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Corneal Neovascularization: An Anti-VEGF Therapy Review

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Abstract

Corneal neovascularization is a serious condition that can lead to a profound decline in vision. The abnormal vessels block light, cause corneal scarring, compromise visual acuity, and may lead to inflammation and edema. Corneal neovascularization occurs when the balance between angiogenic and antiangiogenic factors is tipped toward angiogenic molecules. Vascular endothelial growth factor (VEGF), one of the most important mediators of angiogenesis, is upregulated during neovascularization. In fact, anti-VEGF agents have efficacy in the treatment of neovascular age-related macular degeneration, diabetic retinopathy, macular edema, neovascular glaucoma, and other neovascular diseases. These same agents have great potential for the treatment of corneal neovascularization. We review some of the most promising anti-VEGF therapies, including bevacizumab, VEGF trap, siRNA, and tyrosine kinase inhibitors.

Keywords

anti-VEGF therapy; corneal neovascularization; bevacizumab; ranibizumab; VEGF Trap; silencing RNA

I. Introduction

The cornea is normally an avascular, transparent connective tissue that serves as a mechanical barrier and the anterior refractive surface of the eye. It is supplied by the ciliary arteries, branches of the ophthalmic artery that divide and end in the pericorneal plexus near the limbus. Corneal clarity is the direct result of a very intricate balance between its cellular components and layers.[34] The cornea is also immunoprivileged, which is a major protective feature of the highly organized structure of the eye and also contributes to the high success rates of corneal transplants.[123]

Angiogenesis is the process of new blood vessel growth from pre-existing vascular structures. Corneal angiogenesis occurs in several pathological conditions and brings about a variety of unwanted consequences. New vessels, which sprout from the capillaries and venules of the pericorneal plexus, may block light, compromise visual acuity, worsen the prognosis of penetrating keratoplasty, and lead to inflammation, corneal scarring, and edema.[23] The normally avascular cornea may vascularize in situations in which a

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disequilibrium between angiogenic and antiangiogenic stimuli leads to a surplus of pro-angiogenic factors (such as vascular endothelial growth factor [VEGF], basic fibroblast growth factor [bFGF], and matrix metalloproteinases) and a deficiency in antiangiogenic factors (such as soluble VEGF Receptor-2 [sVEGFR-2 or sflt-1], pigment epithelium–derived factor, angiostatin, and endostatin).[3]

VEGF (also known as VEGF-A) is a secreted growth factor peptide that belongs to a gene family that includes VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). Unlike the other family members, which are all mammalian-encoded peptides, VEGF-E is encoded by the poxvirus orf virus.[88] VEGF-A, which is the focus of this review, is the main regulator of hemangiogenesis, whereas VEGF-C and VEGF-D are key regulators of lymphangiogenesis (although recent evidence indicates that VEGF-A has a role in lymphangiogenesis as well).[27] Alternative splicing of the VEGF-A gene (organized into eight exons and seven introns) leads to the expression of many isoforms, with the main isoforms being VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206. These isoforms vary in molecular mass, solubility, and heparin-binding ability, and VEGF165 is the predominant isoform.[46] VEGF promotes vascular endothelial cell proliferation, migration, and tube formation.[23] It also increases vascular leakage and promotes monocyte chemotaxis and B-cell production in mice, indicating the key role of VEGF in inflammation.[46]

VEGF binds to two members of a receptor tyrosine kinase family, VEGF receptor (VEGFR)-1 and VEGFR-2, also known as Flt-1 and KDR, respectively. VEGFR-2 is considered the main VEGF receptor and mediates the proliferative effects of VEGF on vascular endothelial cells. VEGF binding to VEGFR-2 induces the dimerization and subsequent autophosphorylation of receptors by intracellular kinase domains, which leads to a mitogenic and proliferative signal.[79] VEGF-C and VEGF-D bind to VEGFR-3, another member of this family of receptor tyrosine kinases.

Corneal neovascularization may occur secondary to chemical burns, ischemia, infection, trauma, and inflammation and is a major cause of blindness that affects up to 4.14% of patients presenting for eye care or approximately 1.4 million people per year.[9] Reports indicate that infectious diseases, the extended wearing of contact lenses, and a vascular response to corneal transplantation are major causes of corneal neovascularization.[82] Antiangiogenic therapy demonstrates great promise for future treatment of this condition.

The issue of uncontrolled angiogenesis has its roots in cancer research, stemming from Folkman's observation in 1971 that tumor growth depends on angiogenesis.[49] Subsequent studies demonstrated that tumor cells begin promoting angiogenesis early in tumorigenesis, a process characterized by the oncogene-driven expression of pro-angiogenic proteins that include, but are not limited to, VEGF, bFGF, interleukin-8 (IL-8), PlGF, and transforming growth factor-β (TGF-β). Interventions were then aimed at the disruption of tumor-related angiogenesis in hopes that this would lead to destruction of the tumor. Over time, direct angiogenesis inhibitors, which prevent vascular endothelial cells from proliferating and migrating in response to pro-angiogenic proteins, and indirect inhibitors, which block the expression of tumor proteins that promote angiogenesis or their respective receptors, were generated.[73] This led to the eventual development of anti-VEGF agents. These agents have demonstrated mixed efficacy in treating cancer in phase III clinical trials.[69] Although anti-VEGF therapy has potential in cancer treatment,[68] the results from adjuvant studies have been disappointing.[11] Nevertheless, Folkman's work spurred more angiogenesis research, eventually entering clinical specialties such as cardiology, dermatology, and ophthalmology. Angioregressive therapy is now used in the treatment of several ocular diseases, including corneal neovascularization.

