

Pharmacovigilance in Intraocular Antiangiogenic Therapy

Marianne L. Shahsuvaryan

Marianne L. Shahsuvaryan, Department of Ophthalmology, Yerevan State Medical University, Yerevan, Republic of Armenia

Correspondence to: Marianne L. Shahsuvaryan, Department of Ophthalmology, Yerevan State Medical University, 7Ap, 1Entr, 26 Sayat-Nova Avenue, Yerevan, 0001, Republic of Armenia

Email: mar_shah@hotmail.com

Telephone: +374-10523 468

Received: March 26, 2015

Revised: May 13, 2015

Accepted: May 18, 2015

Published online: June 1, 2015

ABSTRACT

Context: Currently several antiangiogenic agents are being widely and successfully used for the treatment of eye diseases like neovascular macular degeneration, retinal vein occlusion and diabetic macular edema. The discovery of anti-VEGF (Vascular endothelial growth factor) agents has revolutionized the treatment of these conditions. Ophthalmology has witnessed an explosion in the number of intravitreal injections delivered to patients over the past 10 years, driven in large part by the introduction and rapid incorporation of therapy with anti-VEGF agents.

Objective: Taking into consideration that VEGF plays an important role not only in the pathophysiology of several sight-threatening retinal disorders such as age-related macular degeneration, retinal vein occlusion, diabetic macular edema and proliferative diabetic retinopathy, but also in the physiology of the eye, and that more data are becoming available in intraocular antiangiogenic therapy, chemical compound-related ocular side effects across multiple antiangiogenic agents should be analyzed critically.

Methods: A comprehensive literature search was conducted on Medline, PubMed, and Google Scholar databases in February 2015. Search temporal limits included articles published from 2005 to 2015 with the purpose of providing the most recent evidence. Studies were queried using the following keywords in various combinations: antiangiogenics in eye diseases, intravitreal pharmacotherapy by anti-VEGF, adverse effects, potential ocular hazards, bevacizumab,

pegaptanib sodium, ranibizumab, aflibercept. The articles of high or medium clinical relevance were selected for review.

Results: Almost uniformly all trial evaluating ocular safety of antiangiogenic agents reveal the serious side effects including geographic atrophy, pigment epithelial tear, ocular hemorrhage despite the fact that the incidence is low. Ocular safety concern in intraocular pharmacotherapy by anti-angiogenic agents has a strong body of clinical evidence, resulting in plenty of peer reviewed clinical articles.

Conclusion: Currently available findings obviate the need to raise awareness about chemical compound-related ocular side effects in patients with eye diseases treated by antiangiogenic agents. Hopefully that noninvasive therapeutic options will become available soon.

© 2015 ACT. All rights reserved.

Key words: Pegaptanib sodium; Ranibizumab; Aflibercept; Bevacizumab; Intravitreal injections; Ocular side effects.

Shahsuvaryan ML. Pharmacovigilance in Intraocular Antiangiogenic Therapy. *International Journal of Ophthalmic Research* 2015; 1(1): 5-10 Available from: URL: <http://www.ghrnet.org/index.php/ijor/article/view/1158>

INTRODUCTION

Vascular endothelial growth factor (VEGF) plays an important role not only in the pathophysiology of several light-threatening retinal disorders such as age-related macular degeneration, retinal vein occlusion, diabetic macular edema and proliferative diabetic retinopathy contributing to increased permeability across both the blood-retinal and blood-brain barrier, but also in physiology of the eye^[1]. 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. VEGF appears to be essential for development and maintenance of functionally efficient retinal vasculature as well as for integrity of the

retinal pigment epithelium (RPE), Bruch's membrane and choroidal endothelial cells^[2]. Research conducted by Saint-Geniez *et al*^[3] revealed an important role for endogenous VEGF in the maintenance and function of adult retina neuronal cells. Although VEGF affects all cells within the retina, its primary targets are vascular endothelial cells.

The discovery of anti-VEGF agents has revolutionized the treatment of aforementioned retinal diseases. Ophthalmology has witnessed an explosion in the number of intravitreal injections delivered to patients over the past 10 years, driven in large part by the introduction and rapid incorporation of therapy with anti-VEGF agents.

