

Aflibercept as a Treatment for Age-related Macular Degeneration

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Abstract

Vascular endothelial growth factor (VEGF) plays a central role in the development of several chorioretinal vascular disorders including exudative age-related macular degeneration (AMD). Detailed understanding of VEGF biochemistry has led to the development of four drugs which specifically inhibit its actions. Bevacizumab and ranibizumab have been the dominant ophthalmic anti-VEGF drugs for seven years and their regular use has significantly decreased vision loss. In late 2011, aflibercept, a high-affinity, fusion protein that acts as a soluble VEGF receptor, was approved for the treatment of exudative AMD. Phase three trials showed that monthly and bimonthly aflibercept maintained vision in 95 % of patients, improved average visual acuity by +8.3 to +9.4 letters, and thinned the macula comparably to monthly ranibizumab. Since its approval, aflibercept has been shown to decrease retinal edema and subretinal fluid, and flatten retinal pigment epithelial detachments in eyes that have responded incompletely to frequent ranibizumab and bevacizumab injections. Aflibercept's longer duration of action coupled with its comparable unit price (versus ranibizumab) promise to decrease the total cost of treatment.

Keywords

Aflibercept, age-related macular degeneration, choroidal neovascularization, vascular endothelial growth factor (VEGF), VEGF Trap, VEGF Trap-eye

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Age-related macular degeneration (AMD) has been a leading cause of blindness in developed nations for several decades.¹ Most patients with AMD have only minor visual disturbances due to dry or non-exudative AMD (drusen and retinal pigment epithelium (RPE) mottling or hyperplasia), however, approximately 10 % of AMD patients develop severe vision loss due to wet or exudative AMD.² Choroidal neovascular membranes (CNVM) grow from the choriocapillaris and extend beneath the RPE (type one CNVM) or retina (type two CNVM), or infiltrate the neurosensory retina as retinal angiomatous proliferation (type three CNVM). Constant exudation, repeated hemorrhage, and progressive fibrovascular growth result in photoreceptor damage and disciform scarring. Previous treatments—laser photocoagulation and ocular photodynamic therapy—sometimes arrested neovascular growth but only infrequently resulted in improved vision (see *Figure 1*).^{3,4}

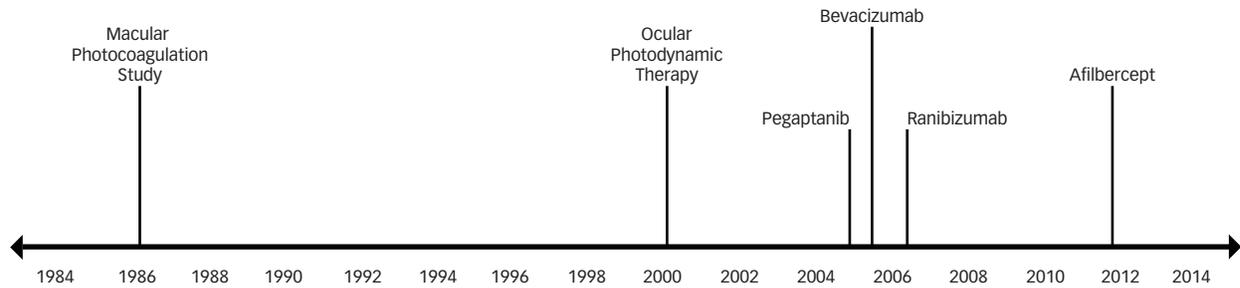
Effective treatment for exudative AMD derived from what at first appeared to be an unlikely source - cancer research. Folkman (1971)⁵ first proposed that tumor growth depended on a soluble angiogenesis factor and Senger (1983)⁶ subsequently discovered the vasopermeability factor. Finally, two independent research groups isolated identical cytokines which became known as vascular endothelial growth factor (VEGF).^{7,8} This discovery kicked off two decades of intense research into the biochemistry of the VEGF families and resulted in the development of four drugs which specifically bind VEGF to prevent it from activating three trans-membrane receptors (VEGFRs). Pegaptanib (Macugen®, Ophthotech, Palm Beach Gardens, FL), an aptamer to VEGF₁₆₅, slows vision loss by approximately 50 % when administered to patients suffering from exudative AMD,⁹ however, only the pan-VEGF-A binding drugs bevacizumab (Avastin®, Genentech, S. San Francisco, CA/

