Corneal Neovascularization and the Utility of Topical VEGF Inhibition: Ranibizumab (Lucentis) Vs Bevacizumab (Avastin)

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Corneal Neovascularization and the Utility of Topical VEGF Inhibition: Ranibizumab (Lucentis) Vs Bevacizumab (Avastin)

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Abstract

Corneal avascularity is necessary for the preservation of optimal vision. The cornea maintains a dynamic balance between pro- and antiangiogenic factors that allows it to remain avascular under normal homeostatic conditions; however, corneal avascularity can be compromised by pathologic conditions that negate the cornea's "angiogenic privilege." The clinical relevance of corneal neovascularization has long been recognized, but management of this condition has been hindered by a lack of safe and effective therapeutic modalities. Herein, the etiology, epidemiology, pathogenesis, and treatment of corneal neovascularization are reviewed. Additionally, the authors' recent findings regarding the clinical utility of topical ranibizumab (Lucentis®) and bevacizumab (Avastin®) in the treatment of corneal neovascularization are summarized. These findings clearly indicate that ranibizumab and bevacizumab are safe and effective treatments for corneal neovascularization when appropriate precautions are observed. Although direct comparisons are not conclusive, the results suggest that ranibizumab may be modestly superior to bevacizumab in terms of both onset of action and degree of efficacy. In order to justify the increased cost of ranibizumab, it will be necessary to demonstrate meaningful treatment superiority in a prospective, randomized, head-to-head comparison study.

Keywords

angiogenesis; bevacizumab; cornea; corneal angiogenic privilege; hemangiogenesis; lymphangiogenesis; neovascularization; ranibizumab; vascular endothelial growth factor; VEGF

I. INTRODUCTION

Corneal transparency and optimal vision require an avascular cornea.[1] The cornea possesses redundant antiangiogenic mechanisms that actively maintain corneal avascularity, collectively accounting for corneal angiogenic privilege.[2] Although the human cornea is avascular under normal homeostatic conditions, corneal angiogenic privilege is not absolute. Corneal neovascularization (NV) is a sight-threatening condition that can develop in response to inflammation, hypoxia, trauma, or limbal stem cell deficiency.[1] A variety of

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therapeutic modalities have been employed in the treatment of corneal NV with variable, and often limited, clinical success.[3]

Vascular endothelial growth factors (VEGFs) regulate the development and maintenance of blood and lymphatic vessels.[4] VEGF neutralizing agents have proven invaluable in the treatment of pathologic conditions such as neovascular age-related macular degeneration and diabetic retinopathy; furthermore, recent findings suggest that VEGF inhibition may be an effective therapeutic modality for corneal NV.[5-7] Because systemic anti-VEGF exposure is associated with severe and potentially life-threatening adverse events, it is prudent to pursue the route of administration that minimizes systemic exposure.[8] Herein, we present a brief review of corneal NV; additionally, we summarize our recent findings regarding the clinical utility of topical ranibizumab (Lucentis®; Genentech, Inc.; San Francisco, CA) and bevacizumab (Avastin®; Genentech, Inc.) in the treatment of corneal NV.

II. BACKGROUND

A. Etiology and Epidemiology

According to the World Health Organization (WHO), approximately 285 million people are visually impaired worldwide; of these, approximately 39 million are blind.[9] Corneal disease is second only to cataract as the leading cause of nonrefractive visual impairment worldwide.[10] Despite aggressive international prevention efforts, corneal disease remains the most common cause of blindness in some developing countries.[11] Corneal NV is a potential sequela of numerous conditions, including infection, injury, surgery, autoimmune disease, limbal stem cell deficiency, neoplasm, dystrophy, and contact lens use.[2] Over a decade ago, it was estimated that there are up to 1.4 million cases of corneal NV in the USA alone.[12] The clinically evident pattern of vessel invasion (eg, vascular pannus, superficial stromal NV, or deep stromal NV) is often indicative of the etiology of corneal NV; for example, deep stromal NV generally develops in response to interstitial keratitis (eg, herpetic stromal keratitis) or significant ocular trauma (Figure 1).[2,12] Corneal NV ultimately alters visual acuity by inducing stromal edema, cellular infiltration, lipid deposition, hemorrhage, and scarring.[13]

Corneal NV is a potential complication of numerous bacterial, parasitic, and viral infections. Trachoma is the world's leading infectious cause of blindness.[14] The WHO estimates that there are 146 million cases of *Chlamydia trachomatis* infection worldwide, and 5.9 million people are blind or at immediate risk of blindness from trachomatous trichiasis.[14] Recurrent episodes of trachoma can damage the eyelid, resulting in eyelash-induced corneal abrasions, ulcerations, NV, and scarring.[15] Onchocerciasis, commonly referred to as river blindness, is the second most common infectious cause of blindness worldwide.[16] The causative filarial nematode, *Onchocerca volvulus*, infects an estimated 37 million people, and 270,000 cases of blindness have been attributed to onchocerciasis.[17,18] Adult worms produce microfilariae that can migrate to the cornea and induce intense inflammation, NV, and opacification upon death of the worm.[19] Herpes simplex virus (HSV) is the most common infectious cause of blindness in the developed world. Extrapolating from the 2010 census, approximately 64,000 episodes of HSV keratitis occur annually in the USA alone.[20,21] Following the primary viral infection, HSV remains dormant in neural ganglia pending episodic reactivation.[22] Recurrent episodes of HSV keratitis can cause NV, opacification, and scarring.

Ocular trauma accounts for approximately 19 million cases of unilateral visual impairment and 1.6 million cases of bilateral blindness worldwide.[23] Corneal wound healing is generally an avascular process; however, corneal NV can develop in response to severe corneal injuries.[2] Chemical burns in particular are known to induce a vigorous inflammatory

response that promotes corneal NV. Furthermore, chemical burns are capable of damaging the corneal limbus, thereby leading to limbal stem cell deficiency.[24] There are numerous potential causes of limbal stem cell deficiency, including inherited (eg, aniridia) and acquired (eg, trauma) etiologies.[24,25] Disruption of the corneal limbus leads to corneal damage and provides an avenue for the extension of conjunctival epithelium and blood vessels into the cornea.

