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Suppression of Neovascularization by Topical and Subconjunctival Bevacizumab After High-Risk Corneal Transplantation

Thomas H. Dohlman, MD,[1,*] Rohan Bir Singh, MD,[1,*] Francisco Amparo, MD,[1] Tatiana Carreno-Galeano, MD,[1] Mohammad Dastjerdi, MD,[1,2] Giulia Coco, MD,[1] Antonio Di Zazzo, MD,[1] Hasanain Shikari, MD,[1] Ujwala Saboo, MD,[1] Kimberly Sippel, MD,[3] Jessica Ciralsky, MD,[3] Sonia H. Yoo, MD,[4] Matheus Sticca, MD,[5] Tais H. Wakamatsu, MD,[5] Somasheila Murthy, MD,[6] Pedram Hamrah, MD,[1,7] Ula Jurkunas, MD,[1] Joseph B. Ciolino, MD,[1] Hajirah Saeed, MD, MPH,[1] Jose A.P. Gomes, MD,[5] Victor L. Perez, MD,[4,8] Jia Yin, MD, PhD, MPH,[1] Reza Dana, MD, MPH[1]

Purpose: To assess the effectiveness of topical and subconjunctival bevacizumab in suppressing vascularization in graft and host bed after high-risk corneal transplantation.

Design: Secondary analysis of prospective, randomized, double-blind, placebo-controlled multicentric clinical trial.

Participants: The study includes patients aged $>$ 18 years who underwent high-risk penetrating keratoplasty, which was defined as corneal vascularization in $\geq$ 1 quadrants of the corneal graft and host bed, excluding the limbus.

Methods: Patients were randomized to treatment and control groups. The patients in the treatment group received subconjunctival injection of bevacizumab (2.5 mg/0.1 ml) on the day of the procedure, followed by topical bevacizumab (10 mg/ml) 4 times per day for 4 weeks. The patients in control group received injection of vehicle (0.9% sodium chloride) on the day of procedure, followed by topical vehicle (carboxymethylcellulose sodium 1%) 4 times a day for 4 weeks.

Main Outcome Measures: Vessel and invasion area of vessels in the corneal graft and host beds.

Results: This study included 56 eyes of 56 patients who underwent high-risk corneal transplantation, with equal numbers in the bevacizumab and vehicle (control) treatment groups. The mean age of patients who received bevacizumab was 61.2 $\pm$ 15.9 years, and the mean age of those treated with vehicle was 60.0 $\pm$ 16.1 years. The vessel area at baseline was comparable in the bevacizumab (16.72% $\pm$ 3.19%) and control groups (15.48% $\pm$ 3.12%; $P =$ 0.72). Similarly, the invasion areas were also similar in the treatment (35.60% $\pm$ 2.47%) and control (34.23% $\pm$ 2.64%; $P = 0.9$) groups at baseline. The reduction in vessel area was significantly higher in the bevacizumab-treated group (83.7%) over a period of 52 weeks compared with the control group (61.5%; $P < 0.0001$). In the bevacizumab-treated group, invasion area was reduced by 75.8% as compared with 46.5% in the control group. The vessel area was similar at 52 weeks postprocedure in cases of first (3.54% $\pm$ 1.21%) and repeat (3.80% $\pm$ 0.40%) corneal transplantation in patients who received bevacizumab treatment. In the vehicle-treated patients, the vessel area was significantly higher in repeat (9.76% $\pm$ 0.32%) compared with first (8.06% $\pm$ 1.02%; $P < 0.0001$) penetrating keratoplasty. In the bevacizumab treatment group, invasion areas at week 52 were comparable in first (11.70% $\pm$ 3.38%) and repeat (11.64% $\pm$ 1.74%) procedures, whereas invasion area was significantly higher in repeat (27.87% $\pm$ 2.57%) as compared with first (24.11% $\pm$ 2.17%) penetrating keratoplasty in vehicle-treated patients.

Conclusions: In patients undergoing vascularized high-risk corneal transplantation, bevacizumab is efficacious in reducing vascularization of corneal graft and host bed, thereby reducing the risk of corneal graft rejection in vascularized host beds.