Current treatments for corneal neovascularization include topical corticosteroid and non-steroid anti-inflammatory medications, photodynamic therapy, laser photocoagulation, fine needle diathermy, and conjunctival, limbal, and amniotic membrane transplantation.[23,82,115] Unfortunately, these all have limited clinical efficacy and also cause a multitude of undesirable side effects, especially elevated intraocular pressure and posterior subcapsular cataracts subsequent to corticosteroid use. Targeting treatment to the anterior segment of the eye requires drugs with unique capabilities, including the ability to pass through an intact outer epithelium. More importantly, none of these treatments target the molecular mediators of angiogenesis.

The requirement of VEGF for corneal neovascularization was first demonstrated in a rat model.[2] Upregulation of VEGF was induced through corneal injury, and neovascularization was subsequently blocked by anti-VEGF antibodies. A later study revealed that VEGF inhibition through the delivery of a murine soluble VEGF receptor protein, mFlt (1–3)-immunoglobulin G, decreased corneal angiogenesis secondary to Herpes simplex virus.[142] This led to the hypothesis that administration of anti-VEGF agents may be an effective therapeutic option for the management of corneal neovascularization.[65] Indeed, anti-VEGF agents are effective in the treatment of other ocular diseases such as neo-vascular age-related macular degeneration, diabetic retinopathy, and neovascular glaucoma.[126] Numerous studies and clinical trials have demonstrated the efficacy and safety of the anti-VEGF agents bevacizumab (Avastin; Genentech/Roche), ranibizumab (Lucentis; Genetech/Roche), and pegaptanib (Macugen; EyeTech, Inc) in the treatment of retinal disorders.[53,110,118,128] Studies demonstrating the efficacy of anti-VEGF agents against corneal neovascularization are less numerous, but more have been published in recent years.

Anti-VEGF agents have demonstrated efficacy in reducing corneal neovascularization in both animal models and clinical trials. Specifically, anti-VEGF antibodies have shown initial therapeutic success. Bevacizumab is a full-length, humanized murine monoclonal antibody that recognizes all isoforms of VEGF. Bevacizumab was initially approved by the U.S. Food and Drug Administration (FDA) to treat meta-static colon cancer,[47] but has also shown efficacy in the treatment of various neovascular ocular diseases and is used off-label to treat neovascular age-related macular degeneration.[5,81] In addition, studies have exhibited partial reduction of corneal neovascularization through topical, subconjunctival, and intraocular application of bevacizumab.[1,6,18,19,25,30,35,39,40,54–58,64,66,67,76,78,80,84,92,102,103,105,134,139,141]

Ranibizumab is the Fab fragment from the same antibody used to create bevacizumab, but it has been affinity-matured so that it binds VEGF-A with significantly higher affinity. Ranibizumab and bevacizumab appear to have similar efficacy profiles in the treatment of neovascular age-related macular degeneration.

Pegaptanib, a 28-base ribonucleic aptamer that specifically binds the VEGF165 isoform with high affinity, was the first FDA-approved anti-VEGF agent for the treatment of neovascular age-related macular degeneration. The fact that it only binds a specific isoform may explain its limited efficacy compared to ranibizumab and bevacizumab. This limited targeting also likely explains its long history of safe use and reduced risk of side effects compared to the other anti-VEGF antibodies. Given its safety record, pegaptanib may be used in the future for maintenance anti-VEGF therapy.[107]

VEGF trap, created by combining the second domain of VEGFR-1 and the third domain of VEGFR-2 with a human IgG Fc fragment,[62] is the highest affinity VEGF blocker currently being studied and acts as a receptor decoy for all isoforms of VEGF-A. Along with binding all isoforms of VEGF-A with high affinity, it also binds PlGF-1 and PlGF-2, which

potentially enhances the antiangiogenic response. Another potential advantage of VEGF trap is its extended duration of action. The VEGF-binding activity of VEGF trap at 79 days after intravitreal injection is comparable to that of ranibizumab at 30 days post-injection.[122] Preliminary clinical trials demonstrated that VEGF trap-eye (aflibercept, EYLEA; Regeneron), a product specially formulated to reduce irritation after direct injection into the eye, has acceptable safety and tolerability when used to treat neovascular age-related macular degeneration[20,36,60,99] and diabetic macular edema.[38] Further clinical trials are needed to compare the effectiveness of VEGF trap to the other anti-VEGF antibodies. Here, we provide preliminary evidence of the demonstrated efficacy of VEGF trap in the prevention of corneal neovascularization in animal models.[104]

