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Anti-VEGF therapy: comparison of current and future agents

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Abstract

With the identification of vascular endothelial growth factor (VEGF) and the confirmation of its pathophysiologic link to retinal and choroidal angiogenesis, numerous agents have been designed to inhibit its activity. It is noteworthy that anatomic and visual benefits have been associated with the use of anti-VEGF agents such as pegaptanib (Macugen) and to a greater extent, ranibizumab (Lucentis) and bevacizumab (Avastin), particularly in the management of neovascular age-related macular degeneration (AMD). Clinical trials and case series have confirmed the utility of these agents. However, shortcomings of the current drugs such as short half-life, intraocular dosing, limited effectiveness in some patients, and potential systemic side effects continue to drive the development of new agents. In this article, we review current anti-VEGF therapies and discuss future developments.
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Introduction

The concept that a vascular growth factor is present in neovascular ocular diseases is not new. In the late 1940s, Michelson published a manuscript outlining a concept that a biochemical factor (factor X) was necessary for the normal developmental and growth of the retinal vasculature.[1] This same growth factor, Michelson proposed, was likely necessary for pathologic angiogenesis as well, and that its presence in this setting was the result of changes in metabolism in the retina. For years ophthalmologists have used pan-retinal laser photocoagulation (PRP) to effectively treat neovascular retinopathy. It has been assumed that the mechanism of action PRP laser has been to reduce the intraocular levels of this, yet unidentified, vascular growth factor. In 1971, Folkman[2] published a paper in the *New England Journal of Medicine* proposing a theory that tumour angiogenesis was necessary for tumour growth and that inhibition of angiogenesis could be therapeutic. His team identified a factor, tumour angiogenic factor that they proposed as a candidate for therapeutic anti-angiogenesis. Many in the field did not initially welcome these concepts, very few others outside of Folkman's laboratory pursued tumour angiogenesis for the next 10 years. In 1983, Senger *et al*[3] identified in tumour ascites fluid a 42 kDA protein and vascular permeability factor. In 1989, Ferrera[4] published results identifying and purifying a novel glycoprotein growth factor specific for endothelial cells that was secreted by pituitary follicular cells. Their glycoprotein is likely the same molecule previously identified by Senger *et al*. Leung[5] and simultaneously Keck *et al*[6]cloned similar molecules: vascular endothelial growth factor (VEGF) and vascular permeability factor. Using antibody techniques specific inhibitors could now be produced ushering in a new era in the treatment of cancer and retinal angiogenesis.

In 2001, The Food and Drug Administration (FDA) approved the first anti-VEGF agent, pegaptanib (Macugen) for the treatment of neovascular age-related macular degeneration (AMD). Though limited in its effectiveness in the treatment of neovascular AMD, its approval signalled the beginning of a new generation in AMD treatment, the era of anti-VEGF therapy.

Vascular endothelial growth factor pharmacologic considerations

Vascular endothelial growth factor (VEGF)-A is a major regulator of angiogenesis and vascular permeability in the eye for physiologic as well as pathologic processes. It also plays a role as a survival factor for many cells. VEGF has been implicated in ocular diseases ranging for diabetic retinopathy to AMD. VEGF-A, the molecule implicated in eye diseases is a member of a gene family which includes: VEGF B, C, D, and placental growth factor. There are also multiple isoforms of VEGF-A based on the number of amino acids included in their structure. Some are bound to the extracellular matrix (ie VEGFs 189 and 206) and cell surfaces, while smaller isoforms (ie VEGF 121) are diffusible. The larger isoforms can be cleaved by fibrinolysis to produce biologically active VEGF 110. VEGF-A binds to two types of receptors, VEGFR1 and VEGFR2, both are protein kinase-activating receptors. It is the binding of the VEGFR2 that is important for ocular neovascularization.

Rational approaches to block VEGF activity would include inhibition of VEGF, VEGFR2 or protein kinase activity amongst others. Two agents have been approved in the United States by the FDA for use in neovascular AMD, pegaptanib (Macugen) and ranibizumab (Lucentis). A third drug bevacizumab (Avastin) has been approved for the use in oncology but is also widely used 'off label' in the treatment of neovascular AMD, diabetic retinopathy and other retinal vascular and proliferative disease processes.