Corneal transplantation is the most common form of solid tissue transplantation.[26] Nearly 40,000 corneal transplantations are performed annually in the USA alone.[27] Surgery-induced corneal injury (eg, refractive surgery) generally provokes an avascular healing process; however, corneal transplantation can involve suture-induced inflammation and alloimmune responses that promote corneal NV.[2,28] In addition to being a potential cause and consequence of corneal transplantation, corneal NV is a known risk factor for graft rejection.[29] Corneal transplantation performed in graft beds devoid of inflammation and vasculature, referred to as low-risk transplantation, enjoys a rate of graft acceptance approaching 90%.[30] However, corneal transplantation performed in previously sensitized, inflamed, or vascularized graft beds, referred to as high-risk transplantation, has a much lower rate of graft acceptance.[31] The risk of corneal graft rejection has been found to correlate with the number of corneal quadrants that exhibit NV.[32] Corneal NV provides the immune system with increased afferent and efferent access to graft alloantigens, thereby increasing the risk of allogeneic immune rejection.[33] Nearly 20% of corneal buttons excised during corneal transplantation exhibit histologic evidence of NV.[34]

Approximately 38 million people in the USA and up to 125 million people worldwide wear contact lenses.[35] Contact lens use is associated with a variety of inflammatory complications, including corneal NV.[36] It has been estimated that 11-23% of contact lens users develop corneal NV.[37] Presumably, contact lenses promote corneal NV by inducing inflammation and hypoxia.[38] Risk factors for corneal NV include prolonged contact lens use, low oxygen permeability-contact lenses, and contact lens contamination. In extreme cases, contact lens use can cause deep stromal NV, hemorrhage, and opacification.[39,40]

B. Pathogenesis

1. Corneal Vessel Formation—Vasculogenesis refers to the *de novo* formation of blood vessels by endothelial precursor cells (angioblasts) or endothelial progenitor cells.[41] Although vasculogenesis primarily occurs during embryologic development, endothelial progenitor cells are capable of giving rise to vascular endothelial cells during the postnatal period.[42-44] Angiogenesis refers to the sprouting or splitting (intussusception) of new vessels from pre-existing vessels.[4] Vasculogenesis and angiogenesis are physiologic processes that occur during normal development and tissue repair; however, these processes can also contribute to pathologic conditions, such as cancer and eye disease.[41] A morphometric analysis of experimental corneal NV described the sprouting and extension of new vessels from pre-existing vessels at the corneoscleral limbal vascular plexus.[45] Vascular endothelial cells in newly developed corneal vessels arise from previously established vessels at the limbal vascular plexus.[46] Interestingly, a majority of the pericytes found in newly formed corneal vessels arise from bone marrow-derived precursor cells rather than the limbal vascular plexus.[46]

2. Corneal Angiogenic Privilege—Avascularity is a unique characteristic possessed by select tissues, such as the cornea and cartilage.[1] Corneal avascularity is maintained despite intermittent exposure to potentially proangiogenic inflammatory stimuli (eg, ocular foreign body) and hypoxic conditions (eg, eyelid closure).[37] Furthermore, the cornea is capable of remaining avascular in the face of significant injury (eg, refractive surgery), and corneal

wound healing is generally an avascular process.[2,37] A dynamic balance exists between the positive and negative regulators of angiogenesis that serves to maintain corneal avascularity (Table 1).[47] In spite of this balance, pathologic conditions can override the cornea's innate antiangiogenic defense mechanisms, thereby compromising the cornea's avascular status.[1,2] The "angiogenic switch," a concept initially postulated to describe the induction of tumor angiogenesis, is relevant in cases of corneal angiogenesis, where it can be used to describe the transition from corneal avascularity to NV that occurs when proangiogenic factors overwhelm the cornea's angiogenic privilege.[48]

3. Promoters of Corneal Angiogenesis

a. Vascular Endothelial Growth Factors: VEGF is one of the most important factors implicated in the pathogenesis of corneal NV. There are multiple members of the human VEGF family, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF).[49] VEGFs interact with the receptor tyrosine kinases VEGF receptor (VEGFR)-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4).[49,50] VEGF-A is considered the most important member of the VEGF family, particularly with regard to pathologic hemangiogenesis. Alternative mRNA splicing allows for the production of pro- and antiangiogenic isoforms of VEGF-A, of which VEGF-A$_{165}$ is the dominant proangiogenic isoform.[51] Inflammation and hypoxia induce the production of VEGF-A by a variety of cell types, including blood vessel-associated pericytes and smooth muscle cells, and inflammation-associated macrophages and T cells.[52-54] The binding of VEGF-A to VEGFR-2 promotes hemangiogenesis by stimulating vascular endothelial cell migration, proliferation, and survival, as well as vessel dilation and permeability.[55-57] The binding of VEGF-C (or –D) to VEGFR-2 or -3 promotes lymphangiogenesis in a similar fashion.[58,59] Furthermore, VEGFs serve as chemoattractants for inflammatory cells (eg, macrophages) that produce additional proangiogenic factors.[60,61] The relevance of VEGF in corneal NV is well established, and VEGF inhibition is currently being investigated as a treatment for corneal NV.[62-65]

b. Fibroblast Growth Factors: Fibroblast growth factors (FGFs) regulate a variety of processes including angiogenesis and wound healing. There are 18 members of the mammalian FGF family that bind to the FGF receptors FGFR1, FGFR2, FGFR3, and FGFR4.[66,67] FGF1 and FGF2, members of the FGF1 subfamily, are potent stimulators of angiogenesis; however, neither FGF1 nor FGF2 is required for normal growth, development, viability, or fertility.[68,69] FGF2, also known as basic FGF (bFGF) or FGF-β, promotes vascular endothelial cell migration, proliferation, and differentiation, and inhibits cellular apoptosis.[70,71] Abrogation of the FGF system leads to the loss of vascular integrity, suggesting that FGF-mediated signaling regulates vessel permeability.[72] FGF2 is synthesized by corneal epithelial cells and passively released in response to epithelial cell injury.[2,73] Once released, FGF2 binds to heparan sulfate polysaccharides expressed on membranes such as Descemet membrane, Bowman membrane, and vascular basement membrane.[74,75] The intrastromal implantation of an FGF2 containing micropellet serves as an important model of experimental angiogenesis.[76]

c. Platelet-Derived Growth Factors: Platelet-derived growth factors (PDGFs) mediate a diverse array of biological processes, including angiogenesis and tissue remodeling.[77] PDGF and VEGF are structurally and functionally related, as evidenced by their evolutionarily conserved PDGF/VEGF homology domain.[78,79] Humans express four PDGF chains (PDGF-A, -B, -C, and -D), that dimerize and interact with receptor tyrosine kinase complexes of PDGFR-α or PDGFR–β.[80] PDGF ligands and receptors are found throughout the human body, and many PDGFs are required for normal growth and development.[77] PDGF-B and PDGFR-β are preferentially expressed by blood vessel-associated cells, and a