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Corneal disorders are the fourth leading cause of blindness globally.[1,2] As of 2020, 43.3 million people were suffering from blindness as per the revised definition of the World Health Organization (best-corrected vision worse than 3/60 [20/400] in the better-seeing eye).[3,4] An estimated 5.5 million people are bilaterally blind or have visual impairment due to corneal disorders globally.[2] Overall favorable clinical outcomes have made corneal

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transplantation the intervention of choice for the management of irreversible corneal blindness.[5] The survival rates of the first transplanted graft in noninflamed and nonvascular hosts are > 90%.[6] However, the high success rates of penetrating keratoplasty are overshadowed by high rates of rejection (> 50%) when a corneal graft is transplanted onto vascularized and inflamed or "high-risk" host beds because of the risk of immune-mediated rejection, despite aggressive immunosuppressive therapy.[7,8]

Corneal transplant failure is most often due to immune-mediated rejection of the transplanted tissue.[9] Several studies have highlighted the deleterious role of host bed vascularization in immune rejection of the transplanted corneal tissue.[10,11] Although the underlying immunopathological mechanisms that disrupt the "immune privilege" of the cornea have not been fully delineated, evidence clearly suggests enhanced allosensitization and subsequent graft rejection are associated with the presence of neovascularization in the host cornea.[12–16] Moreover, mechanisms (such as anterior chamber–associated immune deviation) maintaining the "immune privilege" of the cornea are disrupted because of neovascularization.[17,18] Therefore, strategies for suppressing corneal neovascularization have the potential to inhibit the alloimmune response and prevent corneal graft rejection.

The formation of vessels in inflamed tissue is primarily mediated by VEGF-A, which induces vascular endothelial cells to proliferate and migrate.[14,19–21] In 2004, bevacizumab was approved by the United States Food and Drug Administration for the treatment of advanced metastatic colorectal cancer.[22,23] Bevacizumab is a recombinant, humanized, monoclonal immunoglobulin that binds to VEGF-A and prevents endothelial cell differentiation, proliferation, and migration.[24] It has been used for the treatment of several oncological disorders and was adopted by ophthalmologists for the management of disorders with underlying neovascularization such as proliferative diabetic macular edema and age-related macular degeneration.[25–29] Several preclinical studies have highlighted the efficacy of bevacizumab in inhibiting corneal neovascularization by neutralizing VEGF-A.[30,31] The efficacy of bevacizumab in improving corneal transplantation survival has also been evaluated in the clinical setting in multiple reports.[32,33]

Given the critical contribution of vascularization to driving the alloimmune response in hosts, our group recently performed a multicenter randomized controlled trial evaluating the efficacy of bevacizumab vs. vehicle in improving graft survival in high-risk corneal transplantation.[7] As a follow-up to that randomized controlled trial, in the present study, we have utilized slit-lamp biomicroscopy images and image analysis to determine the effect of bevacizumab on clinical parameters of corneal vascularization in the graft and host bed in high-risk corneal transplantation.

Methods

Study Design

This study is a secondary retrospective analysis of the data collected during the clinical trial which included patients with vascularized host beds who underwent high-risk penetrating keratoplasty over an 8-year period between January 2010 and March 2018. The primary study was a multicenter, prospective, placebo-controlled, double-blinded, randomized control trial assessing the efficacy of perioperative bevacizumab in suppression of vascularization of the graft and host bed in high-risk corneal transplantation. The sample size of the primary study was determined by the methods described previously and included 92 patients who were randomized to receive bevacizumab (n = 48) or vehicle (n = 44).[7] In the present study, patients with slit-lamp photos for all time points were included (56 eyes of 56 patients).

The patients were enrolled from 5 sites (Massachusetts Eye and Ear, Bascom Palmer Eye Institute, LV Prasad Eye Institute, Universidade Federal São Paulo, and Weill Cornell Medical College), and the study was registered at ClinicalTrials.gov (NCT01072357; Table S1, available at www.ophthalmologyscience.org). The study was approved by the institutional review boards of all the participating sites, and it was conducted in strict adherence to the tenets of the Declaration of Helsinki. We obtained a written, informed consent from all patients participating in this study.

The inclusion criterion for enrollment was patients aged > 18 years undergoing penetrating keratoplasty, which was deemed high-risk surgery. For this study, high-risk corneal transplantation was defined as corneal vascularization in ≥ 1 quadrants (≥ 3 clock hours vascularization ≥ 2 mm from the limbus) or extension of corneal vascularization to the graft-host junction in a previously failed corneal transplant. Patients with a history of Stevens–Johnson Syndrome, ocular pemphigoid, ocular or periocular malignancy, nonhealing epithelial defect (> 0.25 mm^2) in the host bed for ≥ 6 weeks preoperatively, uncontrolled glaucoma, or those who had received intraocular or systemic anti-VEGF treatment (in the past 45 days) were excluded from the study. Additionally, patients on dialysis, systemic anti-VEGF agents, systemic immunosuppressive therapy (for other indications), patients with a history of uncontrolled hypertension (systolic blood pressure: ≥ 150 mmHg; diastolic blood pressure: ≥ 90 mmHg) or thromboembolic event (in past 12 months), and pregnant or lactating and premenopausal women not using adequate contraception were not enrolled in the study.

