COVER PAGE

Clinical Protocol Title: A Multi-Center Study of the Safety and Efficacy of Bevacizumab in High-Risk Corneal Transplant Survival

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Participating sites:

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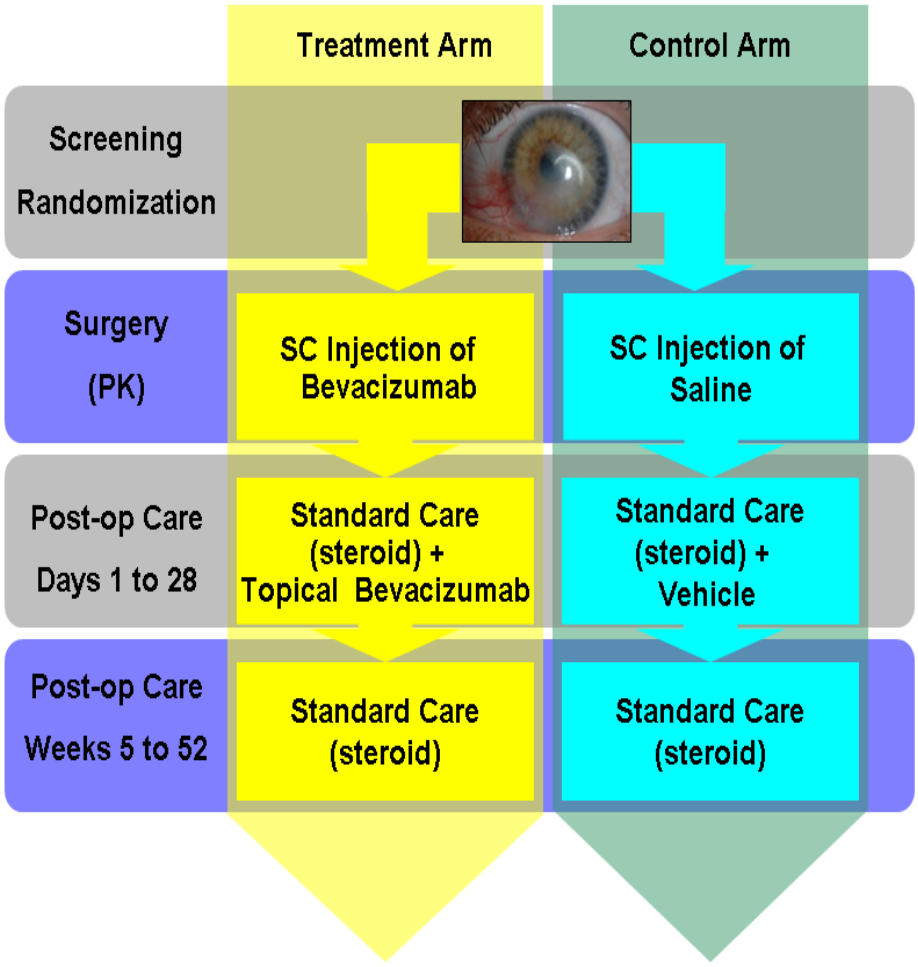
STUDY DESIGN SCHEMATIC



1. CLINICAL PROTOCOL

1.1 Background

The Public Health Relevance of Corneal Blindness: A large analysis of the published literature related to the prevalence and causes of blindness worldwide [1] has concluded that the second leading cause of blindness (after cataract) is corneal disease. Childhood keratitis, trauma, and microbial keratitis among adults, can often lead to vascularized corneal scars, the most frequent causes of corneal blindness. As reported by the Bulletin of the World Health Organization [2] in addition to the many millions of corneally blind individuals are 1.5-2.0 million cases of monocular blindness per year due to corneal pathology. Many of these cases remain unregistered as legal "blindness", since this term refers to vision in the best eye (suggesting bilateral disease). Numerous reports have concluded that in addition to suboptimal eye banking and infrastructural support for transplantation in many areas around the globe, a universal barrier to alleviating the burden of corneal blindness is the poor outcome of grafting in vascularized beds, often requiring repeat procedures, as outlined below. Development of more effective strategies to tackle this unmet need is therefore a priority in ophthalmology. In addition, importantly, corneal disease is the most common type of ocular pathology experienced by deployed military personnel. Significant eye injuries have been experienced by 10-12% of the active-duty personnel who have been deployed since 2002 [26].

The Burden of Transplant Rejection: Corneal transplantation has emerged as the most common form of solid tissue transplantation. Of the >100,000 cases performed annually worldwide, over 38,000 are performed in the US alone [2007 Eye Banking Statistical Report]. In uncomplicated first allografts performed in non-vascular host

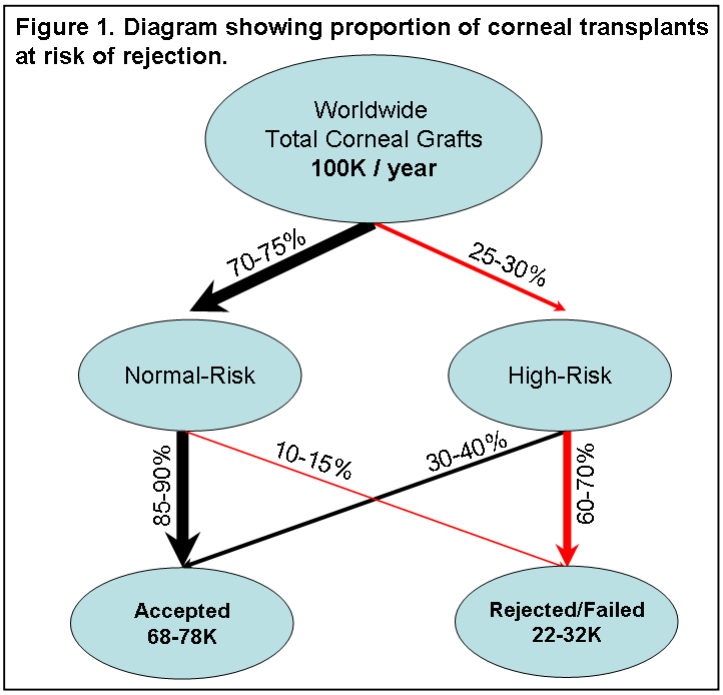


Figure 1. Diagram showing proportion of corneal transplants at risk of rejection.

beds ('normal-risk' grafts), the 2-year survival rate can approach 85-90% under cover of topical steroids, without systemic immune suppression. However, this success rate is completely abrogated in hosts with inflamed and vascularized host beds – in so called 'high-risk' transplantation where rejection rates easily surpass 50% [3]. Many of the features of the healthy cornea that promote transplant acceptance—the avascularity of the tissue, the rarity of professional antigen-presenting cells in the graft bed, and a spectrum of locally produced immunomodulatory cytokines that suppress immunogenic inflammation and complement activation [4]—are lost in high-risk transplants. The frequent rejection of high-risk grafts and the less frequent rejection of the larger pool of normal-risk grafts continues to cumulatively add to the burden of transplant rejection (see Fig. 1) as regrafting a host eye for a rejected transplant is associated with a considerably increased risk of rejection. Accordingly, a significant number of host eyes are getting grafted for a second, third, or fourth time due to the incrementally increased risk of rejection with each surgery. Indeed, repeat grafting due to previous failure has become the second leading indication for corneal transplantation as reported in the 2007 Eye Banking Statistical Report. In the aggregate, as suggested by Fig. 1, close to 30% of corneal grafts (~12,000 cases in US, and ~30,000 cases globally) are at risk of rejection annually.

The Role of Angiogenesis in Exacerbating Transplant Rejection Risk: Allograft rejection is the leading cause of corneal graft failure and thus a leading indication for repeat transplantation [5]. Importantly, stratification of risk factors for immunologic

rejection of corneal transplants has identified recipient vascularization as the leading proximal cause for earlier and more fulminant rejection episodes [3, 6]. Grafting into these vascularized high-risk beds leads to a high rejection rate even with a strict regimen of topical and systemic immunosuppressive drugs [3]. Furthermore, it has been shown that graft rejection in a previously grafted eye relates more to the number of blood vessels in the cornea than to the number of previous grafts [7]. Finally, enhanced angiogenesis into the graft bed often heralds an acute rejection as it enables amplification of the alloimmune response [8]. In summary, vascularization of the cornea is the principal clinically detectable poor prognosticator for corneal graft survival.