Novartis, Basel, Switzerland) and ranibizumab (Lucentis®, Genentech, S. San Francisco, CA/Novartis, Basel, Switzerland) were able to stabilize vision in nearly all patients (91–94 %) and improve vision in the majority.^{10–14} Both drugs possess high binding affinities to all naturally occurring isoforms of VEGF-A (bevacizumab: $K_D=58-1,1000$ pM to VEGF₁₆₅; ranibizumab: $K_D=46-192$ pM to VEGF₁₆₅)^{15–17} as well as the cleaved product VEGF₁₁₀.

Aflibercept Development

Bevacizumab and ranibizumab are full-length antibody and antibody fragments, respectively, both of which were produced from a murine model. The scientists who created the VEGF Trap-eye or aflibercept (Eylea®, Regeneron, Tarrytown, NY), however, decided to employ a different binding strategy to neutralize diffusible VEGF—they created a soluble receptor molecule. Naturally occurring binding domains from native VEGF receptors were fused to the Fc (fragment crystallizable) backbone of a human IgG molecule.¹⁸ The first or 'parent' VEGF-Trap (VEGF-Trap_{R1R1R1}) contained the first three VEGF-binding domains from VEGF receptor one (VEGF_{R1}). Since VEGFR1 has a higher binding affinity for VEGF₁₆₅ than has VEGFR2, this strategy was expected to create the molecule with the highest VEGF binding affinity. Though the resultant trap had a high binding affinity to VEGF₁₆₅ ($K_D=5$ pM), it exhibited unfavorable in vivo pharmacokinetic behavior because its basic amino acid sequences resulted in rapid sequestration within the intercellular matrix. Subsequent molecules (VEGF-Trap_{AB1} and VEGF-Trap_{AB2}) had better pharmacokinetic properties than the parent trap and the fourth and final molecule, which contained the second binding domain from VEGFR1 and the third binding domain from VEGFR2 (VEGF-Trap_{R1R2}), exhibited minimal binding to the matrix. Furthermore, a three-dimensional stoichiometric analysis suggests

Figure 1: The points of introduction for each of the proven treatments for exudative age-related macular degeneration are shown on the timeline. The figure shows when each drug received Food and Drug Administration approval, except for bevacizumab which was first used off-label for the treatment of AMD in mid-2005.



that the drug (aflibercept) simultaneously binds both molecules of the VEGF dimer with a 'two fisted grasp' with a higher binding affinity to VEGF₁₆₅ ($K_D=0.5$ pM) than that of both the parent trap and the native receptors.

Pre-clinical Trials

In pre-clinical studies aflibercept slowed the growth of orthotopic tumors in mice¹⁹ and prevented luteal development in marmosets.²⁰ Aflibercept halted basic fibroblastic growth factor-induced corneal neovascularization,²¹ extended the survival of high risk corneal grafts,²² suppressed CNVM development in transgenic mice that secreted VEGF from their photoreceptors,²³ prevented laser-induced CNVM,²³ and prevented the development of matrigel-induced choroidal neovascular membranes in rats.²⁴

Human Trials

These successes gave rise to early testing of aflibercept in patients with exudative AMD (see *Table 1*). In a dose-escalation trial,²⁵ 25 patients were randomized to receive one of three intravenous doses of aflibercept (0.3 mg/kg, 1.0 mg/kg, or 3.0 mg/kg), followed by a four week observation period, and then three additional doses administered at two week intervals. Lower doses of aflibercept (0.3 mg/kg and 1.0 mg/kg) led to modest improvements in macular thinning (-10–66 %), whereas the highest dose (3.0 mg/kg) resulted in the greatest improvements in vision (+12 letters). Only patients who received 3 mg/kg had detectable serum levels of aflibercept at one month, suggesting that lower monthly doses were incapable of sustaining therapeutic blood levels. Unfortunately, two of the five patients receiving the highest dose experienced dose-limiting hypertension and proteinuria. Therefore, monthly intravenous administration of aflibercept appeared to be incapable of producing sustained therapeutic blood levels with an acceptable safety profile.

