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# Ranibizumab in Circulatory Disorder of Retinal Vein: an Evidence-based Approach

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**ABSTRACT**

*The discovery of anti-angiogenics or vascular endothelial growth factor inhibitors (anti-VEGF) agents is a clear breakthrough with exciting potential in medical management of retinal diseases.Ranibizumab is a humanized, affinity-matured VEGF antibody fragment that binds to and neutralizes all isoforms of VEGF, which has first received FDA approval for the treatment of macular edema due to both central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO). The objective of this review is to evaluate the evidence and discuss the rationale behind the recent suggestions that pharmacotherapy by Ranibizumab may be useful in the treatment of such circulatory disorder of retinal vein as a retinal vein occlusion (RVO). The available evidence suggests that repeated early frequent treatment of RVO with the anti-VEGF agent ranibizumab, gives the best chance of achieving and stabilizing both optimal anatomical and visual outcomes in the short to medium term. There is no standard protocol regarding the optimal timing of initial treatment with ranibizumab and subsequent retreatment is yet to be formulated. Where multiple injections are likely to be required, the effectiveness and safety over longer periods has yet to be determined. With more research and experience into exploring the frequency and safety of currently available agent- ranibizumab , it is also likely that clinicians would achieve the best protocol when dealing with patients sufferring from circulatory disorder of the retinal vein.*
***Keywords****: vascular endothelial growth factor inhibitors, ranibizumab, retinal vein, circulatory disorder, pharmacotherapy.*

## INTRODUCTION

Ranibizumab (Lucentis™; Genentech, South San Francisco, CA, USA) is a humanised antigen binding fragment of a murine full length monoclonal antibody directed against human vascular endothelial growth factor (VEGF). VEGF A. It is produced in Escherichia coli using recombinant DNA technology and has a molecular mass of 48 kD and a single antigen-binding site because it was derived by affinity maturation of a humanized Fab fragment of the original monoclonal anti-VEGF antibody. Ranibizumab binds all active isoforms of VEGF-A and is thus considered a non-selective VEGF-A inhibitor [1,2].

Vascular endothelial growth factor (VEGF) is best known as an endothelial growth and permeability factor [3]. It plays a major role in physiological vasculogenesis and angiogenesis in the embryo [4], and is involved in the formation of pathologic blood vessels, as well as in tumor growth and ocular diseases [5]. The concentration of VEGF is increased in all ocular diseases that involve neovascularization and/or inflammation, such as proliferative diabetic retinopathy [6], neovascular glaucoma[7,8], uveitis [9], and age-related macular degeneration [10]. In addition, VEGF is associated with fibrosis, and with inflammatory diseases such as rheumatoid arthritis [11] and Crohn's disease [12]. A recent study showed an association between VEGF and the healing of cutaneous wounds. Increased levels of VEGF induce scar formation in skin wounds by increasing vascularity and the deposition of collagen, while neutralization of VEGF reduces angiogenesis and cutaneous fibrosis [13].

Two high-affinity VEGF tyrosine kinase receptors have been identified: *fms*-like tyrosine kinase (Flt)-1 and kinase domain receptor (KDR). Both receptors are expressed predominantly in endothelial cells [14], but recently they have also been found in selected non-vascular cells [15-17]. VEGF stimulates inflammation by modulating Flt-1 signaling [11]. The expression of VEGF can be stimulated not only by hypoxia [18] but also by TGF-β [19]. Increased levels of TGF-β have been found in patients with a failing filtration system [20].

Vascular endothelial growth factor (VEGF) plays an important role in the pathophysiology of several light-threatening retinal disorders such as circulatory disorder of the retinal vein- retinal vein occlusion, age-related macular degeneration, diabetic macular edema and proliferative

diabetic retinopathy and contributes to increased permeability across both the blood-retinal and blood-brain barriers. Vascular endothelial growth factor synthesis has been studied in numerous tissues under a myriad of conditions, and although several stimulating factors have been identified, common biochemical pathways lead to VEGF synthesis and emanate from VEGF production. Within the posterior segment of the eye, VEGF is produced by retinal pigment epithelial cells, neurons, glial cells, endothelial cells, ganglion cells, Muller cells, and smooth muscle cells. Although VEGF affects all cells within the retina, its primary targets are vascular endothelial cells.