The exact reasons why the relative immunological quiescence of the eye, which is a central facet of its immune privileged state, is disturbed in a setting of corneal neovascularization (NV) is not fully understood [9]. However, experimental evidence strongly suggests that molecular factors such as the local immunosuppressive cytokine milieu (i.e., transforming growth factor-β, and α-melanocyte stimulating hormone) and functional attributes (i.e., anterior chamber-associated immune deviation) that play a critical role in maintaining the physiologic quiescence in the anterior segment, are subverted in the presence of corneal NV [9]. Thus, treatment of corneal NV in the setting of corneal transplantation can potentially limit both the afferent (sensitization) and efferent (rejection) arms of alloimmunity, and hence reduce the propensity for immuno-inflammatory reactions that can jeopardize graft survival [9].

The use of corticosteroids in the prophylaxis and treatment of corneal transplant rejections has represented the most significant contribution to the prolongation of corneal transplant survival over the last half century [10]. However, the local and/or systemic use of corticosteroids, or alternatively general immunosuppressants, is often associated with significant complications, such as infection, cataracts, glaucoma, and corneal thinning. Therefore, it is apparent that development of molecular strategies that can specifically target a critical ligand may prove to be an effective modality for circumventing the problems inherent in nonspecific treatments.

The Role of VEGF in Corneal NV: Vascular endothelial growth factor (VEGF) is thought to be a key mechanistic mediator of corneal NV. The prominent role of VEGF in the pathophysiology of corneal NV has been demonstrated in experimental models of corneal angiogenesis [11]. In humans, pathological studies have confirmed that VEGF and its receptors are present in higher concentrations in corneal buttons with corneal NV than in normal corneas irrespective of the cause of neovascularization [12]. Furthermore, it has been also shown that systemic inhibition of angiogenesis by neutralization of VEGF can promote corneal graft survival in animals [13]. VEGF inhibitors, including pegaptanib sodium, ranibizumab, and bevacizumab are currently used with considerable success for the treatment of neovascular age-related macular degeneration (AMD) [14].

Bevacizumab (Avastin) is a full-length, recombinant humanized monoclonal immunoglobulin-G1 that binds to and inhibits the activity of VEGF-A thereby inhibiting angiogenesis. It was the first anti-VEGF antibody to be approved by the U.S. Food and Drug Administration specifically for the treatment of metastatic colon cancer, and recently, for non-small cell lung cancer and metastatic breast cancer. Bevacizumab is also used off-label to treat choroidal neovascularization, central retinal vein occlusion, proliferative diabetic retinopathy, and iris neovascularization. Bevacizumab has now been widely adopted and is arguably part of the standard of care for the treatment of neovascular AMD for many participants [14]. Recently, there has been a growing interest in using topical as well as subconjunctival bevacizumab for the treatment of corneal angiogenic pathologies but the use of anti-VEGF strategies in clinical transplantation has not been systematically investigated to date [15-17]. The approach proposed in this application may prove both feasible and of considerable value for several reasons: 1) bevacizumab has shown significant efficacy in treatment of retinopathies and anterior eye diseases (iris neovascularization); 2) it has a good safety profile; and 3) the drug has a relatively low cost, especially for the limited amounts needed for corneal application.

Military Relevance: The significant majority of military personnel with ocular injuries have experienced trauma to the cornea and anterior segment of the eye, inducing scarring, inflammation, and, in severe cases, growth of new blood vessels (angiogenesis) into the normally clear and avascular cornea. Corneal disease constitutes the most common type of ocular pathology experienced by deployed warriors [26]. Importantly, due to changes in munitions use and survival amongst trauma victims, the proportion of surviving military personnel afflicted with serious anterior segment eye injuries has continuously escalated from WWI to WWII, Korea, Vietnam, and now Iraq and Afghanistan.

The highly variable efficacy and myriad side-effects (cataract, glaucoma, and increase risk of infection) with conventional treatments, such as corticosteroids, creates a need for more effective treatments for deployed military personnel. This is particularly the case since the final recourse for civilians and military personnel afflicted with a corneal scar is often a corneal transplant, which when done in a high-risk setting, as often occurs following ocular trauma, has a high potential for failure.

1.2 Rationale

Limited data are available regarding the safety and efficacy of bevacizumab for the prevention of corneal allograft rejection administered either topically or subconjunctivally. In clinical studies to treat corneal NV, bevacizumab has been used topically in different concentration regimens from 0.5% to 2.5% (5 to 25 mg/ml), two to four times a day for durations of 3 weeks to 6 months [15, 16, 19, 20]. In our completed study of 24 eyes of 24 participants, topical bevacizumab 1% in a dose regimen of 4 times a day for 3 weeks has shown significant effect in the treatment of clinically stable corneal NV which was evidenced by a nearly 50% reduction in neovascular area and vessel caliber [16]. None of these participants treated with the same regimen as proposed in this study showed any systemic or local adverse events. Systemic blood pressure remained stable at the baseline level, and no serious side effects occurred during the follow-up period. Similarly, from an ocular standpoint,

topical bevacizumab 1% was tolerated very well in all participants. No local irritation, allergic reaction, or surface epitheliopathy was observed. This is in contrast with a 60% rate of spontaneous loss of epithelial integrity as reported by Kim et al [15]. In their study, the investigators used topical bevacizumab at a higher concentration (1.25%) and for a much longer period (3 months), and adverse effects generally appeared during the second month of treatment. *This suggests that the duration of treatment may well determine the safety of topical bevacizumab* because prolonged blockade of VEGF may impair wound healing and the regeneration of corneal nerves [21], which may cause a loss of epithelial integrity in cornea. Given our systematic prospective analysis of the 24 eyes of 24 participants receiving topical bevacizumab in whom we have noted no local or systemic adverse events, we believe that this pharmacotherapeutic approach is safe.

Bevacizumab has also been used subconjunctivally in the treatment of corneal NV in doses of 1.25 mg/0.05 ml, 2.5 mg/0.1 ml, and 5.0 mg/0.2 ml [17]. You et al. have shown that one time subconjunctival injection of 2.5 mg or greater reduces corneal NV significantly without any serious systemic side effects. However, they reported minor localized side effects including injection site pain, subconjunctival hemorrhage and ocular irritation. In a comparative experimental study to determine the effects of topical and subconjunctival bevacizumab in a mouse model of high-risk corneal graft survival, our group has shown that both topical and subconjunctival bevacizumab treatment can diminish the severity of corneal NV [27]; however, the regression of corneal NV is more profound when treated subconjunctivally. Moreover, only subconjunctival bevacizumab treatment significantly promotes graft survival in the high-risk setting. It is likely that the limited efficacy of topical bevacizumab in corneal graft survival is due (at least in part) to a lack of adequate penetration through the corneal epithelium. Relatedly, bevacizumab is a *humanized* monoclonal antibody, and thus its potency in murine models would be significantly impaired—suggesting that the effect of bevacizumab in the clinical setting could be appreciably more than that suggested by murine data referenced from our laboratory. Given the encouraging clinical results of topical and subconjunctival bevacizumab in the treatment of

corneal NV and our experimental results in high-risk corneal transplantation, it would be reasonable to employ a mixed subconjunctival/topical bevacizumab treatment regimen as proposed herein. We thus propose that a one-time subconjunctival injection of 2.5 mg /0.1 ml bevacizumab at the conclusion of corneal transplantation surgery followed by 4 weeks of topical bevacizumab 1%, four times a day starting at post-operative day 1 following transplantation surgery would combine safety, efficacy and convenience of both routes of drug administration.