Randomization of the Patients

The patients enrolled in the study were randomized and equally divided into treatment or control groups during the screening visit. The patients were randomized by a pseudorandom number generator based on a predetermined randomization scheme. Every patient was given a unique identification code and treatment assignment was coded as well to ensure that investigators as well as patients were masked. The statistician labeled the drugs (both treatment and vehicle) dispensed by the institutional pharmacies using the unique identification codes.

Treatment and Postoperative Management

All patients enrolled in the study underwent full-thickness corneal transplantation (using an interrupted suture technique) as per the standardized surgical protocol. The donors were not age or tissue (ABO blood grouping or human leukocyte antigen) matched with recipients. At the conclusion of the procedure, the patients received subconjunctival injections of either 0.1 ml (2.5 mg) of 2.5% bevacizumab or vehicle (0.9% sodium chloride) at the 12 o'clock position, 1 mm posterior to the limbus. On the following day, patients were started on topical bevacizumab (1% solution) or vehicle (carboxymethylcellulose sodium 1%, Refresh Liquigel) 4 times per day (every 4 hours while awake) for 4 weeks, dispensed by the institutional pharmacies. The patients in both the groups received the standard postprocedure treatment including topical

corticosteroids (1% prednisolone acetate instilled 6 times every day for the first 2 weeks postprocedure). The topical steroid instillation frequency was gradually tapered over the next 6 months, and the frequency reduced to once per day indefinitely. Additionally, patients in both the groups received topical antibiotic eye drops, which were instilled 4 times per day until full epithelialization of the graft was achieved. After the transplant, the patients were clinically evaluated on day 1 and weeks 4, 8, 16, 26, and 52.

Assessing Vessel Area and Invasion Areas

The extent of neovascularization was analyzed by quantification of corneal vessel and invasion areas using slit-lamp images, in all patients (recruited at all the centers) by a single examiner (R.B.S.), in a blinded manner. Total vascular area was defined as the percent area of the cornea (graft and host bed excluding the limbus) occupied by the blood vessels themselves. Total invasion area was defined as the percent area of the cornea (graft and host bed excluding the limbus) with neovascularization. Analysis of vascularization metrics was performed on Adobe Photoshop Version 24 (Adobe Corp) using a standardized protocol. Multiple slit-lamp images were taken at each visit to ensure that best quality images were available for assessment. The step-by-step methodology is outlined in Figure 1. In-focus images with the least glare and with a linear alignment with the objective lens were used. Images of the same alignment and area were selected for baseline and subsequent visits for each patient. In Adobe Photoshop, the "elliptical marquee tool" was used to demarcate the 4 cardinal points (12, 3, 6, and 9 clock hours) of the cornea with exclusion of the limbus. To ensure uniformity in the selected area of the cornea, the baseline image was used as a reference for orientation of subsequent images. Additionally, vessel origins in the peripheral cornea were used as landmarks.

Images were projected onto an Apple iPad (Apple Computing Inc), and the black and white filter was applied to enhance the vessels in the images. An Apple Pencil was used to trace the vessels (with active pressure function, to ensure that the entire girth of the vessels was traced) from the periphery to the center of the cornea. The total vessel and invasion areas were traced in 2 separate layers in Adobe Photoshop. The vessel area was demarcated as the area of vessels assessed using length and girth on the cornea. Invasion area was demarcated as the area of the cornea with vessels and was demarcated starting from the peak of the longest vessel and connecting the vessel peaks unless the gap between the vessels was $>$ 2 clock hours. The separate images of the traced vessel and invasion areas were generated and analyzed using Image J Version 1.53 (National Institutes of Health). The percentages of vessel and invasion areas were calculated using the following formula:

$$= \frac{Black\ pixel\ count}{Total\ pixel\ count} \times 100$$

Statistical Analysis

Demographic data, clinical presentations, and best-corrected visual acuity at different time points were compared using the Fisher exact test or chi-square test. The data were analyzed using Prism 9 software, version 8.5.1 (GraphPad Inc). The data are presented as mean ± standard deviation. Mann−Whitney U tests were used to compare the vessel and invasion percentage areas at different time points after surgery between bevacizumab and the control group. The 1-way analysis of variance test was used to compare the repeated measures. P values of < 0.05 were considered statistically significant.