After 2 decades of extensive research into the VEGF families and receptors, specific molecules have been targeted for drug development, and several medications have received US Food and Drug Administration (FDA) approval. Ranibizumab have been developed specifically for intraocular use.

The objective of this review is to evaluate the evidence and discuss the rationale behind the recent suggestions that pharmacotherapy by Ranibizumab may be useful in the treatment of such circulatory disorder of retinal vein as a retinal vein occlusion (RVO).

## CIRCULATORY DISORDER OF THE RETINAL VEIN

Retinal vein occlusion is one of the most common circulatory disorder of the retinal, with no particular ethnic preference. The Beaver Dam Eye study found that the overall 15-year cumulative incidence of retinal vein occlusion (RVO) was 2.3% and associations with RVO were noted for age, glaucoma, higher serum creatinine/phosphorus levels, lower serum ionized calcium levels, evidence of retinal focal arteriolar narrowing and the use of barbiturates [21]. The Blue Mountains Eye study observed that the prevalence for each age-specific participant was as follows: 0.7% in individuals younger than 60 years of age; 1.2% in those aged between 60 and 69 years; 2.1% in those aged between 70 and 79 years of age; and 4.6% in those aged 80 years or over [22]. They found no significant sex difference in prevalence.

Depending on the location of the obstruction, the RVOs can be divided into central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO). In CRVO the obstruction is located in the central vein, at the level of the optic nerve, so most of the retina is affected. Anatomic features make the central retinal vein vulnerable to occlusion at this location. As the optic nerve and the accompanying central retinal artery and vein pass through the sieve-like connective tissue of the lamina cribrosa, the central retinal vein normally narrows, and the dense connective tissue of the lamina cribrosa limits any expansion of the traversing optic nerve and vessels within. Any thickening of the central retinal artery, which shares a common fibrous tissue sheath with the vein, might easily compress the lumen of the adjacent central retinal vein and start in motion the sequence of events that lead to thrombus formation [23]. In BRVO, the obstruction is located in one of the branches of the central vein, affecting only part of the posterior pole and the portion of the peripheral retina drained by occluded branch [24].

Macular edema is the main reason for decreased visual acuity in RVO. Macular edema is a common sight –threatening response of the retina. It involves the breakdown of the inner blood-retinal barrier due to a restriction of the flow of blood leaving the retina with increased pressure and consists of an abnormal vascular permeability resulting in fluid accumulation and macular thickening, detectable by optical coherence tomography (OCT). Recently the vitreous cavity has increasingly been used as a reservoir of drugs for the direct treatment of macular edema through intravitreal injection route.

## PHARMACOTHERAPY BY RANIBIZUMAB

The discovery of anti-angiogenics or vascular endothelial growth factor inhibitors (anti-VEGF) agents has revolutionized our treatment of eye diseases.

Application of vascular endothelial growth factor (VEGF) inhibitors represents a treatment option for macular edema secondary to RVO that targets the disease at the causal molecular level. Tissue hypoxia, due to either primary vascular occlusive disease or anaerobic tumor metabolism, is the most common driver of VEGF synthesis.

In retinal vein occlusions, VEGF levels are increased proportionate to the degree of retinal ischemia and severity of macular edema. Primary studies have reported favorable results using

ranibizumab[ 25,26]. Anti- VEGF drugs are potent agents for reduction of edema; they reduce vasopermeability but cannot eliminate the pathologic process completely.

Over the past years, ophthalmologists have attempted to treat RVO-associated edema triggered by hypoxia- induced expression of VEGF with ranibizumab (Lucentis). Ranibizumab is a humanized, affinity-matured VEGF antibody fragment that binds to and neutralizes all isoforms of VEGF.

Ranibizumab has first received FDA approval for the treatment of macular edema due to both CRVO and BRVO.