Pegaptanib

Pegaptanib (Macugen) was the first anti-VEGF agent approved. Pegaptanib is a 28-base ribonucleic aptamer, a small fragment of RNA that binds proteins with high affinity. *In vivo*, pegaptanib binds to the extracellular VEGF165 isoform. This interaction inhibits the VEGF from binding and activating the VEGFR2 receptor. Pegaptanib selectively binds to only the 165 isoform. This may explain its limited efficacy compared to agents that are capable of pan-isoform suppression. This selective targeting might also be advantageous in reducing suppression of systemic or ocular VEGF necessary for normal function, making it a safer choice. This concept however has not been validated.

Two concurrent phase III randomized multicentre dose-ranging sham-controlled clinical trials demonstrated the efficacy of pegaptanib in the treatment of neovascular AMD. (VISION Trials).[7] In total, 1186 patients were enrolled in these two trials in which the patient received pegaptanib or sham intravitreally every 6 weeks for 48 weeks. The primary efficacy end point of these trials were the percentages of patients in each group losing less than 15 letters of visual acuity at 1 year. This was achieved in 70% of the pegaptanib treated (0.3 mg dose) *vs* 55% of the sham-treated patients ($P<0.001$) However, this treatment resulted in improvements in visual acuity in few patients. On average, patients continued to lose vision during the first 2 years of the study, but significantly less vision as compared to the sham group. Though pegaptanib was well tolerated, with few serious local or systemic side effects, the visual outcomes were disappointing. In fact, the outcomes were similar to the existing standard treatment, photodynamic therapy with verteporfin. Pegaptanib however was approved for a larger percentage of patients presenting with neovascular AMD, and did not appear to be limited by angiographic subtype. With the advent of much more efficacious agents (ranibizumab and bevacizumab), the use of pegaptanib has fallen precipitously. Given its long record of safe use and theoretical reduced risk of side effects associated with pan-VEGF suppression, some have proposed a role for pegaptanib as maintenance therapy following induction with a pan-VEGF agent. Such an approach, though rational, has not been proven efficacious or safer to date. Trials are ongoing.

Ranibizumab and bevacizumab

It is impossible to talk about ranibizumab in isolation of bevacizumab in the treatment of neovascular AMD. Currently in the United States, according to the preferences and trends (PAT) survey of the American Society of Retina Specialist, these drugs are used in equal frequency to treat neovascular complications of AMD. However, only ranibizumab has received FDA approval, though Medicare has agreed to pay for either drug for appropriate AMD patients. The availability of both drugs has generated some controversy as Genentech has attempted to limit access of bevacizumab for ocular indications, favouring the significantly more expensive alternative ranibizumab. Whether the efficacy and safety of bevacizumab equals that of ranibizumab remains to be determined but there is rational to presuppose that any differences may be small.

Both ranibizumab and bevacizumab were derived from the same murine antibody to VEGF. Bevacizumab is the humanized full-length antibody, whereas ranibizumab is the Fab fragment that is humanized and affinity maturated, so that its binding affinity is approximately 20 times that of bevacizumab. Bevacizumab (Avastin) was developed by Genentech to be used to treat cancer and initially approved by the FDA for use as an adjunct in patients with metastatic colon cancer.