Results

This study included 56 eyes of 56 patients who underwent high-risk corneal transplantation, with 28 patients in the bevacizumab treatment group and 28 patients in the vehicle-treated control group. The mean ages of patients who received bevacizumab (61.2 ± 15.9 years) and vehicle (60.0 ± 16.1 years; $P = 0.81$) were comparable. The bevacizumab treatment group included 10 men (36%) and 18 women (64%), whereas the control group included 16 men (57%) and 12 women (43%). The mean corneal thickness at baseline was similar in both bevacizumab- (632 ± 235 μm) and vehicle-treated (637± 227 μm, $P = 0.64$) patients. The mean best-corrected visual acuity ($P = 0.58$) and intraocular pressure ($P = 0.29$) at presentation were similar in both groups as well. In the treatment group, 17 patients (61%) underwent repeat penetrating keratoplasty due to prior graft failure, whereas the procedure was repeated in 19 patients (68%) in the control group. The indications for corneal transplantation in the patients were graft failure (36; 64%), herpetic keratitis (7; 13%), bullous keratopathy, infectious keratitis (4; 7%), corneal scarring (1; 2%), keratoconus (3; 5%), interstitial keratitis (1; 2%), limbal stem cell deficiency (2; 4%), and Fuchs dystrophy (1; 2%). The demographics and clinical characteristics of patients are summarized in Table 2.

The vessel area at baseline was comparable for patients in the bevacizumab (16.72% ± 3.19%) and control groups (15.48% ± 3.12%; $P = 0.72$). Similarly, the invasion areas were also similar in the treatment (35.60% ± 2.47%) and control (34.23% ± 2.64%; $P = 0.9$) groups at baseline. The reduction in vessel area was significantly higher in the bevacizumab-treated group (83.7%) over a period of 52 weeks compared with the control group (61.5%; $P < 0.0001$). A similar effect was observed in invasion area; in the bevacizumab-treated group, the invasion area was reduced by 75.8%, compared with 46.5% in the control group. We assessed the vessel and invasion areas at day 1 and weeks 1, 4, 8, 16, 26, 39, and 52 (Figs 2, 3). The vessel (22.77% ± 2.74 vs. 22.73% ± 2.91%; $P = 0.9$) and invasion (48.11% ± 5.65 vs. 47.50% ± 5.40%; $P = 0.68$) areas were comparable for both the treatment and control groups 1 day post procedure. At week 1, the vessel area (20.91% ± 3.86% vs. 14.68% ± 2.50%; $P = 0.08$) was moderately reduced at week 1 in the treatment group compared with controls. However, at week 1, the invasion area was significantly lower in bevacizumab-treated patients (33.11% ± 4.28% vs. 47.07% ± 6.30%; $P = 0.009$) compared with controls. We observed significantly lower vessel areas in the grafted corneas at weeks 4 (10.90% ± 1.54% vs. 17.60% ± 2.56%; $P < 0.0001$), 8 (9.83% ± 1.44% vs. 17.32% ± 2.46%; $P < 0.0001$), 16 (6.86% ± 1.09% vs. 13.24% ± 1.83%; $P < 0.0001$), 26 (5.72% ± 1.00% vs. 11.57% ± 1.56%; $P < 0.0001$), 39 (3.84% ± 0.78% vs. 8.93% ± 1.12%; $P < 0.0001$), and 52 (3.71% ± 0.77% vs. 8.76% ± 3.71%; $P < 0.0001$; Fig 4A). Similarly, we observed significantly lower invasion areas in grafted corneas at weeks 1 (33.11% ± 4.28% vs. 47.07% ± 6.30%; $P = 0.009$), 4 (27.99% ± 3.10% vs. 44.48% ±

