HHS Public Access

Author manuscript

Surv Ophthalmol. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

Surv Ophthalmol. 2015 ; 60(6): 524–535. doi:10.1016/j.survophthal.2015.04.003.

Angiogenesis in Glaucoma Filtration Surgery and Neovascular Glaucoma-A Review

Megan Kim, BS, **Chelsea Lee, BS**, **Rachael Payne, BS**, **Beatrice Y.J.T. Yue, PhD**, **Jin-Hong Chang, PhD**, and **Hongyu Ying, PhD***

Department of Ophthalmology and Visual Sciences, Illinois Eye and Ear Infirmary, University of Illinois at Chicago, USA

Abstract

Angiogenesis may pose a clinical challenge in glaucoma, for example during the wound healing phase after glaucoma filtration surgery and in a severe form of secondary glaucoma called neovascular glaucoma (NVG). Up regulation of vascular endothelial growth factor (VEGF), a key mediator of angiogenesis, occurs in eyes that have undergone glaucoma filtration surgery, as well as those with NVG. This has led to studies investigating the ability of anti-VEGF therapy to improve outcomes, and we examine their findings with respect to the safety and efficacy of anti-VEGF agents, mainly bevacizumab and ranibizumab, in eyes that have undergone glaucoma filtration surgery or have NVG. Combining conventional therapies—such as anti-metabolites after filtration surgery and panretinal photocoagulation in NVG—and anti-VEGF drugs may achieve a synergetic effect, although further studies are required to evaluate the long-term efficacy of combination treatments.

Keywords

Angiogenesis; Glaucoma; Glaucoma filtration surgery; Neovascular glaucoma; Anti-VEGF therapy; Bevacizumab; Ranibizumab; Anti-metabolites

I. Introduction

A. Angiogenesis

Angiogenesis is the process of new blood vessel growth from existing blood vessels. This essential process occurs naturally in growth, reproduction, and wound healing to supply nutrients and oxygen to tissues.[1,5] Pathologically, aberrant angiogenesis is associated with rheumatoid arthritis,[108] tumor growth and metastasis,[7,34] and eye disorders such as diabetic retinopathy, retinopathy of prematurity, retinal vein occlusions, and age-related macular

Corresponding author: Hongyu Ying, PhD (hying@uic.edu), Department of Ophthalmology and Visual Sciences, Illinois Eye and Ear Infirmary, University of Illinois at Chicago, 1855 W. Taylor Street, Chicago, Illinois 60612, USA.

degeneration.[25,37,121] The vascular endothelial growth factor (VEGF) family of cytokines promotes angiogenesis in both normal development and disease.[1,7,25,34,37,102,108,121]

The endogenous members of the VEGF family are placenta growth factor (PlGF) and VEGF-A, -B, -C, and –D. VEGF-A serves as the principal ligand, and soluble forms of VEGF-A include VEGF-121, VEGF-145, and VEGF-165.[116] The various members of the VEGF family and their isoforms bind to various VEGF receptors (VEGFR)-1, -2, or -3.[47,93] The signaling pathways that are activated after association of VEGFRs with their ligands and the cellular responses are summarized in Table I. The important roles of the VEGFs in angiogenesis have been demonstrated in cancers, where inhibition of the VEGF pathway inhibits the angiogenic process in various tumors.[52,73,129] Additionally, hypoxia promotes VEGF transcription, indicating that the metabolic requirements of tissues can regulate angiogenesis in order to maintain the delivery of vital nutrients to hypoxic tissues through the proliferation of new capillaries.[90,122] Because angiogenesis plays a major role in a variety of pathological conditions, angiogenic inhibitors have been the focus of numerous clinical studies,[10,18,31,32,57,125,127] with a recent focus on anti-VEGF therapies including antibodies such as bevacizumab and ranibizumab, VEGF trap/aflibercept, and small interfering RNA directed against VEGF or VEGF receptors.[2,19,88]

B. Glaucoma

Glaucoma, one of the leading causes of irreversible blindness worldwide, is normally associated with aging.[26] The number of people with glaucoma is predicted to increase from 64.3 million in 2013 to 111.8 million in 2040, disproportionately affecting Asian and African populations.[112] Glaucoma is not a single entity, but rather a term that describes a group of ocular disorders of diverse etiologies that are clinically defined as intraocular pressure (IOP)-associated optic neuropathy.[16] All forms are potentially progressive and may lead to blindness,[16] but the most prevalent form is primary open-angle glaucoma (POAG). POAG is characterized by changes to the optic nerve head with corresponding defects in the visual field, but retention of a normalanterior chamber.[45] Other types of glaucoma include angle-closure glaucoma, normal tension glaucoma, and secondary glaucoma such as neovascular glaucoma (NVG), exfoliative glaucoma, and uveitic glaucoma.

Normal IOP in human is between 10 and 20 mmHg. The IOP is mainly determined by the production of the aqueous humor and its drainage mainly through the trabecular meshwork at the chamber angle (so called conventional outflow pathway). [9,16] Some aqueous humor, however, also leaves the eye via the ciliary body, through the uveoscleral or non-conventional outflow pathway.[40] The pressure gradients and resistance to the aqueous outflow are likely altered in the various types of glaucoma. POAG is frequently associated with elevated intraocular pressure (IOP).

As many as half of glaucoma cases are diagnosed in later stages of disease, because most forms of chronic glaucoma are asymptomatic.[28] Current therapy is targeted at the reduction of IOP to slow the progression of glaucoma.[9] Because of their efficacy and tolerability, the conventional first-line drugs are β-blockers and prostaglandin analogs, which reduce IOP by decreasing aqueous formation and increasing uveoscleral aqueous outflow, respectively. Other antihypertensive glaucoma medications include carbonic anhydrase inhibitors,

cholinergic agonists, and α2-adrenoceptor agonists. In patients who do not respond to any of the antihypertensive medications, laser trabeculoplasty or glaucoma filtration surgery may be performed to control IOP.[21] Trabeculectomy, the most common type of glaucoma filtration surgery, is considered the mainstay of incisional antiglaucomatous surgeries.[58] The surgical goal is to bypass the TM by allowing aqueous humor to exit through a subconjunctival bleb, thereby relieving IOP.[91]

With the advent of anti-VEGF therapies, many clinical studies have focused on targeting VEGF in ocular disorders, including glaucoma. Anti-VEGF therapy is expected to be an effective addition to the glaucoma treatment regimen because angiogenesis occurs in the wound healing phase after glaucoma filtration surgery to maintain the intentionally created bleb and is fundamental to the underlying pathophysiology of NVG. Inhibition of angiogenesis through anti-VEGF therapy has therefore the potential to improve the success of glaucoma filtration surgery, as well as the outcome in NVG. We describe the angiogenic events that occur following glaucoma filtration surgery and in NVG and summarize pertinent findings from recently published studies evaluating the use of anti-VEGF therapy.

II. Angiogenesis in the Medical Management of Glaucoma

A. Angiogenic response to glaucoma filtration surgery

Although glaucoma is often controlled with antihypertensive medications, surgical intervention becomes necessary in certain situations such as poor patient compliance, progression of disease despite maximum medical therapy, or both.[98] Unlike most other types of surgery, a completely healed wound after filtration surgery constitutes failure. The surgery aims to create a filtering bleb that functions to drain the intraocular fluid through the sclera, which enhances the aqueous outflow and thereby reduces the IOP. Postoperative conjunctival scarring at the site of the filtering bleb, however, promotes adhesion to episcleral tissue, which leads to resealing of the bleb and thus inhibition of the aqueous flow and poor control of IOP.[39,67] The failure to maintain the bleb occurs via increased angiogenesis and fibroblast migration in the conjunctiva, leading to fibroblast proliferation with collagen deposition.[100]

Seet et al[99] developed a systematic spatio-temporal analysis of the phases in the wound healing response to glaucoma filtration surgery using a mouse model. They observed that this post-surgical tissue response can be separated into two distinctive phases. The early “acute inflammatory” phase is characterized by significantly increased transcriptional expression of VEGF, chemokine (C-X-C motif) ligand (CXCL), and matrix metalloproteinase (MMP), as well as increased infiltration of inflammatory cells. The late “fibrotic” phase is marked by increased expression of transforming growth factor (TGF)-β2 and extracellular matrix genes with a concurrent reduction in the inflammatory cell infiltration. The increase in VEGF expression during the early phase, on both transcription and protein levels, indicates that angiogenesis is an early response in the process of bleb wound healing.

