

Aflibercept ophthalmic solution: drug development and clinical uses

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The recent adoption of drugs that inhibit the actions of VEGF has dramatically changed ophthalmology. The intravitreal injection of potent anti-VEGF medications, particularly bevacizumab and ranibizumab, has become standard-of-care for the treatment of the most common blinding diseases – exudative age-related macular degeneration and diabetic macular edema. The introduction of aflibercept, a high binding affinity fusion protein, gives physicians another valuable tool for the treatment of these conditions. The VIEW 1 and 2 trials showed that monthly and bimonthly aflibercept was equivalent to monthly ranibizumab for the treatment of age-related macular degeneration, and the COPERNICUS and GALILEO trials showed that aflibercept was far superior to sham injections for the treatment of macular edema due to central retinal vein occlusions. Ongoing trials are investigating the efficacy of aflibercept for the treatment of edema due to diabetic retinopathy, branch retinal vein occlusions and myopic choroidal neovascularization.

Keywords: aflibercept • age-related macular degeneration • diabetic macular edema • retinal vein occlusion • VEGF

The recent introduction of drugs that prevent the actions of VEGF has revolutionized the treatment of the most common chorioretinal vascular diseases. Since VEGF mediates both ocular angiogenesis and exudation [1], it comes as no surprise that VEGF antagonists normalize retinal anatomy and improve vision in patients with exudative age-related macular degeneration (AMD) [2–5], diabetic macular edema (DME) [6], and macular edema due to retinal vein occlusions [7–10]. As these are the leading causes of vision loss in patients over the age of 20 years in industrialized countries, effective treatments should improve public health, decrease the incidence of blindness, and lower the costs of social programs that care for the visually handicapped. A 50% decrease in the incidence in AMD-related blindness among the elderly in Denmark has already been observed [11] and the more recent adoption of anti-VEGF therapy for DME and retinal vein occlusions promises to further reduce the incidence of blindness.

The widespread use of successful anti-VEGF therapy, however, does not come without the need for a substantial allocation of resources with significant resultant costs. Both direct (ophthalmologic examinations, retinal imaging studies, intravitreal injection and drugs) and indirect (transportation and loss of productivity by both patients and accompanying persons) costs of current treatment regimens are substantial. The successes with current drug therapy have buoyed the morale of afflicted patients and their families, but have further elevated their expectations and demands. Therefore, now more than ever, the need for more effective therapy with lower overall costs is critical.

For 8 years, off-label, intravitreal injections of bevacizumab (Avastin®, Genentech, San Francisco, CA, USA/Roche, Basel, Switzerland) or ranibizumab (Lucentis®,

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Genentech /Novartis, Basel, Switzerland) have been the standard therapy for exudative AMD. Monthly injections of these drugs prevent moderate vision loss in up to 95% of patients and lead to average vision gains of +8–10 letters [2–5]. More recently, anti-VEGF injections and corticosteroid therapy (triamcinolone, dexamethasone inserts and fluocinolone inserts) have also benefited patients with DME [12–14] and macular edema due to vein occlusions [15,16]. It remains unclear, however, if currently available monotherapies produce the maximal possible improvements in vision, and treatment regimens that incorporate frequent drug injections can be difficult to follow for many patients.

The newest anti-VEGF drug, aflibercept (VEGF Trap-eye, Eylea[®], Regeneron, Rensselaer, NY, USA) was created with the hope that tighter VEGF binding would improve the results of therapy and decrease the frequency of injections in patients with chorioretinal vascular conditions. This manuscript will discuss the evolving role of anti-VEGF therapy in the treatment of chorioretinal blinding disorders with a special focus on the development and clinical adoption of intraocular aflibercept.

VEGF & ocular disease

VEGF was first sequenced by two independent research groups in 1989 [17,18] and was soon recognized to be several closely related glycoproteins that regulate angiogenesis and promote vascular hyperpermeability [1]. VEGF species segregate into seven families (VEGF-A, -B, -C, -D, -E, -F and placental growth factor) that activate three *trans*-membrane receptors (VEGFR1, VEGFR2 and VEGFR3) [19]. Isoforms of VEGF-A, particularly VEGF₁₆₅, are responsible for most cases of ocular angiogenesis. VEGF promotes proliferation and migration of vascular endothelial cells, increases in vascular permeability – 50,000-times more than by histamine – by phosphorylating tight junction proteins, causes vasodilation and swelling of vascular endothelial cells, attracts mononuclear white blood cells and endothelial progenitors, and serves as a survival factor [1].

Tissue hypoxia [20], inflammatory mediators (TNF- α , IL-1 α , IL-6 and prostaglandin E2) [21] and growth factors (EGF, FGF and TGF- β) [22] upregulate VEGF synthesis by neuroglia, muller cells, retinal pigment epithelium and white blood cells. Though vascular endothelial cells are the primary targets for VEGF, all retinal cells express VEGF receptors and become activated by elevated tissue VEGF levels [23].

Since the VEGF dimer's small size (35–45 kDa) enables it to pass easily through the retina, it diffuses down concentration gradients through the vitreous and into the anterior chamber of the eye. Aqueous and vitreous VEGF concentrations are elevated in several

chorioretinal vascular conditions: exudative AMD, DME, retinal vascular occlusions and proliferative diabetic retinopathy [24–26]. Larger cumulative areas of retinal ischemia result in higher VEGF concentrations, thereby increasing the tendency toward angiogenesis rather than vascular hyperpermeability.

