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Anti VEGF Agents for Age Related Macular Degeneration

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1. Introduction

The adult retina is a neural tissue with high metabolism and the highest oxygen consumption per unit weight of all human tissues. Therefore, the choroid, the most vascular layer of the eye also nourishing the retina, has one of the highest blood-flow rates in the body, 800 – 1000 mL/100 g tissue/min [1]. In healthy adults this delicate ocular vascular system is maintained and controlled by the balance between the angiogenic factors and angiogenic inhibitors [2].

Age-related macular degeneration (AMD) is the result of complex interactions between lipofuscinogenesis, drusenogenesis, and inflammation which can lead to choroidal neovascularization (CNV) [3]. An imbalance between the proangiogenic vascular endothelial growth factor (VEGF) and the antiangiogenic pigment epithelium-derived factor (PEDF) [3-4], plays a major role in the pathogenesis of the disease.

Inhibitors of VEGF represent a relatively new treatment for CNV. These agents include the Macugen (aptamer) which was almost completely abandoned with the introduction of the efficient FDA approved Ranibizumab (Lucentis; Genentech, Inc, South San Francisco, CA), in addition to others such as the Bevacizumab (Avastin; Genentech, Inc), and the new FDA approved drug Eylea (VEGF Trap Eye Regeneron, Tarrytown, NY, USA).

2. Vascular endothelial growth factors

Vascular endothelial growth factor (VEGF) plays a key role in ocular angiogenesis and vascular permeability. Several VEGF family members have been discovered (VEGF-A, B, C, D

and PlGF). These isoforms of VEGF have different effects in ocular pathologies and may differ in their neuroprotective abilities [5, 6]. RPE and Müller cells are the major sources of VEGF and they exert their effects through multiple receptors that are mostly expressed on endothelial cells and are also found on monocytes and macrophages [7].

VEGF-A, has been most strongly associated with angiogenesis and thus consists the target of most anti-VEGF treatments [8, 9]. VEGF-A signals through two receptor tyrosine kinases, VEGFR1 and VEGFR2, and is induced by hypoxia, unlike other VEGF isoforms [7, 10].

Alternative exon splicing of the human VEGF-A gene results in at least four major biologically active isoforms, containing 121, 165, 189, and 208 aminoacids (five more are VEGFA-145, VEGFA-162, VEGFA-165b, VEGFA-183, and VEGFA-206) [11].

Different VEGF-A isoforms may have different functions in ocular diseases.

VEGF 121 appears to be essential for normal retinal vascular function [12-13], and VEGFA-165 is the predominant isoform in the human eye. It is a heparin-binding, homodimeric, 45-kDa glycoprotein that is predominantly secreted, although a substantial fraction is bound to the cell surface and to the extracellular matrix [13-14]. It appears to be the isoform responsible for pathological ocular neovascularization.

Both isoforms are found in CNV tissue excised from patients with AMD.

In autopsy studies, VEGF levels were found to be elevated in the retinal pigment epithelium (RPE) and choroidal blood vessels within the macular area of eyes with AMD [15].

In summary VEGF-A acts through various pathways which result in promoting pathologic neovascularization:

- It stimulates angiogenesis by being a potent endothelial cell mitogen [10-11].
- It sustains endothelial survival by inhibiting apoptosis [10-12].
- VEGF is a chemo-attractant for endothelial cell precursors, promoting their differentiation [12-13].
- It is a powerful agonist of vascular permeability which is particularly important in CNV. Increased vascular permeability in response to VEGF may be due to formation of fenestrations in microvascular endothelium [12-14].
- Leukocytes may amplify the effects of VEGF via their own secretion of VEGF. Furthermore, VEGF's pro-inflammatory activity, predominantly through the 164 isoform, contributes to pathological ocular neovascularization [14]. It is therefore a crucial target in combating neovascular and ischemic eye diseases such as: choroidal neovascularization, macular edema secondary to diabetic retinopathy (DME) or retinal vein occlusion and retinal neovascularisation that may develop in retinal vein occlusion (RVO) or diabetic retinopathy (DR).

Several anti-VEGF drugs have been studied and have been shown to be effective. However, effective, long-term drug-delivery remains a challenge. Two multi-center, randomized con-

trolled trials comparing the two most commonly drugs available were published recently. A summary of the available drugs (table 1), their mechanism of action and results from large multicenter trials evaluating their efficacy and safety is presented below.

3. Anti VEGF drugs

3.1. Pegaptanib sodium (Macugen) – OSI/Eyetech

This intravitreal RNA aptamer drug was the first anti-VEGF drug approved by the FDA in 2004 for use in neovascular AMD (nvAMD). It targets VEGFA₋₁₆₅[11] Its efficacy and safety were evaluated in the large VISION trial [16, 17].

Patients with different types of sub foveal CNV secondary to AMD were randomized into four groups. Three groups received an intravitreal injection of pegaptanib sodium at a dose of 0.3mg, 1.0mg, 3.0mg to one eye respectively. The injection was given every 6 weeks for a period of 48 weeks in total. The fourth group was the control group and subjects in this group received a sham injection every 6 weeks. Primary outcome was mean change in visual acuity from baseline.

Results from a combined analysis showed that for all three doses of pegaptanib ($P < 0.001$ for the comparison of 0.3 mg with sham injection; $P < 0.001$ for the comparison of 1.0 mg with sham injection; and $P = 0.03$ for the comparison of 3.0 mg with sham injection) there was a significant difference between the patients receiving treatment and those receiving a sham injection. In the group dosed with pegaptanib 0.3 mg, 70 percent of patients lost fewer than 15 letters of visual acuity (VA), as compared with 55 percent among the controls ($P < 0.001$). The risk of severe loss of VA (loss of 30 letters or more) was reduced from 22 percent in the sham-injection group to 10 percent in the group receiving 0.3 mg of pegaptanib ($P < 0.001$). More patients receiving pegaptanib (0.3 mg), as compared with sham injection, maintained their VA or gained acuity (33 % vs. 23%; $P = 0.003$). As early as six weeks after beginning therapy with the study drug, and at all subsequent points, the mean visual acuity among patients receiving 0.3 mg of pegaptanib was better than in those receiving sham injections ($P < 0.002$). During the second year, patients initially assigned to pegaptanib were re-randomized (1:1) to continue or discontinue therapy for 48 additional weeks (8 injections). Those initially assigned to sham were re-randomized to continue sham, discontinue sham, or receive 1 of 3 pegaptanib doses. The proportion of patients who lost more than 15 letters or more in vision between week 52 to week 96 was double (14 vs 7%), if treatment was discontinued compared to those who continued to receive pegaptanib injections. This suggests that there is a more favorable outcome when continuing treatment for at least two years [18]. The Pegaptanib was found safe and there was no significant difference in serious systemic adverse events or severe ocular inflammation, cataract or glaucoma between the pegaptanib treated groups and the sham treated groups [16, 17].

The VA results of the VISION study are clearly inferior to those of the MARINA and ANCHOR studies evaluating the efficacy of intravitreal ranibizumab for nvAMD (detailed lat-

er). However, VA efficacy is only one of the clinical considerations that must be taken into account. The safety profile of the drug is not less important. The Macugen was proven to be safe in the VISION study as well as in the smaller study by N. Feucht et al. [19]; in both studies no relevant systemic or ocular adverse effects were noted. Cardiovascular incidents and overall mortality in the Pegaptanib sodium group were comparable to those of the sham injection group.

Thus, we can conclude that stable vision can be achieved with repeated injections as frequent as every 6 weeks with pegaptanib. This treatment may still be taken into consideration especially in subjects suffering from cardiovascular diseases.

3.2. Ranibizumab (Lucentis) - Genentech

Ranibizumab is a small 48kDa recombinant humanized monoclonal antibody fragment. Its small molecular weight enables it to penetrate the inner limiting membrane and reach the subretinal space when injected intravitreally [5,8-9]. It binds all biologically active isoforms of VEGF with high affinity. The half-life of ranibizumab is 2 – 4 days [5, 8], resulting in a rapid systemic clearance and good systemic safety profile.

