

The role of aflibercept in the management of diabetic macular edema

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Abstract: Diabetic macular edema (DME) represents one of the leading causes of visual impairment in working-age adults. Although there are several proven treatments available for this condition, pharmacotherapy through the use of intravitreal anti-vascular endothelial growth factor agents has revolutionized the management of DME over the past decade with superior outcomes compared to laser therapy. This review summarizes the pathophysiology and available treatment options for the management of DME, with an emphasis on the efficacy and safety profile of a single particular intravitreal anti-vascular endothelial growth factor agent, aflibercept.

Keywords: diabetic macular edema, aflibercept

Introduction

Diabetic retinopathy is a common microvascular complication of diabetes mellitus, occurring in nearly all type 1 diabetics and 80% of type 2 diabetics after a period of 20 years.¹ The major cause of visual loss in these patients and one of the most common in working-aged adults is diabetic macular edema (DME).²

The pathogenesis of DME is multifactorial and is thought to result from a breakdown of the blood–retinal barrier with the leakage of plasma from macular blood vessels with resultant hard exudates and subsequent thickening of the retina.³ Our improved understanding of the pathogenesis and experience in the treatment of DME has led to the establishment of intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy as the preferred first line for the management of this condition over other therapies, including laser photocoagulation, steroids, and vitreoretinal surgery.⁴

There are several drugs blocking the VEGF pathway through different mechanisms, which have been shown to be effective in the management of DME (Table 1). Aflibercept is notable among these leading to improved anatomical and visual outcomes in those presenting with a poorer visual acuity.⁵

Pathogenesis

The key etiologic factor for the development of DME is hyperglycemia. This is hypothesized to lead to retinopathy through activating one or more biochemical pathways, including increased flux through the polyol pathway,⁶ production of advanced glycation end products,⁷ protein kinase C,⁸ and the hexosamine pathway.⁹ The end result of these biochemical pathways is oxidative stress, inflammation, and vascular dysfunction. This leads to upregulation of growth factors and cytokines, including VEGF, disrupting the blood–retinal barrier, and resulting in tissue edema.¹⁰

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Table 1 Available anti-VEGF agents, mechanisms and indications

Anti-VEGF agent	Type	Mechanism	US FDA-approved indication
Pegaptanib Macugen, Eyetech Pharmaceuticals, Melville, NY/Pfizer, New York, NY ⁶¹	Pegylated anti-VEGF aptamer	Competitively binds to the VEGF-A165 isoform at the heparin binding site	nAMD
Bevacizumab Avastin, Genentech, San Francisco, CA ⁶²	Recombinant humanized monoclonal antibody	Binds to the receptor binding site for all isoforms of VEGF-A	Glioblastoma, metastatic colorectal cancer, non-small-cell lung cancer, metastatic kidney cancer; nAMD
Ranibizumab Lucentis, Genentech, San Francisco, CA ⁶³	Recombinant, humanized monoclonal antigen-binding fragment	Neutralizes all forms of VEGF-A	nAMD, macular edema following RVO, DME
Aflibercept Eylea, Regeneron Pharmaceuticals, Inc., Tarrytown, New York, NY ³³	Human recombinant fusion protein; combination of the second Ig domain of VEGFR-1 and third Ig binding domain of VEGFR-2 with the constant Fc portion of the IgG1	Soluble decoy receptor with high affinity for binding to VEGF molecules VEGF-A and PlGF	nAMD, macular edema following RVO, DME

Abbreviations: VEGF, antivascular endothelial growth factor; nAMD, neovascular age-related macular degeneration; RVO, retina vein occlusion; DME, diabetic macular edema; PlGF, placenta growth factor.

DME treatment options

Laser therapy

In 1985, the Early Treatment of Diabetic Retinopathy Study (ETDRS) demonstrated that focal laser photocoagulation in DME reduced the risk of visual loss of 15 or more letters by half at 1 year.¹¹ This finding established laser therapy as a standard of care for DME. The proposed mechanism for the efficacy of this therapy is decreased production of cytokines, predominantly VEGF,^{12–14} and alteration in barrier properties of the retinal pigment epithelium, resulting in an increase of the active reabsorptive transport of fluids from the retina to the intravascular space.¹⁵