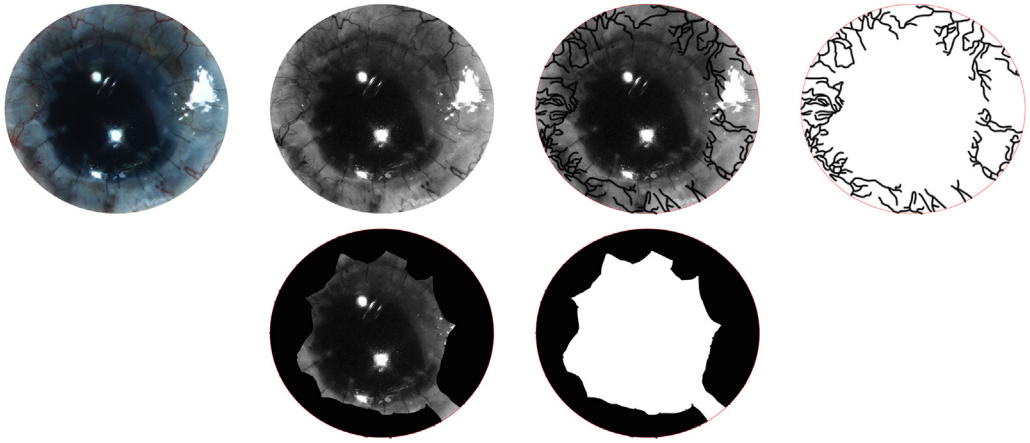

Figure 1. Methodology for assessing vessel and invasion areas in transplanted cornea and host beds. The total vessel and invasion areas were traced in 2 separate layers in Adobe Photoshop. The total vessel area was demarcated as the area of vessels assessed using length and girth on the transplanted corneal tissue. The total invasion area was demarcated as the area of the transplanted graft and host beds with vessels and was demarcated starting from the peak of the longest vessel and connecting the vessel peaks unless the gap between the vessels was > 2 clock hours.

4.50%; $P < 0.0001$), 8 (25.49% ± 3.22% vs. 42.90% ± 4.21%; $P < 0.0001$), 16 (6.86% ± 1.09% vs. 13.24% ± 1.83%; $P < 0.0001$), 26 (5.72% ± 1.00% vs. 11.57% ± 1.56%; $P < 0.0001$), 39 (3.84% ± 0.78% vs. 8.93% ± 1.12%; $P < 0.0001$), and 52 (3.71% ± 0.77% vs. 8.76% ± 3.71%; $P < 0.0001$; Fig 4B).

Next, we performed a comparative analysis of the vessel and invasion areas in patients who underwent first and repeat penetrating keratoplasty in both treatment groups. Interestingly, we observed that the vessel area was similar at 52 weeks postprocedurre in both the cases of first (3.54% ± 1.21%) and repeat (3.80% ± 0.40%) corneal transplantation

Table 2. Demographics and Clinical Characteristics of the Patients Included in This Study

	Bevacizumab (n = 28)	Control (n = 28)	P Value
Age, yrs	61.2 ± 15.9	60.0 ± 16.1	0.81
Sex *n* (%)			
Male	10 (36%)	16 (57%)	0.61
Female	18 (64%)	12 (43%)	
Race n (%)			
White	15 (54%)	11 (39%)	0.55
African American	4 (14%)	6 (21%)	
Hispanic	7 (25%)	4 (14%)	
Asian	1 (4%)	3 (11%)	
Alaskan/Pacific Islander	0	1 (4%)	
BCVA (in logMAR)	1.47 ± 0.83	1.52 ± 0.91	0.58
Intraocular pressure, mmHg	16.9 ± 6.2	15.0 ± 4.6	0.29
Corneal thickness, µm	632 ± 235	637 ± 227	0.64
Corneal transplantation			
First	11 (39%)	9 (32%)	0.71
Repeat	17 (61%)	19 (68%)	
Indication for transplantation			
Graft failure	17 (61%)	19 (68%)	
Herpetic keratitis	3 (12%)	4 (14%)	
Bullous keratopathy	2 (7%)	1 (4%)	
Infectious keratitis	2 (7%)	2 (7%)	
Corneal scarring	1 (4%)	0	
Unknown	1 (4%)	0	
Keratoconus	1 (4%)	2 (7%)	
Interstitial keratitis	1 (4%)	0	
LSCD	1 (4%)	1 (4%)	
Fuchs dystrophy	0	1 (4%)	

BCVA = best-corrected visual acuity; logMAR = logarithm of the minimum angle of resolution; LSCD = limbal stem cell deficiency.

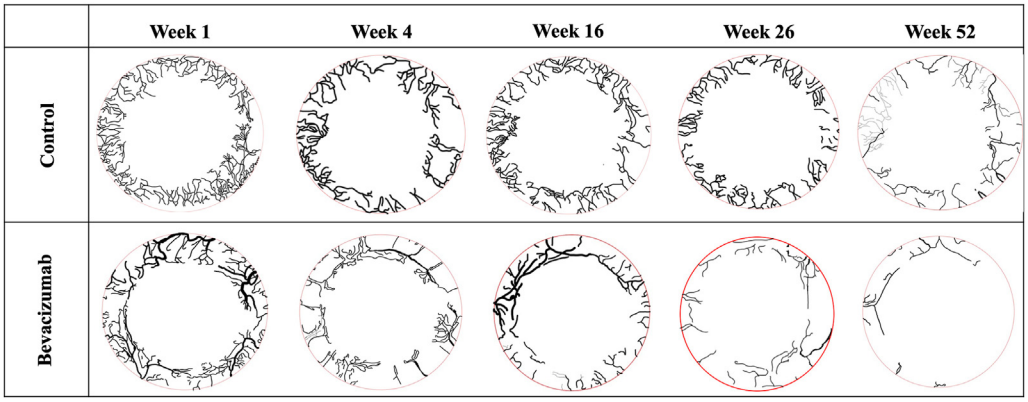

Figure 2. Representative images of vessel area in the grafted tissue and host beds in vehicle and bevacizumab-treated patients.