deficiency in either PDGF-B or PDGFR-β results in fatal cardiovascular and hematological abnormalities.[81,82] Nascent vascular endothelial cells secrete PDGF-B that binds to pericyte and smooth muscle cell-associated PDGFR-β, thereby promoting their migration, proliferation, and survival.[83,84] PDGF-A and -B are detectable in corneal epithelial cells, stromal fibroblasts, and endothelial cells, and PDGF-BB can be isolated from tears.[85,86] PDGFR-α and PDGFR–β are expressed by corneal epithelial cells, stromal fibroblasts, and endothelial cells.[85,87] Inhibition of PDGFR–β leads to vessel-associated pericyte loss and decreased corneal vessel density.[88]

d. Angiopoietins: Angiopoietin (Ang) growth factors regulate angiogenesis, vascular extravasation, and inflammation.[89] The human Ang family consists of Ang-1, -2, -4, and the tyrosine kinase receptors Tie1 and Tie2.[90] Ang-1 deficiency, Ang-2 overexpression, or Tie2 deficiency all result in early embryonic death secondary to vascular abnormalities suggestive of abnormal blood vessel maturation and stabilization.[91,92] Ang-1 promotes vessel assembly, maturation, and stability by facilitating vascular endothelial cell/mural cell (eg, pericyte and smooth muscle cell) interactions.[93] Ang-2 antagonizes the activity of Ang-1, thereby inducing vessel destabilization and facilitating vascular sprouting and branching.[89] The exogenous administration of either Ang-1 or -2 alone does not induce corneal NV; however, Ang-1 and -2 profoundly affect the development of VEGF-mediated corneal NV.[94] Ang-1 promotes vessel maturation as evidenced by increased vessel density and perfusion, whereas Ang-2 promotes vessel destabilization as evidenced by the sprouting of new corneal vessels. Interestingly, Ang-2 induces the regression of blood vessels in the absence of VEGF, suggesting that Ang-2-induced destabilization alone serves an antiangiogenic function.[95]

e. Matrix Metalloproteinases: Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases involved in extracellular matrix remodeling. MMPs often promote angiogenesis by degrading the extracellular matrix and activating proangiogenic molecules.[96] Proangiogenic factors upregulate the production of MMPs, such as MMP-1 (interstitial collagenase-1), MMP-2 (gelatinase A), MMP-9 (gelatinase B), and MT1-MMP (MMP-14), by vascular endothelial cells.[97-100] MMP-2 and MMP-9 are type IV collagenases that degrade the extracellular matrix and liberate proangiogenic molecules.[101-105] MT1-MMP degrades extracellular matrix type I collagen, thereby facilitating vascular endothelial cell invasion, migration, and tubule formation.[106-108] MMP-2, MMP-9, and MT1-MMP are expressed by the cornea, and each of these MMPs is intimately involved in the promotion of corneal NV.[107,109-112] Conversely, MT1-MMP expressed by corneal epithelial cells and keratocytes inhibits angiogenesis, suggesting that MT1-MMP's location defines its function.[113,114] Antiangiogenic MMP functions will be discussed later in this manuscript.

f. Inflammatory Mediators: Inflammation is a characteristic shared by all etiologies of corneal NV. Inflammatory cells are rich sources of VEGF molecules that promote hemangiogenesis and lymphangiogenesis; blood and lymphatic vessels in turn provide the inflamed eye with inflammatory cells that amplify the angiogenic cascade.[33,60,61] Cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), and transforming growth factor (TGF)-β not only activate inflammatory cells, but also upregulate the production of VEGF.[115-118] Chemokines and their receptors are involved in the recruitment of inflammatory cells, and some chemokines (eg, IL-8/CXCL8) can directly stimulate angiogenesis.[119] Promoters of angiogenesis and inflammation often regulate one another; for example, IL-1, IL-6, TNF, and IL-8/CXCL8 enhance MMP expression.[120] Integrins (eg, α1β1, also known as very late antigen-1) facilitate the migration of not only inflammatory cells, but also vascular endothelial cells.[121,122] The importance of these proinflammatory

factors in angiogenesis is well-established, and their significance in corneal NV is becoming increasingly apparent.[121,123,124]

4. Inhibitors of Corneal Angiogenesis

a. VEGF Receptors: Corneal angiogenic privilege relies on a variety of antiangiogenic mechanisms, none more important than the endogenously occurring inhibitors of VEGF. There are several VEGF receptors (VEGFRs) expressed by the cornea that serve as "decoy" receptors for proangiogenic VEGF molecules. Soluble VEGFR-1 (sVEGFR-1/sFLT-1) inhibits hemangiogenesis by sequestering proangiogenic VEGF-A molecules.[125,126] Soluble VEGFR-1 also forms inactive heterodimers with membrane-bound VEGFR-1 and VEGFR-2, further suppressing VEGF-mediated angiogenesis.[127,128] The cornea expresses sVEGFR-2 that binds and sequesters prolymphangiogenic VEGF-C, thereby conserving corneal alymphaticity.[129] The corneal epithelium displays membrane-bound VEGFR-3 that binds and sequesters VEGF-C and -D.[130] This directly suppresses lymphangiogenesis by inhibiting VEGF-C and-D mediated signaling, and indirectly suppresses hemangiogenesis by inhibiting the recruitment of VEGF secreting macrophages.[131] Soluble VEGFR-1 and sVEGFR-2 are essential for maintaining corneal avascularity under normal homeostatic conditions; epithelial expressed VEGFR-3 may be the primary factor responsible for inhibiting inflammation-mediated, pathologic corneal NV.

b. Pigment Epithelium-Derived Factor: Pigment epithelium-derived factor (PEDF) is a secreted glycoprotein that belongs to the serpin superfamily, despite lacking the ability to inhibit serine proteases.[132,133] PEDF possesses potent antiangiogenic, immunomodulatory, and neurotrophic properties.[134-136] PEDF exerts antiangiogenic activity by suppressing VEGF, FGF, and IL-8/CXCL8-mediated vascular endothelial cell migration and proliferation, and inducing vascular endothelial cell apoptosis.[134,137,138] PEDF has been localized to the corneal epithelium and endothelium, and PEDF expression is thought to be an important component of corneal angiogenic privilege.[139,140] The inhibition of PEDF promotes corneal NV, and the exogenous administration of PEDF suppresses corneal NV.[141,142]

c. Angiostatin: Angiostatin is a multifunctional 38 kDa protein fragment generated by the proteolytic cleavage of plasminogen.[143,144] Angiostatin binds to vascular endothelial cell surface-expressed F_1-F_0 ATP synthase, thereby suppressing the production of ATP and inhibiting cell migration and proliferation.[145,146] Angiostatin decreases cell migration and tubule formation upon binding to endothelial cell surface-expressed angiomotin.[147] The binding of angiostatin to integrin $\alpha_v\beta_3$ antagonizes vascular endothelial cell survival and migration.[148] Angiostatin also binds to the hepatocyte growth factor receptor (c-met), resulting in the downstream inhibition of Akt phosphorylation, thereby promoting apoptosis and cell cycle inhibition.[149-151] The antiangiogenic effects of angiostatin may require the presence of IL-12 and be mediated in part by the inhibition ofinflammatory cellrecruitment.[152-154] Endogenous angiostatin can be found in the corneal epithelium and tear fluid.[155,156] The functional relevance of angiostatin in suppressing corneal NV has been demonstrated in models of surgical, mechanical, and alkali-induced corneal injury.[156,157]