Due to concerns over systemic adverse events, aflibercept was administered by intravitreal injections in all subsequent ophthalmic trials. The safety of intravitreal aflibercept had already been established in mice, rats and rabbits so the Clinical evaluation of anti-angiogenesis in the retina intravitreal trial (CLEAR-IT 1) sought to establish the safety, tolerability, maximum tolerated dose and bioavailability of aflibercept in humans.²⁶ Twenty-one patients with neovascular AMD received one of six escalating doses of aflibercept (range: 0.05 mg to 4.00 mg). Six weeks after the injection, the mean decrease in macular thickness was -104 μ m and the average improvement in vision was +4.43 letters.

Among patients receiving the highest doses of aflibercept (2 mg and 4 mg) the mean improvement in vision was +13.5 letters and three of these six patients required no additional therapy through 12 weeks. No drug-related safety issues were identified and neither hypertension nor proteinuria was seen. The maximum tolerated intraocular dose of aflibercept was not determined.

The phase two CLEAR-IT 2 trial evaluated the 12-week effects of a fixed-dose regimen of aflibercept.²⁷ Patients were randomized to receive aflibercept every 12 weeks (0.5 mg, 2.0 mg, or 4.0 mg) or every four weeks (0.5 mg, 2.0 mg). At week 12, the average reduction in central retina/lesion thickness was -119 μ m ($p<0.0001$) whereas patients receiving monthly injections (0.5 mg or 2.0 mg) had average thickness reductions of -53.5 μ m and -169.2 μ m. The average improvement in vision was +5.7 letters, but patients receiving monthly injections improved by > +8 letters. At eight weeks, patients receiving 2 mg q4wk and 2 mg q8wk had similar improvements in vision. After the required injection at week 12, all groups either maintained or improved vision and macular thickness through week sixteen.

The second phase of the CLEAR-IT 2 trial determined the required treatment frequency, and anatomic and visual outcomes when the eyes were switched from a fixed-dose schedule to PRN dosing between weeks 12 and 52.²⁸ During this period, patients were evaluated monthly and retreated with the original dose of aflibercept if they showed clinical or tomographic signs of active CNVM. The average time to first injection after the initial 12 weeks was an impressive 129 days and patients received an average of only two injections (19 % required no injections and 45 % required 1 or 2). At 52 weeks, patients had improved by an average of +5.3 letters (compared to +5.7 letters at week 12). Interestingly, patients who received monthly injections of aflibercept 2 mg from baseline through week 12 had the greatest visual improvement (+9 letters) at week fifty-two.

Data from the early CLEAR-IT trials suggested that higher doses of aflibercept (> 0.5 mg) and three monthly loading doses produced the greatest improvements in vision and longest durations of clinical action. Furthermore, clinically significant durations of action up to two and a half months had been predicted by mathematical modeling.²⁹ Therefore, investigators designed the double-masked, multi-center, active-control phase three VEGF trap-eye: investigation of efficacy and safety in wet age-related macular degeneration trials to include higher doses

Table 1: Major Conclusions from the Four Ophthalmic Clinical Trials with Aflibercept, One Intravenous and Three Intravitreal, are listed.

Study Phase # of Patients	Primary Aims of Study	Major Findings
Intravenous Trial		
Phase 1 25 Patients	To assess the safety, pharmacokinetics, and biological activity of intravenous (0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg) aflibercept	Improvements in excess retinal thickness were -10 %, -66 %, and -60 % at day 15 Improvements in excess retinal thickness were +47.1 % and -63.3 % (0.3 mg/kg, 1.0 mg/kg) at day 71 Two of five patients receiving 3 mg/kg experienced hypertension and proteinuria
CLEAR-IT 1 Trial		
Phase 1 21 Patients	To assess the safety, tolerability, maximum tolerated dose, and bioavailability of intravitreal aflibercept Dose escalation study from 0.05 mg to 4 .00 mg	Average decrease in macular thickness at week 12 was - 119 µm but was greater in q4wk groups Average increase in vision at week 12 was +5.7 letters but was > 8 letters in monthly groups Average time to reinjection was 129 days Average increase vision (2 mg and 4 mg groups) at 52 weeks was +9 letters
CLEAR-IT 2 Trial		
Phase 2 159 Patients	To assess the efficacy and safety of intravitreal aflibercept during a 12-week fixed dosing period followed by a 40-week PRN dosing period Dosing: 0.5 mg or 2.0 mg q4wk; 0.5 mg, 2.0 mg or 4.0 mg q12wk	Average decrease in macular thickness at week 12 was -119 µm but was greater in q4wk groups Average increase in vision at week 12 was +5.7 letters but was > 8 letters in monthly groups Average time to reinjection was 129 days Average increase vision (2 mg and 4 mg groups) at 52 weeks was +9 letters
VIEW 1 and 2 Trials		
Phase 3 2419 Patients	To assess the safety and efficacy of intravitreal aflibercept compared to ranibizumab during a 52-week fixed dosing period followed by a 46-week PRN (12-week cap) dosing period Monthly ranibizumab or aflibercept (0.5 mg or 2.0 mg), aflibercept 2 mg q8wk (after three monthly injections)	Comparable gains in vision among aflibercept groups (+8.3 to +9.4 letters) compared to ranibizumab (+8.7 letters) Comparable improvements in macular thickening among aflibercept (-115.8–6.6 µm) compared to ranibizumab (-127.6 µm) Average injections during year 2 (aflibercept 2 mg q8wk: 4.2; ranibizumab: 4.7) Excellent maintenance of vision (-0.8 letters) in aflibercept 2 gm and ranibizumab group.