The term antiangiogenic therapy was born more than 35 years ago by J. Folkman, who hypothesized that cancer may be treated by abolishing the nutrients and oxygen-providing blood vessels^[4] by agents that could block the angiogenic cascade. Monoclonal antibodies against VEGF were first developed as an intravenous treatment for metastatic colorectal cancer^[5,6].

Consequently, several antiangiogenics have been developed for the treatment of sight-threatening retinal diseases, including neovascular age-related macular degeneration, retinal vein occlusions and diabetic macular edema in diabetic retinopathy.

There are 4 anti-VEGF agents that are either approved or in common use in ophthalmology, namely pegaptanib (Macugen, Pfizer), ranibizumab (Lucentis, Novartis), aflibercept or VEGF Trap-Eye (EYLEA, Bayer) and bevacizumab (Avastin, Roche).

Pegaptanib

Pegaptanib is a selective VEGF inhibitor, targeting only one isoform of the VEGF molecule, leaving other isoforms unaffected^[7]. In 2004, pegaptanib (Macugen (Pfizer and OSI/Eyetech Pharmaceuticals, Inc.) was the first anti-VEGF agent to receive FDA approval for the treatment of neovascular age-related macular degeneration (AMD). The use of pegaptanib has declined with the release of newer anti-VEGF agents, such as ranibizumab (Lucentis™, Genentech, Inc., South San Francisco, CA, and Novartis Pharma AG, Basel, Switzerland), aflibercept (VEGF-trap eye, Eylea™, Regeneron Pharmaceuticals, Inc., and Bayer Pharma AG, Berlin, Germany) and bevacizumab (Avastin™, Genentech, Inc., South San Francisco, CA, and Roche, Basel, Switzerland).

Ranibizumab

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 A. Ranibizumab binds all active isoforms of VEGF-A and is thus considered a non-selective VEGF-A inhibitor^[8].

Aflibercept

Aflibercept or VEGF Trap-Eye (EYLEA, Bayer) is a fully human fusion protein, consisting of soluble VEGF receptors 1 and 2, that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Aflibercept acts as a decoy receptor binding-free VEGF^[9].

Bevacizumab

Bevacizumab (Avastin, Roche), is a full-length, humanized monoclonal antibody directed against all the biologically active isoforms of vascular endothelial growth factor (VEGF) –A.

Bevacizumab binds to the receptor-binding domain of all VEGF-A isoforms^[10].

Bevacizumab is FDA-approved for the treatment of colorectal cancer. However, because the agent costs substantially less per dose than ranibizumab, it has been widely used off-label since 2004 to treat several retinal diseases.

Currently more data are becoming available due to wide antiangiogenic agents use for the treatment of eye diseases, and safety data review for intravitreal injection of common anti-VEGF agents was conducted^[11].

VEGF plays an important role in adult homeostasis. It is essential for postnatal angiogenesis and other non- angiogenic functions, such as the modulation of tissue repair and organ regeneration, vascular homeostasis by regulating key vascular proteins^[12,13], and inflammation.

Taking into consideration that VEGF plays an important role in the physiology of the eye, ocular safety data across multiple antiangiogenic agents should be analyzed critically and chemical compound-related ocular side effects will be highlighted.

CHEMICAL COMPOUND-RELATED OCULAR SIDE EFFECTS

Clinically proved effects

Geographic atrophy: Geographic atrophy (GA) occurs with natural history of AMD, which marks progression of disease, but on the other hand according to the results of the MARINA trial^[14] and further in the CATT trial^[15], it was revealed that ranibizumab causes deterioration of vision in treated patients. When ranibizumab and bevacizumab have been compared at two years^[15] researchers evidenced more prominent harmful effect on retinal pigment epithelium cells in case of ranibizumab.

In the CATT trial^[15,16] it has been shown that frequency of geographic atrophy was higher in monthly treated patients subgroup. Evidences from clinical trials evaluating effectiveness of different antiangiogenic agents-Ranibizumab and bevacizumab in AMD therapy suggest significantly increased frequency of developing geographic atrophy in the range of 20-29% of cases, specifically 20% in the CATT Trial^[15], and 28% and 29% in IVAN and HARBOR trials respectively^[17-19].