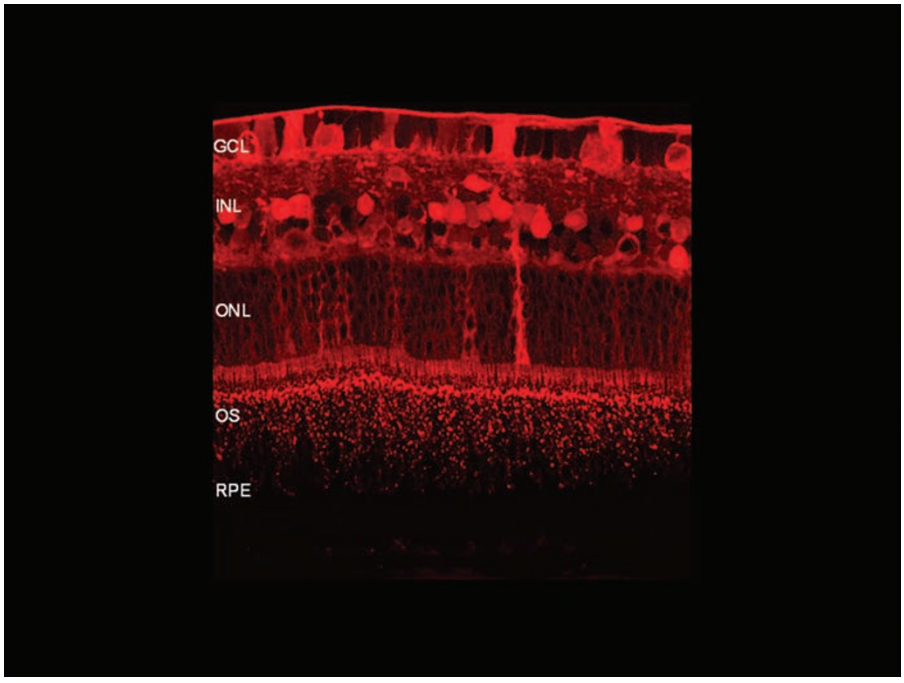

Figure 1 Laser scanning confocal microscope image of a rabbit eye at 24 h after intravitreal injection of bevacizumab. Specific antibody labelling was present along the internal limiting membrane (ILM), the ganglion cell (GC), inner nuclear layer (INL) as well as inner and outer segment (OS) layers of photoreceptors. Courtesy of Robert Avery, MD (California Retina Consultants and UCSB Neuroscience Research Institute Retinal Cell Biology Lab).

Early penetration studies using full-length antibodies and the Fab fragment ranibizumab (Lucentis) seemed to indicate that the full-length antibody penetrated the retina poorly.[8] Conversely, the high-affinity Fab fragment penetrated the neurosensory retina, suggesting that this molecule would be more effective in the treatment of neovascular AMD. Recent penetration studies refute the earlier studies of Mordenti, demonstrating rapid full-thickness penetration[9,10] (Figure 1). Despite this, the Fab fragment (ranibizumab) may offer a few additional advantages over bevacizumab including higher affinity binding and potentially less immunogenicity as it lacks the Fc portion of a full-length antibody.

Recent half-life data suggest an advantage of the larger full-length antibody with increased half-life in the vitreous, retina, and choroid.[11,12] On the other hand, when considering toxicity and systemic exposure, the longer systemic exposure of bevacizumab may be a disadvantage.

Ranibizumab

A number of phase III clinical trials (ANCHOR, MARINA, PIER) have validated the use of ranibizumab in the treatment of all angiographic subtypes of choroidal neovascularization.[13,14]

The MARINA trial investigated ranibizumab (0.3 and 0.5 mg doses) *vs* placebo in patients with occult and minimally classic subfoveal choroidal neovascularization.[14] The primary efficacy end point of this trial was the percentage of patients losing less than 15 early treatment of diabetic retinopathy study (ETDRS) letters with 90% of patients meeting this criteria at 2 years following enrollment. More striking however, on average, patients receiving ranibizumab experienced 6.5 ETDRS letters of improvement at 2 years, while the placebo group lost nearly 15 letters. Equally exciting, 1 of 3 of patients experienced improvement of 3 or more lines of vision improvement and at 2 years, 42% of the patients had vision of 20/40 or better Snellen equivalent. These results demonstrated for the first time, average visual improvement, a new milestone in AMD care (Figure 2).