Clinically, increased bleb vascularity is associated with a poorer prognosis for trabeculectomy.[14,75] This prompted the hypothesis that decreasing vascularity via inhibition

of angiogenesis could improve the outcome. Currently, anti-fibrotic medications are used as adjuncts to inhibit bleb healing; however, despite their efficacy, these are associated with several sight-threatening complications.[98] Interestingly, VEGF expression was shown to be increased in the Tenon tissue of patients who experienced failed glaucoma filtration surgeries compared to patients in whom the surgery was successful and patients without glaucoma.[72] Such results confirm that a significant correlation exists between VEGF expression and the outcome of glaucoma surgery and suggest the potential usefulness of anti-VEGF therapy in promoting the success of glaucoma filtration surgery.

B. Angiogenesis in NVG

NVG is an aggressive form of secondary glaucoma commonly associated with proliferative diabetic retinopathy (PDR), ischemic central retinal vein occlusion (CRVO), and ocular ischemic syndrome.[44] NVG often results in poor visual outcomes. The term “neovascular glaucoma” was first coined by Weiss et al in 1963 to describe glaucoma associated with the presence of new iris and angle vessels.[85] NVG occurs when new fibrovascular tissue proliferates onto the iris and chamber angle structures including the trabecular meshwork, usually in response to ischemia of various etiologies.[105] In approximately 97% of NVG cases, neovascularization of the iris and angle occurs in response to retinal ischemia, whereas only a small fraction of the cases is caused by inflammation without ischemia.[105] Retinal ischemia induces production of pro-angiogenic factors that diffuse into the anterior segment and promote neovascularization of the iris, the angle, or both.[3] The fibrovascular membrane that is created inhibits the aqueous flow and leads to an increase in IOP. Contraction of this abnormal tissue induces the development of peripheral anterior synechiae and progressive angle closure, which further increases IOP to harmful levels that cannot be controlled via conventional antiglaucoma therapy.[3]

III. Therapy in Glaucoma Filtration Surgery

A. Current therapy

In order to maintain the bleb created in filtration surgery, medications are used as adjuncts to inhibit wound healing of the bleb. 5-Fluorouracil (5-FU) and mitomycin C (MMC) are anti-metabolites commonly used to limit wound healing via the induction of widespread fibroblast cell death.[106] MMC is preferentially used as the intraoperative antifibrotics, more than twice as often as 5-FU,[60] and has been validated by Cochrane meta-analysis to reduce significantly the IOP and the risk of surgical failure in eyes that have undergone no previous surgery and in eyes at high risk of failure.[120] Despite their efficacy in reducing post-operative scarring,[67] however, anti-metabolites are associated with complications such as hypotony with maculopathy, cystic avascular bleb, bleb leak, bleb infection, and endophthalmitis.[39,53,95]

Because TGF-β2 has been reported to stimulate proliferation and migration of human Tenon fibroblasts,[22] CAT-152, a human, monoclonal antibody against TGF-β2, was recently evaluated for its efficacy in a large, multicenter, randomized clinical trial.[43] Although the effects of CAT-152 in animal models have been encouraging,[78] the clinical study revealed no significant efficacy for the prevention of bleb failure over a 12-month follow-up period.[43]

The limitations regarding the safety and efficacy of anti-fibrotic pharmaceutical agents as adjuncts have thus motivated researchers to continue searching for better alternatives.

Several other types of adjunctive agents such as Rho-associated protein kinase inhibitor,[49] β irradiation,[92] and triamcinolone acetonide[48] have been investigated as potential alternatives, but are not yet routinely used in clinical settings for trabeculectomies. Anti-VEGF therapy, on the other hand, has demonstrated clinical effectiveness. Anti-VEGF adjuncts have been tested in preliminary *in vitro* and animal studies, as well as small clinical trials, that have shown promising results in reducing postoperative scar formation.

B. Anti-VEGF approach

a. Role of VEGF in bleb scarring—VEGF is most commonly known as a stimulator of endothelial growth and vascular permeability, but it is also an important mediator in wound healing and scar formation. To achieve these functions, VEGF stimulates the angiogenic cascade to provide conduits for oxygen, nutrients, and other mediators involved in wound healing,[6] which is required for the formation of granulation tissue.[68] There is enhanced healing upon stimulation of angiogenesis,[38,82,118,124] as well as delayed healing when angiogenesis is inhibited.[8,76,94]

VEGF not only regulates fibrosis via angiogenesis, but also acts as a mediator in a signaling pathway that promotes fibroblast migration, proliferation, and collagen production.[6,119] VEGF has been shown to induce proliferation of Tenon fibroblasts *in vitro* during the post-trabeculectomy wound healing process.[69]

VEGF directly stimulates both vascular endothelial cells and fibroblasts and may be the link between angiogenesis and scar formation.[119] Among different VEGF isotopes, VEGF-A is the only one showing significantly decreased expression in later stages of wound healing.[99] This suggests that VEGF-A may be involved in the transition from the early to late phases of wound healing. Among the different isoforms of VEGF-A, VEGF-121, VEGF-165, and VEGF-189 are expressed in rabbit Tenon fibroblasts.[69] *In vitro* the addition of VEGF-121 and VEGF-165 stimulates endothelial cell proliferation, whereas the addition of VEGF-121 and VEGF-189 increases fibroblast growth.[113] Although VEGF-121 stimulates proliferation of both endothelial cells and fibroblasts, its effect is more prominent in endothelial cells. This suggests that VEGF-121 and VEGF-165 predominantly affect blood vessel growth, whereas VEGF-189 may be more important in fibrosis. Since VEGF signaling is involved in both angiogenesis and fibrosis, two critical processes in scar formation, inhibition of all isoforms of VEGF may delay bleb healing after glaucoma filtration surgery.

b. Anti-VEGF therapy—A number of studies, including small clinical trials, have investigated anti-VEGF antibodies such as bevacizumab and ranibizumab as potential adjunctive agents in glaucoma filtration surgery. Both antibodies bind to all of the isoforms of VEGF-A; however, ranibizumab is a mature antibody designed to have a significantly stronger binding affinity than bevacizumab.[88]

1. Bevacizumab: Li et al[69] reported that administration of bevacizumab significantly inhibited VEGF-induced Tenon fibroblast proliferation in human (P=0.04) and rabbit

(P=0.02) in a dose-dependent manner. Similarly, O'Neill et al[84] demonstrated, in an *in vitro* model of wound healing with human Tenon fibroblasts, that bevacizumab disrupted fibroblast proliferation, inhibited collagen gel contractility, and induced fibroblast death at concentrations greater than 7.5 mg/mL in serum-free conditions. Li et al[69] also showed that a single application of bevacizumab into the subconjunctival space and anterior chamber at the time of trabeculectomy resulted in a larger bleb area in a rabbit model (n=34; P<0.05). The IOP, however, was similar in the treated and control eyes 29 days after surgery. Memarzadeh et al[79] reported similar results in a larger animal study in which 42 randomized rabbits received seven subconjunctival injections of bevacizumab, 5-FU, or balanced salt solution during the first 14 days after trabeculectomy. There was no significant difference in the mean IOP, but bevacizumab did more than double the bleb survival time (P<0.05) compared to the other two treatments. Ozgonul et al[86] also demonstrated the efficacy of anti-VEGF therapy through a study that compared the efficacy between subconjunctival and intravitreal applications of bevacizumab in rabbit models. They reported that subconjunctival injection of bevacizumab resulted in a greater area and height of the bleb and lower mean IOP compared to intravitreal bevacizumab, 5-FU, and control groups. Inflammation (P=0.030), neovascularization (P=0.004), and fibrosis were also lower in the subconjunctival bevacizumab group. Although achieving significant differences in IOP is difficult in animal models, the bleb morphologic features and bleb survival time support the efficacy of bevacizumab in improving outcomes of glaucoma filtration surgery.