Several lines of evidence emphasize the importance of VEGF in the genesis of chorioretinal vascular conditions. VEGF-A, VEGF-B and placental growth factor have all been detected in excised choroidal neovascular membranes [27–29]. Both VEGF and VEGF RNA are upregulated in both clinical and experimental choroidal neovascular membranes [30,31]. Increased VEGF expression from the retinal pigment epithelium characterizes the early stages of AMD [32]. Exogenously administered VEGF induces neovascularization in primate eyes [33].

Anti-VEGF drug development

Drug developers have employed a variety of recombinant strategies to create molecules that bind soluble VEGF. Ophthotech developed a pegylated aptamer (pegaptanib [Macugen[®]], Ophthotech, New York, NY, USA) that attaches to the heparin binding site (amino acids 110–165) of soluble VEGF₁₆₅, and the longer isoforms VEGF₁₈₃, VEGF₁₈₉ and VEGF₂₀₆, which are primarily bound to matrix and membrane proteoglycans [34]. Developers had hoped that VEGF₁₆₅ specific blockade would effectively suppress the actions of VEGF while avoiding the adverse events that could accompany excessive pan-VEGF-A suppression [35].

Genentech developed a murine antibody (bevacizumab), intended for the treatment of advanced solid tumors, that bound to the receptor binding sequence (amino acids 81–92) of all isoforms of VEGF-A. Developers were concerned that the antibody's large size (149 kDa) would prevent it from penetrating the inner retina and that its fragment crystallizable (Fc) fragment would induce inflammation and prolong the systemic half-life, so they cleaved one of the binding fragments (Fab) from a related antibody, and humanized and affinity enhanced it, to create the intraocular drug ranibizumab [36]. Only years later was bevacizumab first injected intravitreally and found to effectively reduce macular edema in patients with AMD and central retinal vein occlusion (CRVO) [37,38].

Pegaptanib, ranibizumab and bevacizumab are said to possess 'high binding affinity' for VEGF₁₆₅, but the scientists at Regeneron sought to develop a drug with greater potency and extended duration of action by further increasing the binding affinity for VEGF-A. They synthesized a fusion protein by attaching natural binding sequences from the native VEGF receptors to a soluble Fc segment backbone from a human IgG1 [39]. Since VEGFR1 has a higher binding affinity

for VEGF₁₆₅ ($K_D = 10\text{--}20$ pM) than has VEGFR2 ($K_D = 75\text{--}150$ pM), they created the 'parent VEGF trap' (VEGF Trap_{R1R1R1}) with three binding domains from VEGFR1. Unfortunately, this high affinity molecule ($K_D = 5$ pM) rapidly bound matrix proteoglycans, resulting in low serum concentrations and a poor pharmacokinetic profile. The negatively charged protein sequences were modified, resulting in two intermediate compounds (VEGF Trap_{AB1} and VEGF Trap_{AB2}) with improved but still inadequate pharmacokinetic profiles. Finally they paired the second binding domain from VEGFR1 and the third domain from VEGFR2 to create the high affinity ($K_D = 0.5$ pM) VEGF Trap_{R1R2} or aflibercept, which did not bind matrix proteoglycans. Furthermore, molecular spectroscopy indicated that the molecule's two binding arms created a favorable 3D configuration which held the VEGF homodimer in a 'two-fisted' grasp. The use of VEGFR1 binding sequences meant that aflibercept bound not only all isoforms of VEGF-A, but also VEGF-B and placental growth factor.

In rabbit eyes aflibercept has a half-life of 4.6–4.7 days as measured by both ELISA assays [40] and positron emission tomography-CT scans [41]. This compares favorably with the durations of ranibizumab (2.8 days) [42,43] and bevacizumab (4.3 days) [43,44], which were measured with similar techniques. Pharmacokinetic testing of aflibercept in human eyes has not yet been performed, but mathematical modeling suggests that it falls between 7.1 and 9.1 days [45]. After intravitreal injection, aflibercept appears to diffuse through the vitreous and retina before leaving the eye via the choroidal circulation and trabecular meshwork. Aflibercept is not believed to undergo metabolism within the eye, but since it forms a nearly unbreakable bond with VEGF, the complex has a half-life of 18 days in the systemic circulation, compared with a systemic half-life of only 1–3 days for unbound aflibercept [46]. Aflibercept is removed from the systemic circulation by Fc-mediated proteolytic mechanisms [47]. **Table 1** summarizes the important physical characteristics and biochemical behaviors of aflibercept, bevacizumab and ranibizumab.

When aflibercept was formulated for intraocular use, several buffers were added to the systemic solution in order to avoid ocular toxicity [48]. Aflibercept was nontoxic to growing human trabecular meshwork cells, scleral fibroblasts and retinal pigment epithelial cells when tested in an *in vitro* cell assay [49]. In preclinical mouse trials, aflibercept prevented the induction of choroidal neovascular membranes after both laser photocoagulation and the placement of subretinal matrigel [50]. In addition, in mice, it prevented corneal neovascularization after exposure to FGF [51], and decreased the failure rates of high-risk penetrating keratoplasties [52].

In a monkey model, both intravenous and intravitreal aflibercept prevented the development of choroidal neovascularization when administered before laser photocoagulation [53].