FDA has approved the use of ranibizumab for treatment of all angiographic subtypes of subfoveal CNV due to AMD. The phase III MARINA trial evaluated the efficacy and safety of ranibizumab for the treatment of minimally classic or occult with no classic CNV associated with AMD. This 2-year, prospective randomized, double-masked, sham-controlled trial enrolled 716 patients. Patients were randomized in a 1 : 1 : 1 ratio to receive intravitreal ranibizumab at a dose of either 0.3 mg or 0.5mg or sham injection monthly in one eye for 2 years [9]. The primary outcome was the proportion of patients losing fewer than 15 letters from baseline visual acuity at 12 months.

At 24 months, 92% of patients who received 0.3 mg of ranibizumab and 90% of patients who received 0.5 mg ranibizumab lost fewer than 15 letters, compared with 52.9% in the sham group. The proportion of patients who gained at least 15 letters on the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart from baseline to 24 months was 33.3% in the 0.5mg group, 26.1% in the 0.3mg group, and 3.8% in the sham group. The mean change in ETDRS VA from baseline to 24 months was a gain of 6.6 letters in the 0.5mg group, a gain of 5.4 letters in the 0.3mg group, and a loss of 14.9 letters in the sham-injection group [9].

The ANCHOR study evaluated the efficacy of Ranibizumab for treatment of Predominantly Classic subfoveal CNV due to AMD. The ANCHOR trial was a multicenter, randomized double-blind trial that enrolled 423 patients to compare the efficacy and safety of ranibizumab vs PDT with verteporfin[20]. Patients were assigned randomly to receive either 0.3 or 0.5mg of ranibizumab plus sham verteporfin, or sham intravitreal injection plus active verteporfin therapy. Ranibizumab or sham intravitreal injections were given monthly, and the verteporfin or sham was administered on day 0 and then as needed at months 3,6, 9, and 12.

At 12 months, 94.3% of patients in the 0.3mg group and 96.4% in the 0.5mg group lost fewer than 15 letters from baseline compared with 64.3% in the verteporfin group. The proportion of patients who gained at least 15 letters from baseline to 12 months was

40.3% in the 0.5mg group, 35.7% in the 0.3mg group, and 5.6% in the verteporfin group. The mean change in VA from baseline to 12 months was a gain of 8.5 letters in the 0.3mg group, a gain of 11.3 letters in the 0.5mg group, and a loss of 9.5 letters in the verteporfin group [20]. Rates of serious ocular or systemic adverse events were low in both the MARIMA and the ANCHOR trials [20].

Both studies showed no difference in the percentage of patients losing 15 letters in vision between the 0.3 and 0.5mg. However, the 0.5 mg was statistically significantly superior to the 0.3 mg in achieving 15 letters or more in vision. This difference in favor of the 0.5 mg led to its approval by the FDA, and the routine use of 0.5 mg ranibizumab.

The PIER study [21] evaluated the efficacy of 3 consecutive monthly injections of ranibizumab followed by fixed re-treatments only every 3 months. Mean changes from baseline VA at 12 months were -16.3, -1.6, and -0.2 letters loss for the sham, 0.3 mg, and 0.5 mg groups, respectively ($P < \text{or} = .0001$, each ranibizumab dose vs sham). Ranibizumab reduced the growth and leakage from the CNV. However, the treatment effect achieved following the first 3 consecutive injections declined in the ranibizumab groups during quarterly dosing (e.g., at three months the mean changes from baseline VA was a gain of 2.9 and 4.3 letters for the 0.3 mg and 0.5 mg doses, respectively). Results of subgroup analyses of mean change from baseline VA at 12 months by baseline age, VA, and lesion characteristics were consistent with the overall results. Overall, in this study, the proportion of gainers of more than three lines was significantly lower than in MARINA or in ANCHOR trials, and this is due to the fact that following the first 3 consecutive injections patients were shifted to a significant less frequent dosing of quarterly injections instead of monthly.

The EXCITE study evaluated the efficacy and safety of monthly versus quarterly ranibizumab treatment in nvAMD [22]. Patients were randomized (1:1:1) to 0.3 mg quarterly, 0.5 mg quarterly, or 0.3 mg monthly doses of ranibizumab. Treatment comprised of a loading phase (3 consecutive monthly injections) followed by a 9-month maintenance phase (either monthly or quarterly injection). In contrast to the PIER study in which patients were examined and injected only every 3 months following the first 3 consecutive monthly injections, in the EXCITE study, patients were followed monthly, but in the 2 quarterly groups they could receive an injection only every 3 months. BCVA increased from baseline to month 12 by 4.9, 3.8, and 8.3 letters in the 0.3 mg quarterly (104 patients), 0.5 mg quarterly (88 patients), and 0.3 mg monthly (101 patients) dosing groups, respectively. The noninferiority of a quarterly regimen was not achieved with reference to 5.0 letters, meaning dosing with ranibizumab only every 3 months is inferior than dosing every month, and results in a significant less favorable final visual outcome. The safety profile was similar to that reported in prior ranibizumab studies.

Following the results of the PIER and EXCITE study it can be concluded that monthly injections is definitely superior to quarterly injections, and that the quarterly regimen should not be applied.

3.3. Bevacizumab (Avastin) - Genentech

This drug is a full-length recombinant humanized monoclonal antibody (149kDa). It binds to all VEGF-A isoforms, whereas Ranibizumab has only one binding site to VEGF, bevacizumab has two. Bevacizumab in addition has a longer acting effect *in-vitro*; however, it may penetrate less effectively the retina [23-26]. Its half life time in the vitreous is approximately 8-10 days [24-25].

It was first approved by the FDA for metastatic colorectal cancer and is used off-label in ocular disease. Although systemic administration of bevacizumab was shown to be associated with increased systemic cardiovascular adverse events, these appear to be rare following intravitreal administration [8, 24].

Many ophthalmologists until recently offered intravitreal bevacizumab to nvAMD patients based on multiple forms of evidence: results from several retrospective case series, [27-30] extrapolation from the outcomes reported in the MARINA and ANCHOR studies, the structural similarity between ranibizumab and bevacizumab, and mostly the clinical experience of rapid resolution of morphological abnormalities on optical coherence tomography (OCT) and fluorescein leakage from CNV after treatment with bevacizumab.

However, treatment with bevacizumab can nowadays be based on the 2-year results of the Comparison of Age-Related Macular Degeneration Treatment Trial (CATT) and one year results of IVAN study (Inhibit VEGF in Age-related choroidal Neovascularisation) which compared the efficacy of bevacizumab and ranibizumab for nvAMD and will be discussed in detail later in this chapter.

3.4. Aflibercept (VEGF trap)

VEGF Trap-Eye (EYLEA; Regeneron, Tarrytown, NY, USA) (VTE) is a soluble fusion protein consisting of 2 extracellular cytokine receptor domains and a human Fc region of immunoglobulin G (IgG). This 110kDa soluble receptor binds with high affinity to all VEGFA isoforms and VEGF B, and not to VEGF-C and D [5]. The binding affinity of VEGF Trap to VEGF is 10 times higher than bevacizumab. The 2mg dose of VTE at 83 days has been proven to have a similar biologic activity to ranibizumab at 30 days [5, 31]. The CLEAR-IT is a phase II trial, which was recently published and evaluated anatomic outcomes and VA, injection frequency, and safety. The study consisted of 2 phases; the first was a 12-week fixed dosing period followed by an as-needed (PRN) treatment phase to week 52 with VEGF Trap-Eye for nvAMD [31]. Patients were randomly assigned to 1 of 5 intravitreal VEGF Trap-Eye treatment groups: 0.5 mg or 2 mg every 4 weeks or 0.5, 2, or 4 mg every 12 weeks during the fixed-dosing period (weeks 1-12). From weeks 16 to 52, patients were evaluated monthly and were retreated PRN with their assigned dose (0.5, 2, or 4 mg). The decrease in CR/LT (central retinal/lesion thickness) at week 12 versus baseline remained significant at weeks 12 to 52 (-130 μm from baseline at week 52) and CNV size regressed from baseline by 2.21 mm^2 at 48 weeks. After achieving a significant improvement in BCVA during the 12-week- fixed-dosing phase for all groups combined, PRN dosing for 40 weeks maintained the im-

provement in BCVA to 52 weeks (5.3-letter gain; $P < 0.0001$). The robust improvement and consistent maintenance of VA mainly occurred in patients initially dosed with 2 mg every 4 weeks for 12 weeks, demonstrating a gain of 9 letters at 52 weeks. Overall, a mean of 2 injections was administered after the 12-week fixed-dosing phase, and the mean time to first reinjection was 129 days; 19% of patients received no injections and 45% received 1 or 2 injections. Treatment with VEGF Trap-Eye was generally safe and well tolerated, with few ocular or systemic adverse events. They concluded that PRN dosing with VEGF Trap-Eye at weeks 16-52 maintained the significant anatomic and visual improvements established during the 12-week fixed-dosing phase with a low need for re-injections. Repeated dosing with VEGF Trap-Eye was well tolerated over 52 weeks of treatment.