With ranibizumab, Pieramici et al.[27] designed a study following the scheme of the PIER Study, i.e. the first 3 injections monthly and then after 6 and 9 months, if needed (persistent macular edema). They found that ranibizumab is generally well tolerated and may improve best corrected visual acuity (BCVA) and decrease central retinal thickness (CRT) in optical coherence tomography (OCT). But the efficacy was lost after the loading phase, so an interval of 3 months between injections may be too long. In addition, Spaide et al. [28] and Rouvas et al. [29] demonstrated in two prospective studies that the patients with RVO have an improvement in VA, but with a mean of 7.4–8.5 injections in 1 year of follow-up.

Two phase III multicenter, prospective clinical trials assessing the safety, tolerability and efficacy of intravitreal ranibizumab injections in the treatment of macular edema secondary to BRVO and CRVO[30] were finished . They are called BRAVO (study of the efficacy and safety of ranibizumab injection compared with sham in patients with macular edema due to BRVO)[31] and CRUISE (study of the efficacy and safety of ranibizumab injection compared with sham in patients with macular edema due to CRVO) [32].

In the BRAVO study[31], 397 patients with macular edema following branch retinal vein occlusion (BRVO) were randomized to receive monthly intraocular injections of 0.3 mg (n = 134) or 0.5 mg (n = 131) of ranibizumab or sham injections (n = 132). Patients were eligible if they had foveal-involved macular edema from a BRVO occurring within 12 months of study entry, BCVA of 20/40 to 20/400, and CST ≥250 μm (Stratus OCT3). Exclusion criteria were the same as those in the CRUISE trial. Baseline characteristics were well balanced among the three groups; mean BCVA was 20/80, the mean time from diagnosis of BRVO was 3.5 months, and the mean CPT was 520 μm. Starting at month 3, patients were eligible for grid laser treatment if hemorrhages had cleared sufficiently to allow safe application of laser and the following criteria were met: Snellen equivalent BCVA ≤ 20/40 or mean center subfield thickness (CST) ≥ 250 μm, and compared with the visit 3 months before the current visit, the patient had a gain of <5 letters in BCVA or a decrease of <50 μm in mean CST. If rescue laser was not given at month 3, the same criteria were applied at month 4, and if rescue laser was not given at month 4, the criteria were applied at month 5.

At month 6, the primary endpoint, mean change from baseline BCVA letter score was 16.6 and 18.3 in the 0.3 mg and 0.5 mg ranibizumab groups and 7.3 in the sham group $P$ (< 0.0001). The percentage of patients who gained ≥ 15 letters in BCVA was 55.2% (0.3 mg) and 61.1% (0.5 mg) in the ranibizumab groups and 28.8% in the sham group ($P$ < 0.0001). The percentage of patients with a Snellen equivalent BCVA of 20/40 or better was 67.9% (0.3 mg) and 64.9% (0.5 mg) compared with 41.7% in the sham group ($P$ < 0.0001). The percentage of patients with a Snellen equivalent BCVA of 20/200 or worse was 1.5% (0.3 mg) and 0.8% (0.5 mg) compared with 9.1% in the sham group ($P$ < 0.01). Based upon the NEI VFQ-25 survey, patients who received ranibizumab felt they had greater improvement (improvement from baseline in NEI VFQ score: 9.3, 0.3 mg; 10.4, 0.5 mg: 5.4, sham). There was greater reduction of macular edema in the ranibizumab groups because CPT was reduced by 337.3 μm (0.3 mg) and 345.2 μm (0.5 mg) compared to 157.7 μm in the sham group. The percentage of patients with CPT ≤ 250 μm at month 6 was 91% (0.3 mg), 84.7% (0.5 mg), and 45.5% (sham, $P$ < 0.0001). More patients in the sham group (54.5%) received rescue grid laser therapy than in the 0.3 mg (18.7%) or 0.5 mg (19.8%) ranibizumab groups. There were no safety signals identified in either trial.

In the CRUISE Study [32], 392 patients with macular edema following central retinal vein occlusion (CRVO) were randomized to receive monthly intraocular injections of 0.3 mg (n = 132) or 0.5 mg (n = 130) of ranibizumab or sham injections (n = 130).