in patients that received bevacizumab treatment. In contrast, among patients in the vehicle treatment group, the vessel area was significantly higher in repeat (9.76% ± 0.32%) compared with first (8.06% ± 1.02%; $P < 0.0001$) penetrating keratoplasty patients. Moreover, the vessel area in both first and repeat penetrating keratoplasty patients was significantly lower than their vehicle-treated counterparts (Fig 5A). We observed a similar effect of bevacizumab treatment on invasion areas in first and repeat corneal transplantation. In the bevacizumab treatment group, invasion areas at week 52 were comparable in first (11.70% ± 3.38%) and repeat (11.64% ± 1.74%) procedures. On the contrary, invasion area was significantly higher in repeat (27.87% ± 2.57%) as compared with first (24.11% ± 2.17%) penetrating keratoplasty in vehicle-treated patients (Fig 5B).

Discussion

In a double-blind, multicenter, placebo-controlled trial, we assessed the clinical effectiveness of bevacizumab on suppression of corneal neovascularization in high-risk corneal transplantation over a 52-week period. Topical and subconjunctival treatment with bevacizumab significantly lowered corneal vascularization area and invasion areas in the transplanted graft compared with vehicle treatment.

Several studies in the past have also used bevacizumab as a preconditioning treatment before corneal transplantation.[34–36] Additionally, bevacizumab has been used in combination with argon laser to reverse corneal neovascularization after transplantation.[37,38] Until recently, the evidence pertaining to the antiangiogenic effect in the setting of high-risk corneal transplantation was limited to assessment of corneal graft survival.[39,40] This is the first study to objectively assess the efficacy of bevacizumab in inhibiting corneal neovascularization after high-risk transplantation at different time points for 52 weeks after transplantation.

The primary efficacy outcome measures in this study were vessel and invasion areas in the graft tissue and host beds. We adopted a unique, reliable, and standardized method for quantitative assessment of corneal vessels. Because slit-lamp image acquisition has inherent variability because of differences in operator technique and equipment, we assessed vessel percentage area. In the patients who received bevacizumab, the vessel area was significantly decreased in controls at 52 weeks post procedure ($P <$

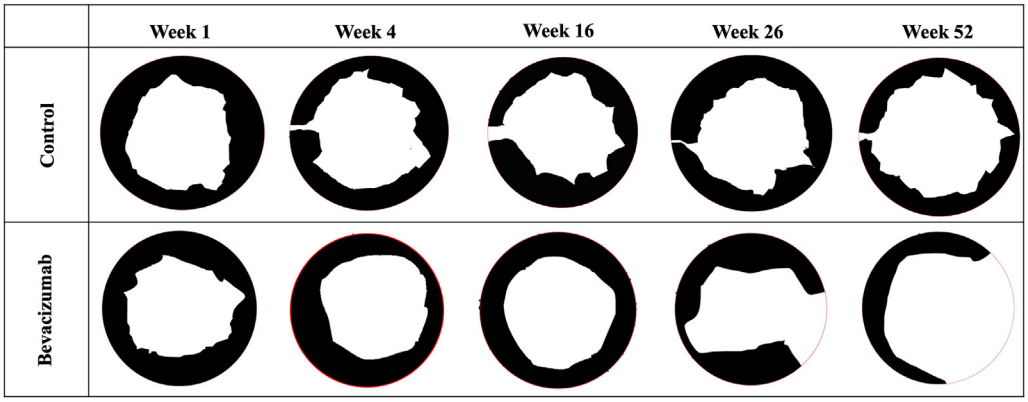

Figure 3. Representative images of invasion area in the grafted tissue and host beds in vehicle and bevacizumab-treated patients.