Human trials

■ AMD

In patients with exudative AMD, pegaptanib (2005) was shown to decrease the rate of vision loss [34] and then ranibizumab (2006) was the first drug able to improve vision [2,3]. Both of these drugs were already well into Phase III trials before aflibercept became ready for human testing. Since the initial focus of aflibercept development was on intravenous therapy for oncologic disease, the first exudative AMD trial studied the efficacy of intravenous infusions [54]. In the Phase I dose-escalation trial, 25 patients received single intravenous infusions of one of three doses of aflibercept (0.3, 1.0, or 3.0 mg/kg), were observed for 4 weeks to monitor safety, were then treated with three doses at 2 week intervals, and were followed for 6 weeks. The average improvement in vision was +12 letters and the average improvement in excess retinal thickening was -10%, +66% and +60%, respectively. Only patients receiving the 3.0 mg dose maintained therapeutic serum aflibercept levels for 1 month. Two patients treated with the highest dose developed hypertension and proteinuria, adverse events that are characteristic of systemic VEGF suppression. Since the only dose that supported monthly administration had an unfavorable safety profile, intravenous infusions were subsequently reserved for patients with advanced tumors while patients with ophthalmologic conditions were switched to intravitreal injections.

The CLEAR-IT 1 trial evaluated the tolerability, safety, bioactivity, and maximum tolerated dose of intravitreal aflibercept [55]. In total, 21 patients each received a single injection of 0.05, 0.15, 0.5, 1, 2 or 4 mg in a dose-escalation manner. The study's primary end point was a 6-week safety analysis, though patients were followed through 12 weeks. No evidence of ocular toxicity was seen among any of the doses and, as a result, the maximum tolerated dose was not determined. Patients achieved an average improvement of +4.43 letters of vision but those receiving the two highest doses (2 and 4 mg) improved by an average of +13.5 letters, with three out of these six improving by more than 15 letters, and three remained stable through 12 weeks without additional injections. Patients experienced an average improvement in foveal thickness of -104 μm whereas those in the two high-dose groups improved by -216 μm . Many of the eyes had pre-existing subretinal fibrosis, so changes in fluorescein angiography could not be universally determined; nonetheless, some eyes demonstrated contraction or regression of the neovascular complex.

Table 1. Important structural characteristics and biochemical properties of aflibercept in contrast with bevacizumab and ranibizumab.

Drug	Molecular weight (kDa)	Dissociation constant (VEGF ₁₆₅ ; pM)	Binding properties	VEGF binding sites	Serum half-life	Intraocular half-life (days)			Ref.
						Rabbits	Monkeys	Humans	
Aflibercept	115	0.5	VEGF-A VEGF-B PGF	1 (binds both VEGF molecules)	1–3 days (unbound) 18 days (bound)	4.6–4.7	Unknown	9.0 [†]	[39–41]
Bevacizumab	149	58–1,000	VEGF-A	2	20 days	4.2–4.3	5.6 [†]	9.8	[36,42–45]
Ranibizumab	48	46–192	VEGF-A	1	6 h	2.6–2.9	3.2	7.1	[36,42,43]

[†]Estimated.

The study suggested a dose-dependent response with patients receiving higher doses experiencing a greater and more prolonged clinical effect through 12 weeks.

The Phase II CLEAR-IT 2 trial assessed the efficacy and safety of aflibercept when administered monthly (0.5 or 2 mg) or quarterly (0.5, 2 or 4 mg) for 12 weeks, followed by *pro re nata* (PRN) treatment through 52 weeks [56,57]. In total 159 patients were randomized equally to the five treatment groups. The primary end point of the first phase of the trial was at 12 weeks, at which time patients were reinjected and then re-evaluated at 16 weeks. All patients gained an average of +5.7 letters, while those receiving monthly injections improved by an average of +8.5 letters. Patients in all groups had similar improvements in vision at week 8, after which the monthly treated groups continued to improve whereas the quarterly treated groups experienced a mild decline. The average improvement in central retinal/primary lesion thickness improved by -103 µm as early as 4 weeks and further improved to -119 µm at 12 weeks. Patients treated monthly experienced the greatest improvement in macular thickness (0.5 mg: -153.3 µm and 2 mg: -169.2 µm).

During the second phase of the trial from weeks 16 through 52, all patients were examined monthly and treated if they exhibited signs of active neovascularization (an increase in macular thickness of >100 µm, loss of vision of >5 letters, persistent or new subretinal fluid, new hemorrhage, or active choroidal neovascular membrane on fluorescein angiography). Moderate vision loss (-15 letters) was prevented in 93% of patients and 73% experienced stable or improved vision (>0 letters gained). The average improvement in vision was +5.3 letters while patients initially treated with 2 mg monthly improved by +9 letters. The average decrease in central retinal/primary lesion thickness was -130 µm, while patients initially treated with 2 mg monthly had an improvement of -143 µm. On fluorescein angiography, the lesion size decreased by an average of 2.21 mm².

Aflibercept demonstrated an impressive duration of action as patients received an average of only two

injections between weeks 16 and 52, with a mean time to first injection of 129 days. Patients originally treated with 2 mg monthly experienced the longest median time to reinjection (150 days). In total, 19% of patients required no additional injections and 45% required only one or two.

The CLEAR-IT 2 trial suggested that patients receiving 2 mg monthly experience superior gains in vision and macular thickness compared with those receiving lower doses and less frequent injections. The trial indicated that quarterly injections may produce suboptimal gains, so the Phase III trials were designed to compare monthly with bimonthly injections.