Intravitreal steroids

Despite the benefit of laser treatment in helping to prevent the progression of DME, there remained a significant subgroup of the EDTRS participants who lost 15 or more letters at the conclusion of the study.¹¹ Given experimental animal data suggesting steroid medication may stabilize the blood–retinal barrier,¹⁶ subsequent trials were conducted demonstrating the benefits in preserving visual acuity and reducing central macular thickness with injections of intravitreal steroids.¹⁷ This supports the role of inflammation and inflammatory cytokines in the pathogenesis of DME. Further studies have demonstrated the effects of intravitreal steroids in the inhibition of blood–retinal barrier breakdown with the prevention of leukostasis, through downregulation of angiogenic VEGF (and its receptors)¹⁸ and inflammatory cytokines, including interleukin-6, interleukin-8, interferon-induced protein-10, monocyte chemotactic protein-1, platelet-derived growth factor-AA, and intercellular adhesion molecule-1.^{19–21}

However, intravitreal corticosteroid therapy has a notable adverse event profile, including elevation of intraocular pressure and cataract development, which may threaten the desired visual outcomes.^{17,22} Importantly, when the effect of cataract is accounted for, visual acuity outcomes for pseudophakic eyes treated with triamcinolone may be comparable to eyes treated with anti-VEGF drugs.²³ Careful patient selection for these therapies is important and relevant.

Other treatment modalities

Therapies targeted at other cytokines have been investigated in preclinical and clinical trials, including inhibitors of multiple growth factors, nonsteroidal anti-inflammatory drugs, targeted chemokine, and cytokine inhibitors.^{24–28} Though some of these therapies have shown promise in early testing as primary or adjunctive therapy, further investigation and comparison with current treatments is required.

Anti-VEGF therapy

The discovery of elevated VEGF levels in angiogenic diseases such as metastatic cancer, neovascular age-related macular degeneration (nAMD),²⁹ branch and central retinal vein occlusion (RVO),^{30,31} and DME^{32,33} led to the revolutionary development and the use of anti-VEGF therapies in these pathologies. In recent years, the expanding use of anti-VEGF agents for the treatment of angiogenic retinal diseases such as DME, nAMD, and RVO has resulted in improved outcomes compared to previously used treatment options.^{34–37}

The role of VEGF

VEGF is a protein, which plays a major role in the regulation of angiogenesis. There are five types of VEGF that have

been identified in humans; these include VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF), with VEGF-A strongly implicated in ocular angiogenic diseases.³⁸ It binds to and results in the activation of two tyrosine kinase receptors: VEGF receptor (VEGFR)-1, which plays a key role in conditions such as inflammation, ischemia, and cancer, and VEGFR-2, by mediating endothelial growth and survival signals.³⁹

In vitro studies have demonstrated increased retinal endothelial cell permeability, proliferation, cell survival, and migration due to exposure to VEGF-A.^{40,41} However, stimulation of endothelial cell permeability and the modulation of VEGF-A function were not shown with PlGF or VEGF-B.^{42–45}

Research from animal models studies suggests that PlGF may play a critical role in the development of diabetic retinopathy.⁴⁶ Genetic deletion of PlGF in a diabetic mouse strain demonstrated protection of retinal damage through prevention of diabetes-induced retinal cell death, capillary degeneration, pericyte loss, and blood–retinal barrier breakdown.⁴⁶

VEGF pathway activation and inhibition

The VEGF pathway is activated when VEGF binds to VEGFR-1 and VEGFR-2 on endothelial cells. VEGFR-1 is a 180 kDa high-affinity receptor of VEGF-A, VEGF-B, and PlGF,⁴⁵ whereas VEGFR-2 is a 200–230 kDa high-affinity receptor for VEGF-A.⁴⁵ Laboratory techniques such as alternative mRNA splicing have produced up to nine further isoforms of VEGF-A.⁴⁵ The smaller VEGF-A isoforms (VEGF₁₂₁ and VEGF₁₆₅) are secreted and are freely diffusible, whereas the larger isoforms (VEGF₁₈₉ and VEGF₂₀₆) are bound to heparin-containing proteoglycans of the basement membrane.^{47,48}

Inhibition of the VEGF pathway is achieved by blocking antibodies targeting VEGF^{49–51} or its receptors.^{52,53} Another approach is to use soluble decoy receptors to prevent VEGF binding to its receptors; the mechanism of action by which aflibercept functions.