Another anti-VEGF therapeutic approach involves the use of silencing RNA (siRNA) to silence VEGF genes. siRNA are double-stranded RNA fragments that are homologous to the gene being suppressed. After processing by Dicer, an RNAase III, these double-stranded RNA fragments incorporate into the RNA-induced silencing complex and then degrade mRNA in a sequence-specific manner.[41,48] As siRNA has the ability to traverse cellular boundaries and inhibit post-transcriptional processing, an siRNA may be superior to anti-VEGF-A antibodies and aptamers in that it can target both the intracellular and extracellular effects of VEGF and its receptors.[143] siRNA is not replicated in mammalian cells[41] and may have only transient effects. Nevertheless, siRNA therapeutic agents, bevasiranib and SIRNA-027 (which target VEGF and the VEGF receptor-1, respectively), are currently being tested. A trial of SIRNA-027 in neovascular age-related macular degeneration demonstrated positive results.[71] In addition, VEGF siRNA reduced VEGF expression and secretion in human corneal cells in vitro and reduced corneal neovascularization in mice in vivo.[75,95,119,143]

Recently, research on the development of new anti-VEGF agents has focused on the inhibition of various steps of the VEGF signaling pathway (Table 1). A few target the downstream tyrosine kinase pathway initiated by VEGFRs. Pazopanib (Votrient; GlaxoSmithKline) is a tyrosine kinase inhibitor currently undergoing clinical trials for the treatment of retinal disorders. This idea has been applied to corneal angiogenesis, and the application of a tyrosine kinase inhibitor has improved graft survival and corneal transplantation in mice.[63] In the following section, we discuss the various anti-VEGF therapeutic agents (Table 1) with regard to their use as interventions/treatments for corneal neovascularization and assess their outcome and safety profiles.

II. Corneal Diseases Involved in VEGF

Various infectious, inflammatory, and traumatic disorders may lead to neovascularization of the cornea.[127] Neovascular patterns can be separated into three clinical groups: deep neovascularization overlying Descemet's membrane (seen in herpetic and luetic interstitial keratitis), stromal neovascularization (as a result of stromal keratitis), and vascular pannus (from ocular surface disorders).[42] Infectious keratitis typically leads to neovascularization and is frequently caused by an infection from the herpes virus family—Herpes simplex and herpes zoster. The mechanism by which a herpes virus initiates neovascularization and the upregulation of VEGF[142] is uncertain, but evidence suggests that IL-6 and matrix metalloproteinase-9 play a role.[17,83] Bacterial and fungal agents may also induce keratitis.[23] Other causes of corneal neovascularization include the overuse of contact lenses, chemical burns, and limbal stem cell deficiency.[19] Corneal neovascularization may also occur in degenerative diseases, such as pterygium and Terrien marginal degeneration.[87]

One major issue related to the topic of corneal neovascularization is allograft rejection after corneal transplantation. Grafting onto vascularized corneal beds is known as high-risk

transplantation because of the frequent occurrence of immune rejection.[90,131] Recipient neovascularization may more than double the risk of graft rejection.[8] Moreover, corneal neovascularization may also be induced postoperatively, further compounding the problem.[9] The normally avascular cornea is an immuno-privileged area of the body, and the precise reason why this tolerance is disturbed during neovascularization is not fully understood. In typical immune responses, lymphatic vessels act as the afferent (sensitization) arm of the response by allowing antigen-presenting and other immune cells to enter the regional lymph nodes, and blood vessels act as the efferent (rejection) arm by allowing effector cells access to the target tissue.[28] Therefore, a reduction of neovascularization should minimize the immunoinflammatory response after corneal transplant and increase graft survival.

III. Anti-VEGF Therapy as an Intervention against Corneal Angiogenesis (Tables 1 and 2)

A. ANTI-VEGF ANTIBODY (BEVACIZUMAB AND RANIBIZUMAB)—OUTCOME AND SIDE EFFECTS

Topical bevacizumab partially reduces corneal neovascularization in experimental animal models.[18,55,92] In the first reported human use of topical bevacizumab therapy for the reduction of corneal neovascularization, two patients were given 1% topical bevacizumab four times a day, and this resulted in significant reductions in superficial and deep stromal neovascularization.[35] Koenig et al[80] delivered topical bevacizumab to 30 eyes of 27 patients who were not responding to traditional anti-inflammatory therapy and reported a 61% reduction in the mean vascularized area and a 24% reduction in vessel diameter. They also suggested that maximal effects were observed when bevacizumab was administered early in the course of neovascularization, which is consistent with animal studies.[85,105] Other human studies have confirmed the efficacy of topical bevacizumab in reducing corneal neovascularization.[19,30,76] Dastjerdi et al reported a 47.1% decrease in mean neovascular area ($p= 0.0014$) and a 54.1% decrease in vessel caliber ($p = 0.00009$) in 10 eyes from 10 patients with clinically stable neovascularization after treatment with bevacizumab.[30] Topical administration of bevacizumab carries an increased risk of corneal epithelial side effects that will be discussed subsequently.