d. Collagen Derivatives: Endostatin is an endogenously occurring 20 kDa fragment of collagen XVIII that inhibits angiogenesis.[158] Collagen XVIII is a component of most epithelial and endothelial basement membranes, including vascular basement membrane.[159-162] The proteolytic cleavage of type XVIII collagen's C-terminal noncollagenous domain (NC1) results in the liberation of biologically active endostatin.[163] Endostatin not only inhibits vascular endothelial cell migration and proliferation, but also promotes endothelial cell apoptosis and cell-cycle arrest.[158] Endostatin directly binds to

VEGFR-2, thereby inhibiting VEGF-A-mediated angiogenesis.[164] Endostatin is not required for the normal development and function of most major organs; however, endostatin deficiency results in aberrant hyaloid vessel regression and retinal vessel development reminiscent of the abnormalities observed in Knobloch syndrome.[165,166] Collagen XVIII has been immunolocalized to the corneal epithelium, epithelial basement membrane, and Descemet membrane.[167] Endostatin inhibits experimental FGF- and VEGF-mediated corneal NV, implicating endostatin in corneal angiogenic privilege.[168] MMP-7 and MT1-MMP-mediated cleavage of collagen-XVIII produces the antiangiogenic collagen fragments neostatin-7 and -14, respectively.[169,170] Type IV collagen, a component of all basement membranes, can be cleaved to produce arrestin, canstatin, and tumstatin.[171] These collagen derivatives negatively regulate angiogenesis, and their importance in corneal angiogenic privilege is becoming increasingly apparent.[2,172]

e. Matrix Metalloproteinases: The regulation of corneal angiogenesis is complex and multifaceted, as evidenced by the conflicting pro- and anti-angiogenic functions of many MMPs. For example, MMP-7 (matrilysin) stimulates the production of VEGF and directly promotes the proliferation of vascular endothelial cells independent of VEGF.[173,174] Moreover, VEGF-mediated angiogenesis is augmented by the degradation of sVEGFR-1 by MMP-7.[175] In spite of these proangiogenic functions, MMP-7 expressed by the basal epithelial layer of the cornea is thought to inhibit angiogenesis because MMP-7 deficiency dramatically increases the angiogenic response to corneal wounding.[176,177] This may be a function of the MMP-7-mediated cleavage of type XVIII collagen, the precursor of antiangiogenic endostatin.[167,178]

f. Thrombospondins and CD36: Thrombospondins (TSPs) are multifunctional endogenous proteins known to suppress angiogenesis. TSPs inhibit vascular endothelial cell migration, proliferation, and tubule formation, and induce vascular endothelial cell apoptosis.[179-182] TSP-1 has been immunolocalized to the cornea's epithelial basement membrane, posterior Descemet membrane, and endothelium.[183] TSP-1 deficiency augments the cornea's angiogenic response to suture-induced inflammation.[184] The antiangiogenic effects of TSP-1 are mediated by the ligation of TSP-1 to the transmembrane glycoprotein CD36 scavenger receptor found on vascular endothelial cells and inflammatory cells.[185,186] The activation of vascular endothelial cell-expressed CD36 directly suppresses corneal angiogenesis.[185] The activation of macrophage-expressed CD36 indirectly suppresses corneal hemangiogenesis and lymphangiogenesis by inhibiting the secretion of VEGF-A, -C, and -D.[185,186] Genetic ablation of CD36 results in the development of age-related corneal NV, demonstrating the functional relevance of TSP and CD36 in corneal angiogenic privilege.[187]

g. Death Signaling Pathways: The Fas (CD95)/FasL (Fas ligand) system inhibits corneal NV by regulating the infiltration of inflammatory cells and vascular endothelial cells.[188-190] Corneal epithelial- and endothelial-expressed FasL induces the apoptosis of infiltrating inflammatory cells, thereby safeguarding ocular immune privilege.[188,189] The Fas/FasL system serves as a barrier against immune cells that invade the cornea in response to inflammation and secrete proangiogenic molecules. Moreover, vascular endothelial cells express Fas, and corneal-expressed FasL suppresses the proliferation of corneal NV by inducing the apoptosis of vascular cells.[190] Programmed death-ligand 1 (PD-L1), a member of the B7-CD28 superfamily, binds to the receptors PD-1 and CD80 and has been shown to regulate inflammatory cell activity by inducing apoptosis.[191] PD-L1 and CD80 have been identified in vascular endothelial cells, and PD-L1 is expressed by corneal epithelial cells.[192] Vascular endothelial cell and corneal epithelial cell-expressed PD-L1 suppresses corneal NV through an inflammation-independent pathway.[192]

C. Conventional Treatment Modalities

1. Medical Therapy—Several medical therapies have been employed in the treatment of corneal NV, including corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs).[193] Corticosteroids (eg, dexamethasone) are the standard medical treatment for patients with actively proliferating corneal NV, particularly in cases of corneal transplantation-associated corneal NV.[194] Corticosteroids inhibit angiogenesis by suppressing inflammatory cell recruitment, proinflammatory cytokine (eg, IL-1, IL-6) expression, and arachidonic acid release.[195-199] Corticosteroids may suppress the development of inflammation-induced corneal NV, but they are generally ineffective at treating stable corneal NV. Moreover, the side effects associated with long-term corticosteroid use (eg, cataract, ocular hypertension, corneal thinning, and opportunistic infections) generally make this an untenable choice. NSAIDs (eg, nepafenac) are another class of anti-inflammatory agents that have been utilized in the treatment of corneal NV.[200,201] NSAIDs inhibit the production of prostaglandins that stimulate angiogenesis.[201,202] Unfortunately, the variable efficacy and sometimes serious side effects associated with the use of topical NSAIDs (eg, corneal ulceration and perforation) have limited their clinical utility, particularly in the setting of patients with concomitant ocular surface disease.[203]

2. Laser-Induced Photocoagulation—Laser-induced photocoagulation is a procedure that can be employed in the treatment of corneal NV. To perform this procedure, a beam of light is selectively focused on the vessel of interest, resulting in heat-induced coagulation and vessel occlusion.[204] Argon, Nd:YAG, and yellow lasers have all been used for this purpose with some success.[205-213] Unfortunately, laser-induced tissue damage can stimulate the release of proangiogenic factors that promote the development of collateral circulation.[212] The combined use of laser-induced photocoagulation and angiogenesis-specific medical treatments (eg, anti-VEGF agents) may overcome this limitation.[212] Laser-induced photocoagulation is associated with a number of uncommon complications, including corneal hemorrhage, corneal thinning, iris atrophy, and necrotizing scleritis.[209-211,213]