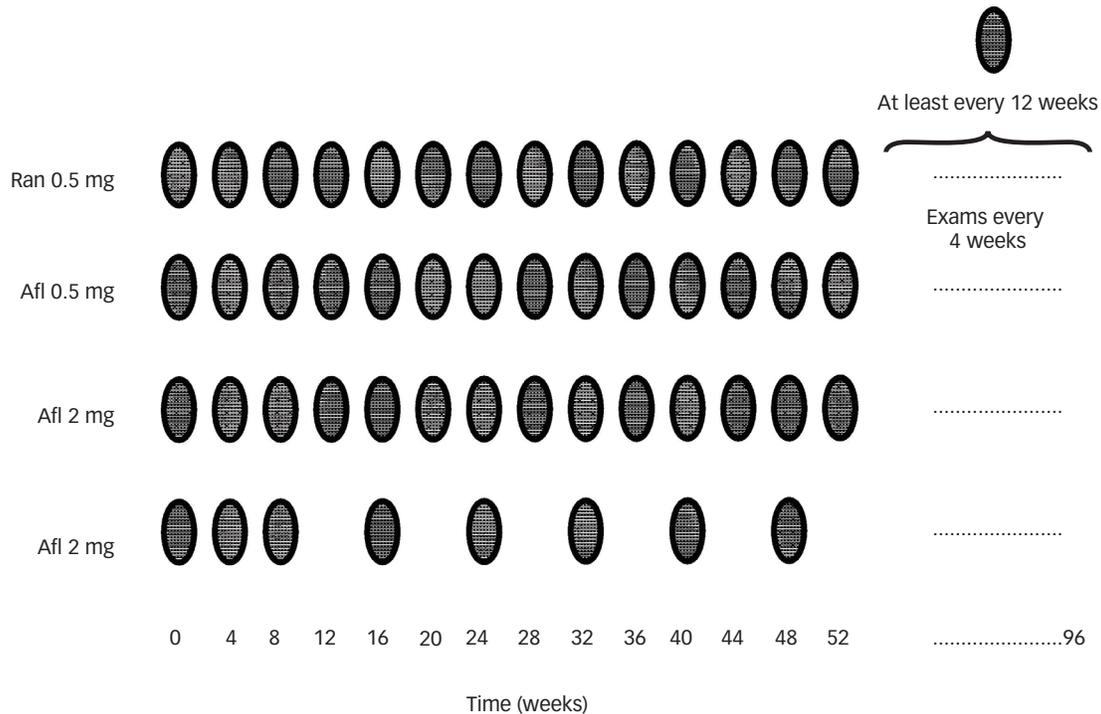
of aflibercept (2 mg) and longer treatment intervals (two months).³⁰ The parallel VIEW 1 (North America) and VIEW 2 trials (South America, Europe, Asia and Australia) randomized a total of 2457 patients, with only 212 (8.6 %) withdrawing before the 52 week endpoint. Patients were treated according to one of four protocols: aflibercept 0.5 mg monthly (0.5 mg q4wk), aflibercept 2.0 mg monthly (2.0 mg q4wk), aflibercept 2.0 mg bimonthly (2.0 mg q8wk) after three monthly loading doses, and ranibizumab 0.5 mg monthly (0.5 mg q4wk) (see *Figure 2*).