Treatments: Participants in the treatment arm will receive a one-time subconjunctival injection of 2.5 mg/0.1 ml of bevacizumab (vs. 0.9% saline [NaCl] in the control group) in the treatment eye at the conclusion of corneal transplantation surgery. Participants will initiate topical bevacizumab (1.0% solution) or vehicle (Refresh Liquigel) four times a day on post-op day-1 and will continue its application for 4 weeks.

Study Population: *Ninety eyes from 90 adults* with corneal NV who are candidates for corneal transplantation will be recruited at the following study centers. The name of the center is followed by the site's target enrollment. Target enrollment for each site is 30 subjects, however, enrollment distribution can increase or decrease by a maximum of 10 subjects in order to meet the overall enrollment goal.

- Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA: 30 subjects +/- 10 subjects
- New York Presbyterian Hospital, Weill Cornell Medical College, New York, NY: 30 subjects +/- 10 subjects
- Bascom Palmer Eye Institute, University of Miami, Miami, FL: 30 subjects +/- 10 subjects

1.3 Potential Limitations of Approach

Similar to any study, this proposal has certain limitations, and we acknowledge these and intend to incorporate our understanding of these in our analyses.

Drug bioavailability and pharmacokinetics. As described above, there is limited information on the pharmacokinetics of topical bevacizumab. However, our laboratory studies (unpublished data) indicate that a single subconjunctival injection of bevacizumab leads to appreciable drug retention by the cornea for at least 3 weeks. It is conceivable that from the standpoint of NV regression, a longer period of treatment or more frequent applications of topical bevacizumab would be more effective than the regimen proposed here. However, (i) our clinical data suggest that significant benefit can be seen after a 3-week treatment, and (ii) this has an acceptable safety profile [16].

Age disparity between donors and recipients. There is no mandatory guideline in our protocol for age matching between donors and recipients. We understand that there is some concern that tissue from older donors will be more apt to lead to failure (due to on average lower endothelial cell counts). We do not believe this will be a major issue for this study: (i) We have instituted guidelines (detailed above) that limit death to preservation time <12 hours and storage time <5 days to ensure particularly high quality tissue; (ii) participating surgeons may each have their own standards for tissue age selection but given the randomized and masked design of the study there is no reason why this would preferentially bias the results in one direction; (iii) interim data from the Cornea Donor Study Investigator Group [25] suggest that high donor age alone is not a significant predictor of graft failure.

Limited Follow-up to 1 year. This study is limited to a *minimum* follow-up of one year. It is conceivable that the intervention will have benefit, but this does not reach statistical significance within this time frame. This is possible but unlikely for the vast majority of high-risk graft rejections occur in the first year [The Australian Corneal Graft Registry 2007 Report]. If we notice that there is a beneficial effect to the treatment at 12 months, but this is of borderline statistical significance, the involved

sites will report on the subjects' clinically indicated follow-up visits in the 2nd year after surgery (as increasing the number of follow-up visits will give added statistical power) and we will report on these data in a follow-up report.

Effect of Confounders. As in any clinical study, there are a large number of potential confounders, including concurrent ocular and systemic morbidities and the heterogeneity of subjects (with variable immune reactivity) and transplant indications. While all of these factors cannot be controlled for, the randomized and masked design of the trial will lower the chances of significant confounding. Additionally, variables with known confounding effect on the primary endpoint (1st vs. regraft) will be managed through stratification of randomization as detailed above. Finally, certain variables may confound results, but these can be accounted for in our analyses. For example, an endothelial rejection episode will lead to increasing corticosteroid usage (as detailed above), and this could affect the degree of NV and other parameters. However, this does not affect the primary endpoint of interest (endothelial rejection rate) and will be considered in our analyses of secondary endpoints.

1.4 Risk/Benefits Assessment

Risks: Systemic administration of bevacizumab is reported to have a low incidence of adverse effects such as hypertension and thrombosis [23]. However, a report by the International Intravitreal Bevacizumab Safety Survey suggested that small doses of drug injected intravitreally would not produce serious systemic effects [24]. There has not been any pharmacokinetics data on use of topical and subconjunctival bevacizumab. It is noteworthy that no drug related serious systemic adverse events, definitively linking the drug to the adverse events, have been reported in any of the studies using bevacizumab for the treatment of ocular neovascularization, including for age-related macular degeneration. This issue remains somewhat controversial however and we will 'err' on the side of being too conservative—hence all the exclusion criteria listed and detailed above. It seems reasonable that a very low dose

of bevacizumab instilled over the surface of the eye or injected subconjunctivally would be safer overall than higher doses given systemically for the purpose the drug was originally approved (colorectal cancer). However, low levels of bevacizumab may reach the systemic circulation. The significance of this is not well understood. Therefore, the risk of the side effects of bevacizumab, which were described in participants taking the drug systemically for the treatment of colon or lung cancer, is unknown, but expected to be very low for the regimen proposed in this study.

The potential ocular surface side effects of bevacizumab, including local irritation, visual disturbances, allergic reaction, or surface epitheliopathy and other ocular surface toxicity responses, are yet to be fully known. In a completed study at MEEI, 24 eyes of 24 participants were treated with topical bevacizumab 1% in frequencies of two and four times a day for 3 weeks did not show any ocular surface side effects [16]. None of these participants experienced any ocular adverse events; however, in a study using topical bevacizumab at a higher dose (1.25%) for a much longer period (3 months) did report did report ocular adverse events (loss of corneal epithelial integrity) during the second month of treatment [15]. These data strongly suggest that the lower concentration of bevacizumab given for a short duration of three weeks will be safe.

Potential Benefits: Considering the encouraging results of clinical studies using subconjunctival and topical bevacizumab for the treatment of corneal NV combined with the promising results of animal studies of bevacizumab in promoting corneal graft survival in high-risk setting, some therapeutic benefits are most likely anticipated to the participating individuals. It is hoped that the information from this study will help us to bring a new treatment modality in prevention of corneal graft rejection in high-risk corneal transplantation.

2. STUDY OBJECTIVES

2.1 Primary Objective

Determine the **safety** of local (subconjunctival + topical) bevacizumab treatment in participants who have undergone high-risk corneal transplantation using the following measures:

- **Systemic safety**: Incidence and severity of systemic adverse events during the study (based on physical examination, subject self-reporting, and changes in vital sign).
- **Ocular safety**: Incidence and severity of ocular adverse events during the study (based on ophthalmic examination and subject self-reporting).

2.2 Secondary Objective(s)

Determine the efficacy of local (subconjunctival + topical) bevacizumab treatment in preventing graft rejection in subjects who have undergone high-risk corneal transplantation. This will be tested using the following outcomes measures:

- **Primary Endpoints**:
  - Twelve-month endothelial rejection rate
- **Secondary Endpoints**:
  - Time from surgery to occurrence of any rejection episode (endothelial, epithelial, subepithelial)
  - Time from surgery to overall graft failure (regardless of cause)
  - Incidence of delayed epithelial healing at Day 7
  - Endothelial cell density at Weeks 26 and 52

- Central corneal thickness at Weeks 4,16, 26, and 52
- Frequency of primary graft failure
- Corneal NV Metrics at the Weeks 4,16, 26, and 52
    - Neovascular Area (NA), measuring the area of the corneal vessels themselves
    - Vessel Caliber (VC), measuring the mean diameter of the corneal vessels
    - Invasion Area (IA), measuring the fraction of corneal area in which vessels are present

3. STUDY DESIGN

3.1 Study Design Description

This is a Phase I/II multicenter, randomized, double masked, vehicle-controlled study evaluating the safety and efficacy of locally delivered bevacizumab (in addition to standard treatment with corticosteroids) in subjects who are candidates for high-risk corneal transplantation. Investigators and subjects will be double-masked to treatment.

As detailed in the 'Statistical Methods' section below, we aim to enroll 90 subjects into this study (45 per arm). Subjects will be followed in an outpatient setting for safety and efficacy for 52 weeks (12 months) following the corneal transplant surgery. A detailed layout of subject visits and study procedures can be found in the Schedule of Events (Appendix 6).