Evaluating impact of treatment schedule in CATT^[15] and IVAN^[17] trials it has been demonstrated that monthly injections predispose to geographic atrophy comparing to as-needed (pro re nata-PRN). The HARBOR trial asseccing the risk factors for geographic atrophy^[18,19] indicated that absence of subretinal fluid in the study eye and atrophic changes in the fellow eye could serve as a predictors for aforementioned complication.

The latest findings by Schütze *et al*^[20] evidenced that in neovascular AMD progressive RPE atrophy and GA developed in the majority of eyes treated by ranibizumab over two years.

Retinal pigment epithelial tear

Tears of the retinal pigment epithelium occur with natural history of AMD associated with choroidal neovascularization, but at the same time could be a consequence of thermal laser therapy, photodynamic therapy and intraocular pharmacotherapy by antiangiogenics^[21].

The general consensus is that retinal pigment epithelium tear is the ocular side effect of intraocular antiangiogenic pharmacotherapy and is not related with any specific anti-VEGF drug^[22-24].

The latest findings by Leon *et al*^[25] evidenced that in 40% of AMD cases the RPE tears occurred within two years of anti-VEGF

injections. In 86.6% of cases the RPE tear was associated with pigment epithelial detachment (PED), and in 46.6% the RPE tear occurred in the central area of the retina and involved the fovea. In all cases visual acuity decreased at the end of the follow-up period (24 weeks) independently of the type or the topographical location of the lesion.

In conclusion, though RPE tears can occur spontaneously with natural history alone, the risk of RPE tears with antiangiogenic therapy is enhanced by preexisting large and high pigment epithelial detachments.

Ocular hemorrhage

Few studies have focused on ocular hemorrhage following the use of intravitreal injections of antiangiogenic agents^[26-30]. Based on Ladas *et al*^[26] findings subconjunctival hemorrhage has been documented approximately in 10% of injections recipients, and was more common in patients simultaneously receiving aspirin^[26]. For bevacizumab it has been documented a case of a massive choroidal detachment/hemorrhage following intravitreal injection^[27]. According to the reports of Karagiannis *et al*^[28] and Modarres *et al*^[29] massive subretinal hemorrhage has been evidenced after switching from bevacizumab to ranibizumab and only in bevacizumab injections^[28,29].

The latest research conducted by Azar *et al*^[30] also revealed that macular hematoma may follow intravitreal anti-VEGF injection for exudative AMD with large occult neovascularization, especially if a large RPE tear is found. In contrast to earlier study^[25] in authors' opinion the occurrence does not seem to be linked to anticoagulation treatment or the presence of vitreomacular traction.

The long-term data from the randomized, double-blind study^[31] evaluating the effectiveness and safety profile of the latest anti-VEGF agent aflibercept in treatment of macular edema secondary to central retinal vein occlusion (CRVO) also evidenced that the most frequent ocular serious adverse event from baseline to week 100 was vitreous hemorrhage.

IMPACT ON OCULAR BLOOD FLOW

Anti-VEGF therapy may therefore have adverse effects on ocular blood flow. Von Hanno *et al*^[32] presented two cases of retinal artery occlusion after intravitreal injection of bevacizumab (Avastin) and ranibizumab (Lucentis) respectively and concluded that the therapeutic principle may be associated with an increased risk of retinal arterial occlusions.

Increased risk of ocular blood flow disturbances was noted specifically in case of bevacizumab injections—retinal venous occlusions^[33], anterior ischemic optic neuropathy^[34,35] development or exacerbation of ocular ischemic syndrome^[36], decrease in retrobulbar blood flow^[37].

Leung *et al*^[38] presented a series of three patients of the nearly 200 patients with CRVO who suffered apparent macular infarction within weeks of intravitreal administration of bevacizumab. The authors stated that this has not been described in the natural history of the disease and is associated with poor visual outcomes.

Few studies have focused on macular ischemia, after intravitreal injection of anti-VEGF agents^[39,40].