VEGF in retinal disease

Excessive activation of VEGF occurs in angiogenic retinal diseases, leading to proliferation and inflammation of vascular endothelial cells, margination of leukocytes, and vascular permeability with subsequent vascular leakage.⁴⁵ Similarly, intravitreal injection of VEGF in primate experiments resulted in findings resembling diabetic retinopathy,

including macular edema and retinal neovascularization.^{54–56} Hypoxia is a major regulator of VEGF-A expression,^{13,47} distinguishing VEGF-A from other growth factors postulated to play a role in ocular neovascular diseases (such as insulin-like growth factor-1, fibroblast growth factors, epidermal growth factor, PlGF, and VEGF-B).⁵⁷ Other stimuli contributing to VEGF expression include estrogen, thyroid-stimulating hormone, transformation, p53 mutation, tumor promoters, and nitric oxide.⁴⁵

These findings led to the development and use of anti-VEGF agents to treat and inhibit aberrant angiogenesis in retinal diseases such as DME and nAMD. Unlike laser therapy, which was previously a mainstay of therapy for these disorders, intravitreal anti-VEGF therapy has been shown to be effective in not only reducing the risk of visual loss, but also in improving vision among patients with nAMD, RVO, and DME.^{36,37,58–60}

Anti-VEGF agents

There are several available anti-VEGF therapies for ophthalmic diseases, including pegaptanib,⁶¹ bevacizumab,⁶² ranibizumab,⁶³ and aflibercept.⁵³ These are summarized in Table 1.

Aflibercept

Aflibercept (Eylea, Regeneron Pharmaceuticals, Inc., Tarrytown, New York, NY)⁶⁴ is a 115 kDa recombinant fusion protein produced from hamster ovary cells and is composed of the combination of a fusion of the second Ig domain of human VEGFR-1 and the third Ig binding domain of human VEGFR-2 with the constant fragment crystallizable portion of the human IgG1.⁵³ It is US FDA approved for the treatment of nAMD, central RVO, and DME.

Mechanism of action

Aflibercept was engineered to act as a soluble decoy receptor with high affinity for binding to all isoforms of VEGF-A, VEGF-B, and PlGF molecules.⁵³ This blocks VEGF pathway activation by inhibiting the binding and activation of the cognate VEGFRs, preventing the undesirable effects of VEGF-A such as neovascularization and increased vascular permeability. Additionally, given the implication of PlGF in the pathogenesis of diabetic retinopathy, blockade of this may also account for potential benefits in the use of aflibercept compared with other anti-VEGF agents.⁴⁶

Similar to other anti-VEGF agents, aflibercept contains an Fc domain as part of its structure. However, it forms stable, inert 1:1 homogeneous complexes with VEGF,

unlike antibodies like bevacizumab, which form heterogeneous multimeric immune complexes.⁶⁵ Such multimeric immune complexes are rapidly cleared from the systemic circulation and can deposit in tissues, causing undesirable off-mechanism effects, such as the platelet aggregation seen in bevacizumab.⁶⁶ These complexes have been postulated to be a possible mechanism for arterial thromboembolic events seen in clinical trials involving bevacizumab^{65,66} and may also cause renal damage when complexes accumulate in the kidney glomeruli. By remaining in the systemic circulation, aflibercept does not appear to induce platelet aggregation or deposit in tissues, providing a theoretical safety benefit compared with bevacizumab.

However, increased risk of thromboembolic events has also been reported in trials of other anti-VEGF agents using ranibizumab, yet it does not form complexes, suggesting other factors may be involved.⁶⁷ Given the role of VEGF in endothelial cell proliferation and survival, one suggestion is that by blocking this effect there is exposure of subendothelial collagen resulting in activation of prothrombotic pathways.^{65,68,69}

Pharmacokinetics

The systemic pharmacokinetics of intravitreal aflibercept (2.0 mg) have been compared to that of ranibizumab (0.5 mg) and bevacizumab (1.25 mg) in a study of 56 patients with nAMD.⁷⁰ This study revealed that systemic exposure to aflibercept was higher than that of ranibizumab, with maximum serum concentration five and sevenfold greater after the first dose and third doses respectively and minimum serum concentration 37- and 53-fold greater after the first and third doses, respectively. This pharmacokinetic study also demonstrated there was accumulation of both aflibercept and bevacizumab after three intravitreal injections but not ranibizumab. Aflibercept was also the most potent of these three drugs in reducing plasma-free VEGF with levels undetectable from 3 hours postdose to greater than 1 week postdose.⁷¹ It is postulated that the Fc fragment present in both the bevacizumab and aflibercept molecules extends their serum half-life, accounting for these differences.⁷² The clinical significance of this is, however, yet to be elucidated.