3.2 Allocation to Treatment

Ninety subjects will be randomized according to a predetermined randomization scheme within each of the three participating centers. A stratified, blocked, randomization scheme will be determined by our trial statistician, Dr. Debra Schaumberg. Randomization will be stratified by center, as well as first graft vs. regraft. Thus there will be a total of 6 strata. Stratification will protect against chance departures between the two arms of the study. A number of blocks of varying size will be determined (e.g. 5 blocks of size 6, 6 blocks of size 4, and 7 blocks of size 3) and randomization assignments will be prepared accordingly. Since we have 6 strata, we plan to use relatively small block sizes to minimize the chance of large departures from the desired allocation ratio that could occur if some of the blocks in individual strata remained unfilled by the time of completion of participant recruitment.

Although the ability of the clinical staff to predict future treatment assignments is increased with smaller block sizes, we do not think that this will be a major issue in the present study.

Description of the Recruitment Process: This study will be conducted in accordance to the Department of Defenses (DOD) regulations for research involving human subjects. All institutions and research staff will comply with the guidelines described in Title 32 Code of Federal Regulations (CFR) Part 219, "Protection of Human Subjects", and title 45 CFR 46 Subparts B, C and D. This study will be using an investigational medication and, therefore, will abide by the Food and Drug Administration's (FDA) regulations outlined in 21 CFR Parts 50, 56, 312 and 812. In addition, all institutions involved in this study will follow the guidelines set forth in the Health Insurance Portability and Accountability Act (HIPAA), which is written in 45 CFR 160-164.

All institutions and investigators involved in this research study understand the regulations and responsibilities for human research with volunteers from the Department of the Army as described in the DoDi directive 3216.02.

Potential study participants will be identified by the study investigators at the designated study sites (Massachusetts Eye and Ear Infirmary, Boston, MA; New York Presbyterian Eye Hospital, New York, NY; Bascom Palmer Eye Institute, Miami, FL). Participants in each investigator's clinic will be pre-screened for possible eligibility in the study. A research technician, study coordinator, research fellow and/or investigator will identify participants who are considering a corneal transplant and are considered at high-risk for rejection. Once identified, the study team will evaluate whether or not the participant may be able to participate in the study based on their ophthalmic medical record and the study eligibility criteria. If no definite reasons to exclude the participant are found during the review, the participant will be approached by their physician (investigator). A recruitment script can be found in Appendix 2.

Other physicians within the institutions will be able to refer participants to the participating investigators who they anticipate may be eligible for participation. Per HIPAA regulations, participants referred to an enrolling site must contact the site themselves. Outside physicians may not refer eligible participants to the enrolling site by name.

All qualified subjects who meet the below inclusion/exclusion criteria will be invited to participate in the study. Those participants who volunteer to participate (after signing the written informed consent) will be enrolled consecutively during routine visits to the study sites. The study eye will be identified at the screening visit. If both eyes are eligible for the study, the worse eye will be selected for entry into the study. Only one eye of each participant will be enrolled into the study. An enrolled participant may re-enroll with the 2nd eye at the end of his/her study participation with the 1st eye. Volunteers will not be given a stipend for participation in the research study. Study medications and all non-standard assessments will be covered by the study funds.

This study does not exclude, or intentionally recruit, subjects based on race or ethnicity, as data does not support any significant differences between these subgroups' susceptibility to the risk of rejection or failure post transplantation [28]. The subjects in this study will therefore, most likely reflect the full range of gender and minorities seen at the participating hospitals which is quite broad given that they are in urban centers.

A sample recruitment script can be found in Appendix 2.

3.2.1 Randomization Procedures

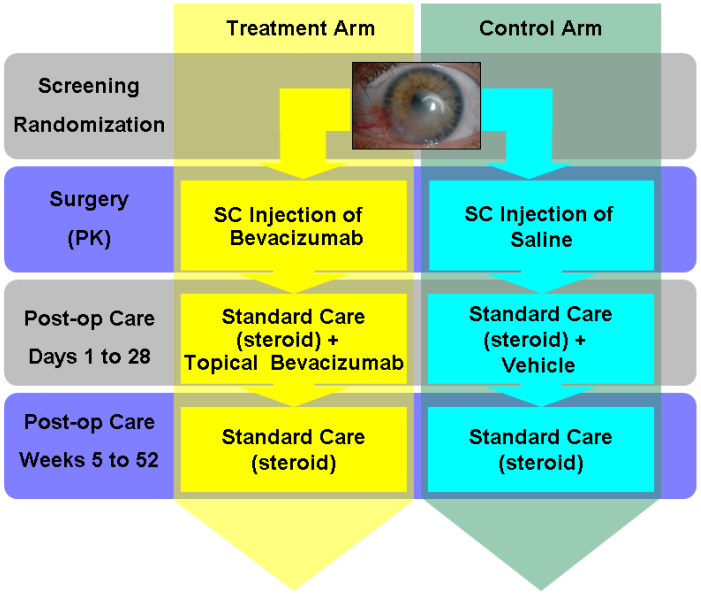
Assignment sheets for randomization will be prepared by the statistician and maintained by MEEI study staff. When a participant is enrolled into the study, the Coordinator at each site will notify MEEI and obtain a masked randomization assignment, which will be communicated to the Mass. Eye and Ear's Pharmacist, who will be in charge of preparing the appropriate medication (active or vehicle) and dispensing to all sites. Codes will be used to indicate different treatment assignments

so that the Site Coordinator and Investigators will remain masked to the subjects' assignment.

The statistician will use each randomization assignment in order according to a set of predetermined rules. Briefly, allocation of active and vehicle treatments will be determined in 4 steps: 1) arrange the initial treatment assignments arbitrarily, 2) generate via computer a list of pseudorandom numbers, 3) link the initial treatment codes with the pseudorandom numbers, 4) order the pseudorandom numbers (e.g. smallest to largest) with associated treatment codes to determine the final order of treatment assignments. Codes will be used to indicate different treatment assignments so that the participants and study investigators will remain masked to the subjects' assignment. For the first participant enrolled, the statistician will mark the first sheet according to the stratum of this participant (center and first vs. regraft), and this participant's ID number will be entered on the line next to the first treatment assignment. Each consecutive participant who falls into the same stratum will be assigned from the next treatment assignment on this sheet until all assignments have been used.

Whenever a participant is enrolled who falls into a stratum for which no sheets have been started or for which all previous sheets have been completed, the next sheet will be used and marked according to the appropriate stratum, and so on.

Figure 2. Randomization scheme and treatment



The examining ophthalmologist will not be involved in allocating the randomization assignments. The Mass. Eye and Ear pharmacist will provide the masked treatment packages to the site study staff to distribute to the study participant. Thus,

it is extremely unlikely that the clinical staff would be able to alter the treatment assignments in such a way as to bias the outcome of the study. Subjects will be randomized to bevacizumab or vehicle with 1:1 allocation at the baseline visit (Screening Visit) (Fig. 2) provided they have satisfied all entry criteria.

3.2.2 Masking Procedures

Identifiers & Masking: Ninety subjects will be randomized according to a predetermined randomization scheme within each of the three participating centers. A stratified, blocked, randomization scheme will be determined by trial epidemiologist and statistician Dr. Debra Schaumberg. Randomization will be stratified by center, as well as first graft vs. regraft. Thus there will be 6 strata. Stratification will protect against chance departures between the two arms of the study. A number of blocks of varying size will be determined (e.g. 5 blocks of size 6, 6 blocks of size 4, and 7 blocks of size 3) and randomization assignments will be prepared accordingly. Since we have 6 strata, we plan to use relatively small block sizes to minimize the chance of large departures from the desired allocation ratio that could occur if some of the blocks in individual strata remained unfilled by the time of completion of participant recruitment. Although the ability of the clinical staff to predict future treatment assignments is increased with smaller block sizes, we do not think that this will be a major issue in the present study.