In Manousaridis and Talk^[40] opinion worsening of macular ischemia in the long term cannot be definitely excluded, particularly in eyes with significant ischaemia at baseline and after repeated intraocular anti-VEGF injections. The decision to offer prolonged anti-VEGF treatment in cases of significant coexisting macular ischaemia should not be based only on measurements of macular

thickness; instead repeat fluorescein angiograms should be performed.

For bevacizumab in CRVO also it has been demonstrated that ischemic retinal injury may be severe adverse vascular reaction^[41].

In conclusion, available findings demonstrated significant impact of antiangiogenics on ocular blood flow.

Tractional retinal detachment

Few studies have focused on the development or progression of tractional retinal detachment after intravitreal injection of bevacizumab before vitrectomy for advanced proliferative diabetic retinopathy^[42-44].

It is recognized that intravitreal injection of anti-VEGF agents should be used with extreme caution in extensive neovascularization secondary to diabetic retinopathy or retinal vein occlusion, and the patient should be followed closely for the possible need for vitrectomy.

The general consensus is that ocular complications are higher in patients treated by anti-VEGF agents^[11,45].

IMPACT ON LONG-TERM EFFICACY

Tachyphylaxis/tolerance

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/tachyphylaxis^[46]. In 2008, two papers suggested for the first time possible tachyphylaxis/tolerance with chronic ranibizumab^[47] and bevacizumab treatment^[48]. Binder S.^[46] 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).

EXPERIMENTALLY PROVED EFFECTS

Impact on retinal pigment epithelium

Malik *et al*^[49] in the experimental study evaluated the safety profiles of antiangiogenics on human retinal pigment epithelium cells. The researchers concluded that bevacizumab and ziv-aflibercept showed mild mitochondrial toxicity at clinically relevant doses, in contrast to ranibizumab.

Impact on photoreceptors and choriocapillaris

Avci *et al*^[50] obviated a significant increase in apoptotic activity in rabbit photoreceptor cells after bevacizumab and pegaptanib sodium injections.

Kurihara *et al*^[51,52] in the experimental study revealed an essential role of VEGF-A in regulating choroid vasculature and cone photoreceptors, responsible for central and color vision and evidenced that VEGF inhibition causes damage and loss of endothelial cells of the choriocapillaries and severe vision loss secondary to cone cell death in mice.

Sene *et al*^[53] also emphasized the critical role of chronic VEGF suppression as a cause of the loss of pro-survival and neurotrophic capacities of VEGF ending by vision loss.

Multiple experimental studies were conducted on rat^[54], rabbit^[55] and primate eye^[56] to evaluate bevacizumab impact on choriocapillaris.

The general consensus^[54-57] is that intravitreal injection of

bevacizumab causes a loss of choriocapillaris endothelial fenestrations and therefore VEGF is required for the maintenance of the choroidal homeostasis^[57].

Impact on ciliary body

The role of VEGF-A in ciliary body homeostasis was explored by Ford *et al*^[58] in the experimental study.

Authors discovered that inhibiting anti-VEGF might have a harmful effect on the ciliary body, as a tissue responsible for producing aqueous humor, by degeneration of capillary beds, particularly fenestrated capillaries found in the ciliary body. Sugimoto *et al*^[55] reconfirmed by electron microscopy decrease of endothelial cell fenestrations in the choriocapillaris and ciliary body after intravitreal injections of bevacizumab.

CONCLUSION

The discovery of anti-VEGF agents has revolutionized the treatment of neovascular macular degeneration, retinal vein occlusion and diabetic macular edema. Ophthalmology has witnessed an explosion in the number of intravitreal injections delivered to patients over the past 10 years, driven by the introduction and rapid incorporation of therapy with antiangiogenics. At the same time taking into consideration that VEGF plays an important role not only in the pathophysiology of several sight-threatening retinal disorders, but also in the physiology of the eye, there is a growing body of evidences on ocular side effects in intraocular antiangiogenic therapy. Currently available findings obviate the need to raise awareness about chemical compound-related ocular side effects in patients with eye diseases treated by antiangiogenic agents. Hopefully that noninvasive therapeutic options will become available soon.

CONFLICT OF INTERESTS

The author has no conflicts of interest to declare.