VIEW1 was a phase III non-inferiority trial conducted in North America that randomized 1217 patients to VTE 0.5 mg monthly dosing (0.5q4wk), VTE 2 mg monthly (2q4wk), VTE 2 mg every two months following 3 initial monthly doses (2q8wk), or ranibizumab 0.5mg monthly (Rq4wk). The primary endpoint was the proportion of patients who lost fewer than 15 ETDRS letters from baseline to week 52 [32].

Secondary endpoints included mean change in BCVA at week 52. The percentage of participants in the Rq4wk, 0.5q4wk, 2q4wk, and 2q8wk treatment arms who gained at least 15 letters in vision were: 31%, 25%, 38%, and 31%, respectively.

The proportions of patients maintaining vision at 52 weeks were 94.4%, 95.9%, 95.1%, and 95.1% for Rq4wk, 0.5q4wk, 2q4wk, and 2q8wk, respectively. All VTE groups were noninferior to ranibizumab. Mean improvement from baseline to week 52 in ETDRS letter score was 8.1, 6.9, 10.9, and 7.9 letters for Rq4wk, 0.5q4wk, 2q4wk, and 2q8wk, respectively.

There was a small significant difference in visual improvement at 52 weeks, between the 2q4wk and the Rq4weeks in favor of the 2q4weeks, however this was not found in the parallel VIEW 2 trial that will be discussed later. Differences between other VTE groups and Rq4wk were nonsignificant. The difference in the mean reduction in central retinal thickness was not significant among the groups. The incidence of adverse events was similar across all treatments, with no increase in blood pressure noted.

Overall, dosing monthly or every two months with VTE was non-inferior to monthly ranibizumab and was well tolerated [32]. The VIEW2 study was a parallel study to VIEW 1 that enrolled 1240 patients from Europe, Latin America, Asia, and Australia and yielded similar results [33]. However, minor differences exist. In the VIEW 2 study there was no statistically significant difference between all treatment arms in ETDRS letter score at week 52, and unlike VIEW1 the 2q4wk group was not superior to Lucentis.

The VIEW 1 and VIEW 2 results demonstrated non-inferior efficacy of VTE 2mg dosed at a fixed regimen every 8 weeks compared to ranibizumab 0.5mg dosed every 4 weeks. The EYLEA was approved by the FDA for injection every 8 weeks for nvAMD, and therefore may lower the injection burden on the patient as well as the medical system.

4. Different regimens for Bevacizumab and Ranibizumab

4.1. Ranibizumab: As-needed regimen

Numerous studies evaluated the effect of PRN intravitreal ranibizumab for the treatment of nvAMD.

The Prospective OCT Imaging of Patients with nvAMD Treated with Intraocular Ranibizumab (PrONTO) study was the first open-label, prospective, uncontrolled study to investigate a variable-dosing of intravitreal ranibizumab over two years [34]. Thirty-seven patients received 3 consecutive monthly injections of 0.5 mg ranibizumab and were then followed monthly and re-treated if there was an increase in OCT central retinal thickness (CRT) of at least 100 microns or a loss of BCVA of 5 letters or more. During the second year, the retreatment criteria were amended to include re-treatment if any qualitative increase in the amount of fluid was detected on OCT. At 24 months (end of 2 years study), mean VA improved by 11 letters with an average of 9.9 injections. In the PrONTO study therefore we can conclude that VA outcomes were nearly comparable with those reported in the MARINA and ANCHOR, but these results were achieved with less than half number of intravitreal injections given in the MARINA and ANCHOR [34].

The SUSTAIN trial was a phase III multicenter open-label single arm study that assessed the safety and efficacy of ranibizumab in patients with sub foveal CNV secondary to AMD using a dosing regimen individualized to patient characteristics. 513 patients who were either ranibizumab treatment-naïve (69 patients) or had completed treatment with ranibizumab or verteporfin PDT in the ANCHOR trial participated in this study [35].

Patients received three consecutive monthly injections of ranibizumab 0.3mg (or 0.5mg for the ANCHOR patients) (the “loading phase”), followed by monthly monitoring visits. Further treatment was administered if VA decreased by >5 letters or if CRT increased by >100 μm . Compared with baseline, mean VA at month 12 increased by approximately 7 letters. VA reached a maximum level after the first 3 monthly injections, decreased slightly when shifting to PRN during the next 2 to 3 months and was then sustained throughout the treatment period. Over 12 months, the mean standard deviation (SD) number of ranibizumab injections received by the 69 ranibizumab naive patients was 5.3 (± 2.2), including the three “loading” injections. This study demonstrated that flexible, guided dosing with fewer injections and monthly monitoring can be efficient and result in good visual outcome in at least some patients [35].

The SAILOR (Safety Assessment of Intravitreal Lucentis for age-related macular degeneration) study, a Phase IIIb study of Lucentis for patients with all subtypes of new or recurrent active sub foveal CNV due to AMD, was a twelve-month randomized (cohort 1) or open-label (cohort 2) multicenter clinical trial [36]. 4300 subjects were recruited. Cohort 1 subjects were randomized 1:1 to receive 0.3 mg (n = 1169) or 0.5 mg (n = 1209) intravitreal ranibizumab for 3 monthly loading doses, followed by monthly visits. Cohort 2 subjects received 1 single open-label 0.5 mg intravitreal ranibizumab, and then continue the monthly follow up visits. Those groups were stratified by AMD treatment history (treatment-naïve vs. previ-

ously treated). Cohort 1 subjects were retreated on the basis of OCT or BCVA criteria. Cohort 2 subjects (n = 1922) received an initial single intravitreal dose of 0.5 mg ranibizumab and were retreated at physician discretion. Safety was evaluated at all visits. It concluded that Intravitreal ranibizumab was safe and well tolerated in a large population of subjects with neovascular AMD. Ranibizumab had a beneficial effect on VA but quarterly visits were insufficient to monitor and capture disease progression [36] If a fixed regimen of monthly injections is not applied, than monthly visits are recommended and injectios performed as needed usually guided by visual acuity and OCT findings.

The HORIZON study was an open-label multicenter extension study that included 853 patients (600 had been previously treated with ranibizumab initially, 184 had crossed over to treatment with ranibizumab, and 69 had not been treated with ranibizumab) who had completed one of the three 2-year, randomized, controlled trials of monthly intravitreal ranibizumab treatment (MARINA, ANCHOR or FOCUS trial). Of the 853 patients, two-year VA data were available for 384 [37]. These patients could receive 0.5 mg ranibizumab at 30-day or longer intervals as needed. Of the patients who received initial treatment with ranibizumab during the ANCHOR, MARINA, and FOCUS trials, there was a mean 10.2-letter increase in VA during the first 2 years of the studies Patients that did not receive anti-VEGF therapy in those trials had worse outcomes. During the first year of the HORIZON study and the third year of the original trials, there was a 5.1-letter loss The initial VA gain decreased by a mean of 8 letters with less frequent dosing in years 3 and 4. During the as needed dosing phase, the mean number of injections in the group initially treated with ranibizumab was 3.6. compared to 4.2 injections for patients that were treated with sham in the original trials.