The VIEW trials tested for non-inferiority of aflibercept against ranibizumab, with prevention of severe vision loss—defined as < 15 Early treatment of diabetic retinopathy study (ETDRS) letters—as the primary endpoint. All aflibercept treated groups maintained vision comparably to those treated with ranibizumab (95.1–6.3 % vs. 94.4 %) and the majority of patients in all treatment groups experienced improved vision (aflibercept: 78–84 %; ranibizumab: 79 %). Similar proportions of patients receiving aflibercept and ranibizumab improved by > 15 letters (25–38 % vs. 31–4 %).

Regarding the major secondary endpoints, letters of vision gained and amount of macular thinning, there were no significant differences between aflibercept and ranibizumab treated patients. Average vision gains in each of the aflibercept treatment arms (+6.9 letters to +10.9 letters) were comparable to gains in the two ranibizumab arms (+8.1 letters and +9.4 letters). Compared to the ranibizumab arm, the only aflibercept treated group that gained significantly more letters was the 2 mg q4wk arm in the VIEW 1 (+8.1 vs. +10.9; $p < 0.001$), but this group improved the least (+6.9 letters) in the VIEW 2, suggesting that the outlying performance in VIEW 1 was a statistical anomaly. Integrated data from both trials showed similar average vision gains in all groups (+8.3 letters to +9.4 letters). Patients in each of the aflibercept arms experienced average macular thinning similar to those receiving ranibizumab (-115.6–56.8 µm vs. -116.8–38.5 µm). Furthermore, between 56.7 % and 80.3 % of eyes showed no edema or subretinal fluid at the end of one year.

Both aflibercept and ranibizumab exhibited excellent safety profiles. Severe ocular complications included RPE tears (ranibizumab: 0.3 %;

Figure 2: The Figure Shows the Treatment Schedule Through two Years for Each Treatment Arm in the VIEW Trials. During the Second Year, Patients were Examined Every four Weeks and Received Injections When Needed, but at Intervals Not to Exceed 12 Weeks.



aflibercept: 0.1 %), and endophthalmitis (ranibizumab: 0.5 %; aflibercept: 0.1 %). Deaths occurred in 1.1 % and 1.2 % of patients receiving aflibercept and ranibizumab, and Anti-platelet trialists’ collaboration (APTC) events, including cerebrovascular accidents and myocardial infarctions, occurred in 1.7 % of aflibercept and ranibizumab treated patients.

Patients received the same drug and dose during year two of the VIEW trials, were examined monthly, but were treated as-needed (PRN) with intervals that could not exceed 12 weeks. Patients maintained vision gains from the first year with those initially receiving aflibercept 2 mg q8wk experiencing the same (-0.8 letters) mild loss of vision during year two (from +8.4 to +7.6 letters) as did patients receiving ranibizumab (+8.7 to +7.9 letters). Aflibercept treated patients required an average of 4.2 injections, compared to 4.7 injections for patients receiving ranibizumab. Compared to those treated with ranibizumab, fewer patients receiving aflibercept 2 mg required the most intensive treatment (> 6 injections: 26.5 % vs. 15.9 %; > 11 injections: 3.0 % vs. 1.9 %) but more received the fewest (three) allowable number of injections (48 % vs. 40 %).

Taken together, these results demonstrate that aflibercept possesses a longer duration of action than ranibizumab. Unfortunately, the 12-week capped PRN regimen did not enable an accurate determination of aflibercept’s average duration of action. However, the data suggest that the aflibercept treatment intervals could be extended to an average of 13 weeks, compared to approximately 10 weeks for ranibizumab [Author Calculations], which is slightly longer than was seen in either the CATT13 or inhibit VEGF in age-related choroidal neovascularization (IVAN).³¹ Thus it appears that aflibercept possesses a longer duration of action than