Assignment sheets for randomization will be prepared by the statistician and maintained by MEEI study staff. When a participant is enrolled into the study, the Coordinator at each site will notify MEEI and obtain a masked randomization assignment, which will be communicated to the Mass. Eye and Ear's Pharmacist, who will be in charge of preparing the appropriate medication (active or vehicle) and dispensing to all sites. Codes will be used to indicate different treatment assignments so that the Site Coordinator and Investigators will remain masked to the subjects' assignment.

Each clinical site will be given a unique study site number. When a participant is enrolled, the study site will then assign the participant a study identification number (ID) prefaced with the study site number and completed with the subject ID, which is assigned sequentially starting from 01. All sites' first subject ID will end with 01 (e.g., the first subject enrolled at MEEI will have subject ID 101 because MEEI's study site ID is 1).

If a participant signs a consent form and does not complete surgery within 90 days, he/she will be considered lost to follow-up. If the participant reschedules surgery and elects to participate, he/she must re-screen and sign a new consent form. The participant may use the study ID number and randomization code assigned in the first screening visit.

The statistician will use each randomization assignment in order according to a set of predetermined rules. Briefly, allocation of active and vehicle treatments will be determined in 4 steps: 1) arrange the initial treatment assignments arbitrarily, 2) generate via computer a list of pseudorandom numbers, 3) link the initial treatment codes with the pseudorandom numbers, 4) order the pseudorandom numbers (e.g. smallest to largest) with associated treatment codes to determine the final order of treatment assignments. Codes will be used to indicate different treatment assignments so that the participants and study investigators will remain masked to the subjects' assignment. For the first participant enrolled, the statistician will mark the first sheet according to the stratum of this participant (center and first vs. regraft), and this participant's ID number will be entered on the line next to the first treatment assignment. Each consecutive participant who falls into the same stratum will be assigned from the next treatment assignment on this sheet until all assignments have been used. Whenever a participant is enrolled who falls into a stratum for which no sheets have been started or for which all previous sheets have been completed, the next sheet will be used and marked according to the appropriate stratum, and so on.

3.2.3 Breaking the Mask

The study drugs (subconjunctival and drops) will be labeled by the pharmacy with a unique identifier provided by the statistician, instructions for storage, the name of the study, an expiration date, and a notice that the bottle may contain active or vehicle solution for investigational use. The pharmacy will maintain a master list of the unique identifier, the coded treatment assignment, and participant ID. The "key" for the coded treatment assignments will also be provided to the pharmacy by the statistician, but the key will be kept separate from the master list to guard against the chance that the participant, coordinator, or other study staff will inadvertently become unmasked to the participant's treatment assignment. However, a participant's medication may be unmasked at any point for a medical emergency or to supplement a serious adverse event (SAE) report.

4. SUBJECT SELECTION

4.1 Subject Inclusion Criteria

The following eligibility criteria are designed to select subjects for whom the protocol treatment is considered appropriate. The criteria must be met at the screening visit. All relevant medical and non-medical conditions will be taken into consideration when deciding whether this protocol is suitable for a particular subject. Subjects who have provided informed consent will be screened using the following inclusion and exclusion criteria.

A. Inclusion Criteria:

- Age > 18 years
- Participant willing and able to provide written informed consent
- Willing and able to comply with study assessments for the full duration of the study
- High-risk characteristics for penetrating keratoplasty:
- Presence of corneal NV in one or more quadrants (≥ 3 clock hours NV ≥ 2mm from the limbus) OR
- Extension of corneal NV to graft–host junction in a previous failed graft
- In generally good stable overall health

4.2 Subject Exclusion Criteria

- History of Stevens-Johnson syndrome or ocular pemphigoid
- Ocular or periocular malignancy
- Non-healing epithelial defect of at least 0.5x0.5 mm in host corneal bed lasting ≥6 weeks preoperatively

- Uncontrolled glaucoma
- Currently on dialysis
- Has received treatment with anti-VEGF agents (intraocular or systemic) within 45 days of study entry
- Concurrent use of systemic anti-VEGF agents
- Change in topical corticosteroid regimen within 14 days of transplantation
- Use of systemic immunosuppressive for indication other than corneal graft rejection
- Pregnancy (positive pregnancy test) or lactating
- Pre-menopausal women not using adequate contraception (Reliable intrauterine devices, hormonal contraception or a spermicide in combination with a barrier method)
- Uncontrolled hypertension defined as systolic blood pressure (BP) ≥150 or diastolic BP ≥90 mmHg
- History of thromboembolic event within 12 months prior to study entry
- Participation in another simultaneous medical investigation or trial

5. STUDY DRUG

5.1 Study Drug Information

Group 1: ***Active Treatment***

Subconjunctival injection of bevacizumab 2.5mg/0.1ml

Topical bevacizumab (1.0% solution) prepared with Refresh Liquigel

Group 2: ***Control Treatment***

Subconjunctival injection of 0.9% saline [NaCl]

Topical Refresh Liquigel

Bevacizumab (Avastin) is a full-length, recombinant humanized monoclonal immunoglobulin-G1 that binds to and inhibits the activity of VEGF-A thereby inhibiting angiogenesis. It was the first anti-VEGF antibody to be approved by the U.S. Food and Drug Administration specifically for the treatment of metastatic colon cancer, and recently, for non-small cell lung cancer and metastatic breast cancer. Bevacizumab is also used off-label to treat choroidal neovascularization, central retinal vein occlusion, proliferative diabetic retinopathy, and iris neovascularization. Bevacizumab has now been widely adopted and is arguably part of the standard of care for the treatment of neovascular AMD for many participants [14]. Recently, there has been a growing interest in using topical as well as subconjunctival bevacizumab for the treatment of corneal angiogenic pathologies but the use of anti-VEGF strategies in clinical transplantation has not been systematically investigated to date [15-17]. The approach proposed in this application may prove both feasible and of

considerable value for several reasons: 1) bevacizumab has shown significant efficacy in treatment of retinopathies and anterior eye diseases (iris neovascularization); 2) it has a good safety profile; and 3) the drug has a relatively low cost, especially for the limited amounts needed for corneal application.

In this study, participants are randomized to Avastin or placebo and receive either receive either:

- AVASTIN 2.5mg/0.1ml Subconjunctival Injection and AVASTIN 1% Study Ophthalmic Solution
- Sodium Chloride 0.1ml Subconjunctival Injection and Refresh Liquigel Study Ophthalmic Solution

Injection of Bevacizumab or Placebo occurs once at the conclusion of surgery. Administration of Avastin or placebo topical drops is four times per day for four weeks.

5.2 Study Drug/Device Compliance/Adherence

During participant follow-up visits at Day 7 and Week 4, participants will be queried on compliance of study and concomitant medications. The decision to withdraw a participant due to non-compliance or adherence to study medications, standard-of-care medications, or study visits will be made by the principal investigator, Dr. Reza Dana.

If withdrawn, participants will not be replaced. They will be asked to return for the follow-up care of the corneal transplantation.

5.3 Study Drug Supplies

- **Formulation and Packaging**

  Subconjunctival bevacizumab will be aseptically prepared from commercially available intravenous bevacizumab by the hospital pharmacy. One tenth of milliliter containing 2.5 mg of bevacizumab will be transferred into a sterile insulin syringe and refrigerated at 2˚C - 8˚C (36˚F - 46˚F) in an amber colored (light-protected) plastic bag till the time of use. One tenth of 1mL of normal saline, aseptically transferred into a sterile insulin syringe, will be used as the subconjunctival 'placebo' control injection.

  Topical bevacizumab solution will be formulated and aseptically prepared from commercially available intravenous bevacizumab (Avastin; Genentech Inc., San Francisco, CA), and transferred into a sterile, light-protected dropper container by the hospital pharmacy. A formulation of 1.0% (10 mg/ml) concentration of bevacizumab with 1% (10 mg/ml) Refresh Liquigel (Allergan, Irvine, CA) will be used. The participants will be instructed to only refrigerate the study drugs. The participants will be instructed to never freeze the study medication.