The results of the HORIZON trial demonstrate that a delay in the initiation of treatment is associated with poorer visual outcomes and continued but less frequent dosing in years 3 and 4 was associated as well with visual decline [37].

PRN regimen of ranibizumab guided by monthly BCVA and other ophthalmic examinations, as detailed before, appears effective in sustaining the BCVA gained with 12 monthly injections with a significant lower number of injections during the extension phase [38].

Each one of these studies evaluated PRN regimens and had its own retreatment criteria, most of them retreated patients with a 100 μ m increase in CRT from the thinnest measurement, and/or a Decreased VA >5 letters compared with VA score from the previous scheduled study visit, but each study had its particular criteria, and follow up regimen. All those studies mentioned previously have used the Time Domain OCT which is less accurate than the Spectral Domain OCT (SD-OCT) – therefore re-treatment criteria usually used the 100 microns increase in thickness. Nowadays by using the SD OCT, residual or recurrent fluid which is less than 100 microns in height can be detected, so patients are re-treated earlier – which may account for a better visual outcome using the PRN regimen. Strengths of PRONTO and SUSTAIN include monthly follow-up, but the PRONTO trial consists of only a small cohort of patients. The SAILOR trial is the largest, but mandated only quarterly follow up visits. Overall, these studies support frequent follow up and individualized retreatment to achieve the best visual acuity gains with the as-needed treatment regimen as an alternative

to the traditional monthly treatments used in the ANCHOR and MARINA trials. Furthermore, the CATT study (detailed later) showed that ranibizumab given as needed with monthly evaluation had effects on vision that were equivalent to those of ranibizumab administered monthly.

4.2. Ranibizumab: Treat-and-extend regimen

Treat-and-extend dosing regimen was first described by Freund et al. for the treatment of retinal angiomatous proliferation with an anti-VEGF agent. It involved increasing intervals between intravitreal injections up to 10 weeks as long as no fluid is present on OCT. If fluid is present, the interval between treatments is shortened [39]. The treat-and-extend regimen is quite variable in terms of treatment criteria, which can include vision loss and/or macular hemorrhage [39], and the time between treatment, which can extend up to 12 weeks [40, 41]. Unfortunately, there are no large, randomized, prospective trials that investigated the efficacy of this regimen compared to the PRN protocol.

Oubraham et al. compared two ranibizumab retreatment strategies; as-needed (PRN) and treat-and-extend, in a retrospective review of 90 patients, 52 in the PRN group, and 38 in the treat-and-extend group. Their treatment regimen included 3 loading doses monthly for the first three months in both groups, and the decision to re-treat was based only on the existence of fluid on OCT. They found that at one year, mean gain in VA was greater in the treat-and-extend group than in the PRN (+10.8 versus +2.3 letters, resp.). Eyes in the treat-and-extend group received significantly more injections (7.8 versus 5.2). Patients in the PRN group were followed every 4-5 weeks and the number of follow-up visits was similar in both groups (8.5 versus 8.8) [40].

Gupta et al. published a retrospective case series of 92 eyes treated with the treat-and-extend ranibizumab regimen. After 2 years, 32% had gained at least 3 lines of vision and received 8.36 and 7.45 injections during the first and second years, respectively. In his study, this regimen was associated with fewer patient visits, injections, and direct annual medical costs compared with monthly injections [41].

4.3. Bevacizumab: As-needed regimen

The ABC trial is a prospective, double-masked, multicenter, randomized-controlled trial that included 131 patients randomized to 3 loading doses of bevacizumab at 6-week intervals followed by as-needed treatment at six week intervals or an alternate treatment at the start of the trial (PDT, pegaptanib, or sham). Thirty-two percent of patients in the bevacizumab group gained at least 15 letters with a mean VA increase of 7 letters vs a mean decrease of 9.4 letters in the alternate group. The median number of injections during the 12 months was 7 injections [42].

Other smaller, retrospective studies note a substantial improvement in VA using a protocol of three loading doses of bevacizumab followed by a PRN regimen, based mostly on OCT findings [43, 44].

Several retrospective studies demonstrated stabilization or improvement in VA following PRN treatment regimen with bevacizumab without a loading phase [45, 46]. One prospective, open-label, nonrandomized clinical study reported a mean VA gain of 8.6 letters in 51 eyes after their second year of PRN bevacizumab treatment with a mean of only 1.5 injections given during year 2 [47].

4.4. Bevacizumab: Treat-and-extend regimen

Gupta et al. retrospectively reviewed 74 eyes of 73 patients with treatment-naive nvAMD. The patients were treated monthly with intravitreal bevacizumab until no intraretinal or subretinal fluid was observed on OCT. The treatment intervals then were lengthened sequentially by 2 weeks until signs of exudation recurred and then was reduced accordingly to maintain an exudation-free macula. Main outcomes measured included mean change from baseline visual acuity, proportion of eyes losing fewer than 3 and gaining 3 or more Snellen visual acuity lines at 1 year of follow-up, annual mean number of injections, OCT (Zeiss stratus) mean central retinal thickness change from baseline, mean maximum period of extension, adverse events, and mean direct annual medical cost. The mean follow-up period was 1.41 years. Mean Snellen VA improved from 20/230 at baseline to 20/109 at 12 months ($P < .001$) and 20/106 at 24 months ($P < .001$). The mean number of injections over the first year was 7.94. The mean OCT central retinal thickness decreased from 316 to 239 μm at 12 months ($P < .001$). The mean direct medical cost over the first year was \$3493.85.

The treat and-extend regimen in their study, was associated with significant visual improvements with fewer patient visits and injections along with lower costs when compared to the MARINA, ANCHOR, and PrONTO protocols [48].

5. Comparison of AMD treatment trials (CATT and IVAN trials)

Several retrospective studies have tried to evaluate the efficacy of ranibizumab as compared to bevacizumab, however they were not powered enough to show the differences in efficacy or safety between the 2 drugs. The CATT and the IVAN trials are two prospective large scale randomized controlled trials that compared the two drugs in different regimens of treatment. The CATT trial is a multicenter, single-blind, non-inferiority trial that collectively enrolled 1208 patients with nvAMD [49]. Patients were randomized to 4 treatment groups: monthly bevacizumab, monthly ranibizumab, as-needed bevacizumab and as needed ranibizumab. In the as needed groups, retreatment was performed if at least one of the following criteria was met: fluid present on Time Domain OCT, decreased VA as compared to previous exam, new or persistent hemorrhage detected on clinical exam, or dye leakage or increased lesion size visible on fluorescein angiography. The primary outcome measure was mean change in VA at one year. The results at 12 months showed that Bevacizumab administered monthly was equivalent to ranibizumab administered monthly, with 8.0 and 8.5 letters gained, respectively. Bevacizumab administered as needed was equivalent to ranibizumab as needed, with 5.9 and 6.8 letters gained, respectively. Ranibizumab as need-

ed was equivalent to monthly ranibizumab, although the comparison between bevacizumab as needed and monthly bevacizumab was inconclusive. Ranibizumab as-needed was found to be equivalent to monthly ranibizumab, but the comparison between bevacizumab as-needed and monthly ranibizumab was inconclusive [49]. This could be due to the less durable treatment effect of bevacizumab in a subgroup of patients [50]. Ranibizumab given as needed was equivalent to bevacizumab given monthly. The comparison between bevacizumab given as needed and ranibizumab given monthly was also inconclusive.

The mean decrease in central retinal thickness was greater in the ranibizumab-monthly group (196 μm) than in the other groups (152 to 168 μm , $P=0.03$ by analysis of variance) Although not powered sufficiently to compare adverse event rates associated with the two drugs, the rates of death, arteriothrombotic events, and venous thrombotic events were similar for patients receiving bevacizumab or ranibizumab. The rate of serious systemic adverse events, primarily hospitalizations, was higher among the patients who had received bevacizumab, but rates of adverse events did not increase with increased exposure to the drug [49].