Subconjunctival bevacizumab therapy has also shown promising results. Experiments conducted in rabbits, mice, and rats have consistently demonstrated statistically significant reductions in corneal neovascularization and ocular tissue VEGF levels after subconjunctival bevacizumab administration. Chu et al[25] administered monthly injections of subconjunctival bevacizumab to 18 patients with lipid keratopathy secondary to corneal neovascularization. They measured the extent, centricity, and percentage of involved corneal surface of the neovascularization. All parameters of corneal neovascularization and lipid deposition improved significantly with bevacizumab (all $p < 0.05$). A study that involved 10 patients with major and minor corneal vessel neovascularization determined that the subconjunctival administration of 2.5 mg (0.1 ml) bevacizumab was well tolerated and led to decreases in the total area and extent of neovascularization for up to 3 months after the injection.[141] Further human studies continue to demonstrate that subconjunctivally injected bevacizumab reduces neovascularization[39,54,139] and also suggest that the efficacy of bevacizumab increases at higher doses (5.0 mg vs 2.5 mg in the You et al[139] study).

It remains unclear whether subconjunctival administration is more effective than topical administration. In a murine corneal graft model, one study showed an increase in corneal graft survival rate when bevacizumab was administered to mice either topically or subconjunctivally after high-risk corneal transplant.[31] It was determined that the reduction in neovascularization was more profound in the subconjunctival administration group.

Moreover, only subconjunctival administration was able to decrease the graft rejection rate, with 33% of the corneal grafts surviving in the subconjunctival group compared to 0% in both the control and topical groups ($p < 0.01$). From these results, it was concluded that topically applied bevacizumab is unable to adequately penetrate through the corneal endothelium. On the other hand, several reports have demonstrated the clinical efficacy of topical bevacizumab in the reduction of corneal neovascularization.[19,30,76,80] Perhaps a sufficient dose was not applied in the murine study (as bevacizumab may need to be given at a higher dose when administered topically to achieve the effects of subconjunctivally applied bevacizumab),[56] or perhaps there are inherent differences between a cornea with inflammation-induced neovascularization and a newly grafted cornea. Nevertheless, Rocher et al achieved results similar to the just-mentioned murine study in a study conducted in rats that used rat anti-VEGF antibodies instead of bevacizumab.[109] (The authors felt that the results would have more merit and be more transferable to the clinical setting if an antibody specific to rat VEGF was used.) Compared with the control group, subconjunctival injection reduced the neovascularization area by more than 20%, whereas topical application decreased it by 15%. In contrast to the previous study, the researchers found that subconjunctival injections and the topical application had the same effect on the rejection rate.

In sum, experimental models indicate that the two methods are useful in reducing corneal neovascularization, but differ in efficacy, as the subconjunctival injection of a lower dose is comparable to the topical administration of a higher dose.[56] Subconjunctival administration does have its own unique risks, which will be described. Clearly, more studies that compare the safety of the two methods are needed before any conclusions can be drawn.

A few studies have investigated the intraocular administration of bevacizumab. Recent comparative studies in mice indicated that intraocular administration (intravitreal, intracameral, or anterior chamber administration) is more effective at reducing corneal neovascularization than subconjunctival administration.[6,40] Avisar et al determined that intravitreal and anterior chamber injection more effectively reduces neovascularization in mice for up to 10 days post-injection.[6] Subconjunctival administration delivered an earlier peak response, perhaps due to the anatomic proximity of the subconjunctival tissue to the limbus. Mouse strains differ in their response to the administration of anti-angiogenic agents in experimental angiogenic models due to their genetic heterogeneity and the fact that size is among the many differences between mouse and human eyes.[103,134] In mice topically applied bevacizumab is not able to penetrate corneas with intact epithelium.[32] Therefore, these studies must be replicated in humans.

These studies achieved the partial, but incomplete, elimination of corneal neovascularization. One reason for this is that cytokines/growth factors other than VEGF also induce angiogenesis in the cornea (FGF, TGF-β, etc.). Further, bevacizumab is solely an antibody against VEGF-A. Thus, it does not block the activities of the other members of the VEGF family. Also, bevacizumab is seemingly only effective against actively growing blood vessels, because established vessels are thought to not require VEGF for proliferation. The covering of blood vessels by pericytes marks the end of a sensitive period in which the absence of angiogenic factors like VEGF can lead to selective apoptosis and the regression of vessels. As most new vessels are covered by pericytes within 2 weeks, treating established vessels with angioregressive anti-VEGF therapy often leads to suboptimal results.[29]

A deep intrastromal injection of bevacizumab was performed because extensive neovascularization was found deep in the stroma achieved both the dramatic regression of neovascularization and the lack of reoccurrence for 6 months.[58] The regression most likely

occurred because this provided the stroma with much better therapeutic levels than other methods of injection. Of course, the safety of intracorneal injection still needs to be established, as exposure of the stroma to higher levels of bevacizumab could lead to more side effects. Corneal neovascularization also regressed after intracorneal injection in two other cases, although in one the patient experienced an intracorneal hemorrhage that spontaneously cleared.