The ANCHOR trial investigated ranibizumab *vs* photodynamic therapy (PDT) (Visudyne) for the treatment of subfoveal classic choroidal neovascularisation (CNV) in AMD.[13] The results of the ANCHOR trial were similar with the ranibizumab group experiencing an average vision improvement of 11.3 letters at 12 months *vs* the PDT group experiencing a loss of 9.5 letters, similar to the previously reported treatment of AMD with PDT trial results[15] (Figure 2). At 2 years,

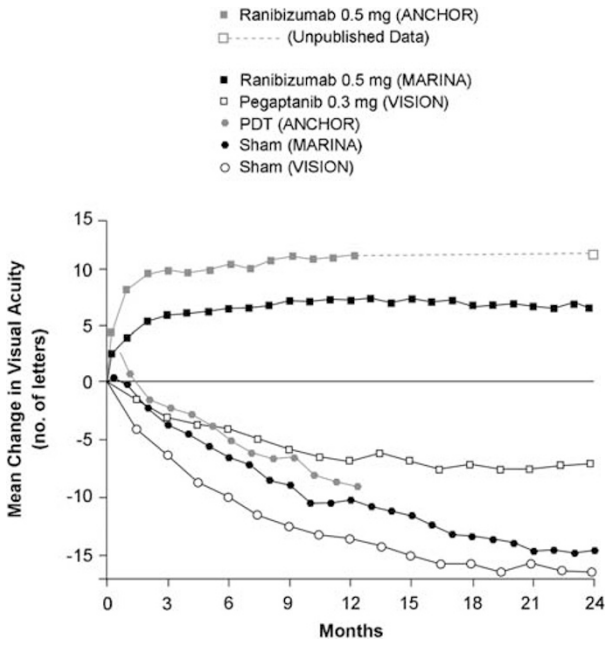

Figure 2 Mean change in visual acuity through 24 months for subjects in MARINA, ANCHOR, TAP and VISION trials.

over 40% of the ranibizumab *vs* 6% of the PDT-treated patients experienced three or more lines of vision improvement, with 38 *vs* 6% or the ranibizumab and PDT groups respectively obtaining vision of 20/40 or better.[13]

In the ANCHOR and MARINA trials, patients were treated every month for 2 years with an intravitreal injection. The PIER trial investigated ranibizumab given every month for the first three injections followed by quarterly injections (unpublished). The vision improved on average in the PIER ranibizumab group similar to the patients treated in the ANCHOR and MARINA trials during the monthly injection phase. However, much of the initial improvements in vision were lost during the quarterly injection period so that at year one the average vision returned to baseline. Nonetheless, the sham injection group lost 16 letters during the first year.

In a small investigator sponsored study (PRONTO), Rosenfeld *et al* demonstrated that results similar to the ANCHOR and MARINA trial could be obtained at 1 and 2 years using three initial monthly injections followed by monthly PRN (as needed) injections (unpublished). During the PRN period re-treatment was determined by vision, clinical evaluation, and optical coherence tomography (OCT) findings. On average, patients treated following the PRONTO protocol required five to six injections during the first year (*vs* 12 in the ANCHOR and MARINA). Although this trial included only 40 patients and no control group, the results have compelled most retina specialists to follow a similar protocol when treating their patients.

The safety of intravitreal ranibizumab has been confirmed in the initial clinical trials and the phase IV SAILOR trial (unpublished). The main ocular risk is the development of endophthalmitis. In a cumulative study (ANCHOR and MARINA data combined), investigating the risk of adverse ocular events, endophthalmitis occurred in 0.5–1.6% of the ranibizumab-treated patients during the first 2 years of treatment ($N=754$) and serious non-infectious uveitis occurred in 0.8–1.1% of the treated patients (unpublished data).

Serious systemic side effects such as systemic hypertension and arterial thromboembolic events are of concern following high-dose intravenous administration of anti-VEGF agents such as bevacizumab. Whether such complications are possible with intravitreal delivery of drugs that are dosed at levels hundreds of times lower remains a concern. In the combined analysis of the ANCHOR and MARINA trials, the rates of hypertension and arterial thromboembolic events were similar between the treatment and control groups. In this 2-year analysis there was however an increased rate of non-ocular haemorrhages in the treated patients (9%, $N=754$) *vs* (5%, $N=379$) the control group (unpublished). Overall the rates of thromboembolic events reported amongst patients receiving ranibizumab in all the trials including the phase IV SAILOR trial appear very similar to age-matched controls. It will require significantly larger numbers of patients to determine whether or not small differences exist.

