

# Angiogenesis and Role of Anti-VEGF Therapy

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Vascular endothelial growth factor (VEGF) is the main culprit to initiate the cascade of angiogenesis in a variety of chorioretinal neovascular disorders such as neovascular age-related macular degeneration (ARMD) and various proliferative retinopathies. Although VEGF has an important role in maintaining the adequate blood flow to retinal pigment epithelium (RPE) and choriocapillaries, however its over-expression in response to hypoxia causes the growth of new vessels, These new vessels are fragile causing leakage and bleeding with promotion of scar tissue formation resulting in the visual loss. Currently there are three anti-VEGF agents available in the market, namely pegaptanib (Macugen), ranibizumab (Lucentis) and bevacizumab (Avastin).

In this article we look at all these drugs available with review of international literature to determine their usefulness.

Vascular endothelial growth factor (VEGF) is a prime regulator of angiogenesis with diverse roles, both physiological as well as pathological during development and adulthood<sup>1</sup>. The physiological response is seen in healing wounds with restoration of blood flow, maintaining female reproductive cycle and formation of placenta with fetal development. While tumor growth, neovascular age related macular degeneration (ARMD) and various proliferative retinopathies are the prime example of its pathological response<sup>2</sup>. On cellular level, angiogenesis depends on a balance between positive regulators such as Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Tumor necrosis factors - alpha (TNF- alpha), Interleukin - 8 (IL-8) and negative regulators which are Thrombospondin-1 (TSP-1), Angiostatin and Endostatin and Plasminogen activator inhibitor - 1 (PAI-1)<sup>3</sup>.

Angiogenic cascade is believed to be initiated due to hypoxia with release of VEGF, FGF and other growth factors. These factors bind to endothelial cells of nearby capillaries thereby activating them. The activated endothelial cells proliferate, migrate and release various proteases. These enzymes increase

permeability of basement membrane. The migrating endothelial cells also form new blood vessels in formerly avascular space. These newly formed vessels are fragile causing leakage and bleeding with promotion of scar tissue formation resulting in visual loss.

The VEGF family consists of structurally related growth factors namely VEGF - A, VEGF - B, VEGF - C, VEGF - D, VEGF - E and Placenta growth factor, all encoded by separate genes<sup>4</sup>. VEGF - A, commonly referred to as VEGF only, is the primary driver in angiogenesis and is the target of most current anti - VEGF agents. Apart from angiogenesis, it also increases the vascular permeability around 50,000 times more than histamine by inducing conformational changes at the tight junctions in the outer blood - retinal barrier. Nine major VEGF - A isoforms have been identified in humans: VEGF 121, VEGF 145, VEGF 148, VEGF 162, VEGF 165, VEGF 165B, VEGF 183, VEGF 189 and VEGF 206. They all differ in property and functions and the number assigned to them actually correspond to the number of amino-acids present in their molecule. VEGF 165 is the most abundantly expressed isoform and VEGF 121 although less abundant is more mitogenic than VEGF 165<sup>5</sup>.

VEGF is naturally expressed in retinal tissue with high levels in the retinal pigment epithelium (RPE). It plays protective role by maintaining adequate blood flow to RPE and photoreceptors. In diseased eyes, its over-expression with increased level occurs due to decrease in blood flow to choriocapillaries and retinal capillaries, resulting in oxidative stress and alteration in Bruch's membrane.

Recent development of anti - VEGF agents has marked a major breakthrough in the treatment of chorioretinal neovascular disease. Currently available anti - VEGF agents include Pegaptanib, ranibizumab and bevacizumab.

**Pegaptanib sodium** (Macugen® Eyetech/ Pfizer) was the first anti angiogenic therapy to be approved by US Food and Drug Administration (FDA) for the treatment of all types of neovascular ARMD in December 2004. It is a RNA aptamer, a modified oligonucleotide with a molecular mass of 50 KDa and produced synthetically to block specifically VEGF 165<sup>6</sup>.

In VISION study (VEGF Inhibitor Study In Ocular Neovascularization), a total number of 1186 patients were treated with all types of neovascular ARMD in different doses of pegaptanib given every 6 weeks intravitreally. At 54 weeks, loss of fewer than 15 letter was seen in 70% of patients with 0.3mg, 71% of patients with 1mg and 65% of patients with 3mg dose, compared with 55% receiving sham injection. A gain of 15 letters were seen in 18% of patients receiving 0.3mg, 20% of patients getting 1mg and 13% of patients receiving 3mg compared with only 6% of patients with sham injection. The ocular complications included, endophthalmitis in 1.3%, iatrogenic cataracts in 0.7% and retinal detachment in 0.6% of patients<sup>7</sup>.

Currently for the treatment of neovascular ARMD, 0.3mg of pegaptanib is used intravitreally every 6 weeks for at least 1 year.