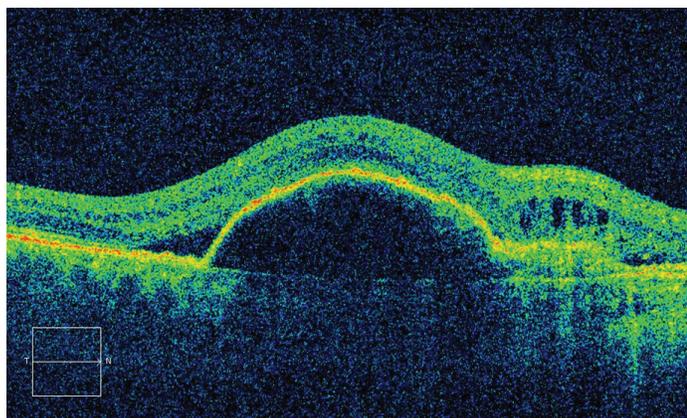
ranibizumab—probably by three weeks—but not by a factor of two, as might be interpreted from the first year of the VIEW trials. Aflibercept has not been compared directly with bevacizumab, but since bevacizumab and ranibizumab performed comparably in head-to-head trials,^{13,31} aflibercept’s head-to-head performance against bevacizumab would probably be similar to that with ranibizumab.

Post-Approval Experience

The favorable results from the first year of the VIEW trials convinced the United States Food and Drug Administration (FDA) on November 18, 2011 to approve aflibercept 2 mg for the treatment of subfoveal and juxtafoveal CNVM due to AMD. The approval allowed aflibercept dosing every four or eight weeks but stated that monthly injections had not produced superior results to bimonthly. Soon after approval, the Centers for Medicare and Medicaid Services approved wholesale reimbursement of aflibercept at a price of \$1850 per dose.

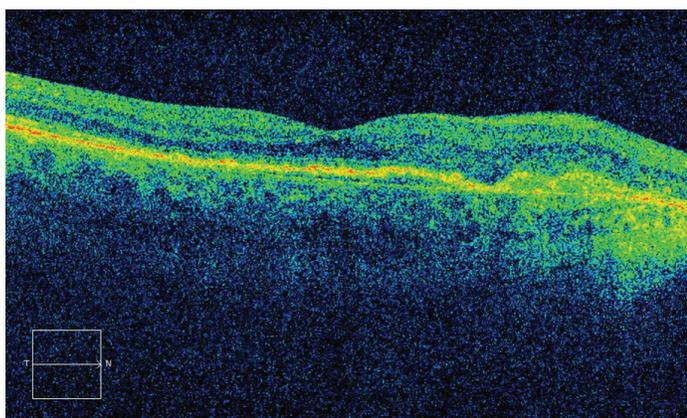
The CLEAR-IT and VIEW trials suggest that extended duration of action is aflibercept’s major therapeutic advantage, since injections can be administered every eight weeks while producing visual and anatomic results comparable to monthly ranibizumab. Previous attempts to extend ranibizumab intervals to three months led to steadily declining vision through one year,³² and a 2-month hiatus after three monthly loading doses resulted in a loss of 3 to 4 letters.³³ A year of bimonthly aflibercept (after three loading doses) would save 42 % compared to monthly ranibizumab, though it would still cost nearly 400 % that of monthly bevacizumab. Most physicians, however, individualize therapy by using either PRN or treat-and-extend strategies, both of which would allow fewer injections. The resultant

Figure 3a: Incomplete response to Bevacizumab



This SD-OCT Scan of the Macula Shows Persistent Intraretinal and Subretinal Fluid, and a Retinal Pigment Epithelial Detachment Despite six Monthly Injections of Bevacizumab.

Figure 3b: Macular Drying After Aflibercept



This SD-OCT Scan of the Eye in Figure 3a was Taken 1 Month after the First Injection of Aflibercept. The Retinal Fluid has Completely Resorbed and the Pigment Epithelial Detachment has Flattened.

savings with aflibercept over ranibizumab would be considerably more modest (PRN: 15 %; treat-and-extend: 26 %) [Author's Calculations].