Two parallel Phase III registration trials were conducted in North America (VIEW 1), and South America, Europe, Asia and Australia (VIEW 2) [5]. A total of 2458 patients – the most of any AMD trial – were randomized to receive one of three dosing regimens of aflibercept (0.5 mg every 4 weeks [0.5q4], 2 mg every 4 weeks [2q4], or 2 mg every 8 weeks [2q8]) or ranibizumab every 4 weeks (Rq4). The primary end point of this noninferiority trial was the proportion of patients avoiding moderate vision loss (>15 letters) at one year, with key secondary end points that included gains in vision and improvement in macular thickness. The primary end point was easily met as 95–96% of patients receiving aflibercept, compared with 94% of those receiving ranibizumab, lost fewer than 15 letters of vision. Integrated data from both trials showed that at 52 weeks patients in the Rq4 and aflibercept groups (0.5q4, 2q4 and 2q8) had similar improvements in vision (+8.7, +8.3, +9.3 and +8.4 letters). No significant differences among the groups were seen in the proportions of patients experiencing stable or improved vision (79 vs 81%), improvement of vision by at least +15 letters (32 vs 30–33%) and by at least +30 letters (6 vs 5–6%). Only the 2q4 group from VIEW 1 had a significantly greater gain than the ranibizumab group (+10.9 vs +8.1 letters; *p* < 0.001) but the modest gains achieved by patients receiving 2q4 in VIEW 2 (+7.9 vs +9.4 letters) did not support a true difference between

the treatment groups, suggesting that the VIEW 1 outperformance by 2q4 was simply due to chance.

All treatment groups (Rq4, 0.5q4, 2q4 and 2q8) had impressive average thinning of the macula (-127.8, -122.9, -137.4 and -139.1 μm). Patients receiving 2q8 in the VIEW 2 experienced a 17 μm 'saw-tooth' fluctuation in macular thickness that decreased to 8 μm by week 52. Further analysis showed that this oscillation was not due to large fluctuations in a small group of patients and the visual acuity (VA) of this group of patients was not compromised. Sequential fluorescein angiograms showed that the average lesion size decreased in all groups (-4.2, -3.5, -4.6 and -3.4 mm^2). Functional improvement scores ranging from 4.5–6.7 on the National Eye Institute Visual Function Questionnaire were seen in all groups while only the Rq4 group in the VIEW 2 demonstrated significantly more improvement than the 2q4 group (6.3 vs 4.5; $p = 0.01$).

Aflibercept had an acceptable safety profile with the most common adverse events, those occurring in >5% of eyes, being unrelated to the disease process. Fortunately, serious adverse ocular events such as endophthalmitis and traumatic cataract were equally distributed between patients receiving ranibizumab and aflibercept (eight patients in both), and occurred after less than 0.1% of injections. The incidence of serious adverse systemic events, fatal events and adverse events leading to withdrawal from the trials were also infrequent and equally spread among treatment groups. Systemic arterial hypertension was seen in only 0.3% of patients.

During the second year of the VIEW trials patients were examined monthly and retreated with the same drug and dose from the first year if signs of neovascular activity, including retinal edema, subretinal fluid, new hemorrhage or loss of vision were present [101]; however, the treatment interval was capped at 12 weeks. On average, 91.5–92.4% of patients lost fewer than 15 letters of vision and patients in all groups lost an average of only 1–2 letters of VA, similar to that seen in other trials when patients were switched from fixed to PRN dosing. For patients originally receiving Rq4, 0.5q4, 2q4 and 2q8, average gains in vision from baseline were +7.9, +6.6, +7.6 and +7.6 letters, respectively, and average increases in central retinal thickness (CRT) from week 52 were 10, 10, 10, and 6 μm , respectively. The average numbers of injections during year 2 were 4.7, 4.6, 4.1 and 4.2. Fewer patients originally randomized to 2q8, compared with Rq4, received 11 injections (1.8 vs 3.7%) and at least six injections (15.9 vs 26.5%). At the end of year 2 a higher percentage of 2q4 patients had dry retinas compared to those receiving Rq4. Similar proportions of patients from all groups achieved 15 letters of visual

improvement from baseline (28.1–33.4%). As defined by the antiplatelet trialists group, the incidences of thromboembolic complications were similar in all groups (2.4–3.8%).

Recent publications have shown that aflibercept is an effective therapy for patients who are recalcitrant or demonstrate a suboptimal response to other anti-VEGF drugs. One patient with polypoidal choroidopathy that received 48 doses of ranibizumab over 5 years experienced a dramatic improvement in macular thickness – 797 μm to 320 μm – after a single dose of aflibercept [58]. In a small series of patients, serous pigment epithelial detachments that were refractory to bevacizumab and ranibizumab therapy resolved after three injections of aflibercept [59]. In the largest published series, 34 eyes with treatment resistant AMD after an average of 28.6 injections over 44.7 months experienced improved vision (20/75 to 20/60), decreased central foveal thickness (416–248 μm), and decreased retinal pigment epithelial detachment height (260–214 μm) after being switched to aflibercept [60].

