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NEW APPROACH IN THE TREATMENT OF OPHTHALMIC NEOVASCULAR DISORDERS: USING FUSION PROTEIN AFLIBERCEPT

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The aim of this review is to appraise the usage of a newly approved anti-vascular endothelial growth factor (anti-VEGF) fusion protein, aflibercept, in ocular neovascular disorders such as diabetic retinopathy and age-related macular degeneration. Aflibercept is a soluble fusion protein, which combines ligand-binding elements taken from the extracellular domains of VEGF receptors 1 and 2 fused to the Fc portion of IgG. This protein contains all human amino acid sequences, which minimizes the risk for immunogenicity in human patients. In this short review we investigate the available literature and data from clinical studies on the efficacy, pharmaceutical and pharmacological properties of aflibercept, and identify its possible advantages over commercially available anti-VEGF drugs. ***Biomed Rev*** **2014; 25: 59-65**

Key words: diabetic macular edema, vascular endothelial growth factor, anti-vascular endothelial growth factor drugs, aflibercept

INTRODUCTION

Diabetic macular edema (DME) is a complication of diabetic retinopathy and is a leading cause of blindness. Nowadays more people suffering from diabetes are diagnosed with DME. Diabetic macular edema, defined as retinal thickening within 2 disc diameters of the center of the macula, results from retinal microvascular changes that compromise the blood-retinal barrier, causing leakage of plasma constituents into the surrounding retina and, consequently, retinal edema (1). Anti-vascular endothelial growth factor (anti-VEGF) drugs and steroids have been introduced as potential alternatives to laser photocoagulation, the basis of treatment. Chronology of the development of the new drugs is shown on Figure 1.

Ocular angiogenesis can be physiological or pathological. The physiological ocular angiogenesis is occurring primarily during embryonic development (2). Ocular angiogenesis in adults is usually pathological and is a major cause of vision loss and blindness due to conditions as presented in Table 1.

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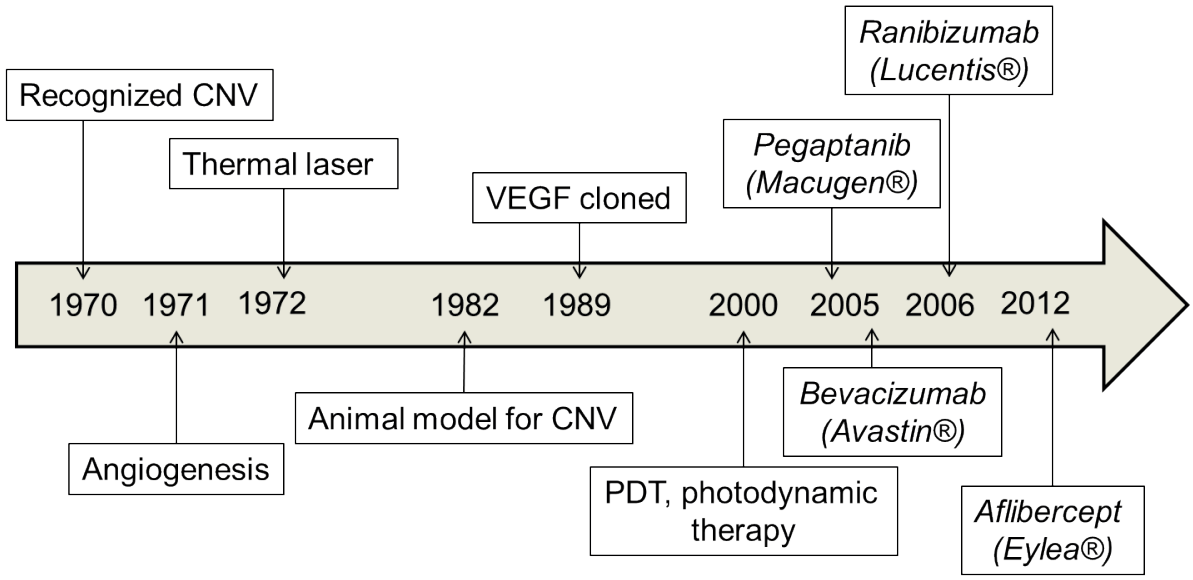

Figure 1. *Timeline of discovery of therapy for neovascular age-related macular degeneration. CNV, choroidal neovascularization; VEGF, vascular endothelial growth factor; PDT, photodynamic therapy.*

Table 1. *Examples of ophthalmic neovascular disorders*

Choroidal neovascularization (CNV) bounded to
Age-related macular degeneration (AMD),
Diabetic retinopathy,
Neovascular glaucoma,
Corneal neovascularization,
Retinopathy of prematurity.