Since its approval, aflibercept use has exceeded most people's expectations but it remains too early to determine how aflibercept's

extended duration of action will translate into clinical practice. At the time of this writing, no post-approval, peer-review publications have confirmed the VIEW trials' primary conclusions. However, early after approval, physicians began injecting aflibercept into eyes which had persistent macular edema, subretinal fluid, and retinal pigment detachments despite frequent injections of ranibizumab and bevacizumab. Many of these 'incomplete responders' had rapid and complete drying of the maculas after aflibercept injections. The 2012 American Society of Retina Specialists meeting featured six papers that described aflibercept as 'salvage therapy' for these difficult-to-treat eyes. A crude analysis of the merged results of these papers revealed that most patients experienced an improvement in visual acuity (20/68 to 20/61) and a decrease in central macular thickness (336–275 μm). Reasons for aflibercept's effectiveness as a salvage therapy are unclear but may include its high binding affinity to VEGF, binding of placental growth factor and VEGF-B, 100 % human protein composition, or tachyphylaxis avoidance (see Figures 3a and 3b).

Aflibercept's excellent safety profile from the VIEW trials appears to have carried over into post-approval use. Early reports of post-injection, sterile endophthalmitis prompted a full investigation by Regeneron, a report to the FDA, and an information letter to interested retina specialists.³⁴ Thirteen cases of post-injection inflammation (eleven from one group practice, nine from the same physician) cleared after treatment without permanent sequelae. An investigation into the manufacturing, packaging, transportation and storage of the drug, as well as the physicians' injection techniques, failed to identify an etiology for the inflammation. No adverse reactions had been noted when doses from the same drug lot were administered by other physician groups and no subsequent clusters of inflammation have been reported. After the first 30,000 injections of aflibercept, the incidence of intraocular inflammation (0.05 %) was similar to that in published reports involving ranibizumab and bevacizumab.

Summary and Future Use

Physicians' overall clinical impression of aflibercept for exudative AMD after the first year of use in the United States has been very positive. Aflibercept has recently been granted regulatory approval in Australia, Japan, and Europe so it appears likely that its world-wide use will increase as physicians take advantage of its role as salvage therapy and increase its use as first-line treatment. ■

- Congdon N, O'Colmain B, Klaver CC, et al., Eye Diseases Prevalence Research Group, Causes and prevalence of visual impairment among adults in the United States, *Arch Ophthalmol*, 2004;122:477–85.
- Ferris FL III, Fine SL, Hyman L, Age-related macular degeneration and blindness due to neovascular maculopathy, *Arch Ophthalmol*, 1984;102(11):1640–2.
- Macular Photocoagulation Study Group, Argon Laser photocoagulation for neovascular maculopathy, *Arch Ophthalmol*, 1986;104:694–701.
- TAP study group, Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin, One-year results of 2 randomized clinical trials – TAP report 1, *Arch Ophthalmol*, 1999;117:1329–45.
- Folkman J, Tumor angiogenesis: therapeutic implications, *N Engl J Med*, 1971;285(21):1182–6.
- Senger DR, Galli SJ, Dvorak AM, et al., Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid, *Science*, 1983;12:983–5.
- Ferrara N, Henzel WJ, Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells, *Biochem Biophys Res Commun*, 1989;161:851–8.
- Connolly DT, Heuvelman DM, Nelson R, et al., Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis, *J Clin Invest*, 1989;84:1470–8.
- Gragoudas ES, Adamis AP, Cunningham ET, et al., Pegaptanib for neovascular age-related macular degeneration, *N Engl J Med*, 2004;351:2805–16.
- Rosenfeld PJ, Brown DM, Heier JS, et al., Ranibizumab for age-related macular degeneration, *N Engl J Med*, 2006;355:1419–31.
- Brown DM, Kaiser PK, Michels, et al., Ranibizumab versus verteporfin for neovascular age-related macular degeneration, *N Engl J Med*, 2006;34:1432–44.
- Busbee BG, Yee W, Li Z, Rubio RG, Efficacy and safety of 2.0 mg or 0.5 mg ranibizumab in patients with subfoveal neovascular AMD: HARBOR Study, *American Academy of Ophthalmology Annual Meeting, Orlando FL*, October 24, 2011.
- Martin DF, Maguire MG, Ying G-S, Ranibizumab and bevacizumab for neovascular age-related macular degeneration, *N Engl J Med*, 2011;364(20):1897–908.
- Heier JS, Brown DM, Chong V, et al., VIEW 1 and VIEW 2 Study Groups, Intravitreal aflibercept (VEGF Trap-Eye) in wet age-related macular degeneration, *Ophthalmology*, 2012;Oct 16 [Epub ahead of print].
- Papadopoulos N, Martin J, Ruan Q, et al., Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab, *Angiogenesis*, 2012;15(2):171–85.
- Ferrara N, Damico L, Shams N, et al., Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration, *Retina*, 2006;26:859–70.
- Presta LG, Chen H, O'Connor SJ, et al., Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders, *Cancer Res*, 1997;57:4593–9.
- Holash J, Davis S, Papadopoulos N, et al., VEGF-Trap: a VEGF blocker with potent antitumor effects, *PNAS*, 2002;99(17):11393–8.
- Byrne AT, Ross L, Holash J, et al., Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model, *Clin Cancer Res*, 2003;9:5721–8.
- Taylor PD, Wilson W, Hillier SG, et al., Effects of inhibition of vascular endothelial growth factor at time of selection on