Patients were eligible if they had foveal-involved macular edema from a CRVO occurring within 12 months of study entry, BCVA of 20/40 to 20/320, and center subfield thickness (CST) ≥250 μm

(Stratus OCT3). Patients were excluded if they had a brisk afferent pupil defect, had scatter laser photocoagulation within 3 months, an intraocular injection of steroid or a VEGF antagonist within 3 months, or had an improvement of ≥10 ETDRS letters in BCVA between screening and baseline.
Baseline characteristics were well balanced among the three groups; the mean age was 68 years, mean BCVA was 20/100, the mean time from diagnosis of CRVO was 3.3 months, and the mean center point thickness (CPT) was 685 μm. At 6 months, the primary endpoint, mean change from baseline BCVA letter score was 12.7 and 14.9 in the 0.3 mg and 0.5 mg ranibizumab groups and 0.8 in the sham group ($P < 0.0001$). The percentage of patients who gained ≥ 15 letters in BCVA was 46.2% (0.3 mg) and 47.7% (0.5 mg) in the ranibizumab groups and 16.9% in the sham group ($P < 0.0001$). The percentage of patients with a Snellen equivalent BCVA of 20/40 or better was 43.9% (0.3 mg) and 46.9% (0.5 mg) compared with 20.8% in the sham group ($P < 0.0001$). The percentage of patients with a Snellen equivalent BCVA of 20/200 or worse was 15.2% (0.3 mg) and 11.5% (0.5 mg) compared with 27.7% in the sham group ($P < 0.005$). Based upon the 25-item National Eye Institute Visual Function Questionnaire NEI VFQ-25 survey, patients who received ranibizumab felt they had greater improvement (improvement from baseline in NEI VFQ score: 7.1, 0.3 mg; 6.2, 0.5 mg: 2.8, sham) [64]. There was greater reduction of macular edema in the ranibizumab groups because CPT was reduced by 433.7 μm (0.3 mg) and 452.3 μm (0.5 mg) compared to 167.7 μm in the sham group. The percentage of patients with CPT ≤ 250 μm at 6 months was 75.0% (0.3 mg), 76.9% (0.5 mg), and 23.1% (sham, $P < 0.0001$). This study demonstrated that six sessions of monthly injections of 0.3 mg or 0.5 mg reduced macular edema and provided substantial visual benefit in patients with CRVO.
After the primary endpoint in the CRUISE and BRAVO trials, patients were evaluated every month and if study eye Snellen equivalent BCVA was ≤20/40 or mean CST was ≥250 μm, they received an injection of ranibizumab; patients in the ranibizumab groups received their assigned dose and patients in the sham group received 0.5 mg. In patients with CRVO, the mean number of ranibizumab injections during the observation period was 3.9, 3.6, and 4.2 in the 0.3 mg, 0.5 mg, and sham/0.5 mg groups; and the percentage of patients that did not receive any injections during the observation period was 7.0, 6.7, and 4.3, respectively [34]. At month 12 in the ranibizumab groups, the improvement from baseline in ETDRS letter score was 13.9, very similar to the month 6 results, indicating that vision is well maintained when injections are given only if there is recurrent or residual macular edema. Patients in the sham group showed substantial improvement during the observation period when they were able to receive ranibizumab; improvement from baseline in letter score was 0.8 at month 6 and 7.3 at month 12. The percentage of patients who had an improvement from baseline BCVA letter score ≥ 15 at month 12 was 47.0% (0.3 mg) and 50.8% (0.5 mg) in the ranibizumab groups, almost identical to the month 6 results. In the sham group, 33.1% of patients improved from baseline ≥ 15 in letter score at month 12 compared to 16.9% at month 6. At month 12, 43% of patients in the two ranibizumab groups had a Snellen equivalent BCVA of 20/40 compared to 35% in the sham/0.5 mg group.
In patients with BRVO, the mean number of ranibizumab injections during the observation period was 2.9, 2.8, and 3.8 in the 0.3 mg, 0.5 mg, and sham/0.5 mg groups; and the percentage of patients that did not receive any injections during the observation period was 17.2, 20.0, and 6.5, respectively [35]. At month 12 in the ranibizumab groups, the improvement from baseline in ETDRS letter score was 16.4 (0.3 mg) and 18.3 (0.5 mg), very similar to the month 6 results, indicating that vision is well maintained when injections are given only if there is recurrent or residual macular edema. Patients in the sham group showed substantial improvement during the observation period when they were able to receive ranibizumab; improvement from baseline in letter score was 7.3 at month 6 and 12.1 at month 12. The percentage of patients who had an improvement from baseline BCVA letter score ≥ 15 at month 12 was 55.2% (0.3 mg) and 61.1% (0.5 mg) in the ranibizumab groups, almost identical to the month 6 results. In the sham group, 43.9% of patients improved from baseline ≥ 15 in letter score at month 12 compared to 28.8% at month 6. At month 12, 67.9% (0.3 mg) and 64.4% (0.5 mg) of patients in the ranibizumab groups had a Snellen equivalent BCVA of 20/40 compared to 56.8% in the sham/0.5 mg group. Thus, in both CRUISE and BRAVO, patients in the sham groups showed a substantial improvement in vision during the second 6 months when they were able to receive ranibizumab as needed, but their