The safety and efficacy of bevacizumab in trabeculectomy have in addition been tested in a number of clinical trials. Vandewalle et al[114] studied the effect of a single intracameral bevacizumab injection in a 12-month prospective, randomized trial with 138 patients. The IOP at 1 year postoperatively was significantly lower than baseline in the placebo (P<0.01) and the treated (P<0.01) group. The bevacizumab-treated group, however, had a higher absolute success rate (P=0.02) and required less IOP-lowering interventions (P=0.003). Grewal et al[41] evaluated the effect of subconjunctival bevacizumab through a nonrandomized, open-label, prospective, interventional case series of 12 patients. After a follow-up of 182 days, IOP control was observed in 92% of the eyes, with an average IOP reduction of 52%. An interesting observation in their study was that the bleb vascularity began to increase 3 months after administration of bevacizumab. While this might have decreased the development of cystic avascular blebs that frequently develop following MMC-augmented surgery, it also raised a concern for possible future bleb failure.

To gauge its value, anti-VEGF therapy was compared to the commonly used anti-metabolites, 5-FU and MMC. Sengupta et al[101] analyzed the conventional MMC and both subconjunctival and topical administrations of bevacizumab in 38 patients undergoing single-site phacotrabeculectomy. All three groups had a significant reduction in mean IOP that was sustained at 6 months. However, 90% of patients treated with subconjunctival bevacizumab showed complete success, which was defined as IOP <18 mm Hg or at least a 20% reduction from the baseline IOP at the end of the follow-up period, as opposed to 60% in the other two groups (P=0.04). They also observed a gradual increase in the vascularity over a 6-month period in blebs of patients who received bevacizumab. Nilfoushan et al[83] compared the outcome of trabeculectomy with subconjunctival bevacizumab (n=18) versus

MMC (n=18) in a prospective, randomized study. Although IOP was significantly reduced in both groups compared to baseline, the reduction was more prominent in the MMC-treated group at 12 months, with 34% and 56% IOP reductions in the bevacizumab- and MMC-treated groups, respectively. Similarly, Jurkowska et al[54] compared the effects of bevacizumab and 5-FU in a nonrandomized, prospective, interventional case study in which 21 patients received intraoperative 5-FU and 32 patients received subconjunctival bevacizumab immediately before and after surgery and again 1 and 7 days after surgery. A significant reduction in IOP occurred in both group, but a greater percentage of 5-FU–treated patients (86.7%) experienced a 30% reduction in initial IOP compared to bevacizumab-treated patients (78.1%) at the end of 12-month follow-up (P=0.38). There were no notable differences between the two groups in terms of visual field indices and postoperative complications; however, more patients in the bevacizumab-treated group required additional medical interventions to successfully control IOP.

2. Ranibizumab: Ranibizumab significantly induces human Tenon fibroblast death compared to serum-free control condition *in vitro* ($P<0.05$)[77] and also significantly reduced collagen type 1 α1 (COL1α1) mRNA, but not fibronectin (FN) mRNA, expression. COL1α1 and FN protein levels, however, were upregulated in the cells treated with ranibizumab compared to the untreated control ($P<0.01$ and $P<0.05$, respectively), and such opposing results for protein and mRNA expression following ranibizumab administration hinders any attempts to draw conclusions from the study regarding VEGF's effects on collagen expression. Nevertheless, these results confirm the direct effect of anti-VEGF antibodies on Tenon fibroblast proliferation and possibly collagen production. The effects of bevacizumab and ranibizumab in filtration surgery are summarized in Table II.

3. Combination therapy: As a number of studies have shown that anti-VEGF therapy alone may not be sufficient to prevent scar formation, researchers have investigated whether combined therapy can offer additive or synergistic effects. How et al[50] analyzed the effect of combining bevacizumab and 5-FU and found that this combination results in a superior antifibrotic effect compared to monotherapy with 5-FU or bevacizumab in rabbit models, as evidenced by a decrease in FN and mature COL1 expression and deposition ($P<0.05$). Additionally, 100% bleb survival at 28 days was observed in the combined treatment group, whereas bleb survival in the monotherapy groups (50% bevacizumab [$P<0.05$] and 25% 5-FU [$P<0.001$]) was significantly lower. A significant reduction in conjunctival vascularity also was observed in the combined therapy group as well as the bevacizumab-only group.

Clinical studies, however, did not show any additive advantages of bevacizumab and 5-FU combination therapies. Suh et al[107] recently performed a clinical study comparing outcomes in 12 patients who received combination therapy with intracameral and subconjunctival bevacizumab and subconjunctival 5-FU to those in 24 patients who received only subconjunctival 5-FU at the time of trabeculectomy. They found no significant difference between the two groups with regards to visual acuity, postoperative IOP, and anti-glaucoma medication use after surgery ($P>0.05$), indicating that the addition of bevacizumab did not have a significant additive benefit compared to 5-FU monotherapy. Similarly, Chua et al[20] analyzed a combination therapy of subconjunctival bevacizumab and 5-FU (n=22) versus

monotherapy of 5-FU (n=21) and observed no significant differences in terms of visual acuity, IOP, or postoperative interventions between the two groups. By 18 months, central bleb avascularity was more frequent in the combined group than the 5-FU group (47.4% and 21.1%, respectively; P=0.17), but this effect was not statistically significant.

By contrast, Kahook et al[55] reported positive results from their pilot study in which 10 patients were randomized to either trabeculectomy with only MMC or trabeculectomy with a combination of intravitreal ranibizumab and MMC. There were statistically significant differences in peripheral bleb area (P=0.02), peripheral bleb vascularity (P=0.02), and non-bleb-related peripheral conjunctiva vascularity (P=0.0003), with the combined group exhibiting more diffuse blebs with a lower degree of vascularity (Table II). The positive results, however, should be regarded with caution because it was insufficiently powered for a long-term follow-up and the conclusions drawn based mainly on bleb morphology, not mean IOP.

4. Other anti-VEGF agents: In addition to bevacizumab and ranibizumab, other relatively uncommon anti-VEGF agents also have been explored. Van Bergen et al[113] evaluated the effect of specific VEGF inhibition *in vitro* using pegaptinib, an antibody specifically targeting the VEGF-165 isoform. Although proliferation of human umbilical vascular endothelial cells was effectively inhibited by pegaptinib in a dose-dependent manner, growth of human Tenon fibroblasts was only significantly reduced by the highest dose of this selective inhibitor. They also tested the effect of pegaptinib in a rabbit trabeculectomy model. A single administration of pegaptanib at the time of surgery significantly reduced angiogenesis during the initial phase (P≤0.03) compared to the control, but there were no significant differences in inflammatory responses and collagen deposition.

Additionally, a preliminary study was performed using trehalose, an anti-angiogenic agent that indirectly targets VEGF. After discovering that trehalose inhibits conjunctival neovascularization and fibroblast proliferation in their previous study[110], Takeuchi et al[109] investigated the effect of trehalose on VEGF-stimulated angiogenesis and myofibroblast proliferation *in vitro*. They observed a significant dose-dependent inhibition of neovascularization (P<0.01), as well as a partial inhibition of myofibroblast proliferation via stimulation of mesenchymal-epithelial transition. Although trehalose is a rather unconventional and indirect anti-VEGF agent, the promising outcomes from the *in vitro* study suggest the possibility of its use as an adjunctive antiscarring agent in the future.

IV. Anti-VEGF Therapy in NVG

A. Current therapy

Treatment of NVG involves reducing IOP as well as treating the underlying disease process responsible for NVG. Panretinal photocoagulation (PRP) is the current gold standard treatment for NVG. PRP reduces the global retinal oxygen demand by destroying the nonessential ischemic retinal tissue to remove the stimulus for production of vasoproliferative factors.[4] A decrease in VEGF levels was reported in patients who received PRP, along with a resultant decrease in neovascularization.[17] Although PRP has shown effectiveness in treating NVG, this treatment leads to death of healthy cells and permanently

diminishes visual fields.[36] PRP also does not result in rapid regression of iris and angle neovascularization, and patients continue to experience high IOP and discomfort for a period of time after the procedure. Moreover, PRP is difficult to perform in patients with NVG with media opacities such as corneal edema and cataract. Hence, treatments aimed at reducing the pro-angiogenic factors directly, such as the use of anti-VEGF antibodies, could help reduce and possibly reverse the neovascularization. Although anti-VEGF therapy does not directly address the underlying ischemia in NVG, it can control the pathologic process without destroying healthy retinal cells.