■ Macular edema due to CRVO

Aflibercept was evaluated for the treatment of CRVO related macular edema in the Phase III, double-masked, 2-year registration trials COPERNICUS and GALILEO. The trials randomized 189 patients from 70 sites in the USA, Canada, Columbia, India and Israel (COPERNICUS) [9] and 177 patients from Europe, Japan and Australia (GALILEO) [10] to receive 2-mg intravitreal aflibercept injections (IVT-AFL) or sham injections (3,2). Enrollment criteria included BCVA from 24–73 letters and CRT >250 μm by time-domain OCT. Patients were treated every 4 weeks through week 24, and from weeks 24 through 52 all patients were examined monthly and treated PRN if the CRT was >250 μm , new cysts or subretinal fluid were seen on OCT, or vision dropped by 5 letters from the best previous result. If none of these criteria were met, patients were given a sham injection. Beginning at week 24, patients originally randomized to sham injections in COPERNICUS were eligible to receive IVT-AFL but those in GALILEO continued with sham injections. The primary end point was the proportion of patients who improved by at least 15 letters of vision by week 24, while additional end points included visual, anatomic and quality of life (NEI VFQ-25) measurements at weeks 24 and 52. Panretinal photocoagulation was performed if any neovascularization of the anterior or posterior segments developed. Patient retention was good.

At week 24 in the COPERNICUS and GALILEO trials, visual improvement of 15 letters was achieved by

56.1 and 60.2% of IVT-AFL-treated patients compared with 12.3 and 22.1% of sham patients ($p < 0.001$), and average gains in vision were +17.3 and +18.0 letters compared with -4.0 and +3.3 letters ($p < 0.001$). In GALILEO, more IVT-AFL patients than sham patients improved by >0, >10 and >30 letters (89.3, 79.8 and 16.5% vs 60.3, 30.9 and 2.9%, respectively) and they had better mean improvements in CRT (-448.6 vs -169 μm). After the sham group in the COPERNICUS trial became eligible to receive IVT-AFL at week 24, patients improved by an average of +7.8 letters by week 52. Therefore, by 52 weeks in COPERNICUS, patients improved by an average of +16.2 letters (IVT-AFL) and +3.8 letters (sham + IVT-AFL) from baseline. Only 7.9% of patients originally randomized to IVT-AFL lost vision, whereas 31.5% of patients originally in the sham group lost vision. Even the most severely affected eyes gained vision when treated with IVT-AFL, as 19.9% of patients with initial VA <20/200 gained 15 letters, and 48.6% of those with more than ten disc areas of retinal nonperfusion gained at least 15 letters. If patients were enrolled within 2 weeks of diagnosis, 64.1% of IVT-AFL and 34.6% of sham patients gained 15 letters, compared with 42.9 and 19%, respectively, if the diagnosis was delayed by >2 months. The mean reductions in CRT at 1 year were similar between IVT-AFL (-413 μm) and sham (-381.8 μm) patients. The mean number of injections between weeks 24 and 52 was 2.7 for the IVT-AFL + PRN group and 3.9 for the sham + IVT-AFL group.

The most common adverse events were conjunctival hemorrhage, eye pain, reduced vision and increased intraocular pressure. No patients in the IVT-AFL group developed neovascularization, compared with six patients in the sham group ($p < 0.001$). The incidence of antiplatelet trialists collaboration events was 2.7% in the sham group and 0.9% in the IVT-AFL group; there were two vascular deaths in the sham group (one due myocardial infarction, one due to cardiac arrhythmia), both of which occurred before week 24.

The decreased frequency of injections between weeks 24 and 52 points to aflibercept's extended duration of action as was seen in the VIEW [5,101] and DA VINCI [61] trials but it also emphasizes the continued need to monitor and treat these patients. Eyes benefitted from crossover to IVT-AFL after 6 months and though the maculas dried well, their VA improvements lagged behind eyes that received aflibercept from the start. In addition, a higher proportion of eyes randomized within 2 months of the onset of symptoms achieved >15 letter gains compared with those randomized after more than 2 months of symptoms. This suggests that delaying anti-VEGF therapy limits visual potential, but it cannot be conclusively determined that crossover

patients would not have done better with 6 monthly injections as was initially given to the IVT-AFL group.

During the second year of COPERNICUS [62] and GALILEO [63], all patients received mandatory evaluations quarterly or bimonthly and were treated with IVT-AFL PRN. From week 52 to week 100 in COPERNICUS, the proportions of IVT-AFL and sham + IVT-AFL patients improving by >15 letters decreased from 55.3 and 30.1% to 49.1 and 23.3%, respectively, the average gains in vision decreased from +16.2 and +3.8 to +13.0 and +1.5 letters, and the average improvements in CRT worsened from -413.0 vs -381.8 μm to -390.0 vs -343.3 μm . The average numbers of required injections were 6.0 and 7.1. From week 52 to week 76 in GALILEO, the proportions of IVT-AFL and sham + IVT-AFL patients improving by >15 letters decreased from 60.2 and 32.4% to 57.3 and 29.4%, the average gains in vision decreased from +16.9 and +3.8 to +13.7 and +6.2 letters, and the average improvements in CRT worsened from -423.5 and -219.3 μm to -389.4 and -306.4 μm . The average numbers of required injections were 1.3 and 1.7.

Patients in all treatment arms tolerated therapy well with few adverse events noted. Unfortunately, reduced frequency of monitoring during the second year in both COPERNICUS and GALILEO coincided with declining vision and worsening macular edema, findings similar to those from the second year of the CRUISE/HORIZON trial [64]. It remains unclear if the declining VA is due to reduced monitoring and treatment frequency or to progressive retinal damage from the underlying vein occlusion; however, the accompanying increase in CRT suggests that patients were undertreated.