REFERENCES

1. Quaggin SE. Turning a blind eye to anti-VEGF toxicities. *J Clin Invest* 2012; 122(11): 3849–3851. Published online 2012 Oct 24. doi: 10.1172/JCI65509 PMID: PMC3498935
2. Homayouni M. Vascular Endothelial Growth Factors and Their Inhibitors in Ocular Neovascular Disorders. *J Ophthalmic Vis Res.* 2009; 4(2): 105–114.
3. Saint-Geniez M, Maharaj ASR, Walshe TE, Tucker BA, Sekiyama E, Kurihara T, Darland DC, Young MJ, D'Amore P. Endogenous VEGF Is Required For Visual Function: Evidence for a Survival Role on Müller Cells and Photoreceptors. *PLoS ONE* 2008; 3(11): e3554. doi:10.1371/journal.pone.0003554
4. Stewart MW. The expanding role of vascular endothelial growth factor inhibitors in ophthalmology. *Mayo Clinic Proceedings*, January 1, 2012
5. Homsí J, Daud AI. Spectrum of activity and mechanism of action of VEGF/PDGF inhibitors. *Cancer Control.* 2007;14(3), 285–294
6. Los M, Roodhart JM, Voest EE. Target practice: lessons from Phase III trials with bevacizumab and vatalanib in the treatment of advanced colorectal cancer. *Oncologist* 2007;12(4), 443–450
7. Singerman LJ, Masonson H, Patel M, Adamis AP, Buggage R, Cunningham E, Goldbaum M, Katz B, Guyer D. Pegaptanib sodium for neovascular age-related macular degeneration: third-year safety results of the VEGF Inhibition Study in Ocular Neovascularisation (VISION) trial. *Br J Ophthalmol* 2008; 92(12):1606-11. doi: 10.1136/bjo.2007.132597. Epub 2008 Jul 9.
8. Rosenfeld PJ, Schwartz SD, Blumenkranz MS, Miller JW, Haller JA, Reimann JD. Maximum tolerated dose of a humanized anti-vascular endothelial growth factor antibody fragment for treating neovascular age-related macular degeneration. *Ophthalmology* 2005; 112: 1048–62.
9. Pieramici DJ, Rabena MD. Anti-VEGF therapy: comparison of current and future agents. *Eye* 2008;22:1330–36.
10. Ferrara N, Hillan KJ, Nowotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. *Biochem Biophys Res Commun.* 2005;333:328–35.
11. Falavarjani KJ, Nguyen QD. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. *Eye* 2013; 27(7): 787–94. Published online 2013 May 31. doi: 10.1038/eye.2013.107 MCID: PMC3709385
12. Luo J, Xiong Y, Han X, Lu Y. VEGF non-angiogenic functions in adult organ homeostasis: therapeutic implications. *J Mol Med (Berl)* 2011;89(7):635-45. doi: 10.1007/s00109-011-0739-1. Epub 2011 Mar 2
13. Guangqi EG, Cao Y, Bhattacharya S, Dutta S, Wang E, Mukhopadhyay D. Endogenous vascular endothelial growth factor-A (VEGF-A) maintains endothelial cell homeostasis by regulating VEGF receptor-2 transcription. *J Biol Chem* 2012;287(5):3029-41. doi: 10.1074/jbc.M111.293985. Epub 2011 Dec 13.
14. Rosenfeld PJ, Shapiro H, Tuomi L, Webster M, Elledge J, Blodi. MARINA and ANCHOR Study Groups. Characteristics of patients losing vision after 2 years of monthly dosing in the phase III ranibizumab clinical trials. *Ophthalmology* 2011;118:523-30.
15. Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group. Martin DF, Maguire MG, Fine SL, Ying GS, Jaffe GJ, Grunwald JE, Toth C, Redford M, Ferris FL., 3rd Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology* 2012;119(7):1388–98.
16. CATT Research Group. Ranibizumab and Bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 2011;364:1897–908.
17. IVAN Study Investigators. Chakravarthy U, Harding SP, Rogers CA, Downes SM, Lotery AJ, Wordsworth S, Reeves BC. Ranibizumab versus bevacizumab to treat neovascular age-related macular degeneration: one-year findings from the IVAN randomized trial. *Ophthalmology* 2012;119(7):1399–1411.
18. Busbee BG, Ho AC, Brown DM, Heier JS, Suñer IJ, Li Z, Rubio RG, Lai P; HARBOR Study Group. Twelve-month efficacy and safety of 0.5 mg or 2.0 mg ranibizumab in patients with subfoveal neovascular age-related macular degeneration. *Ophthalmology.* 2013;120(5):1046-56. doi: 10.1016/j.ophtha.2012.10.014. Epub 2013 Jan 23
19. Ho AC, Busbee BG, Regillo CD, Wieland MR, Van Everen SA, Li Z, Rubio RG, Lai P. HARBOR Study Group. Twenty-four-month efficacy and safety of 0.5 mg or 2.0 mg ranibizumab in patients with subfoveal neovascular age-related macular degeneration. *Ophthalmology* 2014;121(11):2181-92. doi: 10.1016/j.ophtha.2014.05.009. Epub 2014 Jul 9.
20. Schütze C, Wedl M, Baumann B, Pircher M, Hitzenberger CK, Schmidt-Erfurth U. Progression of retinal pigment epithelial atrophy in antiangiogenic therapy of neovascular age-related macular degeneration. *Am J Ophthalmol.* 2015 Mar 10. pii: S0002-9394(15)00109-9. doi: 10.1016/j.ajo.2015.02.020. [Epub ahead of print]
21. Cunningham ET Jr, Feiner L, Chung C, Tuomi L, Ehrlich JS. Incidence of retinal pigment epithelial tears after intravitreal ranibizumab injection for neovascular age-related macular degeneration. *Ophthalmology* 2011;118:2447-52.
22. Chang LK, Flaxel CJ, Lauer AK, Sarraf D. RPE tears after pegaptanib treatment in age-related macular degeneration. *Retina.* 2007;27:857–63.
23. Shaikh S, Olson JC, Richmond PP. Retinal pigment epithelial tears after intravitreal bevacizumab injection for exudative age-related