In vitro safety profile studies demonstrated that bevacizumab has no cytotoxic effects on human corneal cell lines at concentrations up to 4 mg/ml.[22,135] In vivo reports of complications are widespread in the literature. In the initial research on bevacizumab, researchers administered the drug systemically to treat neovascular age-related macular degeneration; there were reports that the intravenous administration of bevacizumab led to an increased risk of thrombotic events including stroke and myocardial infarction, however, especially in patients with cancer.[4] The concentrations of bevacizumab used systemically may be 500 times greater than those used for ocular injections. Nevertheless, ocular administration (intravitreal, subconjunctival, topical, etc.) can also lead to side effects. Almost one-quarter (24.1%) of patients experienced at least one serious systemic event while receiving intravitreal bevacizumab injections, although the authors caution that more studies are needed to draw any conclusions about its safety profile.[93] In rabbits, intravitreal and subconjunctival injections of bevacizumab resulted in high plasma concentrations of the drug. Injections in one eye can lead to effects in the other eye as the drug is transported by the systemic circulation.[101]

Topical administration of bevacizumab appears to be associated with an increased risk of corneal epithelial defects that are dependent on the dose and duration of treatment. Kim et al[76] administered 1. 25% topical bevacizumab to 10 eyes of 7 patients twice daily for 3 months. Six of the 10 eyes developed epitheliopathy in the second month of treatment, suggesting a disruption of the adhesion between the epithelium and basement membrane or a disturbance in the wound healing process. Dastjerdi at al, who limited the duration of administration to 3 weeks and used a slightly smaller dose (1.0%), showed promising results (a 47.1% decrease in mean vascular area) and reported no side effects. [30] Koenig et al[80] administered topical bevacizumab at an even lower dose (0.5%) and reported a much smaller incidence of new corneal epithelial defects in their patients than in the Kim et al[76] study (17. 7% vs 60%). Taken together, these data imply that shorter treatment durations, along with a lower dosage, improve the safety profile of this medication. Randomized, controlled clinical trials with larger sample sizes are needed to confirm this and to identify a minimum dose.

One potential explanation for the epitheliopathy found after topical treatment with bevacizumab is the inhibition of corneal wound healing by anti-VEGF agents. VEGF plays a key role in the complex interactions that characterize the healing of a corneal wound.[132] Kim et al demonstrated the slowing of corneal epithelial wound healing in rabbits following the introduction of topical bevacizumab.[77] They found that bevacizumab reduced the expression of surface integrins and collagens, causing the reduced adhesion of corneal cells, and led to the occurrence of spontaneous corneal epithelial defects during the prolonged healing period. This side effect of bevacizumab is problematic in the treatment of corneal neovascularization in corneas with pre-existing wounds or injuries.

Most clinical studies using subconjunctival bevacizumab have reported no serious side effects. A few, however, have reported complications such as subconjunctival hemorrhage and corneal epithelial defects.[54,139] More serious side effects, such as inadvertent globe perforation and oculocardiac reflex, have also been reported after subconjunctival bevacizumab injection.[51,72] Nevertheless, subconjunctival injection is a widely used method

for the delivery of drugs into the eye with relatively few side effects. It is also simple to perform and serves as a viable option for patients who are not responding well to topical treatment. The issues of possible systemic absorption and other intraocular side effects still need to be addressed.

The intravitreal administration of bevacizumab has been accompanied by an even wider range of side effects. Reported complications include, but are not limited to, corneal abrasion, corneal infiltrative keratitis, corneal stromal edema, retinal pigment epithelium tear, acute visual loss, lens injury, vitritis, vitreous hemorrhage, endophthalmitis, hypertension, and myocardial infarction.[15,16,117,121] The majority of these events occurred during the treatment of retinal disorders, so the side effect profile may be different when intravitreal administration is used for the reduction of corneal angiogenesis. In spite of these side effects, the intravitreal administration of bevacizumab has been performed for years with a low incidence of complications. The safety profile of intravitreal bevacizumab was confirmed in the international intravitreal bevacizumab safety survey that found that, after 7,113 injections, none of the adverse event rates exceeded 0.21%.[50] Nevertheless, the possibility of ocular and systemic effects should be explained to patients, as bevacizumab injected intravitreally could migrate into the bloodstream and lead to blood pressure alterations and cerebrovascular accidents.[117]

B. VEGF TRAP

Cursiefen et al used a suture-induced neovascularization model in mice to demonstrate that VEGF trap successfully reduced vessel formation after injury, the area of vascularization being reduced from 49 ± 12% in control mice to 2. 3 ± 1.5% in mice treated with VEGF trap ($p < 0.001$).[27] Oliveira et al confirmed these findings by demonstrating that VEGF trap reduced bFGF-pellet-induced neovascularization.[104] The area of corneal neovascularization in untreated controls was 1.05 ± 0.12 mm^2 and 1.53 ± 0.27 mm^2 at days 4 and 7, respectively. This was significantly greater than that of mice treated with VEGF trap (0.24 ± 0.11 mm^2 and 0.35 ± 0.16 mm^2 at days 4 and 7, respectively; $p < 0.05$). VEGF trap has also been shown to increase corneal graft survival in mice, as will be discussed subsequently. Future studies that compare the safety and efficacy of VEGF trap and bevacizumab would be useful. As VEGF trap binds all isoforms of VEGF-A with high affinity as well as PlGF, and has a longer duration of effect in the eye, it may be found to be even more effective than bevacizumab in the prevention of neovascularization. Two ongoing clinical trials, VIEW-1 and VIEW-2, are comparing VEGF trap to ranibizumab in the treatment of neovascular age-related macular degeneration.[36]