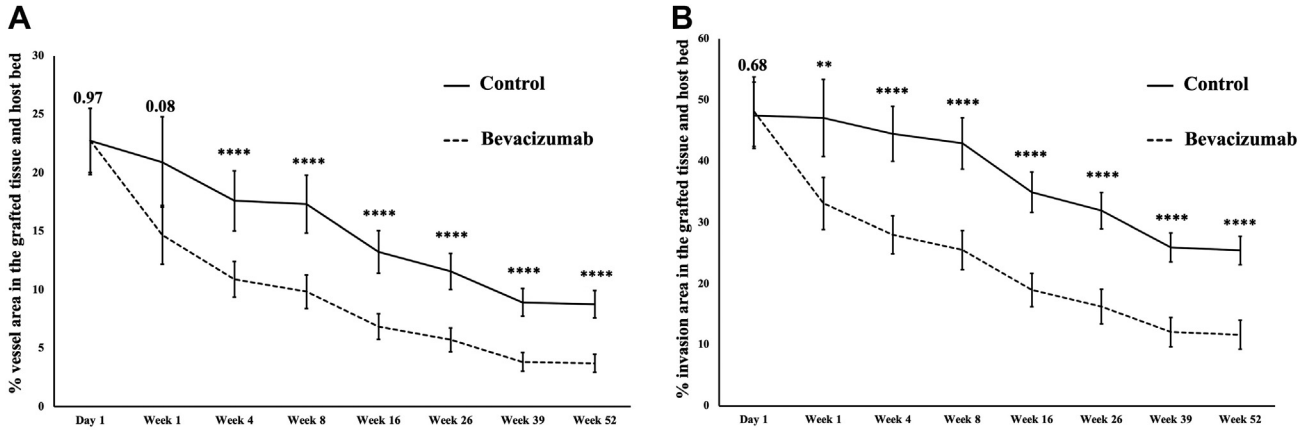

Figure 4. A, Vessel and (**B**) invasion areas in the grafted tissue and host beds in vehicle and bevacizumab-treated patients. ****$P < 0.0001$; **$P < 0.01$.

0.0001). The vessel areas were comparable in both groups at postoperative day 1 ($P = 0.97$), and a moderately lower vessel area was observed after 1 week in bevacizumab-treated patients compared with vehicle-treated controls ($P = 0.08$). However, the vessel area in the bevacizumab treatment group was significantly lower at subsequent time points (weeks 4, 8, 16, 26, 39, and 52). We also observed a moderate reduction in the vessel area in patients who were in the control group. This change might be attributed to the intense topical corticosteroid treatment all patients received because of the high-risk categorization of their corneal transplantation. Thus, the significant reduction in vessel area observed in the bevacizumab group was detectable despite this intense steroid regimen.

Interestingly, we observed a similar reduction in vessel area in bevacizumab-treated patients at 52 weeks regardless of whether patients were undergoing a first or repeat corneal transplant. In contrast, in the vehicle treatment group, vessel area was greater in patients undergoing a repeat corneal transplant as compared with those who had the procedure for the first time.

Corneal invasion areas were comparable in bevacizumab and vehicle-treated group on postoperative day 1. However, we observed a significant decrease in invasion areas area at all subsequent time points (weeks 1, 4, 8, 16, 26, 39, and 52) in patients who received bevacizumab as compared with vehicle treatment. There was a similar reduction in invasion area in bevacizumab-treated patients at 52 weeks, regardless of whether it was the patient's first corneal transplant or a repeat surgery. A similar reduction in neovascular and invasion areas suggests that the antiangiogenic effects of bevacizumab are more pronounced in first-time transplants as compared with repeat transplants, but, by the end of follow-up, the difference between vessel and invasion areas between first and repeat transplants went away.

Bhatti et al evaluated the antiangiogenic effect of bevacizumab in high-risk corneal transplants, showing a significant decrease in corneal vascularization area after treatment in a single center, randomized trial.[41,42] In another study, Krizova et al[43] reported a 38.04% and 22.7% reduction in vascularization area in the peripheral and