3. Photodynamic Therapy—Photodynamic therapy (PDT) is another procedure utilized in the treatment of corneal NV. In this procedure, a photosensitizing agent is delivered to the tissue of interest and activated by a specific wavelength of light, resulting in the production of cytotoxic singlet oxygen.[214,215] In the case of corneal NV, the photosensitizing agent is delivered to corneal vessels and activated by laser light, resulting in vascular endothelial cell damage and vessel thrombosis.[216,217] Photosensitizing agents such as verteporfin, fluorescein, and dihematoporphyrin have all been used to treat corneal NV.[218-221] PDT performed with verteporfin or fluorescein induces the regression of corneal NV with minimal risk.[218-220] PDT performed with dihematoporphyrin also induces the regression of corneal NV, but this photosensitizing agent has been associated with deleterious local and systemic side effects.[221] PDT has been shown to promote both experimental and clinical graft survival and may be an effective treatment for corneal NV following corneal transplantation.[222,223] Combining PDT with angiogenesis-specific inhibitors such as bevacizumab may increase treatment efficacy and decrease the incidence of collateral vessel development.[224] Although PDT performed with an appropriate photosensitizing agent appears to be a safe and effective treatment for corneal NV, the high costs associated with PDT have limited its clinical utility.

4. Fine-Needle Diathermy—Fine-needle diathermy (FND) is a surgical procedure that shows promise in the treatment of corneal NV. In this procedure, a needle is inserted parallel to or within the lumen of the vessel of interest. A diathermy probe in coagulation mode is

then brought in contact with the needle, resulting in vessel cauterization. Clinical investigations have found that FND is a safe and effective procedure for occluding targeted corneal vessels.[225-227] Transient corneal whitening and intra-stromal hemorrhage are the only reported complications. The procedure may need to be repeated because of vessel recanalization or collateral vessel development.[225-227] FND-induced ablation of corneal vessels has successfully aided in the reversal of several cases of corneal graft rejection.[225,226]

5. Ocular Surface Reconstruction—Ocular surface restoration using limbal, conjunctival, or amniotic membrane transplantation can be employed as a final recourse for some patients with otherwise unresponsive corneal NV. Limbal stem cell transplantation has been successfully utilized in the treatment of limbal stem cell deficiency; unfortunately, cases of bilateral limbal stem cell deficiency may require allogeneic transplantation and be complicated by issues with long-term graft survival.[228,229] Cultivated limbal epithelial transplantation is being increasingly used as an alternative to limbal transplantation.[230,231] The transplantation of autologous conjunctival epithelial cells can successfully restore the ocular surface in some settings.[232,233] Human amniotic membrane has long been used to restore the ocular surface because of its ability to suppress inflammation and promote wound healing.[234,235] Amniotic membrane transplantation may be a safe and effective alternative to limbal transplantation; however, additional clinical trials are required to determine its place in the clinical management of corneal NV.[236,237]

III. TOPICAL ANTI-VEGF THERAPY

A. Corneal Penetration of Topical Bevacizumab

Bevacizumab is a humanized monoclonal antibody that binds to isoforms of VEGF-A.[238] Bevacizumab was initially approved for the treatment of metastatic colorectal cancer; however, it has since been used off-label to treat a variety of ophthalmic conditions, including neovascular age-related macular degeneration, diabetic retinopathy, central retinal vein occlusion, and neovascular glaucoma.[5,6,239,240] The systemic administration of bevacizumab has been associated with several severe and potentially life-threatening complications, including hypertension, impaired wound healing, gastrointestinal perforation, bleeding, arteriolar hemorrhage, and arterial thromboembolic events.[241] The route of administration that provides the best combination of safety, efficacy, and practicality should be pursued; with regard to the cornea, the preferred method of administration is generally ocular surface topical instillation.

Bevacizumab is a large full-length immunoglobulin with a molecular weight of 149kD. The corneal epithelial barrier is thought to preclude the penetration of full-length immunoglobulins; nevertheless, topical bevacizumab has been successfully utilized in the treatment of corneal NV.[242-248] To elucidate this incongruity, we investigated the corneal penetration of topical bevacizumab using the bFGF micropellet model of murine corneal NV.[76] Topical bevacizumab 1.0% was administered to the eyes of mice with intact corneas and the eyes of mice with corneal NV. Mice were sacrificed at various time-points, and immunohistochemistry was performed to determine the extent of bevacizumab penetration. As expected, topical bevacizumab did not penetrate beyond the epithelial barrier of healthy corneas (Figure 2A); however, bevacizumab was detected in the corneal stroma of most mice with corneal NV, and staining intensity increased over time despite some variability in the extent of bevacizumab penetration (Figure 2B). These findings suggest that corneal NV diminishes the integrity of epithelial tight junctions, thereby permitting macromolecules such as bevacizumab to penetrate through the corneal epithelial barrier.

B. The Comparison: Topical Ranibizumab (Lucentis) Vs Bevacizumab (Avastin)

1. Introduction—Topical bevacizumab is an effective treatment for corneal NV; however, there is some variability in the clinical response to topical bevacizumab treatment.[244-248] Ranibizumab is a recombinant humanized monoclonal antibody fragment that binds and inhibits VEGF-A isoforms.[249] Ranibizumab has a molecular weight of 48kD, making it approximately one-third the size of bevacizumab and theoretically allowing for better corneal penetration; additionally, ranibizumab has been affinity-matured to optimize its VEGF-A binding potential. These characteristics may enable ranibizumab to treat corneal NV more effectively than bevacizumab. We have completed two separate clinical studies investigating the safety and efficacy of topical bevacizumab and ranibizumab; herein, we will present a brief summary of our study results and compare treatment outcomes.[250,251]

2. Methods—Two prospective, open-label studies were performed investigating the safety and efficacy of topical ranibizumab and bevacizumab in the treatment of corneal NV. These studies were approved by the Institutional Review Boards of Massachusetts Eye & Ear Infirmary or Walter Reed Army Medical Center. Adults that did not meet any of the basic exclusion criteria (Table 2) and exhibited clinically stable corneal NV extending at least 2 mm beyond the corneal limbus were recruited for these studies. Patients were provided with either 1.0% ranibizumab or 1.0% bevacizumab ophthalmic solution and instructed to perform topical administration four times per day over a period of 3 weeks. Punctal plugs were placed in the superior and inferior punctae of treated eyes for the duration of the experiment in order to minimize systemic drug absorption.