If clinical VEGF trap trials are conducted for the prevention of corneal neovascularization, a safe method of injection will have to be determined. Systemic administration of VEGF trap in humans has led to good results in the treatment of neovascular age-related macular degeneration, but also to adverse systemic side effects, including hypertension and proteinuria, at higher doses.[100] Intravitreal injections of VEGF trap-eye are thought to be safer as they are more likely to avoid systemic toxicity. A 4.0 mg injection of VEGF trap-eye was well tolerated in patients with diabetic macular edema,[37] and several different doses have been well tolerated in patients with neovascular age-related macular degeneration.[36] The side effects that can result from localized injection include conjunctival hemorrhage, eye pain, ocular hyperemia, and vitreous floaters.[36] Although studies specific to corneal neovascularization need to be conducted, the current evidence suggests that VEGF trap-eye will reduce or avoid the systemic side effects that occur with the systemic administration of VEGF trap.

C. SILENCING RNA

One of the classic issues regarding the efficacy of siRNA systems is delivery. For example, when siRNA is injected systemically into mice, a significant amount is absorbed by organs such as the spleen and kidney, which reduces the amount of siRNA that reaches the intended target.[120] The ocular delivery of siRNA may be easier because the eye is a closed compartment, and target tissues are close to the area of delivery.[45] siRNA has the potential to be a powerful tool against corneal neovascularization because, unlike other extracellular methods, it blocks the intracellular effects of the VEGF gene. By hindering VEGF secretion and the potential intracellular autocrine loops that make the cells resistant to extracellular blockade, siRNA has the potential to reduce corneal neovascularization to a greater extent than antibodies and decoy receptors.

Kim et al reported that the delivering of a 1:1:1 mixture of siRNA targeting VEGF-A, VEGFR-1, and VEGFR-2 into mice led to decreases in VEGF mRNA and protein levels and a significant reduction in neovascularization.[75] Further, the 1:1:1 mixture was found to be more effective than the administration of an siRNA that targeted only one of the proteins, as it caused a 60% drop in neovascularization ($p < 0.01$). In addition, both the intravenous (administered via the polymer vehicle TargeTran[114]) and subconjunctival administration of this siRNA were effective, with subconjunctival injection being slightly more. Zuo et al induced neovascularization in mice through alkali burn and found that the subconjunctival injection of siRNA targeting VEGF-A led to decreases in VEGF expression, the vascularized area, and the number of new vessels.[143] Singh et al delivered siRNA against VEGF to mice intrastromally and found nearly the same results.[119] They also applied the siRNA in vitro to human corneal cells subjected to hypoxia (hypoxia induces VEGF synthesis) and found decreases in VEGF at the mRNA and protein levels.

As these studies indicate, siRNA directed against VEGF and VEGFR reduces corneal angiogenesis in mice. Future studies are needed to assess the safety of VEGF- and VEGFR-targeted siRNA before this method can be applied in humans. These studies should measure the structure and function of the corneal epithelium and stroma after siRNA injection. In addition, the fact that the effects of siRNA are only transient in mammalian cells[41] must be addressed. Perhaps, in the future, researchers can incorporate siRNA into recombinant viral vectors that would allow for a more prolonged effect.

IV. Corneal Transplantation

Increases in the corneal graft survival rate after anti-VEGF therapy have been demonstrated in animal models.[31,109,133] In an interventional case series, bevacizumab at 2.5 mg/0.1 mL per affected quadrant was injected subconjunctivally, perilimbally, and intrastromally before surgery in two patients with previous graft failure, and subconjunctivally and perilimbally at the end of surgery in 10 patients and during follow-up visits in 4 patients.[130] Even though all the transplantations were high risk, 85.7% of the grafts remained transparent during the follow-up period (mean, 7.1 months). This is consistent with case reports that have demonstrated successful corneal transplantation following bevacizumab injection before and after surgery. These injections, however, were combined with thermal vessel cautery in one report and argon laser coagulation in the other.[52,124] Unfortunately, there is also a case of permanent graft failure following the subconjunctival injection of 2.5 mg/0.1 ml bevacizumab after surgery.[7] Thus, controlled clinical trials with larger sample sizes are needed to determine if bevacizumab can substantially increase graft success rate.

VEGF trap use in murine models of normal and high-risk corneal transplantations has led to high graft survival rates.[9,10,26] Cursiefen et al showed that hemangiogenesis and lymphangiogenesis were induced postoperatively in a normal risk cornea and that both were

reduced by the early postoperative neutralization of VEGF-A through VEGF trap in a dose-dependent manner.[26] This resulted in an improved long term graft survival rate in the treatment group compared to the control group (78% vs 40%; $p < 0.05$). Later studies that modeled high-risk corneas also demonstrated increased graft survival rates following VEGF trap administration. One reported survival rates of 23% in the treatment group and 0% in the control group ($p = 0.007$), whereas the other reported rates of 36% in the treatment group and 9% in the control group ($p < 0.05$).[9,10] Thus it appears that, by reducing neovascularization, the direct contact between the graft and nearby vessels is eliminated (or at least significantly reduced), which prevents the immediate donor-specific sensitization created by nearby draining lymph nodes. This may allow time for tolerogenic mechanisms to develop and promote graft survival.