As opposed to the CRUISE trial with ranibizumab, COPERNICUS and GALILEO did not exclude patients with strong afferent pupillary defects. COPERNICUS enrolled a higher percentage of patients with posterior nonperfusion (15.5 vs 1.5%), and CRUISE sham patients experienced slight improvement (+0.8 letters) during the initial 24 weeks [7], whereas those in COPERNICUS lost 4 letters. No head-to-head comparison between aflibercept and ranibizumab yet exists, so we must await the results of future studies to assess relative potencies for the treatment of CRVO.

■ DME

Aflibercept's first use for DME was in an open-label, prospective pilot trial to assess the safety and bioactivity of a single injection (4 mg) [48]. Five patients with foveal thicknesses >250 μm and VA between 20/40 and 20/320 were enrolled. The trial end point was at 6 weeks with outcomes that included changes in BCVA and foveal thickness. At 4 weeks, patients had median improvements in vision of +9 letters and excess thickness

of $-49\ \mu\text{m}$. At 6 weeks, four out of five patients still had improved foveal thickness (median of $-74\ \mu\text{m}$) and four out of five had improved VA (median of +3 letters). Two out of the five patients experienced improvement in fluorescein leakage through 6 weeks while leakage returned between 4 and 6 weeks in the other three. Injections were well tolerated by all patients with no serious adverse events noted.

After the encouraging results of the pilot trial, a Phase II study (DA VINCI) was designed to compare different doses and treatment regimens of aflibercept with laser photocoagulation for the treatment of DME [64]. Eligible patients had CRT $>250\ \mu\text{m}$ and VA from 20/40 to 20/320. This multicenter trial randomized 221 patients with center-involving DME 1:1:1:1 to five groups: aflibercept 0.5 mg q4wk, 2 mg q4wk, 2 mg monthly $\times 3$ then q8wk, 2 mg monthly $\times 3$ then PRN, and laser photocoagulation. Patients in the laser arm and aflibercept 2 mg q8wk and PRN arms received sham injections at each visit, and all patients in the aflibercept groups received sham laser at 1 week. Patients were eligible for repeat laser at 16 weeks if clinically significant macular edema persisted. Repeat injections were performed in the PRN arm if the CRT was $>250\ \mu\text{m}$, patients lost 5 letters from the best VA with an associated increase in CRT, or lost 5 letters from the most recent VA. Evaluations were performed every 4 weeks with fluorescein angiography at baseline and weeks 12 and 24. The main outcome in this 52-week trial was change in VA, with secondary outcomes that included changes in CRT, proportion of patients improving by 15 letters and the number of laser treatments by 24 weeks.

By week 24, average visual acuities had improved from +8.5 to +11.4 letters in the aflibercept groups (with no significant differences between the groups) compared with +2.5 letters in the laser group ($p < 0.0085$). Gains of 0, 10 and 15 letters were seen in 93, 64, and 34% of patients in the aflibercept groups, respectively, compared with 68, 32 and 21% of patients in the laser group. Loss of 15 letters of vision was experienced by 9.1% of laser patients and 4.5% of patients receiving 0.5-mg aflibercept, but none receiving the 2-mg dose. Mean reductions in CRT ranged from -127.3 to $-194.5\ \mu\text{m}$ for aflibercept groups compared with $-67.9\ \mu\text{m}$ in the laser group ($p = 0.0066$). Compared with other aflibercept groups, the 2 mg q8wk appeared to have less overall improvement, but since these differences were already manifest at 4 weeks and this group had more proliferative disease, it may be that this group had more severe disease than the others. Patients in the aflibercept 2-mg PRN group received a total of 4.4 injections (three monthly + 1.4 PRN).

Conjunctival hemorrhages, seen in 18.9% of aflibercept-treated patients and in 18.2% of laser/sham

patients, were the most common adverse events. There were two cases of endophthalmitis, one due to *Staphylococcus epidermidis* and the other was culture negative, and two retinal tears occurred in aflibercept-treated patients. The major systemic adverse events included systemic arterial hypertension (seen in four aflibercept patients, all of whom had a history of hypertension) and thromboembolic events (in three patients). Seven patients died during the trial: one patient in the laser group (cardiac arrest); one patient in the 0.5 mg q4wk group (multiorgan failure); three patients in the 2 mg q4wk group (one of cerebral infarction, one of non-small-cell lung carcinoma and one of sudden death); two patients in the 2 mg q8wk group (one of renal failure and one of acute coronary syndrome). None of the deaths was attributed to the study drug or study procedure.

Beginning at week 24, aflibercept patients with persistent edema were eligible for rescue laser. Mean changes in VA at week 52 were $+9.7$ – 13.1 letters for aflibercept groups, compared with -1.3 letters for the laser group. Proportions of eyes with 15 letter improvement in vision were 23.8–45.5% in the aflibercept groups and 11.4% for the laser/sham group; this was statistically better for all aflibercept doses except for the 2 mg q8wk group. Greater proportions of patients receiving aflibercept than sham/laser improved by 10 letters (45–71 vs 30%) and experienced improved diabetic retinopathy scores (31–64 vs 12%). Mean reductions in CRT were greater for aflibercept groups than the laser group (-165.4 to -227.4 vs $-58.4\ \mu\text{m}$). On average, the aflibercept groups received fewer laser procedures (one laser from weeks 24 through 48) compared with the laser group (2.5 from baseline to week 48). The mean numbers of aflibercept injections in the 2 mg q8wk (7.4 injections) and 2-mg PRN (7.2 injections) groups in DA VINCI were comparable to the numbers of injections required by patients receiving ranibizumab (7.0 injections) and ranibizumab plus laser (6.8 injections) in the RESOLVE trial. Two patients developed endophthalmitis and one patient had sterile inflammation [65].