Study appointments were held during weeks 1, 3, 8, and 16 of the ranibizumab study, and weeks 1, 3, 6, 12, and 24 of the bevacizumab study. Each patient visit included a detailed medical history review, blood pressure measurement, and a thorough ocular examination, including Snellen visual acuity measurement, slit-lamp biomicroscopy, and central corneal thickness measurement. Digital corneal photography was performed during all visits except week 1.

Treatment efficacy was evaluated by comparing digital slit-lamp pictures taken at baseline with pictures taken at follow-up visits. Analysis of the corneal vessels was performed using Photoshop CS2 (Adobe Systems Inc.; Berkeley, CA) and MATLAB (MathWorks, Inc.; Natick, MA). Three primary metrics of corneal NV (Figure 3) were evaluated: (A) neovascular area (NA), ie, the area of the corneal vessels; (B) vessel caliber (VC), ie, the mean corneal vessel diameter; and (C) invasion area (IA), ie, the fraction of the cornea into which vessel invasion occurred.[247] Secondary measures of safety and efficacy included best-corrected visual acuity, central corneal thicknesses, and systemic blood pressure.

The paired *t*-test was used to compare study metrics for each cohort at follow-up visits with their baseline measures, and the unpaired *t*-test was used to compare study metrics between the cohorts in different studies. Data is expressed as the mean ± standard deviation (SD). *P* values   0.05 were considered statistically significant.

3. Results—Ten eyes from 9 patients were included in the ranibizumab study, and 20 eyes from 20 patients were included in the bevacizumab study. The average patient age was 57.3 ± 14.5 years for the ranibizumab study, and 52.5 ± 14.6 years for the bevacizumab study. The duration of corneal NV was 17.67 ± 19.18 months for the ranibizumab study and 13.69 ± 9.53 months for the bevacizumab study, excluding several cases of unknown duration.

a. Neovascular Area: A statistically significant decrease in NA was observed from baseline to week 3 for the ranibizumab-treated group ($-39.8\% \pm 24.1\%$; $P < 0.001$); meanwhile, a statistically significant decrease in NA was not observed until week 6 for patients treated

with bevacizumab ($-27.9\% \pm 41.2\%$; $P = 0.007$ [Figure 4A]). The average reduction in NA from baseline was 55.3% (SD, 44.4%; $P < 0.001$) at week 16 for the ranibizumab treated group, and 47.5% (SD, 37.5%; $P < 0.001$) at week 24 for patients treated with bevacizumab. Although the decrease in NA at comparable time points was consistently greater for patients treated with ranibizumab, these differences were not statistically significant.

b. Vessel Caliber: The decrease in VC reached statistical significance by week 3 for patients treated with ranibizumab ($-25.8\% \pm 18.8\%$; $P = 0.001$). The decrease in VC did not reach significance until week 12 for patients treated with bevacizumab ($-30.8\% \pm 41.7\%$; $P = 0.006$ [Figure 4B]). At the final study appointments, the average change in VC was -59.0% (SD, 34.9%; $P < 0.001$) for the ranibizumab group and -36.2% (SD, 44.1%; $P = 0.003$) for the bevacizumab group. The decrease in average VC was consistently greater for patients treated with ranibizumab at comparable time points; however, these differences were not statistically significant.

c. Invasion Area: The average change in IA was -12.3% (SD, 54.7%; $P = 0.49$) at week 16 for the ranibizumab-treated group, and -20.0% (SD, 42.0%; $P = 0.06$) at week 24 for the bevacizumab treated-group (Figure 4C). These average decreases were not statistically significant when compared to either baseline measurements or each other.

d. Additional End-points and Adverse Events: Snellen visual acuity measurements were converted to their LogMAR equivalents for analysis. The ranibizumab arm had a corrected LogMAR visual acuity of 0.68 ± 0.62 at baseline and 0.55 ± 0.37 at the final visit (week 16). The bevacizumab arm had a mean corrected LogMAR visual acuity of 0.60 ± 0.78 at baseline and 0.70 ± 0.75 at week 24. There were no statistically significant changes in visual acuity, corneal thickness, or systemic blood pressure in either study. No local (eg, corneal epitheliopathy) or systemic (eg, arteriolar hemorrhage) adverse events were observed or reported.

4. Discussion—Topical ranibizumab and bevacizumab are both effective treatments for corneal NV. Although the average decreases in NA and VC were greater for the ranibizumab-treated cohort, intergroup comparison did not reveal statistically significant differences at comparable time points. Topical ranibizumab was efficacious earlier in the course of treatment than topical bevacizumab, as measured by NA and VC. This may be due to the low molecular weight of ranibizumab allowing for more effective corneal penetration and the establishment of therapeutic concentrations earlier in the course of treatment. These findings are reminiscent of previous comparisons between ranibizumab and bevacizumab in the treatment of neovascular age-related macular degeneration. Ranibizumab was formulated in part because preliminary studies suggested that bevacizumab would not penetrate through the retina.[249] Although it is not uncommon for intravitreal ranibizumab to improve measured outcomes slightly more than bevacizumab (eg, visual acuity, central retinal thickness), these differences are generally not significant.[252]

The results of our comparison suggest that topical ranibizumab may be superior to topical bevacizumab for the treatment of corneal NV; however, it is not possible to definitively reach this conclusion based on our study design and relatively small number of enrolled patients. Neither ranibizumab nor bevacizumab has been approved for the treatment of corneal NV; in order to justify the increased cost of ranibizumab, it will be necessary to demonstrate meaningful treatment superiority.[253] Therefore, a prospective, randomized, head-to-head comparison trial will be required to make authoritative conclusions regarding the long-term efficacy of these medications.

Adverse events were neither observed nor reported for any of our patients who received topical anti-VEGF therapy. However, several clinical studies from other groups have reported local complications (eg, epithelial defects, corneal stromal thinning) associated with the application of topical bevacizumab (Table 3).[245,246,248] Furthermore, ocular surface VEGF inhibition has the potential to induce neurotrophic keratopathy by blocking VEGF-mediated neural growth.[254] Factors that may have contributed to the adverse events noted in previous studies include: 1) use of a bevacizumab concentration greater than 1.0%, 2) duration of treatment greater than 4 weeks, and 3) inclusion of patients with pre-existing corneal epithelial defects. Studies of the safety and efficacy of intravitreal ranibizumab and bevacizumab in the treatment of neovascular age-related macular degeneration have reported comparable rates of systemic adverse events.[252,255] Topical VEGF inhibition has not been associated with any systemic adverse events in the limited number of clinical trials completed to date; however, given the risks, physicians should use discretion when selecting patients and take precautions to minimize systemic anti-VEGF exposure (eg, punctal plugs).