- macular degeneration. *Indian J Ophthalmol.* 2007;55:470–2.
24. Smith BT, Kraus CL, Apte RS. Retinal pigment epithelial tears in ranibizumab-treated eyes. *Retina.* 2009;29:335–9.
 25. Leon EP, Saviano S, Zanei A, Pastore MR, Guaglione E, Mangogna A, Tognetto D. Spontaneous or secondary to intravitreal injections of anti-angiogenic agents retinal pigment epithelial tears in age-related macular degeneration. *Int J Ophthalmol* 2014;7(4):681–85. doi:10.3980/j.issn.2222-3959.2014.04.18
 26. Ladas ID, Karagiannis DA, Rouvas AA, Kotsolis AI, Liotsou A, Vergados I. Safety of repeat intravitreal injections of bevacizumab versus ranibizumab: our experience after 2,000 injections. *Retina* 2009;29 (3):313–38.
 27. Brouzas D, Koutsandrea C, Moschos M, Papadimitriou S, Ladas I, Apostolopoulos M. Massive choroidal hemorrhage after intravitreal administration of bevacizumab (Avastin) for AMD followed by contralateral sympathetic ophthalmia. *Clin Ophthalmol* 2009;3:457–59.
 28. Karagiannis DA, Mitropoulos P, Ladas ID. Large subretinal haemorrhage following change from intravitreal bevacizumab to ranibizumab. *Ophthalmologica* 2009;223 (4):279–82.
 29. Modarres M, Naseripour M, Falavarjani KG, Nikeghbali A, Hashemi M, Parvaresh MM. Intravitreal injection of 2.5 mg vs 1.25 mg bevacizumab (avastin) for treatment of CNV associated with AMD. *Retina* 2009;29:319–324.
 30. Azar G, Mauguet-Fayssse M, Nyouma J, Cohen SY, Coscas F, Français-Maury C, Souied E, Soubrane G. Occurrence of macular hematoma after ranibizumab treatment for age-related macular degeneration. *Eur J Ophthalmol* 2015; 25(2): 163 - 167 DOI:10.5301/ejo.5000529
 31. Heier JS, Clark WL, Boyer DS, Brown DM, Vitti R, Berliner AJ, Kazmi H, Ma Y, Stemper B, Zeitz O, Sandbrink R, Haller JA. Intravitreal aflibercept injection for macular edema due to central retinal vein occlusion: two-year results from the COPERNICUS study. *Ophthalmology* 2014; 121(7):1414 PMID 24679444
 32. von Hanno T, Kinge B, Fossen K. Retinal artery occlusion following intravitreal anti-VEGF therapy. *Acta Ophthalmol* 2010;88(2):263-6.
 33. Mansour AM, Bynoe LA, Welch JC, Pesavento R, Mahendradas P, Ziemssen F, Pai SA. Retinal vascular events after intravitreal bevacizumab. *Acta Ophthalmol* 2010;88 (7):730–5.
 34. Ganssauge M, Wilhelm H, Bartz-Schmidt KU, Aisenbrey S. Nonarteritic anterior ischemic optic neuropathy (NA-AION) after intravitreal injection of bevacizumab (Avastin) for treatment of angoid streaks in pseudoxanthoma elasticum. *Graefes Arch Clin Exp Ophthalmol* 2009;247 (12):1707–10.
 35. Hosseini H, Razeghinejad MR. Anterior ischemic optic neuropathy after intravitreal injection of bevacizumab. *J Neuroophthalmol* 2009;29 (2):160–1.