vision at month 12 was not as good as that in patients in the ranibizumab groups. This raises a question as to whether delay in treatment carries a visual penalty.

The results from open-label extension trial of the 12-month Ranibizumab assessing long-term safety and efficacy in BRAVO and CRUISE trials [36] evidenced that in patients who completed month 12, the mean number of injections (excluding month 12 injection) in the sham/0.5-, 0.3/0.5-, and 0.5-mg groups was 2.0, 2.4, and 2.1 (branch RVO) and 2.9, 3.8, and 3.5 (central RVO), respectively. The incidence of study eye ocular serious adverse events and systemic adverse events potentially related to systemic vascular endothelial growth factor inhibition across treatment arms was 2% to 9% and 1% to 6%, respectively. The mean change from baseline BCVA letter score at month 12 in branch RVO patients was 0.9 (sham/0.5 mg), -2.3 (0.3/0.5 mg), and -0.7 (0.5 mg), respectively. The mean change from baseline BCVA at month 12 in central RVO patients was -4.2 (sham/0.5 mg), -5.2 (0.3/0.5 mg), and -4.1 (0.5 mg), respectively. The authors concluded that no new safety events were identified with long-term use of ranibizumab; rates of systemic adverse events potentially related to treatment were consistent with prior ranibizumab trials. Reduced follow-up and fewer ranibizumab injections in the second year of treatment were associated with a decline in vision in central RVO patients, but vision in branch RVO patients remained stable. Results suggest that during the second year of ranibizumab treatment of RVO patients, follow-up and injections should be individualized and, on average, central RVO patients may require more frequent follow-up than every 3 months.

In addition, the subanalyses in BRAVO and CRUISE study [37-40] generally confirmed that patients with BRVO or CRVO who were younger or who had worse vision and greater retinal thickness at baseline fared better. Patients with BRVO fared better if time from diagnosis to treatment was less than 3 months. Patients with CRVO had similar results regardless of time to treatment.

In general, then, in BRVO, patients who needed fewer therapies, such as laser or other previous treatments, probably had milder RVO requiring less treatment. Patients who were younger did better than those who were older. And patients with CRVO had a more unpredictable course than those with BRVO, and therefore warrant even closer observation than those with BRVO [41].

The worldwide use of intravitreal application of anti-vascular growth factor and the realisation that regular applications over long periods of time are necessary to maintain vision in these eyes, has revealed the problem of tolerance/tachyphylaxy [42]. In 2007, the paper suggested for the first time possible tachyphylaxis/tolerance with chronic ranibizumab [43]. Binder S. [42] recommended different options to prevent tachyphylaxis/tolerance: (1) to increase the dosage or shorten treatment intervals if tolerance has developed; (2) to pause treatment if tachyphylaxis has occurred; (3) to combine drugs with different modes of action; or (4) to switch to a similar drug with different properties (bevacizumab and ranibizumab differ in molecular size, affinity and absorption).