**Ranibizumab** (Lucentis® Genetech - Novartis) is another anti - VEGF agent approved by the FDA for the treatment of patients with neovascular ARMD in June 2006. It is humanized antigen - binding fragment (Fab) of a full length murine monoclonal antibody directed against all isoforms of VEGF - A. It is produced in Escherichia Coli using recombinant DNA technology and has a molecular mass of 49KDa. Due to its smaller molecular size, it can easily penetrate all layers of retina after an intravitreal injection. The average vitreous elimination half - life is also attributed to its smaller size and significant diffusion<sup>8</sup>.

In MARINA study (Minimally classic/ occult trial of the Anti - VEGF antibody Ranibizumab in the treatment of Neovascular ARMD) 716 patients were given ranibizumab intravitreally every 4 weeks for 2 years. At one year, 94.5% of patients with 0.3mg, 94.6% with 0.5mg dose lost fewer than 15 letters compared with 62% of patients having sham injection. A gain of 15 letters were witnessed in 24.8% of patients with 0.3mg and 33.8% of patients receiving 0.5mg ranibizumab compared with 5% of patients having sham injection. All patients showing visual improvement maintained their vision for 2 years. Ocular complications included endophthalmitis in 1% of cases and uveitis in 1.3% of patients<sup>9</sup>.

Another study, Anti - VEGF antibody for the treatment of predominantly Classic choroidal Neovascularization in AMD (ANCHOR) compared the use of intravitreal ranibizumab with photodynamic therapy (PDT) using Visudyne. Either intravitreal ranibizumab was given every month for 2 years or PDT on entry and every 3 months as needed accordingly to protocol. At 1 year 94.3% (0.3mg) and 96.4% (0.5mg) patients lost fewer than 15 letters compared with 64.3% using PDT. Visual acuity also improved for 15 letters in 35.7% (0.3mg) patients and 40.3% (0.5mg) patients against only in 5.6% patients in PDT group<sup>10</sup>.

In PIER Study (Phase IIIb study of ranibizumab efficacy and safety in choroidal neovascularization due to ARMD) efficacy of reduced dosing of ranibizumab was investigated, given monthly for 3 months and thereafter once every 3 months for 12 months. Patients gained vision during first 3 months with monthly injections but lost vision in following 9 months<sup>11</sup>.

In the Prospective OCT imaging of Patients with Neovascular ARMD Treated with Intraocular Lucentis (PrONTO) study, intravitreal injections of ranibizumab was given for 3 months and thereafter only upon sign of disease, actually based on serial OCT findings. Results at 12 months showed that 95% of patients lost fewer than 15 letter of visual acuity and 35% of patients gained 15 letter or more. Although this was a small study but it demonstrated the usefulness of reduced dosing in conjunction with utility of OCT<sup>12</sup>.

The RhuFab V2 Ocular Treatment Combining the Use of Visudyne to evaluate Safety (FOCUS) trial, a phase 1 - II, prospective, randomized, single masked study examined the efficacy of ranibizumab in combination with PDT using visudyne. Patients with

predominantly classic subfoveal choroidal neovascular membrane (CNV) received PDT followed by monthly ranibizumab 0.5mg or sham injection for 2 years. PDT was repeated at 3 monthly interval according to the angiographic findings. At 12 months 90% of patients with combined PDT and ranibizumab lost fewer than 15 letters compared to 68% of patients in the PDT only group. 24% of patients gained 15 letters or more having combination therapy compared to 5% in PDT only group<sup>13</sup>.

**Bevacizumab** (Avastin® Genentech / Roche) is a 149 KDa humanized full – length monoclonal antibody to all isoforms of VEGF – A, approved as intravenous infusion in combination with chemotherapy for metastatic colorectal cancer. It is derived from the same murine anti – VEGF antibody as ranibizumab. Before the FDA approval of ranibizumab, retinal specialist all over the world started using bevacizumab: off label intravitreally in variety of neovascular ocular disease like CNV, macular edema and proliferative diabetic retinopathy (PDR)<sup>14-19</sup>. Intravitreal usage minimizes the risks of potential side effects associated with systemic exposure like arterial thromboembolic events (ATE), hypertension and hemorrhage. Interestingly preliminary studies have shown improvement both visually and anatomically in terms of reduced macular thickness. Bevacizumab has been used intravenously as well as intravitreally in patients with neovascular ARMD and has shown comparable results<sup>20</sup>. Bevacizumab preparation is unpreserved and contains no ingredients that are toxic to eyes. It is used in dose of 1.2mg – 2.5mg, though optimal dosage remains unknown. Bevacizumab has been tested in rabbit eyes with no evidence of retinotoxicity<sup>21</sup>. The serum half life of bevacizumab is 17-21 days. Theoretically, the longer half life of bevacizumab compared to ranibizumab confers higher risk of systemic toxicity but no known serious adverse events have been reported in uncontrolled studies to date. Though, longer half – life may also mean that less frequent administration is required. The short term results of bevacizumab therapy are comparable to that of ranibizumab in patients with neovascular ARMD, with improvement in visual acuity and reduction in retinal thickness<sup>22</sup>. Nevertheless, its cost per dose as compared to ranibizumab is the main driving force compelling retinal specialist of our region to use bevacizumab instead of ranibizumab when patient’s financial issue come in way. The hypothesis that bevacizumab is as safe and effective as ranibizumab has not been established due to

unavailability of controlled, double blind randomized clinical trials. Currently National Eye Institute in US is conducting a “Comparison of Age – related macular degeneration Treatment Trial (CATT). This clinical trial will directly compare ranibizumab and bevacizumab for the treatment of neovascular ARMD and may answer questions regarding the efficacy and dosage required for the bevacizumab.