 36. Huang ZL, Lin KH, Lee YC, Sheu MM, Tsai RK. Acute vision loss after intravitreal injection of bevacizumab (avastin) associated with ocular ischemic syndrome. *Ophthalmologica.* 2010;224 (2):86–89.
 37. Mete A, Saygili O, Mete A, Bayram M, Bekir N. Effects of intravitreal bevacizumab (Avastin) therapy on retrobulbar blood flow parameters in patients with neovascular age-related macular degeneration. *J Clin Ultrasound* 2010;38(2):66–70.
 38. Leung LS, Silva RA, Blumenkranz MS, Flynn HW Jr, Sanislo SR. Macular infarction following intravitreal bevacizumab for treatment of central retinal vein occlusion. *Ophthalmic Surg Lasers Imaging* 2012;43:e73-9.
 39. Papadopoulou DN, Mendrinos E, Mangioris G, Donati G, Pournaras CJ. Intravitreal ranibizumab may induce retinal arteriolar vasoconstriction in patients with neovascular age-related macular degeneration. *Ophthalmology* 2009;116 (9):1755–61.
 40. Manousaridis K, Talks J. Macular ischaemia: a contraindication for anti-VEGF treatment in retinal vascular disease? *Br J Ophthalmol* 2012;96(2):179-84.
 41. Isola V, Pece A, Massironi C, Reposi S, Dimastrogiovanni F. Accelerated ischemic vascular retinopathy after intravitreally injected bevacizumab for central retinal vein occlusion in elderly patients. *Clin Ophthalmol* 2013;7: 455 –60.
 42. Arevalo JF, Maia M, Flynn HW, Jr, Saravia M, Avery RL, Wu L, Eid Farah M, Pieramici DJ, Berrocal MH, Sanchez JG. Tractional retinal detachment following intravitreal bevacizumab (Avastin) in patients with severe proliferative diabetic retinopathy. *Br J Ophthalmol* 2008;92 (2):213–16.
 43. Oshima Y, Shima C, Wakabayashi T, Kusaka S, Shiraga F, Ohji M, Tano Y. Microincision vitrectomy surgery and intravitreal bevacizumab as a surgical adjunct to treat diabetic traction retinal detachment. *Ophthalmology.* 2009;116 (5):927–38.
 44. Yeh PT, Yang CM, Lin YC, Chen MS, Yang CH. Bevacizumab pretreatment in vitrectomy with silicone oil for severe diabetic retinopathy. *Retina* 2009;29 (6):768–74.
 45. Day S, Acquah K, Mruthunjaya P, Grossman DS, Lee PP, Sloan-FA. Ocular complications after anti-vascular endothelial growth factor therapy in Medicare patients with age-related macular degeneration. *Am J Ophthalmol* 2011; 152(2): 266–72. PMID: PMC3143287 NIHMSID: NIHMS303283
 46. Binder S. Loss of reactivity in intravitreal anti-VEGF therapy: tachyphylaxis or tolerance? *Br J Ophthalmol* 2012;96:1-2.
 47. Keane PA, Liakopoulos S, Onhchin SC. Quantitative subanalysis of optical coherence tomography after treatment with ranibizumab for neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2008;49:3115-20.
 48. Schaal S, Kaplan HJ, Tetzl TH. Is there tachyphylaxis to intravitreal anti-vascular endothelial growth factor pharmacotherapy in age-related macular degeneration ? *Ophthalmology* 2008;115:2199-205.