Bevacizumab

Though not specifically developed for intraocular use, bevacizumab has demonstrated biologic activity akin to ranibizumab in the treatment of neovascular AMD. The use of bevacizumab was spawned from the excitement investigators generated from the ranibizumab clinical trials. As the clinical effect of ranibizumab was so apparent, one need not wait for the statistical analysis to be convinced that a positive biologic effect was occurring. Unfortunately, unless the patients were part of the ongoing clinical trials, ranibizumab was not available at that time. Fortunately, bevacizumab had been recently FDA approved and was available for the use in colorectal cancer. This made it available to physicians to be used off-label for other indications. A group of investigators in Miami first demonstrated that intravenous bevacizumab could be useful in the treatment of choroidal neovascularization; however, systemic side effects were a limiting factor.[16] Rosenfeld and co-workers then demonstrated that intravitreal delivery was a possibility in two case reports.[17,18] Following this, a number of

investigators, including our group, became early adaptors of intravitreal bevacizumab.[19,20] Our initial series of patients with neovascular AMD treated with intravitreal bevacizumab was encouraging with rapid reduction in subretinal fluid, macular oedema, and pigment epithelial detachments in most treated patients[19] (Figure 3). This was often associated with visual improvement if the lesions treated were not too mature (Figure 4). Again, the apparent biologic effect generated excitement amongst clinicians and bevacizumab was

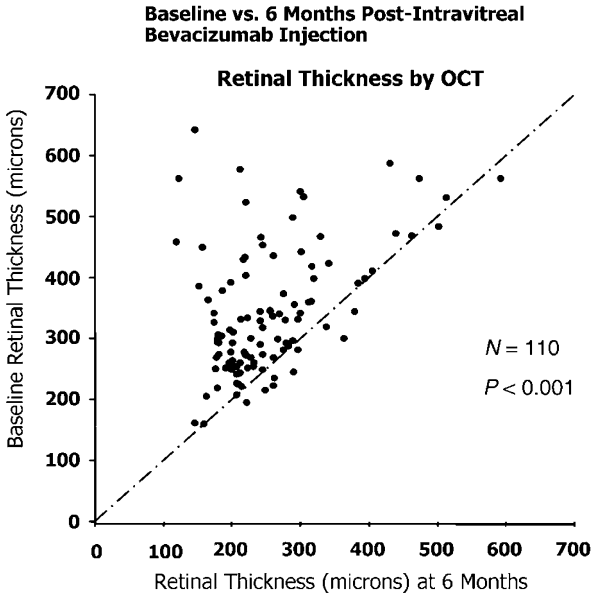

Figure 3 Scatter plot of change in central subfield thickness (microns) 6 months after injection of bevacizumab, as measured by optical coherence tomography.

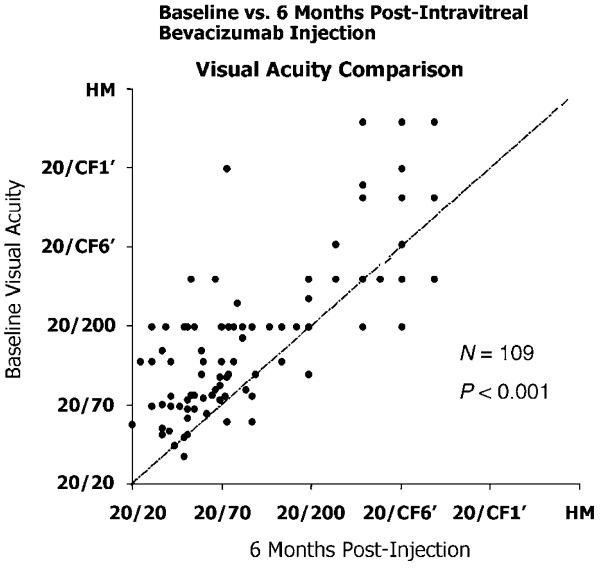

Figure 4 Scatter plot of change in Snellen visual acuity 6 months after injection(s) of bevacizumab.