IV. CONCLUDING COMMENTS

Although the clinical significance of corneal NV has long been recognized, management has been confounded by a lack of effective medical and surgical treatment modalities. A roundtable discussion was recently held to define unmet medical needs and formulate treatment guidelines for the management of corneal NV.[256] The committee recognized the need for steroid-sparing pharmacological treatments for corneal NV and acknowledged the therapeutic potential of procedures such as FND and laser ablation. The committee's proposed guidelines stress the importance of treating clinically relevant corneal NV, particularly in cases of infectious keratitis, keratoplasty, limbal stem cell deficiency, and chemical burns. The presence of corneal NV demonstrably increases the risk of graft rejection; therefore, pre- and post-conditioning of the host bed with anti-VEGF therapeutics is an attractive method of potentially increasing graft survival. Treatment guidelines will undoubtedly become more aggressive as the medical and surgical treatments of corneal NV become more effective.

The targeted inhibition of VEGF promises to provide clinicians with an angiogenesis-specific pharmacological approach to treating corneal NV. Based on our experience to date, topical VEGF inhibition using either ranibizumab or bevacizumab appears to be a safe, effective, and practical method for treating stable corneal NV. Clinicians wishing to integrate topical anti-VEGF in their clinical practice should take care in selecting patients and take precautions to minimize the risk of local and systemic adverse events. Novel anti-VEGF therapeutics (eg, VEGF trap, VEGF siRNA, and VEGFR tyrosine kinase inhibitors) may prove to be more effective than the currently available anti-VEGF agents.[257-259] The concurrent inhibition of VEGF and other proangiogenic factors such as PDGF or Ang-2 promises to be more effective than the inhibition of VEGF alone.[260,261]

Combining angiogenesis-specific medical therapy with surgical procedures may overcome limitations in the efficacy of both.[212,224] Novel antiangiogenic agents such as gene signal (GS)-101, a potent inhibitor of the scaffold protein insulin receptor substrate-1 (IRS-1) have shown promise in preclinical and clinical trials.[262,263] The continued development of antiangiogenic agents such as these promises to provide physicians with new tools to safely and effectively treat this potentially devastating, yet often overlooked, condition.

Acknowledgments

Abbreviations

Ang	Angiopoietin
CD	Cluster of differentiation
FasL	Fas ligand
FGF	Fibroblast growth factor
FND	Fine-needle diathermy
HSV	Herpes simplex virus
IA	Invasion area
IL	Interleukin
MMP	Matrix metalloproteinase
MT-MMP	Membrane-type MMP
mVEGFR	Membrane-bound VEGF receptor
NA	Neovascular area
NSAID	Nonsteroidal anti-inflammatory drug
NV	Neovascularization
PDGF	Platelet-derived growth factor
PD-L1	Programmed death-ligand 1
PDT	Photodynamic therapy
PEDF	Pigment epithelium-derived factor
PlGF	Placental growth factor
sVEGFR	Soluble VEGF receptor
TGF	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinases
TNF	Tumor necrosis factor
TSP	Thrombospondin
VC	Vessel caliber
VEGF	Vascular endothelial growth factor
VEGFR	VEGF receptor
WHO	World Health Organization

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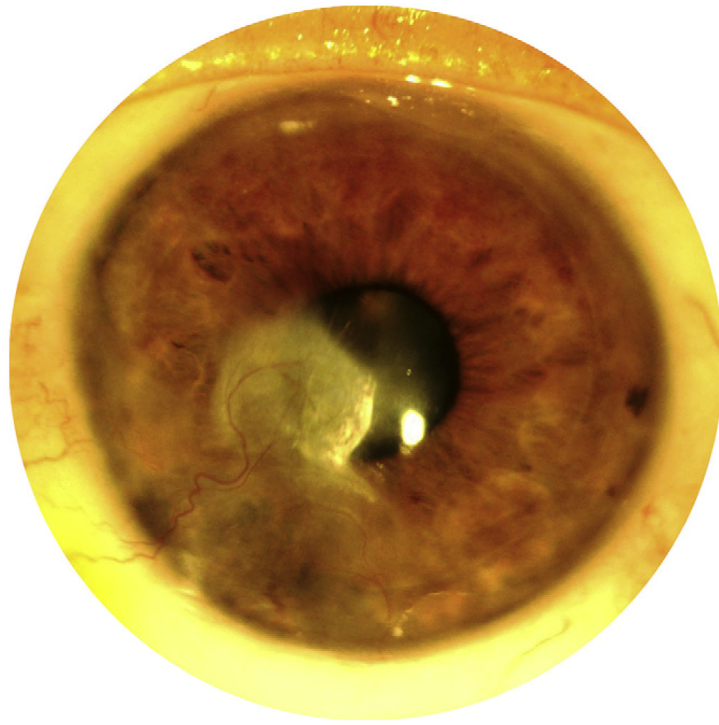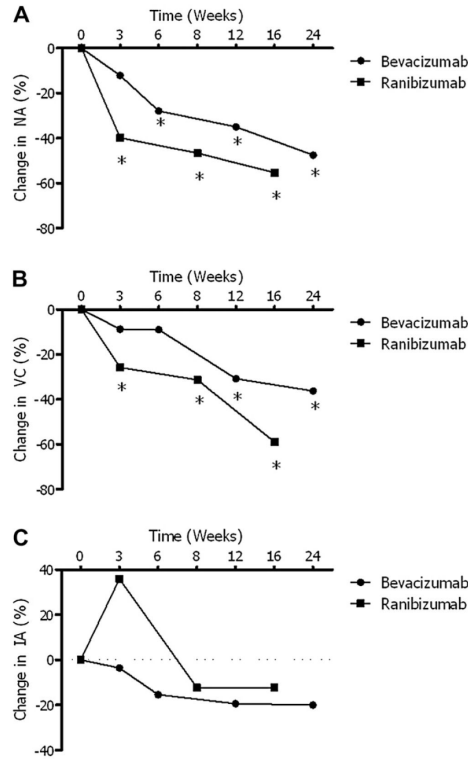
Figure 1.
Clinical appearance of corneal neovasculariztion (NV). Superficial stromal NV, deep stromal NV, and corneal scarring secondary to recurrent herpes simplex virus (HSV) keratitis.
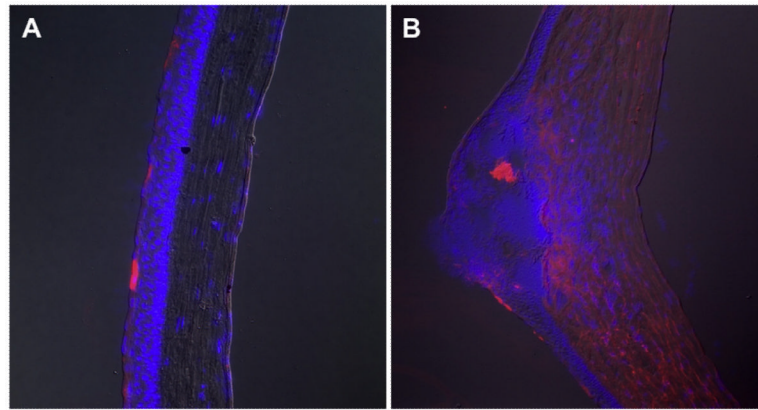
Figure 2.
Corneal penetration of topical bevacizumab. Immunohistochemical staining seven days after the initiation of topical bevacizumab 1% treatment, 3 times per day, in a normal cornea with intact epithelium (A), and a neovascular cornea (B). Immunoreactivity to bevacizumab was limited to the superficial epithelial layers of normal corneas (A), whereas immunoreactivity to bevacizumab was found in all layers of most neovascularized corneas (B) (×200).
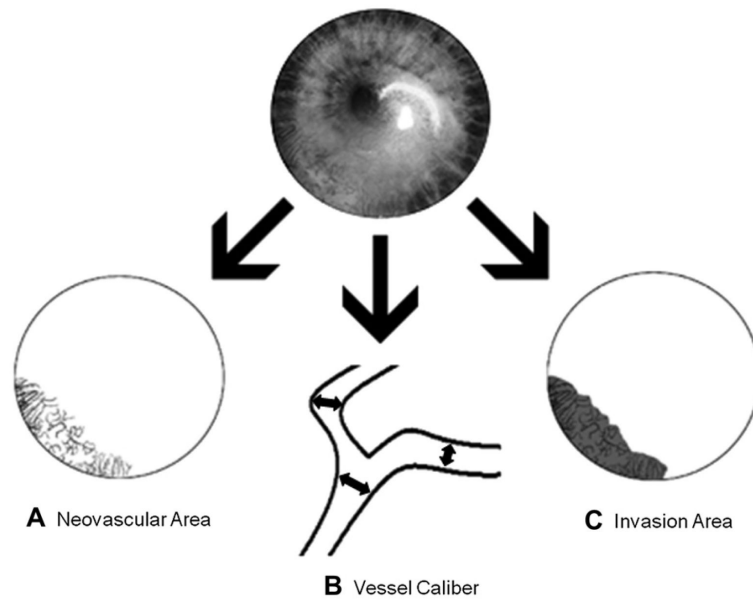
Figure 3.
Primary metrics of treatment efficacy. (A) Neovascular area (NA), ie, the area of the corneal vessels; (B) vessel caliber (VC), i.e., the mean corneal vessel diameter; and (C) invasion area (IA), ie, the fraction of the cornea into which vessel invasion occurs.