The major clinical trials involving aflibercept, along with their important conclusions, are summarized in [Table 2](#).

Expert commentary

Based on the results of the VIEW trials, the US FDA approved aflibercept (18 November 2011) for the treatment of exudative AMD. Subsequently, aflibercept approval for AMD has been granted by regulatory agencies in Australia, Japan, Europe and the UK.

The use of aflibercept by retina surgeons has steadily increased since its approval. Physicians have had insufficient time to verify aflibercept's extended duration of action, and rather than using it as primary therapy

Table 2. Important Phase I, II and III clinical trials evaluating the efficacy of aflibercept for the treatment of age-related macular degeneration, diabetic macular edema and macular edema due to retinal vein occlusions.		
Trial (Phase)	Important findings	Ref.
Age-related macular degeneration		
CLEAR-AMD 1 (I)	Improved vision and macular thickness was seen with higher doses (1 and 3 mg/kg) Only 3 mg/kg resulted in therapeutic serum levels at 1 month Two out of five patients receiving 3 mg/kg developed hypertension and proteinuria	[54]
CLEAR-IT 1 (I)	Doses up to 4 mg were well tolerated Dose dependent improvements in visual acuity and macular thickness Many patients had clinical responses through 12 weeks	[55]
CLEAR-IT 2 (II)	Eyes receiving monthly injections had better improvements in VA and CRT than those receiving quarterly injections Average time to first injection during PRN phase was 4 months	[56,57]
VIEW 1 and 2 (III)	Monthly and bimonthly IVT-AFL (2 mg) was found to be equivalent to monthly ranibizumab IVT-AFL patients treated PRN during year 2 required an average of 4.1 injections No major safety differences between aflibercept and ranibizumab were seen	[5,101]
Macular edema due to central retinal vein occlusion		
COPERNICUS & GALILEO (III)	Monthly IVT-AFL was superior to sham injections Patients with earlier diagnosis and less retinal ischemia improved more, but even chronic and ischemic eyes benefitted from treatment Sham eyes that received IVT-AFL after 6 months benefitted but lagged behind those receiving prompt IVT-AFL Less frequent monitoring and PRN treatment in year 2 led to increased CRT and decreased VA	[9,10]
Diabetic macular edema		
DA VINCI (II)	IVT-AFL was superior to laser/sham treatments for edema Higher and more frequent doses of aflibercept produced better results Compared with sham/laser group, fewer lasers were required by patients receiving IVT-AFL	[65]
VISTA & VIVID (III)	Ongoing trials comparing monthly IVT-AFL (2 mg) with sham/laser for the treatment of diabetic macular edema	[103]
Macular edema due to BRVO		
VIBRANT (III)	Ongoing trial comparing monthly IVT-AFL (2 mg) with sham/laser for the treatment of BRVO related macular edema	[104]

BRVO: Branch retinal vein occlusion; CRT: Central retinal thickness; IVT-AFL: Intraocular aflibercept injection; PRN: *Pro re nata* (as needed); VA: Visual acuity.

for exudative AMD, most physicians use it as therapy for recalcitrant patients, or those who have suboptimal responses to treatment with bevacizumab and ranibizumab. In fact, the 2012 American Society of Retinal Specialists Preferences and Trends survey discovered that although 78% of retinal specialists had used aflibercept, only 10% considered it a first-line therapy for AMD. However, 80% stated that they would readily switch incomplete responders to aflibercept [102].

Aflibercept's perceived extended duration of action for the treatment of AMD has been frequently discussed and debated. The VIEW trials were the first to demonstrate that a bimonthly regimen of anti-VEGF injections in treatment-naive patients is as effective as monthly ranibizumab. Previous attempts to extend the treatment interval for ranibizumab to 3 months resulted in progressive loss of vision [66] and when patients in EXCITE were evaluated 2 months after having received

three monthly ranibizumab injections, their average vision had declined by 3–4 letters [67]. Rather than treating patients according to the strategies employed during the first year of Phase III protocols, most physicians individualize therapy with either PRN or treat-and-extend regimens. The second year of the VIEW trials suggested that the median duration of action of aflibercept in patients who are examined monthly and injected PRN may be as long as 12 weeks, similar to that predicted by mathematical modeling [68]. The average treatment interval that can be achieved with treat-and-extend, the most commonly used strategy, is not known, but the VIEW trials suggest that it may be as long as 3 months.

The successful introduction of frequently administered anti-VEGF drugs for the treatment of AMD and their more recent use for macular edema due to CRVO and DME has swamped retinal physicians'

offices. Effective anti-VEGF therapy incurs significant costs and, with the aging of the population in industrialized nations, threatens to further strain health-care budgets and limit patients' access to physicians. With aflibercept's extended duration of action, the first year of protocol-driven treatment for exudative AMD would result in fewer aflibercept injections and office visits, with a 42% savings compared with ranibizumab. Savings accrued with treat-and-extend regimens are unknown, though results from the second year of the VIEW trials suggest that they will be considerably less. Because the per dose cost of bevacizumab (US\$50) is far less than that of aflibercept (\$1850) and ranibizumab (\$1950), treatment costs with bevacizumab, despite the need for more frequent visits and injections, will remain considerably less than those with aflibercept.