While PRP and/or anti-VEGF interventions may be sufficient to control the IOP in open-angle NVG, subsequent glaucoma therapy (with or without surgery) is usually indicated when most of the angle is closed due to synechiae.[104] Aqueous drainage devices,[80] cyclocryocoagulation,[65] transscleral diode laser,[35] Nd-Yag cyclophotocoagulation,[27] and vitrectomy with PRP and trabeculectomy[62] are the possible methods of treatment. Although the aqueous drainage device has traditionally been preferred over trabeculectomy, advent of anti-VEGF therapy has resulted in increasing use of the latter. The use of anti-VEGF agents has been shown to decrease IOP, possible complications, and hyphema postoperatively, thereby improving the surgical success rates.[61,96]

B. Anti-VEGF approach

a. Role of VEGF in NVG—VEGF is synthesized by multiple types of retinal cells in response to retinal ischemia. Studies have found that VEGF levels are significantly elevated in the aqueous humor of patients with rubeosis and NVG.[51,59,130] In addition, the aqueous and vitreous levels of VEGF are higher in patients with NVG associated with diabetes than in diabetic patients with only proliferative retinopathy.[66] These results confirm the importance of VEGF signaling in neovascularization in NVG. Therefore, the use of VEGF inhibitors to reduce VEGF levels has been explored as a treatment option for NVG, and multiple case series have indicated the treatment efficacy of anti-VEGF agents, predominantly intravitreal injections of bevacizumab and ranibizumab, in reducing neovascularization in the iris and angle in NVG. These are summarized in Table III and described in more detail below.

b. Anti-VEGF therapy

1. Bevacizumab: Many clinical trials have evaluated the use of anti-VEGF antibodies to treat NVG. In one of the first randomized clinical trials evaluating the efficacy of intravitreal bevacizumab, NVG patients treated with three intravitreal bevacizumab injections at 4-week intervals exhibited a significant decrease in IOP compared to their baseline IOP as well as significant regression in iris neovascularization at 1 month (P=0.007 and P=0.01, respectively), 3 months (P=0.058 and P=0.004), and 6 months (P=0.047 and P=0.004) post-treatment time points.[126] An observational case series tracked 50 adults with NVG who could not be treated with PRP. Six months after these patients were treated with intravitreal bevacizumab, they reported a reduction in pain from their baseline pain score starting 1 week after administration (P<0.001).[64] Additionally, a short-term clinical study reported that patients with NVG displayed a considerable reduction in VEGF concentration in the aqueous humor (P=0.04) without a significant change in IOP 2 weeks after receiving an

intracameral injection of bevacizumab.[70] In a similar pilot study evaluating the effect of intracameral bevacizumab before any surgical treatment for NVG, some patients were found to no longer require any surgical intervention, whereas other patients became candidates for filtration surgery.[29] Upon testing the use of bevacizumab as a topical treatment, another pilot study found that after topical application of bevacizumab 4 times a day consistently for 2 weeks the mean IOP of eight patients was reduced by 17.5%.[117]

2. Ranibizumab: The efficacy of ranibizumab was investigated in a clinical series by Luke et al[74] who showed that patients with both rubeosis and NVG experienced considerable iris neovascularization regression ($P<0.001$) and a rapid IOP reduction from their baseline ($P=0.005$) 2 weeks after ranibizumab injection. Moreover, the improvement in IOP was maintained for the follow up period of 12 months ($P<0.05$). They concluded that ranibizumab is effective as an adjuvant treatment for NVG and rubeosis because it preserves the anterior chamber angle.

3. Combination therapy: Brouzas et al[11] published a clinical series evaluating the safety and efficacy of monthly intravitreal bevacizumab with standard NVG treatment. The case series demonstrated that NVG treatment along with monthly injections of bevacizumab was effective in decreasing iris and anterior chamber angle neovascularization. After these combined treatments, however, acceptable IOP levels were not maintained, requiring additional medical interventions. Ehelers et al[30] compared combination therapy consisting of PRP and same-day intravitreal bevacizumab injection to PRP alone. They showed that total neovascular reduction occurred more often ($P<0.001$) and more rapidly ($P<0.0001$) in the combined group. In addition, IOP decreased more rapidly in the combined treatment group ($P=0.03$). Similarly, Vasudev et al[115] compared the effects of intravitreal bevacizumab with PRP treatment to PRP treatment alone on the angle of the anterior chamber. From the 1-year follow-up, patients treated with bevacizumab and PRP were seen to have a longer lasting open angle than those treated with PRP only ($P<0.05$).

In addition to PRP, the value of adding bevacizumab to current NVG treatments was also investigated in retinal ablative procedures[24]. After 12 months, NVG patients who received intravitreal bevacizumab injection after a retinal ablative procedure had reduced vasculature in the iris, better visual acuity ($P=0.0001$), and decreased IOP from their baseline ($P=0.0001$; Table III).

V. Future Directions

A. Glaucoma filtration surgery

Additional studies with larger sample sizes and a more systematic approach are necessary to gain a better understanding of the effect of anti-VEGF agents in patients undergoing glaucoma filtration surgery. More evidence is needed to support the use of VEGF inhibitors for preventing postoperative angiogenic and fibroblastic activities, including determination of the most effective doses and the optimal timing and route of administration (intravitreal, subconjunctival, or intracameral). The gradual increase in bleb vascularity by 3 months after administration of VEGF inhibitors, as observed in multiple clinical studies,[41,101] must be addressed to avoid bleb failure associated with increased bleb vascularity. Multiple

administrations of anti-VEGF agent may be necessary to suppress angiogenesis and achieve long-term preservation of the bleb.

Several comparison studies have shown that the safety and efficacy of anti-VEGF therapy are not significantly different from those of current antiscarring medications. To confirm these results, a larger randomized clinical trial is planned.[54] Although only a few clinical studies have examined the efficacy of bevacizumab combined with an anti-metabolite in comparison to that of an anti-metabolite alone, most of these studies have reported no significant additive effect. A clinical study with a larger cohort and longer follow-up period is required to confirm and better understand these findings.

Moreover, combination therapy using anti-angiogenic and anti-fibrotic agents should be further investigated as these processes occur at different times in wound healing. TGF-β2 exerts anti-inflammatory as well as pro-fibrotic effects in subconjunctival scarring. The difficulty of mediating these contradictory effects may explain, in part, the lack of success to date of anti-TGF-β2 therapy as an adjunctive agent following glaucoma filtration surgery.[42] The use of anti-VEGF agents in combination with anti-TGF-β2 agents not only allows abrogation of both early and late stages of scar formation, but may also enhance the anti-fibrotic effect of anti-TGF-β2 therapy by potentially reducing inflammation. Defining the early and late phases of bleb healing in humans will also be advantageous for improving the specificity of molecularly targeted therapies and outlining the therapeutic windows for optimal use.

B. Neovascular glaucoma

The main concerns regarding clinical trials using anti-VEGF antibodies in NVG are the length of evaluation and sample size. Although bevacizumab and ranibizumab were found to be effective for reducing iris and anterior chamber neovascularization, larger studies evaluating patients over longer time periods will further determine their long-term effect. This is important because reappearance of neovascularization may recur.[11,30,126] More studies are needed to evaluate the effect of these antibodies on IOP, especially given that the finding of Brouzas et al[11] conflicts with those of other studies,[30,117,126] with Brouzas et al reporting that IOP could not be maintained without the use of additional drugs. This suggests that combination therapy with another drug may more effectively treat NVG.

Although there has been a focus on using bevacizumab in combination with PRP, another therapeutic strategy employing bevacizumab involves administering the drug beforehand to allow PRP in patients who initially are unable to undergo PRP.[29] Further studies on this treatment approach are warranted. Likewise, it would be worthwhile to investigate the effects of intracameral bevacizumab injections on IOP and neovascularization, because the decrease in VEGF concentration with this treatment is a promising finding.[70]

In addition to antibodies such as bevacizumab and ranibizumab, aflibercept could potentially be another anti-VEGF agent for treating NVG. The NCT01711879 a 52-week randomized controlled clinical trial investigating the efficacy of intravitreal aflibercept injections in participants with NVG compared to standard PRP, is underway. Aflibercept, which is a novel humanized recombinant fusion protein designed to bind all isoforms of VEGF-A as

well as PIGF,[2] is known to be effective in treating neovascular age-related macular degeneration[46] and is reported to have broader anti-angiogenic effect and higher binding affinity for VEGF-A isoforms than other anti-VEGF therapies.[87] With evidence demonstrating excellent responses to anti-VEGF therapy in NVG, such an addition to the available anti-VEGF agents may improve treatment outcomes.