At 1 year, patients initially assigned to monthly treatment were reassigned randomly to monthly or as-needed treatment, without changing the drug assignment[51] The 2 year results demonstrate that among patients receiving monthly injections for 2 years, mean gain in visual acuity was similar for both drugs (bevacizumab-ranibizumab difference, -1.4 letters; 95% confidence interval [CI], -3.7 to 0.8; $P = 0.21$). However mean gain was greater for monthly than for as-needed treatment (difference, -2.4 letters; 95% CI, -4.8 to -0.1; $P = 0.046$). The proportion of patients without fluid on OCT ranged from 13.9% in the bevacizumab-as-needed group to 45.5% in the ranibizumab monthly group (drug, $P = 0.0003$; regimen, $P < 0.0001$). Switching from monthly to as-needed treatment in the second year resulted in greater mean decrease in vision during year 2 (-2.2 letters; $P = 0.03$) and a lower proportion without fluid (-19%; $P < 0.0001$). Rates of death and arteriothrombotic events were similar for both drugs ($P > 0.60$). The proportion of patients with 1 or more systemic serious adverse events was higher with bevacizumab than ranibizumab (39.9% vs. 31.7%; adjusted risk ratio, 1.30; 95% CI, 1.07-1.57; $P = 0.009$), even though most of the excess events have not been associated previously with systemic therapy targeting vascular endothelial growth factor (VEGF) [51].

The first year results from an NIHR Health Technology Assessment (HTA) programmed funded trial, IVAN (Inhibit VEGF in Age related choroidal Neovascularization) were recently published [52]. The trial compared the efficacy of ranibizumab vs bevacizumab in 610 subjects with nvAMD from 23 hospitals and academic institutions in the UK. In addition blood samples were repeatedly evaluated for the VEGF concentration in the plasma. Patients received injections of the drug into the affected eye every month for the first three months. Groups were then subdivided to receive either injections at every visit thereafter namely the continuous group or only if the specialist decided there was persistent disease – namely the discontinuous group. Whenever re-treatment was performed the patient received a series of 3 monthly consecutive injections as opposed to 1 injection given in the CATT. After 12 months the comparison between the two drugs was inconclusive (-1.99 let-

ters in favor of the Ranibizumab, 95% confidence interval [CI], -4.04 to 0.06). Discontinuous treatment was equivalent to continuous treatment (-0.35 letters; 95% CI, -2.40 to 1.70). Mean foveal total thickness did not differ by drug, but was 9% less using the continuous treatment (geometric mean ratio [GMR], 0.91; 95% CI, 0.86 to 0.97; $P = 0.005$). Fewer participants receiving bevacizumab had an arteriothrombotic event or heart failure (odds ratio [OR], 0.23; 95% CI, 0.05 to 1.07; $P = 0.03$). There was no difference between drugs in the proportion of subjects experiencing a serious systemic adverse event (OR, 1.35; 95% CI, 0.80 to 2.27; $P = 0.25$). Serum VEGF levels were found to be significantly lower in subjects treated with bevacizumab compared to ranibizumab (GMR, 0.47; 95% CI, 0.41 to 0.54; $P < 0.0001$) and higher with discontinuous treatment compared to continuous treatment (GMR, 1.23; 95% CI, 1.07 to 1.42; $P = 0.004$). These results clearly indicate the higher systemic exposure to bevacizumab compared with ranibizumab. Researchers concluded that the comparison of VA at 1 year between bevacizumab and ranibizumab was inconclusive. Visual acuities with continuous and discontinuous treatment were equivalent. Other outcomes are consistent with the drugs and treatment regimens having similar efficacy and safety.

The results of the second year of the IVAN trial and others like the VIBERA (Germany), EQUAL (Netherlands) and MANTA (Austria) studies [53-55] are about to be published in the coming future.

6. Management of nonresponders

As many as 10% of patients demonstrate a significant loss of vision in spite of 2 years of monthly anti-VEGF therapy [9, 20]. Within this group of individuals exist those who progress to disciform scar, RPE tear, massive subretinal hemorrhage, geographic atrophy, and in addition eyes that demonstrate persistent macular fluid/blood and leakage on OCT and fluorescein angiography associated with vision loss. This subgroup of patients is referred to as anti-VEGF non-responders. This variability in anti-VEGF treatment response can be attributed to more aggressive forms of nvAMD, including retinal angiomatous proliferation (RAP), tachyphylaxis to anti-VEGF drugs, mimics of wet AMD [56], and genetic differences among patients [57, 58].

The therapeutic approach in these patients include alternating bevacizumab and ranibizumab, switching to a newer anti VEGF drug- Eylea, combination therapy which is further discussed in the next section, or other treatment options such as brachytherapy and transpupillary thermotherapy.

6.1. Combination therapy

Since the development and progression of nvAMD involve pro-angiogenic factors, vascular permeability molecules, and inflammatory proteins, targeting only one component of this process may be insufficient and temporary, as shown by the data presented above. Anti VEGF agents are very effective in halting vascular leakage, but it has shown to be a temporizing treatment, and there is an increased need for a treatment with longer efficacy dura-

tion..The ideal combination therapy regimen would provide a longer-lasting treatment effect in addition to potentially being more or equally efficacious to monotherapy alone.

The main combination therapies are further discussed.

6.1.1. PDT+ anti VEGF

SUMMIT is a clinical trial program that includes three similarly designed, controlled studies to further examine the safety, efficacy, and treatment burden of combination therapy with PDT and ranibizumab compared with ranibizumab alone: DENALI in the USA, and Canada, examining verteporfin PDT in combination at both standard- and reduced-fluence light doses; MONT BLANC in Europe, examining verteporfin PDT in combination at standard-fluence light dose only, and an Asian study (EVEREST) which is designed to compare standard fluence PDT combined with ranibizumab and ranibizumab monotherapy in the treatment of polypoidal choroidal vasculopathy (PCV). Twelve-month results of the MONTBLANC study showed that combining standard-fluence PDT with ranibizumab 0.5mg results in VA improvement that is noninferior to a ranibizumab monotherapy regimen with three ranibizumab-loading doses followed by injections on a monthly PRN basis (non-inferiority margin of 7 letters). There was no significant difference between the two treatment arms with regard to proportion of patients with a treatment-free interval of at least three months duration after month 2. Adverse event incidence was similar between treatment groups [59]. As monotherapy is not inferior to the combination; they concluded that monotherapy should be the preferred treatment.

Twelve-month results of the DENALI study showed that combining PDT with ranibizumab in a regimen that consists three ranibizumab loading doses followed by additional injections on a monthly PRN basis and PRN PDT every 3 months can improve VA at month 12 in patients with sub foveal CNV secondary to nvAMD [60]. However the combination treatment was found inferior to the monotherapy with ranibizumab alone. At month 12, patients in the standard fluence combination group and the reduced fluence combination group gained on average 5.3 and 4.4 letters from baseline, compared to a more significant gain of 8.1 letters in the ranibizumab monthly monotherapy group. DENALI did not demonstrate non-inferior visual acuity gain for PDT combination therapy compared with ranibizumab monthly monotherapy, meaning the monotherapy with monthly ranibizumab was found superior to the combination therapy.

Six months results of the EVEREST trial were recently published [61]. At Month 6, verteporfin PDT combined with ranibizumab or verteporfin PDT alone was superior to ranibizumab monotherapy in achieving complete polyp regression (77.8% and 71.4% vs. 28.6%; $P < 0.01$); mean change \pm standard deviation in best-corrected visual acuity (letters) was the highest in the combination group although not statistically better than the ranibizumab monotherapy. There was a mean improvement of 10.9 ± 10.9 letters in the verteporfin PDT + ranibizumab, 7.5 ± 10.6 letters in the verteporfin PDT alone, and 9.2 ± 12.4 letters in the ranibizumab monotherapy. There were no new safety findings with either drug used alone or in combination. Based on the results of the EVEREST we can conclude that combination therapy of reduced fluence PDT with ranibizumab should be applied in

cases of PCV, because this combined treatment yields the best VA results and highest rate of anatomic closure of the polyps.