 49. Malik D, Tarek M, Caceres del Carpio J, Ramirez C, Boyer D, Kenney MC, Kuppermann BD. Safety profiles of anti-VEGF drugs: bevacizumab, ranibizumab, aflibercept and ziv-aflibercept on human retinal pigment epithelium cells in culture. *Br J Ophthalmol* 2014;98:i11-i16 doi:10.1136/bjophthalmol-2014-305302
 50. Avci B, Avci R, Inan UU, Kaderli B. Comparative evaluation of apoptotic activity in photoreceptor cells after intravitreal injection of bevacizumab and pegaptanib sodium in rabbits. *Invest Ophthalmol Vis Sci* 2009;50(7):3438-46. doi: 10.1167/iovs.08-2871. Epub 2009 Feb 28.
 51. Kurihara T, Westenskow PD, Bravo S, Aguilar E, Friedlander M. Targeted deletion of Vegfa in adult mice induces vision loss. *J Clin Invest* 2012;122(11):4213–4217.
 52. Kurihara T, Westenskow PD, Friedlander M. Hypoxia-inducible factor (HIF)/vascular endothelial growth factor (VEGF) signaling in the retina. *Adv Exp Med Biol* 2014;801:275-81. doi: 10.1007/978-1-4614-3209-8_35.
 53. Sene A, Chin-Yee D, Apte RS. Seeing through VEGF: innate and adaptive immunity in pathological angiogenesis in the eye. *Trends Mol Med* 2015;21(1):43-51. doi: 10.1016/j.molmed.2014.10.005. Epub 2014 Nov 1.
 54. Shimomura Y, Hirata A, Ishikawa S, Okinami S. Changes in choriocapillaris fenestration of rat eyes after intravitreal bevacizumab injection. *Graefes Arch Clin Exp Ophthalmol* 2009; 247(8):1089-94. DOI: 10.1007/s00417-009-1054-1
 55. Sugimoto Y, Mochizuki H, Miyagi H, Kawamata S, Kiuchi Y. Histological findings of uveal capillaries in rabbit eyes after multiple intravitreal injections of bevacizumab. *Curr Eye Res* 2013;38(4):487-96. doi: 10.3109/02713683.2013.763990. Epub 2013 Feb 1.
 56. Peters S, Heiduschka P, Julien S, Ziemssen F, Fietz H, Bartz-Schmidt KU; Tübingen Bevacizumab Study Group, Schraermeyer U. Ultrastructural findings in the primate eye after intravitreal injection of bevacizumab. *Am J Ophthalmol* 2007;143(6):995-1002. Epub 2007 Apr 23.
 57. Marneros AG, Fan J, Yokoyama Y, Gerber HP, Ferrara N, Crouch

Shahsuvaryan ML. Ocular impact of intravitreal anti-VEGF

RK, Olsen BR. Vascular endothelial growth factor expression in the retinal pigment epithelium is essential for choriocapillaris development and visual function. *Am J Pathology* 2005; 167(5), 1451-59.

58. Ford KM, Saint-Geniez M, Walshe TE, D'Amore PA. Expression and role of VEGF--a in the ciliary body. *Invest Ophthalmol Vis*

Sci 2012 7;53(12):7520-7. doi: 10.1167/iops.12-10098.

Peer reviewer: Yoshihiko Usui, Department of Ophthalmology, Tokyo Medical University Hospital, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo, Japan 160-0023.