VI. Method of Literature Search

In order to prepare this review, we conducted a Medline and PubMed search of the medical literature for the period between 1963 and 2015 using the following key words in various combinations: *angiogenesis, glaucoma, VEGF, neovascularization, anti-VEGF therapy, bevacizumab, pegaptanib, ranibizumab, glaucoma filtration surgery, neovascular glaucoma, topical, subconjunctival, intravitreal,* and *intracameral*. In addition, reference lists from the selected articles were used to identify additional articles not included in the electronic database. Articles were appraised critically and pertinent information was included in this review and cited accordingly.

VII Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article. Publication for this article was supported by National Institutes of Health grants, EY021886, EY023691, VA I01 BX002386 (JHC), UL1TR000050 (UIC CCTS), and EY01792 (UIC core grant); an unrestricted grant from Research to Prevent Blindness, New York, NY; and an award from the Illinois Society for the Prevention of Blindness (HY).

References

1. Adair TH, Montani JP. Angiogenesis. 2010
2. Al-Halafi AM. Vascular endothelial growth factor trap-eye and trap technology: Aflibercept from bench to bedside. Oman J Ophthalmol. 2014; 7:112–115. [PubMed: 25378873]
3. Allingham RR, Shields MB. Ovid Technologies Inc. Shields' textbook of glaucoma. 2011:294–308.
4. Anchala AR, Pasquale LR. Neovascular glaucoma: a historical perspective on modulating angiogenesis. Semin Ophthalmol. 2009; 24:106–112. [PubMed: 19373695]
5. Azar DT. Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc. 2006; 104:264–302. [PubMed: 17471348]
6. Bao P, Kodra A, Tomic-Canic M, et al. The role of vascular endothelial growth factor in wound healing. J Surg Res. 2009; 153:347–358. [PubMed: 19027922]
7. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 2003; 3:401–410. [PubMed: 12778130]
8. Bermudez DM, Xu J, Herdrich BJ, et al. Inhibition of stromal cell-derived factor-1alpha further impairs diabetic wound healing. J Vasc Surg. 2011; 53:774–784. [PubMed: 21211927]
9. Boland MV, Ervin AM, Friedman DS, et al. Comparative effectiveness of treatments for open-angle glaucoma: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2013; 158:271–279. [PubMed: 23420235]
10. Brose MS, Nutting CM, Jarzab B, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. Lancet. 2014; 384:319–328. [PubMed: 24768112]

11. Brouzas D, Charakidas A, Moschos M, et al. Bevacizumab (Avastin) for the management of anterior chamber neovascularization and neovascular glaucoma. Clin Ophthalmol. 2009; 3:685–688. [PubMed: 20054417]
12. Bry M, Kivela R, Holopainen T, et al. Vascular endothelial growth factor-B acts as a coronary growth factor in transgenic rats without inducing angiogenesis, vascular leak, or inflammation. Circulation. 2010; 122:1725–1733. [PubMed: 20937974]
13. Cai J, Jiang WG, Ahmed A, Boulton M. Vascular endothelial growth factor-induced endothelial cell proliferation is regulated by interaction between VEGFR-2, SH-PTP1 and eNOS. Microvasc Res. 2006; 71:20–31. [PubMed: 16337972]
14. Cantor LB, Mantravadi A, WuDunn D, et al. Morphologic classification of filtering blebs after glaucoma filtration surgery: the Indiana Bleb Appearance Grading Scale. J Glaucoma. 2003; 12:266–271. [PubMed: 12782847]
15. Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003; 9:653–660. [PubMed: 12778163]
16. Casson RJ, Chidlow G, Wood JP, et al. Definition of glaucoma: clinical and experimental concepts. Clin Experiment Ophthalmol. 2012; 40:341–349. [PubMed: 22356435]
17. Chalam KV, Brar VS, Murthy RK. Human ciliary epithelium as a source of synthesis and secretion of vascular endothelial growth factor in neovascular glaucoma. JAMA Ophthalmol. 2014; 132:1350–1354. [PubMed: 25079256]
18. Chang JH, Garg NK, Lunde E, et al. Corneal neovascularization: an anti-VEGF therapy review. Surv Ophthalmol. 2012; 57:415–429. [PubMed: 22898649]
19. Chen S, Feng J, Ma L, et al. RNA interference technology for anti-VEGF treatment. Expert Opin Drug Deliv. 2014; 11:1471–1480. [PubMed: 24898870]
20. Chua BE, Nguyen DQ, Qin Q, et al. Bleb vascularity following post-trabeculectomy subconjunctival bevacizumab: a pilot study. Clin Experiment Ophthalmol. 2012; 40:773–779. [PubMed: 22429268]
21. Cohen LP, Pasquale LR. Clinical characteristics and current treatment of glaucoma. Cold Spring Harb Perspect Med. 2014; 4
22. Cordeiro MF, Bhattacharya SS, Schultz GS, et al. TGF-beta1, -beta2, and -beta3 in vitro: biphasic effects on Tenon's fibroblast contraction, proliferation, and migration. Invest Ophthalmol Vis Sci. 2000; 41:756–763. [PubMed: 10711691]
23. Coso S, Zeng Y, Opeskin K, et al. Vascular endothelial growth factor receptor-3 directly interacts with phosphatidylinositol 3-kinase to regulate lymphangiogenesis. PLoS One. 2012; 7:e39558. [PubMed: 22745786]
24. Costagliola C, Cipollone U, Rinaldi M, et al. Intravitreal bevacizumab (Avastin) injection for neovascular glaucoma: a survey on 23 cases throughout 12-month follow-up. Br J Clin Pharmacol. 2008; 66:667–673. [PubMed: 19032174]
25. Crawford TN, Alfaro DV 3rd, Kerrison JB, et al. Diabetic retinopathy and angiogenesis. Curr Diabetes Rev. 2009; 5:8–13. [PubMed: 19199892]
26. Dandona L, Dandona R. What is the global burden of visual impairment? BMC Med. 2006; 4:6. [PubMed: 16539747]
27. Delgado MF, Dickens CJ, Iwach AG, et al. Long-term results of noncontact neodymium:yttrium-aluminum-garnet cyclophotocoagulation in neovascular glaucoma. Ophthalmology. 2003; 110:895–899. [PubMed: 12750086]
28. Deva NC, Insull E, Gamble G, et al. Risk factors for first presentation of glaucoma with significant visual field loss. Clin Experiment Ophthalmol. 2008; 36:217–221. [PubMed: 18412589]
29. Duch S, Buchacra O, Milla E, et al. Intracameral bevacizumab (Avastin) for neovascular glaucoma: a pilot study in 6 patients. J Glaucoma. 2009; 18:140–143. [PubMed: 19225351]
30. Ehlers JP, Spirn MJ, Lam A, et al. Combination intravitreal bevacizumab/panretinal photocoagulation versus panretinal photocoagulation alone in the treatment of neovascular glaucoma. Retina. 2008; 28:696–702. [PubMed: 18463512]
31. Ellenberg D, Azar DT, Hallak JA, et al. Novel aspects of corneal angiogenic and lymphangiogenic privilege. Prog Retin Eye Res. 2010; 29:208–248. [PubMed: 20100589]