6.1.2. Triple therapy

Augustin et al. described a combination therapy involving standard fluence PDT with reduced light duration (70 seconds instead of 83 seconds), with total light dose of 42 J/cm². Sixteen hours after PDT administration patients were taken to the operating room, and underwent limited vitrectomy followed by intravitreal dexamethasone (800 mcg) and intravitreal bevacizumab (1.5 mg) injections [62]. Most patients treated with this triple therapy had long-lasting improvement in VA with only one treatment at final follow-up (The mean follow-up period was 40 weeks (range, 22-60 weeks). Less than one-fourth of the patients treated with this regimen required additional treatment either with repeat triple therapy or anti-VEGF alone during the follow-up period [62].

Bakri et al. retrospectively reviewed the treatment benefit in patients receiving a same-day combination of reduced fluence PDT, intravitreal dexamethasone (200mcg), and intravitreal bevacizumab (1.25 mg). Patients were either treatment naïve or previously treated. At final follow-up, patients treated with this regimen showed stable VA and decreased macular thickness [63].

An average of less than one additional treatment with either repeat triple therapy or anti-VEGF was required in the treatment of naive group while almost four additional treatments were required in previously treated patients [63]. No steroid-related complications were noted in either study [62, 63]. A prospective interventional case series of 17 patients treated with a same-day regimen of standard fluence PDT, intravitreal bevacizumab (1.25 mg), and intravitreal triamcinolone (IVTA) (2 mg), was recently published. Patients treated with this regimen also showed an improvement in VA and reduced central macular thickness [64].

The Reduced Fluence Visudyne Anti-VEGF Dexamethasone in Combination for AMD Lesions (RADICAL) trial is a prospective, multicenter, randomized trial of combination therapy for the treatment of wet AMD that began in 2008. Patients are randomized into four treatment arms including anti-VEGF monotherapy with ranibizumab, half-fluence PDT with ranibizumab, half-fluence PDT with ranibizumab and dexamethasone, or quarter-fluence PDT with ranibizumab and dexamethasone. The dose of ranibizumab used was 0.5 mg and dexamethasone 500 mcg. Patients enrolled in the trial were followed for a total of 24 months. For the first 12 months, patients were followed monthly with decision for retreatment made at each visit. After 12 months, patients were reassessed every 3 months or sooner at physician's discretion. Patients in the anti-VEGF monotherapy arm received mandatory first 3 monthly consecutive injections followed by retreatment as needed thereafter. Retreatments with combination therapy were not administered prior to 8 weeks interval. Twelve-month data released by the sponsor [65], QLT incorporated, showed significantly fewer re-treatments in all combination therapy arms compared with the groups of patients treated with anti-VEGF monotherapy [65].

VA appears to equally improve among all groups, but confidence intervals varied. Of the three combination therapy arms, the triple therapy half-fluence PDT group shared similar mean visual improvement compared with monotherapy and had the fewest re-treatments. After 12 months, three retreatments of triple therapy with half-fluence PDT were required compared to 5.1 re-treatments of monotherapy ($p < 0.001$). Adverse event incidence was similar amongst all treatment groups. The final results of the 24-months trial were not published yet.

7. Potential complications of anti VEGF agents

Safety issues with anti VEGF intravitreal injections include local ocular adverse events (AEs) from the drug or the injection, as well as potential systemic AEs of the drug.

Ocular AEs may be categorized as common but not serious and rare but potentially serious. The AEs that are considered common but not serious include subconjunctival hemorrhage, vitreous floaters from medication or vitreous hemorrhage, and discomfort from antiseptic used to prepare the conjunctiva before the injection [9, 20, 21].

Repeated intravitreal injection of ranibizumab or bevacizumab, over extended time periods, has been demonstrated to result in a low incidence of serious ocular adverse events. In the CATT study, endophthalmitis developed after only two of 5449 injections (0.04%) in 599 patients treated with ranibizumab, and after only four of 5508 injections (0.07%) in 586 patients treated with bevacizumab. Uveitis, retinal detachment, retinal vascular occlusion or embolism, retinal tear, and vitreous hemorrhage each also occurred in less than 1% of patients [49, 50]. Efforts are underway in order to further reduce the incidence of these events, with studies evaluating the effect of needle type and injection technique on patient pain levels, vitreal reflux, and ocular complications [66].

It is unknown if pretreatment antibiotics for several days prior to injection, or only on the procedure's day is necessary in order to reduce the risk of endophthalmitis. Furthermore, it is unknown if post treatment antibiotics are necessary on the day of the procedure or thereafter to reduce this risk furthermore. Although the product insert for ranibizumab indicates that the administration of the intravitreal injection should include the use of sterile gloves and a sterile drape, not all physicians agree that these items are necessary to maintain sterile conditions for the injection. However, all agree that the use of a lid speculum and administration of povidone-iodine to the lids, lashes, and conjunctiva are recommended [67].

Another concern is an allergic reaction to the drug. Since ranibizumab is a recombinant monoclonal antibody that contains both mouse and human derived segments, some patients treated with the drug may develop systemic antibodies [8, 20].

In the ANCHOR trial 3.9% of ranibizumab 0.5-mg subjects had developed antibodies to ranibizumab compared with 0% in the PDT group [20].

In the MARINA trial, after 24 months, 6.3% of subjects treated with ranibizumab 0.5 mg and 1.1% of those in the sham injection group developed antibodies to ranibizumab [8].

Systemic AEs are a concern, since inhibitors of VEGF injected intravitreally, can penetrate the general circulation and compromise functions that rely on VEGF outside of the eye, such as wound healing and the formation of new blood vessels around the heart or brain in cases of ischemia [68, 69]. Patients with AMD already are at higher risk of cardiovascular disease than the general population because of their age and the association of AMD with systemic hypertension [70], consequently, participants in clinical trials of VEGF inhibitors were carefully monitored for possible increases in blood pressure, occurrence of myocardial infarction/stroke, and nonocular hemorrhages [8, 20].

Among participants in the MARINA trial, approximately 16% in both the ranibizumab 0.5 mg and sham injection groups developed hypertension [8] and in the ANCHOR treatment related hypertension was higher in the PDT group (8.4%) than in the ranibizumab group (6.4%) [20].

In the CATT trial there was no evidence that ranibizumab 0.5 mg was associated with increases in either diastolic or systolic blood pressure [49, 50].

Nonocular hemorrhages include events such as cerebral or gastrointestinal bleeding. In the ANCHOR trial, non ocular hemorrhage was more frequent in the 0.5-mg ranibizumab group (6.4%) than in the PDT group (2.1%) [20]. In the MARINA trial, the cumulative frequency of nonocular hemorrhage by month 24 was 5.5% in the sham injection group compared with 8.8% in the 0.5-mg ranibizumab group [8].

Among participants in the MARINA trial, approximately 16% in both the ranibizumab 0.5 mg and sham injection groups developed hypertension [3].

In the CATT trial Gastrointestinal disorders (e.g., hemorrhage, hernia, nausea, and vomiting), occurred in 11 (1.8%) ranibizumab-treated and in 28 (4.8%) bevacizumab-treated patients ($P = 0.005$) [51].

With respect to cardiovascular or cerebrovascular events, during the ANCHOR trial, 1 subject in the PDT group (0.7%) and 3 subjects in the ranibizumab 0.5-mg group (2.1%) developed nonfatal myocardial infarctions, although the events did not occur shortly after treatment. [20]. The frequency of stroke (1 in each group) and cerebral infarction (0 in each group) in the ANCHOR trial were too low to draw meaningful conclusion [20].