- **Preparing and Dispensing**

  The recipes for the treatment formulations have been written by the Massachusetts Eye and Ear Infirmary Clinical Pharmacy. The Mass. Eye and Ear pharmacy will prepare, label and distribute the study medication to all enrolling sites.
  All study medications (topical and subconjunctival injection of Avastin or placebo) for participants at NY Presbyterian (Cornell) and Bascom Palmer (U. Miami) will be shipped to the non-Mass. Eye and Ear sites via overnight Federal Express for arrival prior to surgery. The medications will be packaged and sent in the appropriate packaging to ensure that the medication is kept cool during transportation.

- **Administration**

  Day 0: At the conclusion of surgery, 0.1 mL (2.5 mg) of bevacizumab or vehicle (0.9% NaCl) will be injected subconjunctivally at the 12-o'clock position 1 mm posterior to the limbus.

  Day 1: In addition to standard treatment with corticosteroids, participants will receive topical bevacizumab (1% solution) or vehicle (Refresh Liquigel), four times a day (every 4 hours while awake) for 4 weeks starting on Day 1 post-transplant surgery. Subjects will self-administer study treatment topically in the study eye. Participants will be instructed to stop using the study treatment by the end of week 4, and return all unopened or partially-filled drug bottles to the study site. Any empty bottles may be disposed of by the participant.

  Participants will be given a study medication instruction sheet that will specify which date to start and stop the medication. The sheet will also provide instructions on storing the medication.

  During treatment period: If a subject contracts infectious conjunctivitis, they will be instructed to discard the remainder of the study drug and new drug will be sent from the host site pharmacy, Massachusetts Eye and Ear Infirmary.

5.4 Study Drug/Device Storage and Accountability

- Participants will be asked to protect the study medication from light and will be instructed to refrigerate the study drugs. At the conclusion of the 4-week treatment period, participants will be asked to return any remaining study medication to be destroyed per pharmacy regulations.

5.5 Other Medications

- **Corticosteroid**

  All participants will receive topical 1% prednisolone acetate drops six times a day (every 2-3 hours while awake) for the first 2 weeks after surgery. The frequency of administration of topical steroids will be gradually tapered as follows: 4x/day for 6 weeks, 3x/day for 4 weeks, 2x/day for 6 months, and then once a day indefinitely, unless intervening complications (e.g. acute rejection) require that the regimen be altered (see section 7.3, 'Corneal Graft Rejection'). In addition to standard treatment with corticosteroids, *participants will receive topical bevacizumab (1% solution) or vehicle (Refresh Liquigel), four times a day (every 4 hours while awake) for 4 weeks starting on Day 1 post-transplant surgery*.

- **Antibiotic**

  All participants will receive an antibiotic eye drop four times a day until full graft epithelialization, or as necessary with regard to each participant's needs. Use of bandage contact lens is allowed if necessary. Selective suture removal will be initiated as early as 6 months after the surgery unless the sutures get loose or broken earlier. All subjects will be instructed in the signs and symptoms of rejection (decrease in vision; redness, pain or irritation persisting for more than a few hours; painful sensitivity to strong light) and urged to contact their ophthalmologist immediately should they develop such symptoms.

- **Considerations with Concomitant Medications**

  The use of long-acting forms of corticosteroids (e.g., triamcinolone) injected subconjunctivally at the end of corneal transplant surgery is prohibited (subconjunctival injection of rapid-acting steroids such as dexamethasone at the end of surgery is allowed) in this protocol. Use of systemic

immunosuppressant medications is prohibited throughout the study, except oral steroids which are allowed for treatment of corneal graft rejection episodes as stated above. Concomitant medications other than these prohibited agents are permitted as necessary. Subjects using topical steroid preparations prior to transplantation must be on a stable regimen (i.e., neither intensified nor augmented) for 14 days prior to surgery.

Subjects who develop elevated intraocular pressure secondary to steroids should be managed, if possible, by initiating anti-hypertensive therapy as per standard 'glaucoma' care regimens. If this proves inadequate and the intraocular pressure management becomes difficult, the dose (frequency and potency) of the topical corticosteroid will be reduced, especially if there is a history of steroid-responsiveness. If maximally tolerated medical therapy does not sufficiently control intraocular pressure, alternative approaches to intraocular pressure control should be considered. Elective surgery post transplantation (e.g., for cataract removal) should be deferred if possible until after the 12-month follow-up period has expired.

Therapy with steroids can be altered at the discretion of the investigator for treatment of graft rejection, as described in section 7.3, 'Treatment of Corneal Graft Rejection'.

6. BIOSPECIMEN COLLECTION

No biospecimens will be collected for this study.

7. STUDY PROCEDURES

7.1 Screening Procedures

Prospective participants, as defined by the inclusion/exclusion criteria, will be considered for entry into this study. The study design and treatment regimen will be discussed with each participant. Written informed consent will be obtained before any study-specific screening evaluations are performed. Screening evaluations will be performed within ninety (90) days preceding Day 0 (Surgery Day). The following evaluations and procedures will be performed for all subjects during the screening period:

- Written informed consent
- Record current ocular and systemic medications
- Record significant medical/surgical history in the past 5 years
- Record demographic data, including date of birth, sex, and race/ethnicity
- Review of systems
- Measurement of systolic and diastolic blood pressures
- Urine pregnancy test for women of childbearing potential, if appropriate
- Ocular assessments (OU)
- Best spectacle-corrected visual acuity (BSCVA) (OU)
- Slit-lamp examination (OU)
- Intraocular pressure (OU)
- Dilated funduscopy (Study eye)
- Corneal photography (Study eye)

- Pachymetry (OU)
- Classification of depth of blood vessels (superficial/anterior-, mid-, or deep-stromal)* (OU)

(* if at multiple depths will classify at depth where preponderance of neovessels reside)

Once a participant is deemed eligible and enrolled into the study they will be given a unique study identification number. The Mass. Eye and Ear Pharmacy will assign the treatment code that corresponds with the unique Study ID based on a pre-established randomization scheme created by the trial statistician. Each participant will be randomized to one of two randomization groups in equal allocations.

7.2 Enrollment/Baseline Procedures

A list of baseline procedures can be found in the Schedule of Events.

Description of the Informed Consent Process: Subjects are required to sign an informed consent and Health Insurance Portability and Accountability Act (HIPAA) before participating in the study.

Given that the Investigators, Research Fellows and Study Coordinators are responsible for maintaining a detailed knowledge of the study protocol, safety profile and previous work with the medication, these individuals will discuss the protocol with participants and obtain informed consent. The study team will review the study procedures, visit schedule, risk and benefits, alternative treatments and financial responsibilities with all potential subjects. Each participant will be informed of their right to withdraw at any time from the study without affecting their care or relationship with the treating physician and participating institution. A study member will also explain and discuss with the participant their confidentiality rights as described in the HIPAA form. Consent will be obtained from each participant prior to

any study procedures being performed by the Investigator, Research Fellow or Study Coordinator within the clinic.

A note will be made on the study record that the informed consent was signed by the participant. The informed consent will follow the guidelines set forth by the FDA and relevant site's IRB. A copy of the consent form will be given to the participant.

We do not anticipate a need to obtaining on-going consent or re-assessing capacity over the course of the study. No minors will be eligible to participate.

7.3 Study Drug or Device Procedures

Surgery Day (Day 0): Transplant surgery (penetrating keratoplasty) may occur up to ninety days after the screening visit. If available, study staff will document endothelial cell count of donor tissue.

Penetrating Keratoplasty Procedure: Participants will undergo penetrating keratoplasty under general or retrobulbar/peribulbar anesthesia. Tissue matching for HLA or ABO will not be performed in this study. The donor cornea will be trephined from the endothelial surface. Trephine size will be selected according to recipient corneal size (range 7.0–8.0 mm), and the donor–recipient disparity will be 0.25–0.50 mm. 10-0 nylon suture material will be used. The technique of suturing will be interrupted since the risk of suture-related complications with running sutures is higher in vascularized host beds. The donor endothelium will be protected at all times by maintaining a formed anterior chamber with viscoelastic substances. At the conclusion of surgery, 0.1 mL (2.5 mg) of bevacizumab or vehicle (0.9% NaCl) will be injected subconjunctivally at the 12-o'clock position 1 mm posterior to the limbus. Steroid and antibiotic will be injected subconjunctivally at the 6-o'clock position. Penetrating keratoplasty may be combined with other procedures such as cataract extraction, intraocular lens implantation, and vitrectomy procedures as necessary with regard to each participant's needs.