32. Ellis L, Pan Y, Smyth GK, et al. Histone deacetylase inhibitor panobinostat induces clinical responses with associated alterations in gene expression profiles in cutaneous T-cell lymphoma. Clin Cancer Res. 2008; 14:4500–4510. [PubMed: 18628465]
33. Feng Y, Wang W, Hu J, et al. Expression of VEGF-C and VEGF-D as significant markers for assessment of lymphangiogenesis and lymph node metastasis in non-small cell lung cancer. Anat Rec (Hoboken). 2010; 293:802–812. [PubMed: 20225197]
34. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol. 2002; 29:15–18. [PubMed: 12516034]
35. Fong AW, Lee GA, O'Rourke P, et al. Management of neovascular glaucoma with transscleral cyclophotocoagulation with diode laser alone versus combination transscleral cyclophotocoagulation with diode laser and intravitreal bevacizumab. Clin Experiment Ophthalmol. 2011; 39:318–323. [PubMed: 20973900]
36. Fong DS, Girach A, Boney A. Visual side effects of successful scatter laser photocoagulation surgery for proliferative diabetic retinopathy: a literature review. Retina. 2007; 27:816–824. [PubMed: 17891003]
37. Fruttiger M. Development of the retinal vasculature. Angiogenesis. 2007; 10:77–88. [PubMed: 17322966]
38. Galiano RD, Tepper OM, Pelo CR, et al. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. Am J Pathol. 2004; 164:1935–1947. [PubMed: 15161630]
39. Georgoulas S, Dahlmann-Noor A, Brocchini S, et al. Modulation of wound healing during and after glaucoma surgery. Prog Brain Res. 2008; 173:237–254. [PubMed: 18929113]
40. Goel M, Picciani RG, Lee RK, et al. Aqueous humor dynamics: a review. Open Ophthalmol J. 2010; 4:52–59. [PubMed: 21293732]
41. Grewal DS, Jain R, Kumar H, et al. Evaluation of subconjunctival bevacizumab as an adjunct to trabeculectomy a pilot study. Ophthalmology. 2008; 115:2141–2145. e2142. [PubMed: 18692246]
42. Grehn F, Hollo G, et al. Group CATTS. Factors affecting the outcome of trabeculectomy: an analysis based on combined data from two phase III studies of an antibody to transforming growth factor beta2, CAT-152. Ophthalmology. 2007; 114:1831–1838. [PubMed: 17719641]
43. Khaw P, Grehn F, et al. Group CATTS. A phase III study of subconjunctival human anti-transforming growth factor beta(2) monoclonal antibody (CAT-152) to prevent scarring after first-time trabeculectomy. Ophthalmology. 2007; 114:1822–1830. [PubMed: 17908591]
44. Hayreh SS. Neovascular glaucoma. Prog Retin Eye Res. 2007; 26:470–485. [PubMed: 17690002]
45. Hazin R, Hendrick AM, Kahook MY. Primary open-angle glaucoma: diagnostic approaches and management. J Natl Med Assoc. 2009; 101:46–50. [PubMed: 19245072]
46. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology. 2012; 119:2537–2548. [PubMed: 23084240]
47. Hoeben A, Landuyt B, Highley MS, et al. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev. 2004; 56:549–580. [PubMed: 15602010]
48. Hogewind BF, Pijl B, Hoyng CB, et al. Purified triamcinolone acetonide as antifibrotic adjunct in glaucoma filtering surgery. Graefes Arch Clin Exp Ophthalmol. 2013; 251:1213–1218. [PubMed: 23052714]
49. Honjo M, Tanihara H, Kameda T, et al. Potential role of Rho-associated protein kinase inhibitor Y-27632 in glaucoma filtration surgery. Invest Ophthalmol Vis Sci. 2007; 48:5549–5557. [PubMed: 18055804]
50. How A, Chua JL, Charlton A, et al. Combined treatment with bevacizumab and 5-fluorouracil attenuates the postoperative scarring response after experimental glaucoma filtration surgery. Invest Ophthalmol Vis Sci. 2010; 51:928–932. [PubMed: 19797222]
51. Hu DN, Ritch R, Liebmann J, et al. Vascular endothelial growth factor is increased in aqueous humor of glaucomatous eyes. J Glaucoma. 2002; 11:406–410. [PubMed: 12362079]
52. Inai T, Mancuso M, Hashizume H, et al. Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. Am J Pathol. 2004; 165:35–52. [PubMed: 15215160]

53. Jampel HD, Solus JF, Tracey PA, et al. Outcomes and bleb-related complications of trabeculectomy. Ophthalmology. 2012; 119:712–722. [PubMed: 22244944]
54. Jurkowska-Dudzinska J, Kosior-Jarecka E, Zarnowski T. Comparison of the use of 5-fluorouracil and bevacizumab in primary trabeculectomy: results at 1 year. Clin Experiment Ophthalmol. 2012; 40:e135–142. [PubMed: 21668792]
55. Kahook MY. Bleb morphology and vascularity after trabeculectomy with intravitreal ranibizumab: a pilot study. Am J Ophthalmol. 2010; 150:399–403. e391. [PubMed: 20570237]
56. Karkkainen MJ, Haiko P, Sainio K, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol. 2004; 5:74–80. [PubMed: 14634646]
57. Khasraw M, Pavlakis N, McCowatt S, et al. Multicentre phase I/II study of PI-88, a heparanase inhibitor in combination with docetaxel in patients with metastatic castrate-resistant prostate cancer. Ann Oncol. 2010; 21:1302–1307. [PubMed: 19917571]
58. Khaw PT, Chiang M, Shah P, et al. Enhanced trabeculectomy: the Moorfields Safer Surgery System. Dev Ophthalmol. 2012; 50:1–28. [PubMed: 22517170]
59. Kim YG, Hong S, Lee CS, et al. Level of vascular endothelial growth factor in aqueous humor and surgical results of ahmed glaucoma valve implantation in patients with neovascular glaucoma. J Glaucoma. 2009; 18:443–447. [PubMed: 19680051]
60. Kirwan JF, Lockwood AJ, Shah P, et al. Trabeculectomy in the 21st century: a multicenter analysis. Ophthalmology. 2013; 120:2532–2539. [PubMed: 24070811]
61. Kitnarong N, Chindasub P, Metheetrairut A. Surgical outcome of intravitreal bevacizumab and filtration surgery in neovascular glaucoma. Adv Ther. 2008; 25:438–443. [PubMed: 18425438]
62. Kiuchi Y, Nakae K, Saito Y, et al. Pars plana vitrectomy and panretinal photocoagulation combined with trabeculectomy for successful treatment of neovascular glaucoma. Graefes Arch Clin Exp Ophthalmol. 2006; 244:1627–1632. [PubMed: 16639623]
63. Koch S, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. Cold Spring Harb Perspect Med. 2012; 2:a006502. [PubMed: 22762016]
64. Kotecha A, Spratt A, Ogunbowale L, et al. Intravitreal bevacizumab in refractory neovascular glaucoma: a prospective, observational case series. Arch Ophthalmol. 2011; 129:145–150. [PubMed: 21320957]
65. Kovacic Z, Ivanisevic M, Rogosic V, et al. Cyclocryocoagulation in treatment of neovascular glaucoma. Lijec Vjesn. 2004; 126:240–242. [PubMed: 15918320]
66. Kuzmin A, Lipatov D, Chistyakov T, et al. Vascular endothelial growth factor in anterior chamber liquid patients with diabetic retinopathy, cataract and neovascular glaucoma. Ophthalmol Ther. 2013; 2:41–51. [PubMed: 25135700]
67. Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. Surv Ophthalmol. 2003; 48:314–346. [PubMed: 12745005]
68. Li WW, Talcott KE, Zhai AW, et al. The role of therapeutic angiogenesis in tissue repair and regeneration. Adv Skin Wound Care. 2005; 18:491–500. quiz 501–492. [PubMed: 16365547]
69. Li Z, Van Bergen T, Van de Veire S, et al. Inhibition of vascular endothelial growth factor reduces scar formation after glaucoma filtration surgery. Invest Ophthalmol Vis Sci. 2009; 50:5217–5225. [PubMed: 19474408]
70. Lim TH, Bae SH, Cho YJ, et al. Concentration of vascular endothelial growth factor after intracameral bevacizumab injection in eyes with neovascular glaucoma. Korean J Ophthalmol. 2009; 23:188–192. [PubMed: 19794946]
71. Liu ZY, Qiu HO, Yuan XJ, et al. Suppression of lymphangiogenesis in human lymphatic endothelial cells by simultaneously blocking VEGF-C and VEGF-D/VEGFR-3 with norcantharidin. Int J Oncol. 2012; 41:1762–1772. [PubMed: 22922710]
72. Lopilly Park HY, Kim JH, Ahn MD, et al. Level of vascular endothelial growth factor in tenon tissue and results of glaucoma surgery. Arch Ophthalmol. 2012; 130:685–689. [PubMed: 22332204]
73. Lorenzon E, Colladel R, Andreuzzi E, et al. MULTIMERIN2 impairs tumor angiogenesis and growth by interfering with VEGF-A/VEGFR2 pathway. Oncogene. 2012; 31:3136–3147. [PubMed: 22020326]