Drug clearance from the vitreous of the eye occurs across the retina through the choroidal circulation and through diffusion into the anterior chamber to exit via the trabecular meshwork.⁷³ There are no published reports about the intravitreal half-life of aflibercept in humans; however, there have been two rabbit models estimating this

through immunoassay and radioisotope imaging techniques as between 4.5 days and 4.58 days.⁷⁴ Given the anatomic and biological differences between humans and rabbits, the half-life is expected to be longer in humans, suggested to be 9 days based on its intermediate molecular size between ranibizumab and bevacizumab.⁷³ Further mathematical modeling suggests that aflibercept is able to maintain significant intraocular binding activity up to 10–12 weeks after a single injection.⁷³

Aflibercept in clinical trials of DME

Following the results of a Phase I trial assessing the safety and the bioactivity of aflibercept in DME, Phase II (DA VINCI⁷⁵), and Phase III (VISTA, VIVID⁷⁶, and Protocol T⁵) studies have confirmed its efficacy and safety for this indication (Table 2).

DA VINCI (DME and VEGF Trap-Eye: INvestigation of Clinical Impact)⁷⁵ was a 52-week, multicenter, randomized, double-masked, active-controlled Phase II clinical trial that assessed the safety and efficacy of intravitreal aflibercept in comparison with focal/grid laser photocoagulation in patients with DME. Two hundred and twenty-one patients were randomly assigned to either 0.5 mg aflibercept every 4 weeks (0.5q4), 2 mg aflibercept every 4 weeks (2q4), 2 mg aflibercept for three initial monthly doses and then every 8 weeks (2q8), 2 mg aflibercept for three initial monthly doses and then on an as-needed basis (2PRN), or macular laser photocoagulation as specified by the modified ETDRS protocol.

VISTA (Study of Intravitreal Administration of VEGF Trap-Eye in Patients with DME) and VIVID⁷⁶ (VEGF Trap-Eye in vision impairment due to DME) were two multicenter, randomized, double-masked, active-controlled Phase III trials of 872 eyes with center-involving DME. VISTA was conducted in the US only, whereas VIVID was conducted in Europe, Japan, and Australia. Eyes were randomized for treatment with intravitreal aflibercept injection either 2 mg every 4 weeks (2q4), 2 mg every 8 weeks after five initial monthly doses (2q8), or macular laser photocoagulation.⁷⁶

The Protocol T Study⁵ was a 52 week, multicenter, randomized trial by the Diabetic Retinopathy Clinical Research Network comparing the efficacy and safety of intravitreal aflibercept (2 mg), bevacizumab (1.25 mg), and ranibizumab (0.3 mg). Six hundred and sixty participants were randomized in a 1:1:1 fashion to each arm of treatment, which specified injections at baseline and every subsequent 4 weeks unless visual acuity was 20/20 or better with a central macular

Table 2 Summary of studies assessing the use of aflibercept in the treatment of diabetic macular edema

Study	Study design	Year	Sample size (patients)	VA (letters) study eye	CMT (μm) study eye
Da Vinci (Phase II) ⁷⁵	Multicenter, randomized, double masked, active controlled	2012			
0.5q4			44	11.0 ^a	-165.4 ^a
2q4			44	13.1 ^a	-227.4 ^a
2q8			42	9.7 ^a	-187.8 ^a
2PRN			45	12.0 ^a	-180.3 ^a
Control ^b			44	2.5	-58.4
VISTA (Phase III) ⁷⁶	Multicenter, randomized, double masked, active controlled	2014			
2q4			154	12.5 \pm 9.5 ^a	-185.9 \pm 150.7 ^a
2q8			151	10.7 \pm 8.2 ^a	-183.1 \pm 153.5 ^a
Control ^b			154	0.2 \pm 12.5	-73.3 \pm 176.7
VIVID (Phase III) ⁷⁶	Multicenter, randomized, double masked, active controlled	2014			
2q4			136	10.5 \pm 9.5 ^a	-195.0 \pm 146.6 ^a
2q8			135	10.7 \pm 9.3 ^a	-192.4 \pm 149.9 ^a
Control ^b			132	1.2 \pm 10.6	-66.2 \pm 139.0
Protocol T (Phase III) ⁵	Multicenter, randomized, single masked (subject)	2015			
2q4			224	13.3	-169 \pm 138
Bevacizumab			218	9.7	-101 \pm 121
Ranibizumab (0.3 mg)			218	11.2	-147 \pm 134

Notes: ^a $P < 0.05$ compared to control arm; ^bmacular laser photocoagulation; 0.5q4–0.5 mg aflibercept; every 4 weeks; 2q4–2 mg aflibercept every 4 weeks; 2q8–2 mg aflibercept every 8 weeks following three loading doses; PRN – aflibercept pro re nata following three loading doses.