Vascular endothelial growth factor (VEGF)-A appears to be necessary for growth of blood vessels in a variety of normal and pathological circumstances (3). The VEGF family consists of five related glycoproteins, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF) (4). VEGF has two main receptors in normal biological systems: VEGFR1 and VEGFR2. VEGFR2 mediates most of the endothelial cell proliferating activity of VEGF, whereas VEGFR1 mediates other activities of VEGF, such as a chemoattraction (5). Both receptors are important for the angiogenic-promoting properties of VEGF.

VEGF TRAP-EYE MECHANISM OF ACTION

This new approach to the treatment of wet AMD was developed to enhance the therapeutic activity – significant improvement in visual acuity and reducing frequency of administration. Aflibercept/VEGF trap-eye (VTE) is a soluble fusion protein, which combines ligand-binding elements taken from the second binding domain of the receptor VEGFR1 and the third binding domain of the receptor VEGFR2 fused to the Fc portion of IgG_1 (Fig.2).

Each arm of VEGF Trap-Eye binds to the binding interface on each pole of the active VEGF or PlGF dimer. This forms a stable and inert 1:1 complex with the growth factor (6) uniquely binding the dimer on both sides. The Trap is aptly named, since the molecule isolates (or traps) the dimer, forming inert complexes with the growth factor (6) that do not interact with another VEGF Trap molecule. This blocks and effectively arrests the VEGF angiogenesis cascade. It also prevents the creation of multimeric complexes that can aggregate and cause immune responses in body tissues. VEGF Trap-Eye binds VEGF more tightly than native receptors, blocking cell-surface receptor activation (7).

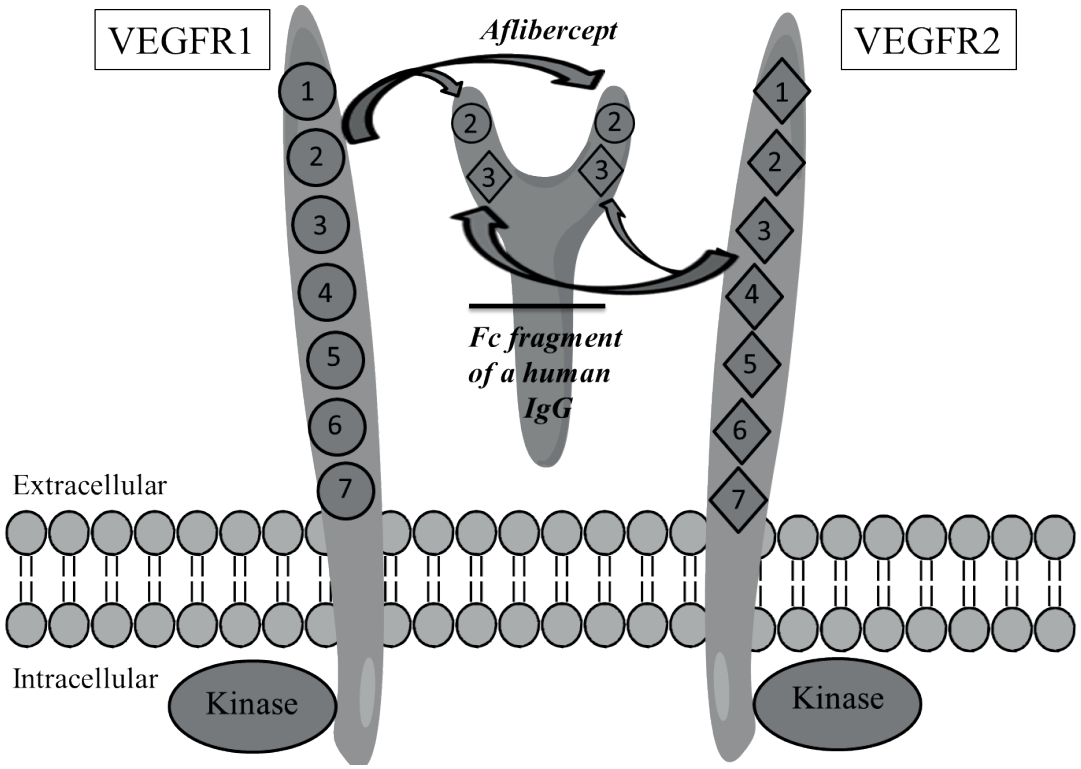

Figure 2. *Creation of fusion protein – aflibercept through fusing the second VEGF-binding domain from VEGFR1 and the third VEGF-binding domain from VEGFR2 to the Fc backbone of an IgG1 molecule.*