74. Luke J, Nassar K, Luke M, et al. Ranibizumab as adjuvant in the treatment of rubeosis iridis and neovascular glaucoma--results from a prospective interventional case series. Graefes Arch Clin Exp Ophthalmol. 2013; 251:2403–2413. [PubMed: 23893090]
75. Marks JR, Clarke JCK, Peto T, et al. Postoperative increased bleb vascularity persists for over one year and has implications for intraocular pressure control. Investigative Ophthalmology & Visual Science. 2004; 45:U377–U377.
76. McBride JD, Jenkins AJ, Liu X, et al. Elevated circulation levels of an antiangiogenic SERPIN in patients with diabetic microvascular complications impair wound healing through suppression of Wnt signaling. J Invest Dermatol. 2014; 134:1725–1734. [PubMed: 24463424]
77. Md Noh SM, Sheikh Abdul Kadir SH, Bannur ZM, et al. Effects of ranibizumab on the extracellular matrix production by human Tenon's fibroblast. Exp Eye Res. 2014; 127:236–242. [PubMed: 25139730]
78. Mead AL, Wong TT, Cordeiro MF, et al. Evaluation of anti-TGF-beta2 antibody as a new postoperative anti-scarring agent in glaucoma surgery. Invest Ophthalmol Vis Sci. 2003; 44:3394–3401. [PubMed: 12882787]
79. Memarzadeh F, Varma R, Lin LT, et al. Postoperative use of bevacizumab as an antifibrotic agent in glaucoma filtration surgery in the rabbit. Invest Ophthalmol Vis Sci. 2009; 50:3233–3237. [PubMed: 19182254]
80. Mosaed S, Minckler DS. Aqueous shunts in the treatment of glaucoma. Expert Rev Med Devices. 2010; 7:661–666. [PubMed: 20822388]
81. Nagy JA, Dvorak AM, Dvorak HF. VEGF-A and the induction of pathological angiogenesis. Annu Rev Pathol. 2007; 2:251–275. [PubMed: 18039100]
82. Nauta A, Seidel C, Deveza L, et al. Adipose-derived stromal cells overexpressing vascular endothelial growth factor accelerate mouse excisional wound healing. Mol Ther. 2013; 21:445–455. [PubMed: 23164936]
83. Nilforushan N, Yadgari M, Kish SK, et al. Subconjunctival bevacizumab versus mitomycin C adjunctive to trabeculectomy. Am J Ophthalmol. 2012; 153:352–357. e351. [PubMed: 21982106]
84. O'Neill EC, Qin Q, Van Bergen NJ, et al. Antifibrotic activity of bevacizumab on human Tenon's fibroblasts in vitro. Invest Ophthalmol Vis Sci. 2010; 51:6524–6532. [PubMed: 20574016]
85. Olmos LC, Lee RK. Medical and surgical treatment of neovascular glaucoma. Int Ophthalmol Clin. 2011; 51:27–36. [PubMed: 21633236]
86. Ozgonul C, Mumcuoglu T, Gunal A. The effect of bevacizumab on wound healing modulation in an experimental trabeculectomy model. Curr Eye Res. 2014; 39:451–459. [PubMed: 24215335]
87. Papadopoulos N, Martin J, Ruan Q, et al. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. Angiogenesis. 2012; 15:171–185. [PubMed: 22302382]
88. Pieramici DJ, Rabena MD. Anti-VEGF therapy: comparison of current and future agents. Eye (Lond). 2008; 22:1330–1336. [PubMed: 18497829]
89. Pipp F, Heil M, Issbrucker K, et al. VEGFR-1-selective VEGF homologue PlGF is arteriogenic: evidence for a monocyte-mediated mechanism. Circ Res. 2003; 92:378–385. [PubMed: 12600898]
90. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol. 2014; 9:142–160. [PubMed: 24610033]
91. Razeghinejad MR, Fudemberg SJ, Spaeth GL. The changing conceptual basis of trabeculectomy: a review of past and current surgical techniques. Surv Ophthalmol. 2012; 57:1–25. [PubMed: 22137574]
92. Rehman SU, Amoaku WM, Doran RM, et al. Randomized controlled clinical trial of beta irradiation as an adjunct to trabeculectomy in open-angle glaucoma. Ophthalmology. 2002; 109:302–306. [PubMed: 11825813]
93. Robinson CJ, Stringer SE. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. J Cell Sci. 2001; 114:853–865. [PubMed: 11181169]
94. Rossiter H, Barresi C, Pammer J, et al. Loss of vascular endothelial growth factor a activity in murine epidermal keratinocytes delays wound healing and inhibits tumor formation. Cancer Res. 2004; 64:3508–3516. [PubMed: 15150105]

95. Saeedi OJ, Jefferys JL, Solus JF, et al. Risk factors for adverse consequences of low intraocular pressure after trabeculectomy. J Glaucoma. 2014; 23:e60–68. [PubMed: 24145291]
96. Saito Y, Higashide T, Takeda H, et al. Beneficial effects of preoperative intravitreal bevacizumab on trabeculectomy outcomes in neovascular glaucoma. Acta Ophthalmol. 2010; 88:96–102. [PubMed: 19775309]
97. Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol. 2008; 26:2013–2019. [PubMed: 18421054]
98. Schwartz K, Budenz D. Current management of glaucoma. Curr Opin Ophthalmol. 2004; 15:119–126. [PubMed: 15021223]
99. Seet LF, Finger SN, Chu SW, et al. Novel insight into the inflammatory and cellular responses following experimental glaucoma surgery: a roadmap for inhibiting fibrosis. Curr Mol Med. 2013; 13:911–928. [PubMed: 23651348]
100. Seibold LK, Sherwood MB, Kahook MY. Wound modulation after filtration surgery. Surv Ophthalmol. 2012; 57:530–550. [PubMed: 23068975]
101. Sengupta S, Venkatesh R, Ravindran RD. Safety and efficacy of using off-label bevacizumab versus mitomycin C to prevent bleb failure in a single-site phacotrabeculectomy by a randomized controlled clinical trial. J Glaucoma. 2012; 21:450–459. [PubMed: 21543993]
102. Shibuya M. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. J Biochem. 2013; 153:13–19. [PubMed: 23172303]
103. Shibuya M. VEGF-VEGFR Signals in Health and Disease. Biomol Ther (Seoul). 2014; 22:1–9. [PubMed: 24596615]
104. Simha A, Braganza A, Abraham L, et al. Anti-vascular endothelial growth factor for neovascular glaucoma. Cochrane Database Syst Rev. 2013; 10:CD007920. [PubMed: 24089293]
105. Sivak-Callcott JA, O'Day DM, Gass JD, et al. Evidence-based recommendations for the diagnosis and treatment of neovascular glaucoma. Ophthalmology. 2001; 108:1767–1776. quiz1777, 1800. [PubMed: 11581047]
106. Spaeth GL, Mutlukan E. The use of antimetabolites with trabeculectomy: a critical appraisal. J Glaucoma. 2001; 10:145–151. [PubMed: 11442174]
107. Suh W, Kee C. The effect of bevacizumab on the outcome of trabeculectomy with 5-Fluorouracil. J Ocul Pharmacol Ther. 2013; 29:646–651. [PubMed: 23621628]
108. Szekanecz Z, Besenyei T, Szentpetery A, et al. Angiogenesis and vasculogenesis in rheumatoid arthritis. Curr Opin Rheumatol. 2010; 22:299–306. [PubMed: 20305562]
109. Takeuchi K, Nakazawa M, Ebina Y. Effects of trehalose on VEGF-stimulated angiogenesis and myofibroblast proliferation: implications for glaucoma filtration surgery. Invest Ophthalmol Vis Sci. 2011; 52:6987–6993. [PubMed: 21778273]
110. Takeuchi K, Nakazawa M, Ebina Y, et al. Inhibitory effects of trehalose on fibroblast proliferation and implications for ocular surgery. Exp Eye Res. 2010; 91:567–577. [PubMed: 20650271]
111. Tchaikovski V, Fellbrich G, Waltenberger J. The molecular basis of VEGFR-1 signal transduction pathways in primary human monocytes. Arterioscler Thromb Vasc Biol. 2008; 28:322–328. [PubMed: 18079407]
112. Tham YC, Li X, Wong TY, et al. Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040: A Systematic Review and Meta-Analysis. Ophthalmology. 2014
113. Van Bergen T, Vandewalle E, Van de Veire S, et al. The role of different VEGF isoforms in scar formation after glaucoma filtration surgery. Exp Eye Res. 2011; 93:689–699. [PubMed: 21907194]
114. Vandewalle E, Abegao Pinto L, Van Bergen T, et al. Intracameral bevacizumab as an adjunct to trabeculectomy: a 1-year prospective, randomised study. Br J Ophthalmol. 2014; 98:73–78. [PubMed: 24158846]
115. Vasudev D, Blair MP, Galasso J, et al. Intravitreal bevacizumab for neovascular glaucoma. J Ocul Pharmacol Ther. 2009; 25:453–458. [PubMed: 19857107]