Figure 4.
Summary and comparison of the primary study metrics. (A) Neovascular area (NA): the ranibizumab treated cohort experienced a significant decrease by week 3, while the bevacizumab treated cohort required 6 weeks to experience a significant decrease; (B) vessel caliber (VC): ranibizumab treated patients experienced an earlier significant reduction (3 weeks) than bevacizumab treated patients (12 weeks); (C) invasion area (IA): neither medication produced a significant decrease at any time point. (*P 0.05 as compared to baseline measures.)

Table 1

Summary of pro- and antiangiogenic factors involved in corneal NV

Proangiogenic Factors	Antiangiogenic Factors
Vascular endothelial growth factors • VEGF-A, VEGF-C, etc.	Vascular endothelial growth factor receptors • sVEGFR1, mVEGFR3, etc.
Fibroblast growth factors • FGF1, FGF2 (bFGF), etc.	Pigment epithelium-derived factor
Platelet-derived growth factors • PDGF-AA, PDGF-AB, etc.	Collagen derivatives • Endostatin, canstatin, etc.
Angiopoietins • Ang-1, (±) Ang-2	Angiopoietins • Ang-2
Angiogenin	Angiostatin
Matrix metalloproteinases • MT-1MMP, MMP-2, etc.	Matrix metalloproteinases • Epithelial MT1-MMP, MMP-7, etc.
Integrins • αVβ3, α1β1, etc.	Tissue inhibitors of matrix metalloproteinases • TIMP-1, TIMP-2, etc.
Cytokines/chemokines • IL-1, IL-8/CXCL8, etc.	Cytokines/chemokines • IL-1RA, IP-10/CXCL10, etc.
Hepatocyte growth factor/scatter factor	Thrombospondins and CD36 • TSP-1, TSP-2, etc.
Insulin-like growth factor	Death signaling pathways • Fas/FasL, CD80/PD-L1
Renin-angiotensin system	Decorin, small leucine-rich proteoglycan
Placental growth factor	Placental growth factor
Leptin	Prolactin
Thrombin	Anti-thrombin
Platelet activating factor	Plasminogen activator inhibitor

Table 2

Exclusion criteria for study enrollment

• **Age 75 years**
• Ongoing or recent (3 months) episode of ocular infection
• Ongoing or recent (3 months) persistent corneal epithelial defect (14 days duration measuring 1 mm^2)
• Ongoing or recent (3 months) contact lens use
• Recent (1 mo) change in dose or frequency of ocular steroids or NSAIDS
• Ocular or periocular neoplasia
• Recent (3 mo) or planned surgery
• Uncontrolled hypertension (systolic blood pressure 150 mmHg or diastolic blood pressure 90 mmHg)
• Diabetes mellitus
• History of a thromboembolic event (eg, myocardial infarction or cerebrovascular accident)
• Coagulation abnormality, including anticoagulation medications (excluding aspirin)

Table 3

Summary of clinical trials investigating topical bevacizumab for the treatment of corneal NV

Author	Corneal Pathology	N	Conc.	Treatment Frequency	Duration (Days)	Percent with Decreased NV	Percent with Ocular Event
DeStafeno	4, 5	2	1.00%	4/day	25	100%	0%
Bock	1, 3	5	0.50%	5/day	108	100%	20%
Kim	1, 2, 3, 6, 9	10	1.25%	2/day	30-90	70%	60%
Dastjerdi	1, 2, 3, 6, 7, 8, 9	10	1.00%	4/day	21	100%	0%
Koenig	1, 2, 3, 6, 7, 8, 9, 10, 11	30	0.50%	5/day	102	80%	16.7%

Corneal pathology: 1) keratoplasty, 2) HSV keratitis, 3) chemical burn, 4) cicatricial pemphigoid, 5) traumatic rupture, 6) pterygium, 7) dry eye disease, 8) limbal stem cell deficiency, 9) Stevens-Johnson syndrome, 10) measles keratitis, 11) Salzmann's nodular degeneration. (Conc. = Concentration).