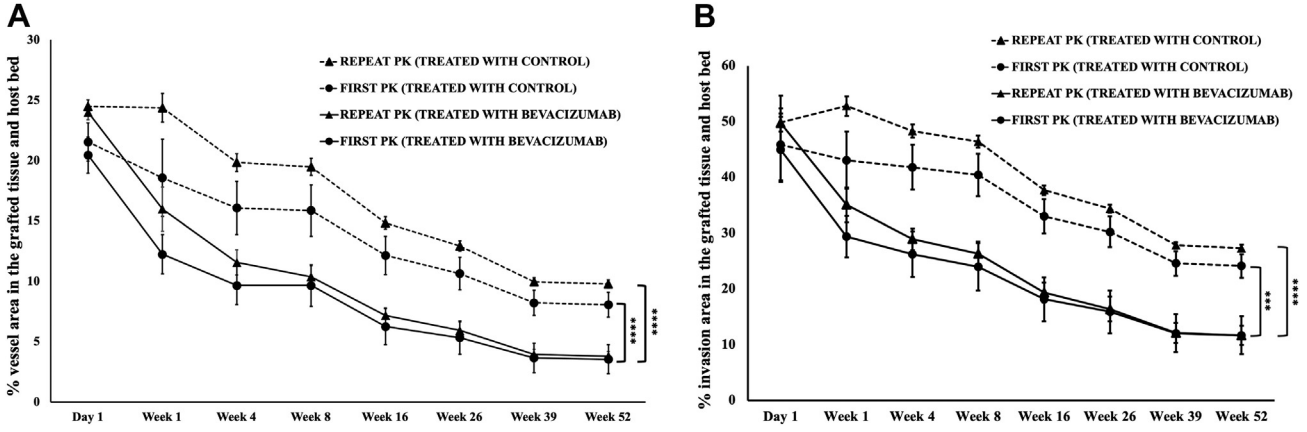

Figure 5. A, Vessel and (**B**) invasion area areas in the grafted tissue and host beds of patients undergoing first or repeat corneal transplantation. ****$P < 0.0001$, ***$P < 0.001$.

central segments of cornea, respectively, in bevacizumab-treated patients compared with only 21.6 and 9.6% reduction vascularization area in central and peripheral corneal segments, respectively, in controls. The present study evaluated the effect of bevacizumab on corneal neovascularization in high-risk corneal transplantation over a 52-week period, demonstrating evidence of its suppressive effects on quantifiable vascularization metrics. Our previous report highlights the role of bevacizumab in preventing and delaying endothelial rejection in high-risk corneal transplantation corroborates the efficacy of bevacizumab in suppressing vascularization in the setting of high-risk corneal transplantation.[7] A larger clinical trial would help to confirm the therapeutic potential of bevacizumab for preventing graft rejection in cases of high-risk transplantation through blockade of VEGF-A. Additionally, future clinical trials to study the effects of other anti-VEGF therapies, such as ranibizumab or aflibercept, administered as a pretreatment (before) or after surgery in high-risk corneal transplantation may also hold potential for improving high-risk corneal transplant survival.[44–48] In addition, the standardized methods for imaging and vessel quantification applied in this study, as well as recent upgrades to previously described semiautomated methods, can be used for future clinical trials that aim to objectively assess and quantify corneal vascularization and invasion areas.[49,50]

Because of its retrospective nature, this study has several limitations. The primary study included 92 patients; however, images were not available for all patients at all time points. Therefore, a comprehensive analysis to assess the efficacy of bevacizumab in suppressing corneal neovascularization in all patients could not be performed. Second, most of the patients included in this study were enrolled at a single center, which can potentially introduce a location bias. Lastly, all images were examined by a single reviewer in a blinded manner. Despite establishment of and adherence to a standardized image analysis protocol, there may be some degree of subjectivity introduced during image analysis and processing.

Corneal vascularization has a deleterious impact on the survival of corneal grafts in high-risk recipients. Although penetrating keratoplasty typically has high success rates, more than half of "high-risk" grafts are rejected, posing major challenges for cornea specialists and their patients. In this study, we assessed the therapeutic efficacy of the anti-angiogenic antibody bevacizumab on vascular area and invasion areas within transplanted grafts and host beds in the high-risk corneal transplantation setting. These findings highlight the therapeutic potential of bevacizumab in reducing the risk of corneal graft rejection when transplanted in vascularized host beds or in cases of repeat corneal transplantation.

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[1] Cornea Service, Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts.

[2] Department of Ophthalmology and Visual Science, Rutgers New Jersey Medical School, Newark, New Jersey.

[3] Department of Ophthalmology, Weill Cornell Medicine, New York, New York.

[4] Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida.

[5] Cornea and External Disease Service, Paulista Medical School/Universidade Federal de São Paulo, São Paulo, Brazil.

[6] Cornea Service, The Cornea Institute, LV Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, Telangana, India.

[7] Cornea Service, New England Eye Center, Department of Ophthalmology, Tufts Medical Center, Tufts University School of Medicine, Boston, Massachusetts.

[8] Foster Center for Ocular Immunology, Duke Eye Center, Duke University School of Medicine, Durham, North Carolina.

*T.H.D. and R.B.S. contributed equally to this work.

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Author Contributions:

Conception and design: Dohlman, Singh, Amparo, Dana

Data collection: Dohlman, Singh, Amparo, Carreno-Galeano, Dastjerdi, Coco, DiZazzo, Shikari, Saboo, Sippel, Ciralsky, Yoo, Sticca, Wakamatsu, Murthy, Hamrah, Jurkunas, Ciolino, Saeed, Gomes, Perez, Yin, Dana

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Correspondence:

Reza Dana, MD, MSc, MPH, Cornea Service, Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, 243 Charles St., Boston, MA 02114. E-mail: Reza_Dana@meei.harvard.edu.

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