116. Vempati P, Popel AS, Mac Gabhann F. Extracellular regulation of VEGF: isoforms, proteolysis, and vascular patterning. Cytokine Growth Factor Rev. 2014; 25:1–19. [PubMed: 24332926]

117. Waisbourd M, Shemesh G, Kurtz S, et al. Topical bevacizumab for neovascular glaucoma: a pilot study. Pharmacology. 2014; 93:108–112. [PubMed: 24556733]

118. Weinheimer-Haus EM, Judex S, Ennis WJ, et al. Low-intensity vibration improves angiogenesis and wound healing in diabetic mice. PLoS One. 2014; 9:e91355. [PubMed: 24618702]

119. Wilgus TA, Ferreira AM, Oberyszyn TM, et al. Regulation of scar formation by vascular endothelial growth factor. Lab Invest. 2008; 88:579–590. [PubMed: 18427552]

120. Wilkins M, Indar A, Wormald R. Intra-operative mitomycin C for glaucoma surgery. Cochrane Database Syst Rev. 2005:CD002897. [PubMed: 16235305]

121. Witmer AN, Vrensen GF, Van Noorden CJ, et al. Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res. 2003; 22:1–29. [PubMed: 12597922]

122. Wu Y, Lucia K, Lange M, et al. Hypoxia inducible factor-1 is involved in growth factor, glucocorticoid and hypoxia mediated regulation of vascular endothelial growth factor-A in human meningiomas. J Neurooncol. 2014; 119:263–273. [PubMed: 24980036]

123. Yang F, Jin C, Jiang YJ, et al. Potential role of soluble VEGFR-1 in antiangiogenesis therapy for cancer. Expert Rev Anticancer Ther. 2011; 11:541–549. [PubMed: 21504321]

124. Yang H, Shin S, Ahn J, et al. Local injection of pulp cells enhances wound healing during the initial proliferative phase through the stimulation of host angiogenesis. J Endod. 2013; 39:788–794. [PubMed: 23683280]

125. Yao JC, Lombard-Bohas C, Baudin E, et al. Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. J Clin Oncol. 2010; 28:69–76. [PubMed: 19933912]

126. Yazdani S, Hendi K, Pakravan M, et al. Intravitreal bevacizumab for neovascular glaucoma: a randomized controlled trial. J Glaucoma. 2009; 18:632–637. [PubMed: 19826393]

127. Ye W, Liu R, Pan C, et al. Multicenter randomized phase 2 clinical trial of a recombinant human endostatin adenovirus in patients with advanced head and neck carcinoma. Mol Ther. 2014; 22:1221–1229. [PubMed: 24662947]

128. Zhang F, Tang Z, Hou X, et al. VEGF-B is dispensable for blood vessel growth but critical for their survival, and VEGF-B targeting inhibits pathological angiogenesis. Proc Natl Acad Sci U S A. 2009; 106:6152–6157. [PubMed: 19369214]

129. Zhang W, Ran S, Sambade M, et al. A monoclonal antibody that blocks VEGF binding to VEGFR2 (KDR/Flk-1) inhibits vascular expression of Flk-1 and tumor growth in an orthotopic human breast cancer model. Angiogenesis. 2002; 5:35–44. [PubMed: 12549858]

130. Zhou M, Chen S, Wang W, et al. Levels of erythropoietin and vascular endothelial growth factor in surgery-required advanced neovascular glaucoma eyes before and after intravitreal injection of bevacizumab. Invest Ophthalmol Vis Sci. 2013; 54:3874–3879. [PubMed: 23674760]

Table I

VEGF Family and VEGF Receptors

Receptors	Known Signaling Pathways	Ligands[15]	Responses
VEGFR-1 (Flt1)	ERK/MAPK PI3K/PKB/AKT[111]	VEGF-A	• Weak angiogenesis[81] • Soluble VEGFR-1 prevents VEGF-A binding to VEGFR-2 (negatively regulates angiogenesis)[123]
		VEGF-B	Sustains newly formed blood vessels[128]
		PlGF	Arteriogenesis[89]
VEGFR-2 (KDR)	Ras/RAFK/ERK/MAPK[63,103]	VEGF-A	• 10-fold stronger angiogenic response than VEGFR-1[12] • Endothelial cell proliferation, survival, migration and permeability[13]
VEGFR-3 (Flt4)	PI3K/AKT[23]	VEGF-C	Lymphangiogenesis[33,56,71]
		VEGF-D	

Surv Ophthalmol. Author manuscript; available in PMC 2016 November 01.

Table II

Application of Anti-VEGF Agents in Glaucoma Filtration Surgery

Anti-VEGF Agents	Mechanism of Action	Method of Administration	Outcome			References
			In vitro	Animal Model	Clinical Trial	
Bevacizumab (Bvb)	Binds to all isoforms of VEGF-A, inhibiting ligand-receptor (VEGFR-1 and VEGFR-2) interaction[97]	Intracameral	Inhibition of rabbit and human Tenon fibroblast proliferation and collagen gel contractility in a dose-dependent manner	--	• No significant effect in terms of IOP control but higher absolute success rate compared to placebo • No significant additive effect when combined with 5-FU	Li et al[69] O'Neill et al[84] Memarzadeh et al[79] Ozgonul et al[86] How et al[50] Vandewalle et al[114] Grewal et al[41] Sengupta et al[101] Nilforushan et al[83] Jurkowska-Dudzinska et al[54] Suh et al[107] Chua et al[20]
		Subconjunctival		• Larger, more diffused, and less vascular bleb with longer bleb survival time but no significant effect on IOP • Combination with 5-FU has better antifibrotic effect than monotherapy	• No significant difference in terms of safety and efficacy compared to 5-FU or MMC • No significant additive effect when combined with 5-FU • Gradual increase in bleb vascularity starting 3 months after treatment	
Ranibizumab (Rbb)	Affinity - matured to stronger affinity than Bvb, Rbb binds to all isoforms of VEGF-A inhibiting ligand-receptor binding (VEGFR-1 and VEGFR-2)[88,97]	Intravitreal	Inhibition of human Tenon fibroblast proliferation and reduction of COL1α1 transcription	--	Larger, more diffuse blebs with lower vascularity when combined with MMC	Noh et al[77] Kahook et al[55]

Table III

Application of Anti-VEGF Agents in Neovascular Glaucoma

Anti-VEGF Agents	Mechanism of Action	Administration	Clinical Trial Results	References
Bevacizumab (Bvb)	*Refer to* Table II	Intravitreal	• Reduced iris neovascularization and IOP • Combination therapy with PRP and retinal ablative procedure showed a longer lasting open angle, improved visual acuity, and decreased IOP • Reduced pain within 1 week after first administration	Yazdani et al[126] Brouzas et al[11] Ehelers et al[30] Vasudev et al[115] Kotecha et al[64] Costagliola et al[24] Lim et al[70] Duch et al[29] Waisbourd et al[117]
		Intracameral	• Decreased concentration of VEGF in aqueous humor • No significant change in IOP • Allowed or eliminated need for angle filtration surgery	
		Topical	Reduction of mean IOP	
Ranibizumab (Rbb)		Intravitreal	Reduced and maintained IOP in patients with rubeosis and NVG better than in patients with rubeosis only	Luke et al[74]