Postoperative Care: All participants will receive topical 1% prednisolone acetate drops six times a day (every 2-3 hours while awake) for the first 2 weeks after surgery. The frequency of administration will be gradually tapered as follows: 4x/day for 6 weeks, 3x/day for 4 weeks, 2x/day for 6 months, and then once a day indefinitely, unless intervening complications (e.g. acute rejection) require that the regimen be altered (see below, 'Corneal Graft Rejection' section). In addition to standard treatment with corticosteroids, participants will receive topical bevacizumab (1% solution) or vehicle (Refresh Liquigel), four times a day (every 4 hours while awake) for 4 weeks starting on Day 1 post-transplant surgery. Subjects will self-administer study treatment topically in the study eye. Participants will be instructed to stop using the study treatment by the end of week 4, and return all unopened or partially-filled drug bottles to the study site. Any empty bottles may be disposed of by the participant. All participants will receive an antibiotic eye drop four times a day until full graft epithelialization, or as necessary with regard to each participant's needs. Use of bandage contact lens is allowed if necessary. Selective suture removal will be initiated as early as 6 months after the surgery unless the sutures get loose or broken earlier. All subjects will be instructed in the signs and symptoms of rejection (decrease in vision; redness, pain or irritation persisting for more than a few hours; painful sensitivity to strong light) and urged to contact their ophthalmologist immediately should they develop such symptoms.

Corneal Graft Rejection. Corneal allograft rejection will be diagnosed and classified according to its clinical manifestations as endothelial rejection ("definite", "probable", and "possible"), epithelial rejection, subepithelial infiltrates, or any combination thereof [18]:

- The diagnosis of 'definite' endothelial rejection will be made when an eye that has had a clear graft for at least 2 weeks shows the onset of inflammatory reaction in the anterior chamber (anterior chamber cells or

keratic precipitates) as well as both stromal edema and an endothelial (Khodadoust) rejection line.

- The diagnosis of 'probable' endothelial rejection will be made when an eye satisfies the diagnosis of 'definite' rejection but *does not have an endothelial rejection line.* 'Probable' rejection may alternatively be diagnosed by the presence of keratic precipitates on the graft endothelium *without* stromal edema.
- 'Possible' endothelial rejection will be defined as acute graft edema without obvious signs of anterior chamber inflammation in a graft that had previously been clear for 2 weeks.
- Epithelial rejection will be diagnosed by the presence of an elevated epithelial line that stains with fluorescein.
- Subepithelial infiltrates will be diagnosed by the appearance in the anterior stroma of grey-white deposits, 0.2 to 0.5 mm in diameter, randomly distributed throughout the graft.

Treatments associated with these clinical manifestations are part of a patient's clinical care, not a research procedure.

Treatment of Corneal Graft Rejection.

All cases of endothelial rejection will be treated with topical 1% prednisolone acetate drops applied hourly when the participant is awake. In the setting of 'definite' or 'probable' endothelial rejection, oral corticosteroids will be added if the endothelial rejection presents with extensive (involving >one-half the en-face surface area) *and* significant (>20% relative increase in corneal thickness compared to baseline) graft edema. These participants will need several additional visits, and corticosteroid therapy will be tailored according to the clinical findings at the time. Epithelial rejection and subepithelial infiltrates will be treated with topical 1% prednisolone acetate drops every 2 hours while participants are awake. These participants will be

reexamined within 1 week, and the corticosteroid drops will be tapered over ensuing weeks.

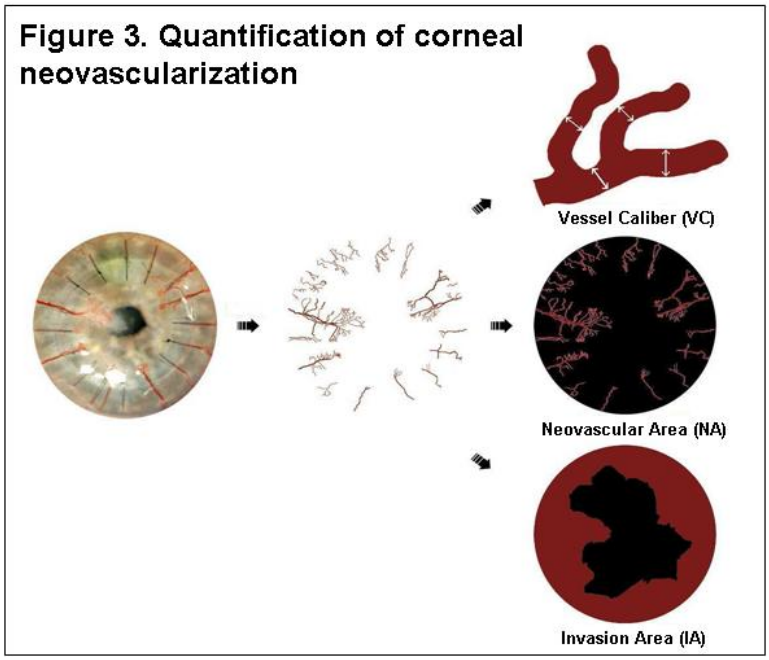
Subjects who experience an endothelial rejection episode have reached the primary study endpoint, but will continue to be followed for other (secondary) endpoints through Week 52. The assessment of subjects being treated for graft rejection at times outside of the prescribed set protocol will be in accord with week 26 endpoints' assessment.

A flow diagram of Classification and Treatment of Graft Rejection can be found in Appendix 4.

Corneal Photography. Prior to corneal transplant surgery at the screening visit, and at Weeks 4, 16, 26, and 52, digital corneal photography will be done using a slit-lamp with a digital camera attachment and a flash-through-the-slit illumination system. By fine focusing on the area of the cornea where NV predominates, the entire cornea will be pictured using diffuse illumination and 10x magnification. If lids are drooping, the photographer will attempt to gently remove them from area of focus with a cotton swab. If the lids occlude the cornea, a lid speculum may be used to facilitate whole-cornea photography. Participants with dark irises, which may prohibit a clear image of the blood vessels, will be dilated with tropicamide only (without use of an adrenergic agent that may induce vascular 'blanching') at each visit. Upon dilation, photos will be taken with retro illumination of the cornea. Further guidance on imaging can be found in the photography protocol (Appendix 5).

Immediately after a study visit, captured image files will be transmitted in a masked fashion (containing only the participant study identification number and visit number) by each site to StudyTrax. Mass. Eye and Ear will serve as the central image repository and analyzing site. The assigned Clinical Research Fellow, trained and experienced in NV quantification, will perform this task. The investigator who performs

the quantification will be masked to the treatment regimen.



Quantification of Corneal NV. Digital slit-lamp corneal pictures will be analyzed using graphics editing software (Photoshop) and a mathematical program (Matlab script). After the total corneal area is delineated, the blood vessels are isolated using Photoshop software. To allow for precise quantification of corneal NV, three metrics will be computed using a Matlab script: Neovascular Area (NA), which measures the area of the corneal vessels themselves; Vessel Caliber (VC), which determines an approximate mean diameter of the corneal vessels; Invasion Area (IA), which measures the fraction of corneal area in which vessels are present [16] (**Fig. 3**).

The investigator will assess the depth of blood vessels during the slit lamp examination and note whether the vessels are considered predominantly superficial/anterior-stromal, mid-stromal or deep-stromal.

Endothelial Cell Density. Endothelial cell density will be assessed at week-26 and week-52 visits via confocal/specular microscopy at each center based on the preferences of the site. All measurements on a given subject and at each site will be made utilizing the same technique throughout the study.

Corneal Thickness. Central corneal Pachymetry will be performed on the study eye at Week-4, 16, 26 and 52 visits using an ultrasound pachymeter. Three readings of central corneal thickness will be obtained at each of these visits.