Inhibiting the downstream tyrosine kinase pathway leads to an increased graft survival rate in mice.[63] PTK/ZK is a tyrosine kinase inhibitor that targets all VEGF receptors (VEGFR-1, VEGFR-2, and VEGFR-3), whereas the tyrosine kinase inhibitor ZK911 has been shown to target VEGFR-2. Treatment with these agents inhibited both hemangiogenesis and lymphangiogenesis, and treatment with ZK911 led to an increased graft survival rate compared to the control group (68% vs 33%; $p < 0.02$). Tyrosine kinase inhibitors such as these may have advantages over other anti-VEGF agents as they inhibit almost every VEGF receptor, have high potency, and are absorbed orally.[98]

V. Future Directions

In spite of the usefulness of anti-VEGF agents, the reality is that VEGF is not the only molecule involved in angiogenesis. Other angiogenic or antiangiogenic molecules in the cornea include FGF-1 and FGF-2, matrix metalloproteases, angiostatin, endostatin, pigment endothelium–derived factor, thrombospondin, insulin-like growth factor, PlGF, and platelet-derived growth factor (PDGF).[23,115] Can combination therapies that target some of these other pathways along with the VEGF pathway be more efficacious than anti-VEGF monotherapy?

PDGF is one of the molecules showing the most promise as a target for inhibition in such combination therapy. Vascular endothelial cells produce PDGF-B, which binds to receptors on pericytes and regulates and recruits these connective tissue cells.[111] As mentioned herein, the recruitment of pericytes enhances the stability of new vessels, partly by reducing their requirement for VEGF.[29] Therefore, the removal of PDGF-B should lead to the elimination of (or at least a reduction in the number of) pericytes and the subsequent regression of vascularization. In accordance with this, decreases in the number of pericytes and the corneal vessel density after the blocking of the PDGF-B receptor (PDGFR-B) have been demonstrated in mice.[24,33] As pericyte coverage may lead to anti-VEGF therapy not being effective in the elimination of established vessels, co-blockade of the VEGF-A and PDGF-B pathways may be a better strategy for the treatment of established vessels as it will cause regression of both the newly formed and established vessels.

Jo et al used an antibody against PDGFR-B along with pegaptanib (an aptimer against VEGF-A, see earlier) to determine if blocking both pathways simultaneously was more effective than blocking each pathway alone.[70] They found that mice treated with both the PDGFR-B antibody and pegaptanib showed a greater reduction in neovascularization than the monotherapy treatment groups. In addition, they demonstrated that there is a window of sensitivity in which chronic, established vessels refractory to VEGF could regain sensitivity to VEGF blockade through the inhibition of PDGF signaling. Pérez-Santonja et al employed a similar co-blockade strategy, but used bevacizumab and sunitinib (Su-tent; Pfizer, New York), a receptor tyrosine kinase inhibitor that blocks the VEGFR-1, VEGFR-2 and PDGF-

B signaling pathways.[106] In rabbits, they found that this combination therapy led to an inhibition of neovascularization that was 2.9 times greater than that found when bevacizumab (which blocks the VEGF pathway, but not PDGF) was administered alone. Sunitinib is FDA-approved for the treatment of renal and gastrointestinal tumors and has been shown to reduce choroidal neovascularization in mice.[125] The combined inhibition of these two pathways may lead to better treatments for corneal neovascularization in the future.

The combination of anti-VEGF treatment and photodynamic therapy has been used synergistically to attack both newly formed and established unwanted vessels.[138] Other molecules that have demonstrated inhibitory effects on corneal neovascularization include suleparoide, thalidomide, suramin, genistein, somatostatin, octreotide, and rapamycin.[115] More recent work has implicated CD36 (an antiangiogenic receptor), brain-specific angiogenesis inhibitor 1, suramab, propolis extract, and various nutrient mixtures as having the capability to reduce corneal neovascularization,[74,86,96,97,116,137] and revealed that the network of molecules involved in corneal angiogenesis is even more extensive than what was previously thought to be. Many of these have been shown to reduce angiogenesis in part by reducing VEGF levels, and some have also shown efficacy in cancer treatment. Platelet-activating factor (PAF) is also a potent inducer of corneal angiogenesis, and PAF antagonists such as LAU-0901 may demonstrate therapeutic potential against corneal neovascularization.[59,89] Further investigations of some of these agents may reveal more possibilities for combination treatments that include anti-VEGF agents.

Anti-VEGF agents have generated enormous hope for the treatment of corneal neovascularization. Large, randomized, controlled clinical trials are essential for the justification of the continued development of these agents. The establishment of safe doses and methods of administration are required before these agents can be used in the clinical setting. Therefore, further investigation is needed before anti-VEGF agents can become key therapeutic agents in the inhibition of corneal angiogenesis.

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 1989 and 2011 using the following key words in various combinations: *VEGF, cornea, neovascularization, anti-VEGF antibodies, bevacizumab, pegaptanib, ranibizumab, siR-NA, VEGF trap, tyrosine kinase inhibitors, corneal transplantation, penetrating keratoplasty, PDGF, topical, subconjunctival, intravitreal*. In addition, reference lists from the selected articles were used to obtain further articles not included in the electronic database. Articles were appraised critically and pertinent information was included in this review and cited accordingly.