- follicular angiogenesis, expansion, development and atresia in the marmoset, *Mol Hum Reprod*, 2007;13(10):729–36.
21. Oliveira HB, Sakimoto T, Javier JAD, et al., VEGF Trap_{R102} suppresses experimental corneal angiogenesis, *Eur J Ophthalmol*, 2010;20:48–54.
 22. Bachman BO, Block F, Wiegand SJ, et al., Promotion of graft survival by vascular endothelial growth factor A neutralization after high-risk corneal transplantation, *Arch Ophthalmol*, 2008;126(1):71–7.
 23. Saishin Y, Saishin Y, Takahashi K, et al., VEGF-trap_{R102} suppresses choroidal neovascularization and VEGF-induced breakdown of the blood-retinal barrier, *J Cell Physiol*, 2003;195:241–8.
 24. Cao J, Zhao L, Li Y, et al., A subretinal matrigel rat choroidal neovascularization (CNV) model and inhibition of CNV and associated inflammation and fibrosis by VEGF trap, *Invest Ophthalmol Vis Sci*, 2010;51:6009–17.
 25. Nguyen QD, Shah SM, Hafiz G, et al., 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, *Ophthalmology*, 2006;113(9):1522–32.
 26. Nguyen QD, Shah SM, Browning DJ, et al., A phase I study of intravitreal vascular endothelial growth factor trap-eye in patients with neovascular age-related macular degeneration, *Ophthalmology*, 2009;116:2141–8.
 27. Brown DM, Heier JS, Ciulla T, et al., Primary endpoint results of a phase II study of vascular endothelial growth factor trap-eye in wet age-related macular degeneration, *Ophthalmology*, 2011;118:1089–97.
 28. Heier JS, Boyer D, Nguyen QD, et al., The 1-year results of CLEAR-IT 2, a phase 2 study of vascular endothelial growth factor trap-eye dosed as-needed after 12-week fixed dosing, *Ophthalmology*, 2011;118:1098–106.
 29. Stewart MW, Rosenfeld PJ, Predicted biological activity of intravitreal VEGF Trap, *Br J Ophthalmol*, 2008;92(5):667–8.
 30. Heier JS, Brown DM, Chong V, et al., VIEW 1 and VIEW 2 Study Groups, Intravitreal aflibercept (VEGF Trap-Eye) in wet age-related macular degeneration, *Ophthalmology*, 2012;Oct 16 [Epub ahead of print].
 31. Chakravarthy U, Harding SP, Rogers CA, et al., Ranibizumab versus bevacizumab to treat neovascular age-related macular degeneration: one-year findings from the IVAN randomized trial, *Ophthalmology*, 2012;119(7):1399–411.
 32. Regillo CD, Brown DM, Abraham P, et al., Randomized, double-masked, sham-controlled trial of ranibizumab for neovascular age-related macular degeneration: PIER Study 1, *Am J Ophthalmol*, 2008;145:239–48.
 33. Schmidt-Erfurth U, Eldem B, Guymer R, et al., EXCITE Study Group, Efficacy and safety of monthly versus quarterly ranibizumab treatment in neovascular age-related macular degeneration: the EXCITE study, *Ophthalmology*, 2011;118:831–9.
 34. Regeneron Pharmaceuticals Inc, – Form 8-K – EX-99,2 – Letter to the FDA dated February 13, 2012, http://www.fda.gov/oc/oc/sec-filings/120213/REGENERON-PHARMACEUTICALS-INC_8-K/exhibit99-2.htm, Accessed October 9, 2012.