INTRAOCULAR AND SYSTEMIC PHARMACOKINETIC OF AFLIBERCEPT

Preclinical studies on intraocular pharmacokinetics are conducted on rabbits. The half-life of aflibercept in these studies was established to be 4.7 days (8). The half-life is close to that of bevacizumab (4.32 days), but is significant higher than ranibizumab (2.88 days) (9, 10). Intraocular pharmacokinetics, and in particular the half-life, is related to the molecular weight of the drugs. The differences in molecular size of aflibercept, ranibizumab and bevacizumab are given in Table 2.

Table 2. *Molecular weight of aflibercept, ranibizumab and bevacizumab*

Drug	Molecular weight
Aflibercept	97 kD
Ranibizumab	47 kD
Bevacizumab	149 kD

Aflibercept is a dimeric glycoprotein with a protein molecular weight of 97 kD, but also contains some percent of glycosylation, which is leading to an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kD. That is why eye pharmacokinetic behavior of aflibercept is similar to bevacizumab. There are limited data in human eyes studies, but since the intraocular half-life of a macromolecule is primarily determined by its molecular size, we could use mathematical equation to predict it. By using such an equation, half-life of aflibercept is estimated to be 7.1 days (11). Experimental studies confirm a very good penetrationin all layers of the retina (12). After intravitreal injection of 2 mg of aflibercept (the highest dose used in pivotal ophthalmologic trials), the maximum plasma concentration detected in 1-3 days is significantly (200-fold) lower than the concentration required for maximal systemic VEGF binding. The detection in plasma is as a free drug (a minor quantity) or in a complex bound with VEGF. The drug is rapidly cleared from circulation via pinocytotic proteolysis and glomerular

filtration after forming a complex with VEGF via the same pathways that metabolize antibodies. The systemic half-life of the unbound aflibercept is 1.5 days, which is inferior to that of bevacizumab (20 days) and close to the systemic half-life of ranibizumab (6 hours) (11).

PHARMACODYNAMIC AND DOSAGE FORMS OF AFLIBERCEPT

The binding affinity of aflibercept to the VGFR-A_{165} assessed by the equilibrium dissociation constant (K_d) was 0.49 pM. (14) Its affinity was 19- and 181-fold higher than the native VEGF receptors fused to Fc (VEGFR-1, 9.33 pM or VEGFR-2, 88.8 pM, respectively). All three anti-VEGF drugs bind with high affinity to theVGFR-A_{165}, but aflibercept have shown approximately 100-fold lower (i.e. the binding affinity was ~ 100-fold tighter) than that for ranibizumab (Kd = 46 pM) and bevacizumab (Kd = 58 pM). (14) In addition, aflibercept possesses a high binding affinityfor PlGF-2 (Kd ~ 45 pM) (15).

Nowadays, aflibercept has ophthalmologic and oncologic use registration. Dosage forms that are used in ophthalmology are preservative-free, sterile, aqueous solution in a single-use, glass vial designed to deliver 0.05 mL (50 microliters) of aflibercept (40 mg/mL in 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% polysorbate 20, and 5% sucrose, pH 6.2). The trade name, under which the product is registered, is Eylea® (Regeneron Pharmaceuticals, Tarrytown, NY, USA and Bayer, Berlin, Germany). In oncology practice the product is registered under another generic name - ziv-aflibercept, with trade name Zaltrap® (Sanofi-Aventis, Paris, France and Regeneron Pharmaceuticals, Tarrytown, NY, USA). It was approved for the treatment of patients with metastatic colorectal cancer who had been previously treated (16). According to the manufacturer, this drug must not be administered intravitreally due to its hyperosmolarity (1000 mOsm). The dosage that is used in ophthalmology is 100-fold lower than the dose allowed in oncology (4–6 mg/kg) (17).

THERAPEUTIC EFFICACY

Aflibercept is currently being approved for the treatment of two eye diseases: neovascular (wet) age-related macular degeneration (AMD) and macular edema following central retinal vein occlusion (CRVO).

Neovascular age-related macular degeneration

In the first clinical experience, the purpose has been to evaluate the maximum tolerated dose, the bioactivity, the safety and tolerability of intravitreally administered aflibercept. This was done in Phase I study named Clinical Evaluation of Anti-angiogenesis in the Retina Intravitreal Trial (CLEAR-IT 1) (18). The dosage range of aflibercept was between 0,05 – 4 mg, which were tested on 21 patients. At 6 weeks after a single injection, most patients experienced an improvement in visual acuity (mean visual gain, 4.4 letters) and showed a decrease in macular thickness (−105 μm). Figure 3 shows the result obtained with our patient suffering from neovascular (wet) AMD: after treatment with aflibercept there was an almost 50% reduction in macular thickness and almost the same percent improvement in visual acuity (unpublished data).