The results from the COPERNICUS and GALILEO trials led to FDA approval of aflibercept for the treatment of macular edema due to CRVO in September 2012. The results from these trials are similar to those from the CRUISE and HORIZON trials, but the improvement and maintenance of vision in IVT-AFL treated eyes, compared with those randomized to sham, is slightly better with aflibercept. Since entry criteria for the trials differed, direct comparisons between the two drugs needs to be made with caution. A head-to-head trial with aflibercept, ranibizumab and bevacizumab, though not currently planned, would be valuable. An application has been submitted to the European Regulatory Authority for the treatment of edema due to CRVO.

Only more recently has anti-VEGF therapy become the standard-of-care for most patients with center involving DME and macular edema due to branch retinal vein occlusions. The FDA has approved ranibizumab for both of these conditions and bevacizumab is available for off-label use. Aflibercept is currently being evaluated in Phase III trials for DME (VISTA and VIVID) and macular edema due to branch retinal vein occlusions (VIBRANT). Both have completed enrollment but results of the trials have not yet been subjected to peer review.

Within 3 months of FDA approval for the treatment of exudative AMD, 15 cases (11 from one practice and nine from one physician) of sterile inflammation had been reported to the manufacturer. Nine of these cases presented with pain, and intraocular fluid cultures and antibiotic injections were performed in five. An investigation discovered that the associated drug doses came from five different manufacturing lots, with three lots accounting for 13 out of the 15 cases. During this three month period, 30,000 intravitreal injections of aflibercept were performed, amounting to a 0.05% incidence of intraocular inflammation. This outbreak of sterile inflammation prompted Regeneron to issue

a 'Dear Doctor' letter, informing retinal physicians of this cluster of cases. No etiology for these cases was discovered and no subsequent clusters of inflammation have been reported [69].

Future perspective

Aflibercept will likely receive FDA approval for DME and edema due to branch retinal vein occlusion within the next 2 years and since physicians have readily accepted aflibercept for the treatment of AMD, its use for vein occlusions and DME can be expected to grow quickly.

Despite the availability of four anti-VEGF drugs, the quest for more effective VEGF blockade continues. DARPin MP0112 is a high affinity ($K_D = 2$ pM) molecule that possesses a long intravitreal half-life (13.5 days in a four patient trial). The drug has completed Phase I and II trials for DME and AMD, and Phase III trials may begin soon. Given the usual pace of Phase III trials, however, regulatory approval would probably not be obtained for at least 4 years.

For years, physicians have advocated combination therapy for the treatment of AMD, but trials that paired anti-VEGF drugs with photodynamic therapy and radiation have yielded disappointing results. Monthly ranibizumab combined with fovista (ARC1950, Ophthalmotech, Princeton, NJ, USA), an aptamer that binds PDGF, produced superior results to ranibizumab monotherapy in patients with exudative AMD. Phase III trials will likely begin in late 2013. Other pharmacotherapies, such as complement inhibitors and integrin antagonists, are still in early trials. A complement 5 inhibitor (ARC1905, Ophthalmotech) was administered with ranibizumab in a dose-escalation trial. Average improvements in vision ranged from +11.7 to +15.3 letters at 6 months with 46–60% improving by at least three lines. A small molecular weight integrin $\alpha_5\beta_1$ antagonist (JSM 6427, Jerini AG, Berlin, Germany) showed a trend toward improved VA after a single dose but further trials have not been announced.

Intravitreal triamcinolone injections have been shown to benefit pseudophakic eyes with DME and CRVOs, but cataracts frequently develop and intraocular pressure elevation occurs in over 30% of patients. The sustained release dexamethasone insert (Ozurdex[®], Allergan, Irvine, CA, USA) has been approved for the treatment of retinal vein occlusions, and testing for DME is ongoing. Sustained release fluocinolone inserts have been approved in the UK for the treatment of DME and an application has been filed with the FDA. Corticosteroids reliably reduce macular edema in DME and retinal vein occlusions but because of their less favorable safety profile, they will continue to be viewed as second-line therapy.

Conclusion

Aflibercept, a high-affinity VEGF binding protein, is an effective therapy for a variety of chorioretinal vascular conditions. It has been approved for the treatment of exudative AMD and macular edema due to CRVO, and is being investigated for DME and macular edema due to BRVO. Because of its potency and favorable duration of action, its use will likely continue to increase for years to come.

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The author has sat on advisory boards for Allergan and Regeneron and served as a consultant for Boehringer-Ingelheim. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Background

■ Chorioretinal vascular diseases, if untreated, are the leading causes of blindness in industrialized nations.

VEGF & ocular disease

■ VEGF is a key stimulator of the neovascularization and blood–retinal barrier breakdown that characterizes common chorioretinal vascular disorders.

Anti-VEGF drug development

■ Aflibercept, a high affinity VEGF blocker, is composed of binding sequences from native VEGF receptors 1 and 2.

Human trials

- Monthly and bimonthly aflibercept was found to be equivalent to monthly ranibizumab for the treatment of exudative age-related macular degeneration.
- Aflibercept was superior to sham injections for the treatment of macular edema due to central retinal vein occlusions.
- Aflibercept is currently in Phase III trials for the treatment of diabetic macular edema and edema due to branch retinal vein occlusions.

Future perspective

■ Though drugs targeting other growth factors and inflammatory mediators are under development, anti-VEGF therapy will remain central to the treatment of retinal vascular diseases for many years to come.

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