Abbreviations: VA, visual acuity; CMT, central macular thickness.

thickness below eligibility threshold and no improvement or worsening in the visual acuity in response to the previous two injections.

All three of these studies demonstrated benefits in the improvement of visual acuity and reduction of central macular thickness from baseline with the aflibercept arms as shown in Table 2. Both DA VINCI and VIVID/VISTA demonstrated superiority to laser photocoagulation and also improvements in the diabetic retinopathy severity score in those participants. DA VINCI demonstrated similar anatomical and visual outcomes in the four treatment arms of aflibercept, despite the differences in dosing.

Protocol T demonstrated that head-to-head there was no clinically meaningful difference between the three anti-VEGF agents in terms of improvement of best-corrected visual acuity. However, when the participants were stratified into their presenting visual acuity, aflibercept resulted in improved visual outcomes compared with both ranibizumab and bevacizumab when the presenting visual acuity was < 69 letters ($\sim < 20/50$). When the presenting visual acuity loss was only mild (between 78 and 69 letters), no apparent differences in mean visual improvement were found. Additionally, reductions in central macular thickness were

significantly more marked with aflibercept compared to both ranibizumab and bevacizumab.

None of these three trials had sufficiently large cohorts to determine definitive statements regarding the systemic safety of aflibercept. Both DA VINCI and VISTA/VIVID demonstrated a similar ocular adverse event profile for aflibercept compared to laser photocoagulation. There were no differences in ocular adverse events between the three treatment arms in Protocol T; however, a post hoc analysis performed demonstrated a statistically significant increased frequency in cardiac and vascular disorders in the ranibizumab group.

There are some limitations with the design of the clinical trials. In VIVID/VISTA, the exclusion criteria included uncontrolled diabetes mellitus defined as $\text{HbA}_{1c} > 12\%$ in VIVID or at the discretion of the investigator in VISTA contributing to a potential selection bias in these cases. Additionally, a second source of selection bias was the exclusion criteria of more than two previous macular laser treatments in the study eye or if in the opinion of the investigator that the patient had no potential to benefit from laser treatment.

In Protocol T, the dose of ranibizumab utilized was 0.3 mg, and a potential issue is raised regarding whether using a 0.5 mg dose would have had a different outcome in

this treatment arm. Previous studies evaluating ranibizumab for DME demonstrated that there was no difference in terms of efficacy between these dosages; however, smaller studies have demonstrated some benefit with higher dosages of ranibizumab for refractory DME.⁷⁷

Additionally, the bevacizumab used in this study was repackaged into single use vials at a central pharmacy, perhaps not reflecting clinical practice whereby these are sourced from various compounding pharmacies. Bevacizumab from the same compounding pharmacy has been shown to have varying protein concentrations raising the suspicion of uniformity amongst the potency of the drug being administered.⁷⁸

Future directions

The clinical trials have shown aflibercept to be an effective treatment for DME. Due to small sample sizes, it is not possible to make definitive statements about the systemic safety of aflibercept. Further Phase IV studies, meta-analyses, and postmarketing surveillance will provide valuable data.

The role of aflibercept in managing DME refractory to the treatment with other intravitreal agents is a relevant clinical question in this subgroup of patients. Although the pathologies are very distinct, studies in nAMD^{79,80} have shown visual and anatomical benefit in switching from between anti-VEGF agents. The intense regime of rigorous clinical review and treatment with anti-VEGF while yielding excellent visual outcomes will be burdensome for the patient, clinics, and treating physicians. It is likely that alternative treatment paradigms including “treat and extend” will be explored in an attempt to reduce this treatment burden.

Conclusion

Previous treatment strategies for DME such as steroid and laser photocoagulation have been shown to effectively preserve the vision. The effectiveness of anti-VEGF therapy to improve the vision in patients with DME is clearly established. Aflibercept in recent trials suggests superior visual outcomes in eyes with worse initial vision. The higher binding affinity to VEGF and additional action of PIGF of aflibercept may explain this difference in efficacy. With continuing experience of this important therapy, clinicians will explore alternative treatment algorithms to modify the injection frequency and treatment burden while maintaining effectiveness and safety in managing their diabetic patients.

Disclosure

Andrew A Chang is a consultant for Bayer, Novartis and Alcon. The authors report no conflicts of interest in this work.

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