far longer than the previously determined VEGF suppression time for ranibizumab of around 37 days for neovascular AMD patients,[6,7] and thus provides a plausible rationale for the 56-day injection intervals recommended by the treatment label. Of the 27 patients in this study, only 1 patient had definitely lost aqueous VEGF suppression after less than 56 days (55 days), but aqueous VEGF was shown to be suppressed for at least 48 days (Patient 25; Figure 2). Six patients had an uncertain suppression status at 56 days, owing to sample availability, but VEGF suppression times of at least 41 days (Patients 21–24, 26, 27; Figure 2).

Individual aflibercept VEGF suppression times were stable for up to 12 months; no signs of tachyphylaxis or rebound effects were observed. No patient had an assayed aqueous VEGF level above detection limit at a time point that showed suppression below detection limit for another specimen of that same patient at an equal time point. We have already shown such stability of VEGF suppression times for ranibizumab to be stable in neovascular AMD patients[7] as well as in diabetic macular edema patients.[10] In both groups, VEGF suppression times vary between different patients but are constant for each patient. These individual differences in VEGF suppression time may be attributable to differences in VEGF production as well as aflibercept decay and may support individualized therapy.

The major limitation of this study was the nonexperimental design, as varying injection intervals were mainly based on disease activity, precluding precise definition of the exact time at which aqueous VEGF suppression was lost. However, as these uncertain VEGF suppression times are longer than the latest sample time for many patients, the true mean aqueous VEGF suppression time will be longer than our currently determined mean of 71 days.

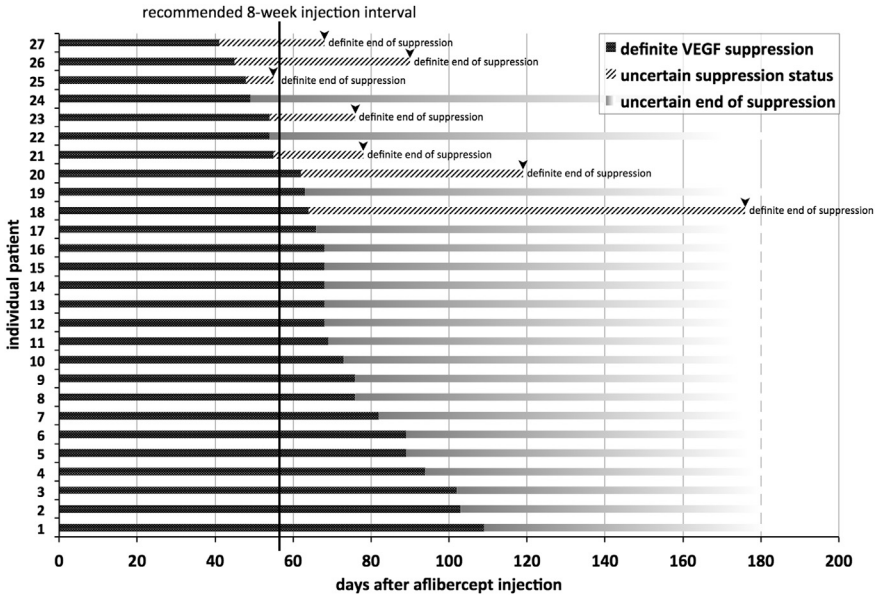

FIGURE 2. Stacked histogram for individual patients with neovascular age-related macular degeneration, showing the duration of complete vascular endothelial growth factor suppression as well as the end of suppression whenever definable. The true duration of suppression is within the time period during which the suppression status is uncertain owing to unavailable sampling.

For the same reason, we were also unable to determine the exact relationship between aqueous aflibercept VEGF suppression time and the reoccurrence of clinical CNV activity. However, for ranibizumab we have shown the sequence of events following the loss of aqueous VEGF suppression until the reoccurrence of morphologic, and ultimately functional, events.[6] It seems plausible that aflibercept treatments will show a similar correlation. This assumption is further supported by the fact that our reinjection regimen was based on clinical disease activity and resulted in injection intervals of often more than 8 weeks, indicating that the observed aflibercept VEGF suppression times correlate to clinical findings.

Our results are in line with the model predictions of Stewart and Rosenfeld,[5] in which a slight increase in elimination half-times from the vitreous, combined with a large increase in binding affinity,[11] are taken to be the likely explanation for differences in functional half-times. However, here, as in our previous work with ranizumab,[6] we have assayed VEGF rather than making deductions based on the properties of the different anti-VEGF medications, permitting a more definite correlation to functional effects. Concentrations of VEGF in aqueous humor are known to be lower than those in vitreous,[9] and those in vitreous are themselves are presumably only a diluted reflection of those within the retina or in the subretinal space, where VEGF is postulated to be functionally angiogenic. Nevertheless, it is plausible that the VEGF concentrations in these various ocular "compartments" are in reasonable equilibrium, so that the deductions we make from the aqueous humor concentrations will reflect those relevant to deeper levels of the ocular architecture.

Importantly, the influence of additional growth factors and cytokines apart from VEGF may have to be taken into account regarding CNV activity. CNV persistence in some patients (never drying up completely) points in this direction.

In conclusion, this work provides clinical data supporting the pharmacokinetic rationale for aflibercept injections every 8 weeks in patients with neovascular AMD, or at least for longer intervals between aflibercept injections than are needed for ranibizumab.

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Ophthalmic Injuries at Hiroshima and Nagasaki

Because he had studied cataracts induced by radiation exposure in radiologists and cyclotron workers, David G. Cogan of the Howe Laboratory in Boston was recruited by the Atomic Bomb Casualty Commission to go to Japan in 1950 and investigate eye injuries in survivors of the atomic bombing of Hiroshima and Nagasaki. Japanese ophthalmologists had already reported the immediate effects of blast and fire injury but the special effects of ionizing radiation were just starting to be understood. Cogan originally thought that ionizing irradiation of the body intense enough to produce eye effects would be incompatible with survival, but later admitted that he had not considered the potential for partial shielding of the body that occurred in crowds. The head, however, was frequently exposed and Cogan did find victims with the after-effects of radiation exposure. Since the development of cataract from ionizing radiation was delayed, often for years, clear radiation-related cataracts were not evident until sometime after the exposure. David Cogan's work at the Atomic Bomb Casualty Commission helped to set safety standards for workers in atomic energy facilities, a crying need in the following decades that saw the building of atomic energy power plants and nuclear medicine laboratories

Submitted by Steven A. Newman from the Cogan Ophthalmic History Society.