rapidly accepted worldwide as a treatment for neovascular AMD. It can be argued that bevacizumab for AMD was one of the most successful drug launches in history and it occurred without the financial support of the pharmaceutical industry. To date, numerous retrospective and a few prospective trials have all suggested positive results in treated patients with reduction in leakage detected by OCT and fluorescein angiography. No obvious safety issue disparate from those reported for ranibizumab have been identified, though a longer systemic half-life might increase the length of time that a patient is exposed to such side effects. A registry of 5228 patients (7113 injections) treated at 70 separate centres with bevacizumab has been collected, failing to demonstrate obvious safety outliers.[21] Retrospective reviews and registry data are at risk of sampling bias, incomplete reporting, and short follow-up, so definite safety conclusions cannot be drawn.

How does one determine whether to suggest ranibizumab or bevacizumab for a given patients with CNV? In reality, most decisions come down to finances with the cost of ranibizumab being 40 times that of bevacizumab in the United States.

No one can argue that the available clinical science favours the use of bevacizumab over ranibizumab. The ranibizumab phase III clinical trials are the best information indicating the safety and efficacy of anti-VEGF therapy for AMD. All other currently available clinical data falls short of these trials. To suggest that bevacizumab or ranibizumab has a clear efficacy or safety advantage is speculative. Two clinical trials, one in England and one in the United States (CATT Trial) are designed to investigate in a head-to-head fashion ranibizumab and bevacizumab in the treatment of choroidal neovascularization. In addition, the CATT trial will also determine whether or not there is a significant difference between monthly or PRN dosing. However, it will likely be 1 or 2 years before preliminary data from these trials are available.

Future anti-VEGF agents

A number of novel anti-VEGF agents are currently in phase III clinical trials and if they demonstrate efficacy similar to ranibizumab, may come to market in the next few years. One such agent is VEGF trap.[22] VEGF trap is essentially a soluble VEGF receptor. When injected into the vitreous, VEGF trap acts as a decoy receptor binding-free VEGF. VEGF trap is smaller than a full-length antibody and should penetrate all layers of the retina. It has a higher affinity than the currently available anti-VEGF agents and blocks all isoforms of VEGF. Phase I trials with intravenous administration demonstrated a positive biologic effect with the reduction of retinal

thickness but also a dose-dependant increase in blood pressure in patients receiving VEGF trap (unpublished data). In a phase I/II study of intravitreal VEGF trap ($N = 21$) there were no systemic or ocular side effects (unpublished data). Treated patients experienced a reduction in macular thickening, lesion size, visual improvement on average of 4.8 ETDRS letters, and 95% avoided 15 or more letters of vision loss at 6 weeks following a single injection. Phase III trials are underway.

Another novel strategy to inhibit VEGF utilizes small interfering RNA technology (siRNA). siRNA involves short double-stranded RNA fragments. These siRNA become incorporated into the RNA-induced silencing complex (RISC). When activated, the RISC binds to the target sequence resulting in mRNA cleavage and silence of the gene; two such siRNA molecules are under investigation bevasiranib and SIRNA-027. Bevasiranib is designed to inhibit production of VEGF, while SIRNA-027 is directed against the VEGF receptor. Phase I/II trials have been encouraging; however, treated eyes did not experience a statistically significant improvement in distant visual acuity (unpublished). A phase III trial of bevasiranib (COBALT) is underway to see if it might be effectively used in conjunction with ranibizumab to reduce the need for additional treatment.

We have entered the era of anti-VEGF therapy in the treatment of choroidal neovascularization in patients with AMD. This treatment has resulted in unprecedented visual and anatomic outcomes far outpacing other available treatments. Today physicians and patients can expect visual stabilization in most patients and visual improvement in many, particularly, if treatment is begun early in the course of the disease. Current research now focuses on ways of increasing the durability of effect, reducing side effects, and facilitating delivery. The bar has been set quite high now with medications such as ranibizumab and bevacizumab, but advances in therapy can be expected in the next 5 years.

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