## POTENTIAL HAZARDS OF ANTI-VEGF THERAPY

### Local adverse effects

Intravitreal injections of various agents have been studied extensively [44]. The overall risk of complications is low when the injection is administered by experienced ophthalmologists [44]. Known risks of intravitreal injections can be vision threatening and require prompt diagnosis and treatment, possibly surgical intervention. The most serious but rarely occurring injection-related complications include acute-onset endophthalmitis,[45-47] pseudo-endophthalmitis, cataract development/progression, retinal detachment, and hemorrhage [44] . The latest study [47] revealed that endophthalmitis following intravitreal injection is associated with an increased incidence of Streptococcus spp. infection, earlier presentation and poorer visual outcomes when compared with endophthalmitis following cataract surgery. Irigoyen et al. [48] concluded that the overall numbers of patients with endophthalmitis following intravitreal injections has risen dramatically over the past years. In contrast to earlier reports of multicentre studies, outcome of patients is relatively poor in the current treatment settings [ 48, 49].

The preparation of the intravitreal injection site with topical povidone-iodine is the preferred prophylactic method to minimize the risk of endophthalmitis. There is no need for topical antibiotic use after intravitreal injection [46].Additional infrequent complications include

hypotony, angle closure, hemiretinal vein occlusion, retinal pigment epithelial tears, iritis/uveitis, optic disc atrophy, corneal epitheliopathy, maculopathy, and anaphylactic reaction to the agent injected in the vitreous.[44,50]. A 2006 national survey in USA Complications reported following complications rate associated with intravitreal injections: endophthalmitis - 31%, increased IOP - 26%, cataract - 11%,other - 16% [51]. The most important adverse local effects related to anti-VEGF agents include uveitis, retinal detachment and cataracts.

In conclusion, the overall risk of complications is low when the injection is administered by experienced ophthalmologists [44].

**Systemic adverse effects**

While used intravitreally, the systemic absorption is minimal, however, a trend has been observed towards a higher risk of stroke among patients with a history of heart disease [53].

There is some evidence that intravitreal anti-VEGF injections may result in systemic absorption, with the potential for injury in organs that are reliant on VEGF, such as the kidney. Pellé et al.[54] reported the first case of a patient who developed an acute decrease in kidney function, nonimmune microangiopathic hemolytic anemia with schistocytes, and thrombocytopenia after 4 intravitreal injections of ranibizumab. Light microscopy of a kidney biopsy specimen showed segmental duplications of glomerular basement membranes with endothelial swelling and several recanalized arteriolar thrombi. Because of the increasing use of intravitreal anti-VEGF agents, ophthalmologists and nephrologists should be aware of the associated risk of kidney disease. Early detection is crucial so that intravitreal injections can be stopped before severe kidney disease occurs. In Sorenson and Sheibani [55] opinion perhaps baseline and renal function during treatment (serum creatinine and urinary protein levels, blood pressure) should be carefully monitored to ensure that the improved visual acuity is not at the expense of renal function.

Patients should discuss the potential risks and benefits of intravitreal pharmacotherapy with their physicians before receiving treatment.

In conclusion, major concerns with anti-VEGF therapy for ocular diseases include: repeat intravitreal injections; risk of cardiovascular complications; possible retinal and neural toxicity due to cumulative dosing; interference with physiologic functions of VEGF; and economic and cost-effectiveness concerns.

## CONCLUSION

The available evidence suggests that repeated early frequent treatment of RVO with the anti-VEGF agent ranibizumab, gives the best chance of achieving and stabilizing both optimal anatomical and visual outcomes in the short to medium term. There is no standard protocol regarding the optimal timing of initial treatment with ranibizumab and subsequent retreatment is yet to be formulated. The general approach from various studies suggests the initial loading dose of one injection per month for the first 3 months. The patients are then reviewed once a month and reinjections are indicated based on anatomical response. Where multiple injections are likely to be required, the effectiveness and safety over longer periods has yet to be determined. With more research and experience into exploring the frequency and safety of currently available agent- ranibizumab , it is also likely that clinicians would achieve the best protocol when dealing with patients sufferring from circulatory disorder of the retinal vein.

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