Aflibercept is well-tolerated with no visible ocular inflammation seen. In the second clinical phase (CLEAR-IT 2), the purpose has been to evaluate dose and dosing range interval, the patients (n = 159) being randomized into five treatment groups: the first two groups received 3 aflibercept injections of 0.5 mg or 2 mg monthly and the other three groups received only one aflibercept injection of 0.5 mg, 2 mg, or 4 mg monthly (19). The results of this clinical stage can be summarized as follows: (i) the reduction in macular thickness experienced by the patients receiving three monthly injections exceeded that of patients treated only once (20), (ii) patients initially treated with 2 mg every 4 weeks had the best visual improvement (mean gain of 9 letters) and also aflibercept can be applied as needed with excellent gains in vision (21). Thus, three different dosing schedules were identified for the clinical phase III studies: 0.5 mg monthly (0.5Q4), 2 mg monthly (2Q4), or 2 mg every 2 months (2Q8) after the loading phase of three initial monthly doses and all of them compared to the standard dosing schedule for ranibizumab 0.5 mg monthly. The studies VEGF Trap-eye Investigation of Efficacy and Safety in Wet AMD (VIEW) 1 and 2, have been conducted to evaluate efficacy of aflibercept to standard treatment – ranibizumab, against which all subsequent drugs should be compared. The View 1 study enrolled 1217 patients in the US and Canada, and the View 2 study enrolled 1240 patients in Europe, Asia, Japan, and Latin America.In both studies, the primary efficacy endpoint has been the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at week 52 compared to baseline. Data are available through week 52. Both aflibercept 2Q8 and aflibercept 2Q4 groups were shown to have efficacy that was clinically equivalent to the ranibizumab 0.5 mg Q4 group.In VIEW 1, patients receiving 2 mg of aflibercept every 4 weeks gained more vision than those receiving ranibizumab (+10.9 letters versus +8.1 letters; p = 0.0054) (22). Improvements in macular thickness were not statisti-