## CONCERNS REGARDING ANTI – VEGF THERAPY

### Frequency, duration and follow up:

It is not known when it’s safe to stop the anti – VEGF treatment. Most of the trials have published their results between 1-2 year period with no long term data available beyond two years. In ANCHOR and MARINA studies, patients received ranibizumab injection every month for 2 years with no designated clinical end point for the treatment. PIER study, on the other hand tried to reduce the frequency of the injections, but results of this study appeared less impressive than the previous studies suggesting that by simply increasing the time period between injections may have compromised the visual end result. The currently ongoing SAILOR trial is expected to provide more information on the benefit and efficacy of variable dosing.

### Cost

Single injection of Pegaptanib in Pakistan cost around Rs. 60,000, while cost of one injection of ranibizumab mounts to Rs. 85,000. Bevacizumab comparatively is cheaper at Rs. 3,500 per single dose. The cost of the injection is the main force driving retinal specialist to opt for bevacizumab, when one clearly understands the benefit and efficacy of ranibizumab proven through multiple international trials. The cost issue also becomes imperative when these injections are given repeatedly.

### Safety

The procedure related ocular complication such as endophthalmitis and retinal detachment are reported around 1% in MARINA and ANCHOR trials. The Pan -American Collaborative Retina Study Group (PACORES) has recently reported 12 months safety data on 4303 injections of intravitreal bevacizumab given in 1310 eyes, with rate of endophthalmitis at 0.16% and retinal detachment at 0.02%. Although these rates of complications are much less than the reported one in the early trials, but one still has to take

strict aseptic precautions giving these injections preferably in the operating room<sup>23</sup>.

The International Intravitreal Bevacizumab Safety Survey, and internet based study from 70 centers in 12 countries has analyzed ocular and systemic adverse events related to more than 7000 injections. The event rates for corneal abrasions, lens injury, retinal detachment, uveitis, endophthalmitis, central retinal artery occlusion, sub retinal hemorrhage, RPE tears, transient ischemic attacks (TIA), cerebro vascular accidents (CVA) and death with no individual event rate exceeding 0.21%<sup>24</sup>.

There are concerns regarding systemic drug related adverse events such as arterial thrombo embolism and myocardial infarction in patients undergoing anti - VEGF treatment. In MARINA trial the rate of ischemic stroke was same in patients receiving ranibizumab and sham injections (1.3% in 0.3mg group, 2.5% in 0.5mg group and 0.8% in sham group). The incidence of myocardial ischemia was reported 2.5% in 0.3mg group, 1.3% in 0.5mg group and 1.7% in sham group.

In SAILOR trial, there was 1.2% incidence of stroke with 0.5mg ranibizumab compared to 0.3% in 0.3mg group, suggesting a dose - dependent increase in the rate of thrombo embolic event.

Alexander and coworkers in retrospective analysis of US Medicare data base from 2001 - 2003 have showed that, in- patient ischemic stroke rate for 15771 patients with neovascular ARMD was 3.5% compared to 3.6% in 44408 matched controls<sup>25</sup>.

Though, there may be a small risk of increased incidence of stroke in patients undergoing anti - VEGF therapy, more data is required to settle this issue. And again benefits of this treatment outweighs any potential risks in that particular patient population.

### Which VEGF Blockage

As discussed earlier, VEGF plays an important role not only in the disease state but also in maintaining the normal physiology of the circulation. So what is going to be the long term effect of this VEGF blockage. Although visual stability and recovery is more impressive with ranibizumab, it dose block all VEGF isoforms compared to pegaptanib which only neutralizes VEGF 165. In long term which anti - VEGF blockage is associated with less complications is not known.

## CONCLUSION

Anti-VEGF therapy has become the first line treatment not only in neovascular ARMD but also in various proliferative retinopathies. As suggested by various trials, ranibizumab has the most impressive results regarding the visual stability and improvement, specially in neovascular ARMD. Unfortunately because of the higher cost, specially in our country, we are seeing more and more use of bevacizumab in patients where anti-VEGF treatment is necessary. Once the results of CATT trial are published, one will know the true effectiveness of bevacizumab compared to ranibizumab. Although intravitreal injections are easy to use but one has to be careful avoiding procedure and drug related complications by using aseptic conditions and reducing the frequency of these injections without compromising the treatment benefits.

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