At 24 months, the overall frequency of cardiovascular systemic events in the MARINA trial was similar in the 0.5-mg ranibizumab and sham injection groups [8]. There were only small differences in the frequency of thromboembolic events between the sham injection group (3.8%) and the ranibizumab 0.5-mg group (4.6%) [8]. The frequency of death (2.5%) was the same in the ranibizumab 0.5-mg and sham injection groups [8]. Two individuals in each group died of stroke.

There was no significant difference in the frequency of myocardial infarction between the 2 treatment groups in the SAILOR trial [36].

In the CATT trial at 2 years, 5.3% assigned to ranibizumab and 6.1% assigned to Bevacizumab had died ($P = 0.62$). The proportion of patients with arteriothrombotic events was simi-

lar in the ranibizumab-treated patients (4.7%) and in the bevacizumab-treated patients 5.0%; ($P = 0.89$). Venous thrombotic events occurred in 3 (0.5%) ranibizumab-treated patients and in 10 (1.7%) bevacizumab-treated patients ($P = 0.054$) [51].

One or more serious systemic adverse events occurred in 255 patients (21.5%), with 53 (17.6%) in the ranibizumab-monthly group, 64 (22.4%) in the bevacizumab-monthly group, 61 (20.5%) in the ranibizumab-as-needed group, and 77 (25.7%) in the bevacizumab-as-needed group ($P = 0.11$ by the chi-square test). Hospitalizations accounted for 298 of the 370 individual serious systemic adverse events (80.5%). When dosing-regimen groups were combined, the proportions of patients with serious systemic adverse events were 24.1% for bevacizumab and 19.0% for ranibizumab ($P = 0.04$). After adjustment for demographic features and coexisting illnesses at baseline, the risk ratio for bevacizumab, as compared with ranibizumab, was 1.29 (95% confidence interval, 1.01 to 1.66; $P = 0.04$). Patients treated as needed had higher rates than patients treated monthly (risk ratio, 1.20; 95% CI, 0.98–1.47; $P = 0.08$). After excluding all events previously associated with systemic treatment with anti-vascular endothelial growth factor drugs, 170 (28.4%) of ranibizumab-treated patients and 202 (34.5%) of bevacizumab-treated patients had experienced events ($P = 0.02$) [51].

Although event rates for these cerebrovascular or cardiovascular events seem to be low with ranibizumab, ophthalmologists should ensure that patients understand the theoretic potential for these risks. Additional studies over time may help to refine understanding of the magnitude, if any, of this risk.

In the recently published IVAN trial at 12 months, 6 participants (1.9%) in the ranibizumab group and 5 (1.7%) in the bevacizumab group ($P = 0.81$) had died; 5 (1.6%) had received continuous and 6 (2.0%) discontinuous treatment ($P = 0.74$) [52]. Fewer participants treated with bevacizumab compared with ranibizumab had an arteriothrombotic event or heart failure (0.7% vs. 2.9%; odds ratio, 0.23; 95% CI, 0.05 to 1.07; $P = 0.03$), but no difference between treatment regimens was found ($P = 0.34$). One or more serious systemic adverse events occurred in 30 (9.6%) in the ranibizumab group and 37 (12.5%) in the bevacizumab group ($P = 0.25$). Similarly, 30 (9.7%) in the continuous and 7 (12.3%) in the discontinuous group had ≥ 1 serious systemic adverse events ($P = 0.32$). More than 10 participant-specific events occurred in 3 MedDRA categories: cardiac disorders, surgical or medical procedure, and any other class (available at <http://aaajournal.org>). Comparisons by drug and regimen for cardiac disorders and surgical or medical procedure showed no differences ($P \geq 0.46$). One case of severe uveitis developed after 1 injection; there was 1 reported traumatic cataract and 3 retinal pigment epithelial tears. Five “other” ocular events were each reported once.

7.1. Safety of Bevacizumab

Data on the safety of intravitreal bevacizumab are more limited than data on Ranibizumab safety, due to the lack of large multicenter trials performed with Bevacizumab. The results of the CATT and IVAN trials were previously presented.

8. New anti VEGF agents under investigation

8.1. RNA Interference (SIRNA)

SIRNA stands for short interfering RNA. SIRNAs are 21 to 25 nucleotide-long double-stranded RNA molecules capable of destroying a corresponding target messenger RNA with high selectivity and efficacy [71]. This leads to post transcriptional gene silencing (PTGS).

SIRNAs work intracellularly, where they are incorporated into a protein complex called RNA-induced silencing complex (RISC) [71]. The RISC has RNA helicase activity, which unwinds the two strands of RNA. The strand of the siRNA that becomes associated to the RISC leads the complex to selectively cleave and degrade messenger RNA molecules containing a complementary sequence. The siRNA is engineered to match the protein encoding nucleotide sequence of the target messenger RNA. Since the translation of messenger RNA into proteins is an amplification step, destroying it is a very potent method of inhibiting protein function.

SIRNA-027 (SIRNA Therapeutics, Inc.) is a short interfering RNA that targets the VEGF receptor 1 (VEGFR-1). Animal experiments have shown that both intravitreal and periocular injections of siRNA directed against VEGFR1 lead to a substantial reduction of VEGFR1 messenger RNA levels [71-72].

The siRNA suppressed the development of CNV at rupture sites in Bruch's membrane and decreased retinal neovascularization in mice with oxygen-induced ischemic retinopathy [72-73].

Acuity Pharmaceuticals has also produced a siRNA called Cand5 or Bevasiranib that targets the messenger RNA of the VEGF protein itself. Animal models have shown prevention of CNV development after laser-induced injury [72].

Bevasiranib sodium was developed for intravitreal administration. Following intravitreal injection, bevasiranib is well distributed within the eye and localizes to the retina [72, 73].

Preliminary results of Phases I and II clinical trials of bevasiranib have shown promising results for the treatment of nvAMD and diabetic macular edema. There are various studies of different phases underway (the COBALT studies although recruitment was stopped). A phase III study evaluating the combination of bevasiranib and ranibizumab in nvAMD (the CARBON study) is currently underway.

The purpose of this study is to compare intravitreal bevasiranib sodium as maintenance therapy for AMD following initiation with three monthly doses of ranibizumab. Preliminary clinical results indicate that the effects of bevasiranib do not appear until six weeks after the initiation of treatment, which suggests that combination therapy with anti VEGF drug might be justified. The late effect of bevasiranib might be linked to its mechanism of action, since bevasiranib inhibits the synthesis of new VEGF, and does not eliminate existing VEGF, a direct anti-VEGF agent may be required to neutralize VEGF already present in the eye before inhibition of new VEGF synthesis. Preliminary results of the carbon and cobalt studies suggested that over 30% of patients on combination ranibizumab-bevasiranib achieve an im-

provement of at least three additional lines of VA than those on ranibizumab alone. The safety and efficacy of this combination awaits the full results of the ongoing clinical trials.

However, the lack of available data from randomized placebo-controlled or comparative studies makes it difficult to evaluate the role of bevasiranib in nvAMD therapy. It is clear from experimental and preclinical studies that anti-VEGF siRNA is capable of down regulating VEGF production, a key goal of anti-VEGF therapy [72].

In summary, bevasiranib exploits an interesting technology [72, 73] and may be a useful addition to the currently available drugs used to treat wet AMD.

8.2. Tyrosine kinase inhibitors

VEGF A signals through two VEGF receptors [7, 10]. VEGF R consist of protein-tyrosine kinases (VEGFR-1, VEGFR-2, and VEGFR-3) and two non-protein kinase coreceptors (neuropilin-1 and neuropilin-2) [10]. New drugs targeting these tyrosine kinases are being investigated.

Vatalanib (PTK787; Novartis)- Vatalanib [74] is a potent tyrosine kinase inhibitor with good oral bioavailability and activity against the VEGFR family, PDGFR β and c-Kit receptor kinases. Preclinical studies [74] suggest that vatalanib induces dose-dependent inhibition of VEGF-induced angiogenesis. A phase I/II trial, ADVANCE [75], to evaluate the safety and efficacy of oral vatalanib combined with PDT with verteporfin in 50 patients has been completed, but the data have not yet been published in a peer-reviewed journal.