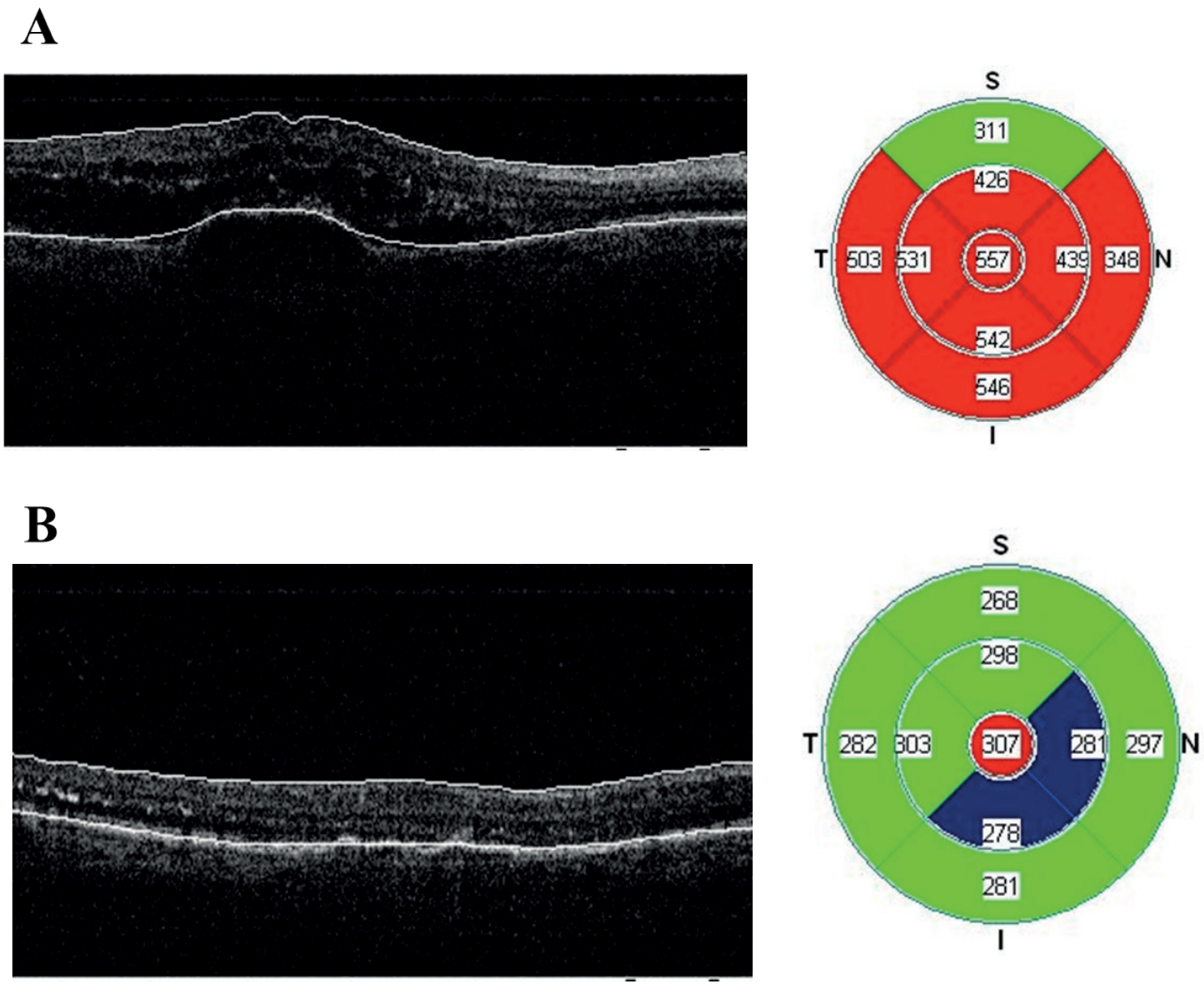

Figure 3.A *Macular thickness before administration of aflibercept – 557,90 µm;* ***B*** *Macular thickness four weeks after administration of aflibercept – 307,00 µm;*

cally different in the treatment groups. VIEW 2 patients receiving 2 mg of aflibercept every 8 weeks showed bimonthly fluctuations in macular thickness without corresponding fluctuations in visual acuity (22). The safety of aflibercept was excellent and was comparable with that of ranibizumab in both the VIEW 1 and VIEW 2 studies. Severe extraocular adverse events such as stroke and myocardial infarction occurred with similar frequencies in patients receiving aflibercept (0.7% and 2.6%, respectively) and in patients receiving ranibizumab (1.6% and 2.6%, respectively) in both VIEW trials. Both studies showed that all 3 regimens of aflibercept were noninferior to the ranibizumab monthly regimen and the advantages of aflibercept's regimen are the fewer injections (no loss of efficacy) and less risk of endophthalmitis.

Macular edema following central retinal vein occlusion (CRVO)

Therapeutic activity of aflibercept in the treatment of macular edema following CRVO was proved in two phase III clinical studies - COPERNICUS (Controlled Phase 3 Evaluation of Repeated intravitreal administration of VEGF Trap-Eye In Central retinal vein occlusion: Utility and Safety) (23) and

GALILEO (General Assessment Limiting Infiltration of Exudates in central retinal vein Occlusion with EYLEA) (24). The first six months of the both studies were identical, patients were randomized into two groups – receiving intravitreal aflibercept 2 mg monthly and those who received intravitreal placebomonthly.In the second six months,GALILEO study was design to continue treatment group with aflibercept on the PRN (provided as needed) basis and the control group with placebo (24), while in the COPERNICUS study (23), all patients were treated with aflibercept on a PRN basis.In both the COPERNICUS and GALILEO studies, aflibercept injection resulted in an improvement in visual acuity of .15 letters in 56.1% and 60.2% of patients, respectively, at week 24 compared with those receiving sham injections (12.3% and 22.1%, respectively). At week 52 in the COPERNICUS study, the improvement in visual acuity was 55.3% in the aflibercept/aflibercept PRN patients compared with 30.1% in the placebo/aflibercept PRN patients. In the GALILEO study, in which control patients did not receive any aflibercept injections, the improvement was 60.2% and 32.4%, respectively. The results of these studies showed that it is possible to maintain an excellent visual outcome and to extend the range of administration while using the PRN strategy. These data indicate that aflibercept provides benefits to patients with CRVO and using this drug as needed may become a first line approach that will reduce the burden of monthly injections.

CONCLUSION

It can be stated that aflibercept represents an attractive alternative to the available anti-VEGF drugs as it leads to similar improvements in visual outcomes and has a longer duration of action, due to stronger and prolonged binding to the VEGF-A receptor, which allows a decrease of the frequency of injections.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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