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TABLE 1

Summary of Anti-VEGF Applications and Properties

Anti-VEGF Agents	Structure	Mechanism of Action	Administration	Efficacy	Side Effects
VEGF antibodies					
-*Bevacizumab (Bvb)*	Recombinant humanized murine mAb against VEGF-A	Blocks interactions between VEGF-A and receptors (VEGFR-1 and VEGFR-2)	Topical (T) Injection: SC, IO, IV	Partial NV reduction High dose of T comparable to lower dose of SC IO more effective that SC in mice	Epithelial defects (T, SC), subconjunctival hemorrhage (SC), corneal abrasion (IO), corneal stromal edema (IO), RPE tear (IO), retinal detachment (IO), vision loss (IO), lens injury (IO), vitritis (IO), vitreous hemorrhage (IO), endophthalmitis (IO), hypertension (IO), thrombosis (IV)
-*Ranibizumab (Rbb)*	Fab against all VEGF-A fragments	Blocks the interaction between VEGF-A and receptors (VEGFR-1 and VEGFR-2) *more matured thus higher affinity than Bvb	Injection: Intraocular (IO)	Partial NV reduction	Shorter half-life (may lead to fewer side effects than Bvb, though data not clear) Few studies on corneal NV Uveitis (IO), endophthalmitis (IO), corneal abrasion (IO), vitreous hemorrhage (IO), retinal tear, lens injury (IO), intraocular inflammation (IO), retinal detachment, etc.
-*Pegaptanib (Pgb)*	RNA aptamer	Blocks the interaction between a specific VEGF-A isoform, VEGF165, and receptors (VEGFR-1 and VEGFR-2)	Injection: Intraocular (IO)	Limited (only binds VEGF165 isoform) Few studies on corneal NV	Reduced risk (limited target) Few studies on corneal NV Endophthalmitis (IO), retinal detachment (IO), lens injury (IO), conjunctival hemorrhage (IO), vitreous hemorrhage (IO), traumatic cataract (IO)
VEGF trap	Fusion of the second domain of VEGFR-1 and the third domain of VEGFR-2 with a human IgG fc fragment	Binds all VEGF-A isoforms and PlGF-1, -2	Injection: S, IO	Partial NV reduction Extended duration in eye *Highest affinity	Hypertension (S), proteinuria (S)
siRNA	Double stranded RNA fragments homologous to target gene (VEGF and its receptors)	Processed by RNAse III (Dicer), fragments incorporated into RNA induced silencing complex (RISC), degrades mRNA sequence-specifically	Intravenous (IV) Subconjunctival (SC)	Targets both the intracellular and extracellular effects of genes Transient effects SC more effective than IV SIRNA-027 human trial completed	Few studies on corneal NV
VEGF Signaling - *tyrosine kinase inhibitors*	Variable Small molecules	Targets the downstream tyrosine kinase pathway initiated by VEGFR	Orally available	Targets all/most VEGF receptors None FDA-approved for ocular use Pazopanib in clinical trials Sunitinib in animal trials	Few studies on corneal NV Skin toxicity (seen in cancer treatment)

IO = intraocular; IV = intravenous; NV = neovascularization; RPE = retinal pigment epithelium; S = systemic; SC = subconjunctival.

TABLE 2

Anti-VEGF Applications in Corneal Neovascularization–related Diseases

Disease/Disorder		Clinical Trial	Animal Model	References
Inflammatory Disorders				
	Ocular pemphigoid	X		DeStafeno et al[35]
				Doctor et al[39]
				Koenig et al[80]
	Atopic conjunctivitis			
	Rosacea			
	Graft rejection	X		Bahar et al[13]
				Erdurmus et al[43]
				Koenig et al[80]
				Saxena et al[113]
				Vassileva et al[130]
	Lyell's syndrome			
	Stevens-Johnson syndrome	X		Doctor et al[39]
				Kim et al[76]
				Uy et al[129]
	Graft versus host disease			Bahar et al[12]
				Koenig et al[80]
				You et al[139]
Infectious keratitis				
Viral	Herpes simplex	X	X	Carrasco[21]
				Chu et al[25]
				Hosseini et al[64]
				Kim et al[75]
				Saravia et al[112]
				You et al[139]
	Herpes zoster			
Bacterial	Pseudomonas			
	Chlamydia trachomatis	X		Zaki et al[141]
	Syphilis			
Fungal	Candida		X	Yuan et al[140]
	Fusarium			
	Aspergillus			
Parasitic	*Acanthamoeba*			
	Onchocerciasis			
Degenerative–Congenital disorders				
	Pterygium	X		Bahar et al[13]
				Banifatemi et al[14]
				Fallah et al[44]
				Kim et al[76]
				Koenig et al[80]
				Mandalos et al[91]
				Mauro et al[94]
				Saxena et al[113]
	Terrien marginal degeneration	X		Qian et al[108,139]
	Aniridia	X		Koenig et al[80]
				You et al[130]