Pazopanib (GW786034; GlaxoSmithKline)- Pazopanib [76] is a second-generation tyrosine kinase inhibitor against all VEGFR, PDGFR α , PDGFR β , and c-kit. A phase I clinical trial using pazopanib as eye drops in 38 healthy volunteers has successfully demonstrated its safety and tolerability. Subsequently, a phase II trial [77] to evaluate its pharmacodynamics, pharmacokinetics and safety has been completed, but the data have not yet been published in a peer-reviewed journal.

8.3. Anti-VEGFR vaccine therapy

This is an immunologic approach to combat CNV. A recent report demonstrated CD8+ cytotoxic T lymphocyte (CTL)-mediated regression of physiologic and pathologic retinal neovascularization [78], thus a possible immunologic therapy for CNV was suggested. It was approved by an animal model [79] which showed that CNV can regress by inducing cellular immunity specific for VEGFR-2.

More recently, a phase I study [80] of anti-VEGFR vaccine therapy has been recruiting participants. The patients will be vaccinated once a week for 12 weeks. On each vaccination day, VEGFR-1 peptide (1 mg) and VEGFR-2 peptide (1 mg) mixed with Montanide ISA 51 will be administered by subcutaneous injection. The study will evaluate the safety and tolerability as well as the immunological and clinical response of the vaccine therapy to treatment of nvAMD.

8.4. Anti inflammatory mediators

As mentioned before, both angiogenic and inflammatory processes are involved in nvAMD, new therapeutics targeting the inflammatory process, besides steroids are being investigated POT-4 (Potentia Pharmaceuticals)- POT-4 [81] is a peptide capable of binding to human complement factor C3 (C3). As C3 is a central component of all known complement activation pathways, its inhibition effectively shuts down all downstream complement activation that could otherwise lead to local inflammation, tissue damage and up-regulation of angiogenic factors such as VEGF.

A phase I single escalating dose study [82] has just released its first results, which indicate that POT-4 IVT is safe, and the data accumulated so far support the continued investigation of POT-4 for the treatment of both dry and wet AMD with a larger randomized phase II trial to further define its efficacy profile.

ARC1905 (Ophthotech Corp.) -ARC1905 [81] is an anti-C5 aptamer, which prevents the formation of key terminal fragments (C5a and C5b-9) by inhibiting human complement factor C5 (C5). C5a fragment is an important inflammatory activator inducing vascular permeability, recruitment and activation of phagocytes. C5b-9 is involved in the formation of membrane attack complex (C5b-9), which initiates cell lysis [81]. Thus by inhibiting these C5-mediated inflammatory, ARC1905 might be beneficial in wet AMD.

A phase I study [83] to evaluate the safety, tolerability, and pharmacokinetic profile of multiple doses of ARC1905 IVT in combination with multiple doses of Lucentis has been completed, but the data have not yet been published in a peer-reviewed journal.

OT-551 (Othera)-OT-551 [84], an Othera Pharmaceuticals' Othera (OT)-551 antioxidant eye drop has the potential for chronic treatment of the dry form of age-related macular degeneration.

A phase I trial [84] demonstrated that when the compound is added to Lucentis or Avastin treatment, there is a synergistic effect in patients with wet AMD. A pilot study [85] of participants with bilateral geographic atrophy is designed to characterize the effect of 0.45% concentration of OT-551 eye drops on the progression of geographic atrophy area over a two-year period.

8.5. AdPEDF - Fovista (GenVec)

Pigment epithelium-derived factor (PEDF) is one of the most potent antiangiogenic proteins found in humans, which were shown to inhibit VEGF-induced proliferation, migration of microvascular endothelial cells, reduce VEGF-induced hypermeability and cause vessel regression in established neovascularization [86]. AdPEDF uses a DNA carrier, to deliver the PEDF gene, resulting in the local production of AdPEDF in the treated eye.

A phase I escalating-dose clinical trial [87] in patients with nvAMD was completed. Three to six months after a single injection, it suggested that 50–94% of patients had a stabilization or improvement in lesion size from baseline, suggesting that antiangiogenesis may last for several months after a single IVT. there were no dose-limiting toxicities or drug-related severe adverse events reported. Further studies investigating the efficacy of AdPEDF in patients with wet AMD are under way.

| Trial | No. of patients | Injection protocol | Drug, dosage, control | Results |
|----------|-----------------|---|---|--|
| VISION | 1186 | IVT every 6 weeks | Pegaptanib; 0.3/1.0/3.0 mg-Sham | 31%–37% stable vision, 4%–6% gained ≥ 3 lines (12 months) |
| MARINA | 716 | IVT monthly | Ranibizumab; 0.3/0.5 mg-sham | 95% stable vision, 26%–34% gained ≥ 3 lines (12 months) |
| ANCHOR | 423 | IVT monthly | Ranibizumab; 0.3/0.5 mg-Sham + PDT every 3 months if needed | 96% Stable vision, 35%–40% gained ≥ 3 lines (12 months) |
| FOCUS | 162 | IVT monthly+PDT | Ranibizumab; 0.5 mg-PDT every 3 months | 90% stable vision |
| HORIZON | 853 | IVT monthly | Ranibizumab; 0.5 mg | Mean loss of vision 2–5 letters, 3% gained ≥ 3 lines, 7%–14% lost ≥ 3 lines (12 months) |
| PIER | 183 | IVT monthly x 3, re-treatment every 3 months | Ranibizumab; 0.3/0.5 mg-sham | 83%–90% stable vision, 12%–13% gained ≥ 3 lines (12 months) |
| PRONTO | 37 | IVT monthly x 3, re-treatment as needed (9.9 injections over 24 months) | Ranibizumab; 0.5 mg | 43% gained ≥ 3 lines (24 months) |
| SUSTAIN | 513 | IVT monthly x 3, re-treatment as needed (5.3 injections for "naïve" patients over 12 months) | Ranibizumab; 0.3/0.5 mg | Mean BCVA increased steadily from baseline to month 3 to reach +5.8 letters, decreased slightly from month 3 to 6, and remained stable from month 6 to 12, reaching +3.6 at month 12 |
| CLEAR-IT | 51 | IVT single | Aflibercept; 0.05/0.15/0.5/1.0/2.0/4.0 mg | 95% stable vision, 50% of 2.0/4.0 mg group gained ≥ 3 lines (3 months) |
| VIEW1 | 1217 | VTE 0.5 mg monthly (0.5q4wk), VTE 2 mg monthly (2q4wk), VTE 2 mg every two months (2q8wk), or ranibizumab 0.5mg monthly (Rq4wk) | Aflibercept (VTE); 0.5/2.0 mg-Ranibizumab; 0.5 mg | All VTE groups were noninferior to ranibizumab. |
| VIEW2 | 1240 | | | |

Table 1. Summary of main clinical trials on anti VEGF treatment for AMD

9. Conclusion

Over the past decade, the treatment of nvAMD improved dramatically with the discovery of anti-VEGF agents that have enabled patients not only to stabilize the vision but to improve and regain vision in this potentially blinding disease.

With the goal of maximizing VA and minimizing the frequency of intravitreal injections and associated risks of treatment, evidence-based management of wet AMD has evolved into individualized anti-VEGF therapy with frequent follow up and retreatment. As a safer and more cost-effective alternative to the traditional monthly treatments used in the ANCHOR and MARINA trials, two individualized anti-VEGF treatment regimens have been described, but neither has been proven superior to date: as-needed (or “PRN”) therapy and the treat-and-extend strategy. Despite a paucity of evidence comparing the as-needed versus the treat-and extend treatment regimens, a possibility exists that the treat and extend regimen will prove to be the most efficacious, cost-saving, and preferred protocol. The current evidence based treatment strategy for the management of wet AMD supports the use of either bevacizumab or ranibizumab either monthly or with a more individualized treatment strategy with close followup. As second generation anti-VEGF agents become available and the stress on our healthcare systems intensifies, increasingly efficacious and cost-conscious treatment strategies will be essential.

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