Both Endothelial cell density and corneal thickness will serve as secondary endpoints. In general, endothelial loss and corneal graft edema are both significantly associated

with graft failure. Tracking these metrics will assist in evaluating the potential graft-protective function of local VEGF blockade.

7.4 Standard of Care Procedures (procedures regardless of study participation)

- Record current ocular and systemic medications
- Record significant medical/surgical history in the past 5 years
- Record demographic data, including date of birth, sex, and race/ethnicity
- Review of systems
- Ocular assessments (OU)
- Best spectacle-corrected visual acuity (BSCVA) (OU)
- Slit-lamp examination (OU)
- Intraocular pressure (OU)
- Dilated funduscopy (Study eye)
- Pachymetry (OU)

7.5 Follow-up Procedures

To determine the safety and efficacy of subconjunctival/topical bevacizumab in promoting graft survival, follow-up visits will be scheduled at Day 1 (≤90 days from screening) and 7 (±6 days), Week 4 (±10 days), 8 (±2 weeks), 16 (±2 weeks), 26 (±2 weeks), 39 (±2 weeks), and 52 (±2 weeks).

A list of follow-up procedures can be found in Appendix 1 and 6.

7.6 Unscheduled Visits

Unscheduled visits have also been built into the study protocol to ensure that participant's care is not limited by the investigational medication or study events. In the event of an unscheduled visit, participants can be seen by the investigator, fellow, or resident and imaging is not required.

7.7 Early Termination

Subjects who voluntarily withdraw from the study prior to completion will be asked to return for an early termination evaluation in 7 days (±3 days) for monitoring of all adverse events. The schedule of assessments for early termination is the same as that for the final visit (Week 52).

7.8 Schedule of Activities

A Schedule of Events can be found in Appendix 6.

8. SAFETY AND EFFECTIVENESS ASSESSMENTS

8.1 Safety Assessments

The safety of the study medication will be evaluated at every visit following surgery and will be defined by the incidence of related adverse events. Specifically, we will evaluate:

- **Systemic safety**: Incidence and severity of systemic adverse events during the study (based on physical examination, subject self-reporting, and changes in vital sign).
- **Ocular safety**: Incidence and severity of ocular adverse events during the study (based on ophthalmic examination and subject self-reporting).

All adverse events will be reviewed by the principal investigator Reza Dana within 24 hours of occurrence and reported on the following schedule:

- Possibly, Probably, or Definitely Related **Expected AE** – Report to IRB on annual basis
- Possibly, Probably, or Definitely Related **Expected Serious AE** – Report to IRB on annual basis
- Possibly, Probably, or Definitely Related **Unexpected AE** – Report to IRB within 30 days of event
- Possibly, Probably, or Definitely Related **Unanticipated Problem** – Report to IRB within 7 days of event (24 hours for death of data loss)
- Possibly, Probably, or Definitely Related **Unexpected Serious AE** – Report to IRB within 7 days of event
- Specify the frequency of safety observation (a number of times, at defined time points, after a certain number of subjects have been recruited or as needed).

8.2 Effectiveness Assessments

The effectiveness of bevacizumab will be judged using the following parameters at specified time points:

- **Primary Endpoints**:
  - Twelve-month endothelial rejection rate
- **Secondary Endpoints**:
  - Time from surgery to occurrence of any rejection episode (endothelial, epithelial, subepithelial)
  - Time from surgery to overall graft failure (regardless of cause)
  - Incidence of delayed epithelial healing at Day 7
  - Endothelial cell density at Weeks 26 and 52
    - As measured by confocal/specular microscopy
  - Central corneal thickness at Screening, Weeks 4,16, 26, and 52
  - Frequency of primary graft failure
  - Corneal NV Metrics at Screening, Weeks 4,16, 26, and 52
    - Neovascular Area (NA), measuring the area of the corneal vessels themselves
    - Vessel Caliber (VC), measuring the mean diameter of the corneal vessels
    - Invasion Area (IA), measuring the fraction of corneal area in which vessels are present

9. ADVERSE EVENT RECORDING AND REPORTING

9.1 Recording Requirements

Investigators and study staff will be asked to report unanticipated problems or adverse events immediately to Dr. Dana to allow for proper reporting. Expected adverse events for the study drug injection during surgery include ocular pain/discomfort, bleeding/bruising beneath the injection site (conjunctiva), and globe penetration. Expected adverse events for the study drug treatment period include corneal thinning, epithelial defect/surface epitheliopathy, delayed epithelial healing, ocular burning upon instillation of medication, hypertension, and symptoms related to allergic reaction (e.g, rash, difficulty swallowing). All expected adverse events directly related to the standard of care surgery (e.g., ocular infection, loss of eye, photophobia, increased tearing) are listed in the surgical consent form and reviewed with the subject by the treating physician.

All participants are informed of the anticipated risks of the trial and asked to report any unusual ocular or systemic problems that occur during their participation in this trial to the investigator or cornea research staff. In addition, all participants will be queried during their visits for occurrence of side effects from the medications, post-surgical complications, and overall well-being. Any event mentioned by the participant will be documented in the CRF.

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects' case histories (source data, case report form). For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the

event should be classified as a serious adverse event) and; 2) an assessment of the causal relationship between the adverse event and the study drug(s) or device(s).

Adverse events or abnormal test findings felt to be associated with the study drug(s) will be followed until the event (or its sequel) or the abnormal test finding resolves or stabilizes at a level acceptable to the Sponsor-Investigator.

Study staff will alert the treating physician immediately of any participant complaints or problems that are expressed to them. Dr. Dana will be made aware of any unexpected problems that have occurred within 24 hours if considered to be an unexpected adverse event or serious adverse event, regardless of the assumed relation to the study medication, Dr. Dana and the study staff will follow the reporting guidelines below.

Recording and Reporting Adverse events to HSC

The study team at NYPH and BPEI and investigators at MEEI will be given the phone number and e-mail address of both Dr. Dana and the Cornea Research staff. If the event is serious or requires immediate attention Dr. Dana will be called on his cell phone or paged. If Dr. Dana is not available, the fellow or attending on call will be paged. All enrolling sites have access to an Emergency Room specializing in ophthalmic care that can be used, as well, 24 hours a day, 365 days a year.

Adverse events and unanticipated problems will be reported to the DOD and IRB as detailed below.

Summary of HSC Reporting Requirements:

Reporting Schedule	Non-Serious AE		Serious AE		Unanticipated Problem
	Expected	Unexpected	Expected	Unexpected	7 Days (24 hours for death or data loss)
Possibly, probably or definitely related	Continuing Review	30 Day	Continuing Review	7 Days	

*Any unexpected and study-related death must be reported to MEEI IRB within 24 hours of the PI's knowledge of the event by e-mail or telephone.

Recording and Reporting Adverse Events

For each subject, AEs and SAEs occurring after informed consent is obtained will be recorded until the subject has completed his/her participation in the study.

Adverse Event recording will be done in a concise manner using standard, acceptable medical terms that reflects the reason for the procedure or the diagnosis based on the abnormal measurement.

Any serious adverse event that is ongoing when a subject completes his/her participation in the Study will be followed until any of the following occurs:

- the event resolves or stabilizes;
- the event returns to baseline condition or value (if a baseline value is available);

- the event can be attributed to agents(s) other than the Study Product, or to factors unrelated to Study conduct.

Any subsequent AE felt by the Investigator to be causally related to the use of the Study Product will be reported.

Abnormal Test Findings

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy
- Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study
- The test finding is considered an adverse event by the Sponsor-Investigator of the IND application

Causality and Severity Assessment

The Sponsor-Investigator of the IND application will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the Sponsor-Investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s)", the adverse event will be classified as

associated with the use of the study drug(s) for reporting purposes. If the Sponsor-Investigator's final determination of causality is "unknown but not related to the study drug(s)", this determination and the rationale for the determination will be documented in the respective subject's case history (source data or case report form).

9.2 REPORTING PROCEDURES

Reporting of Adverse Events to FDA

1. Adverse events will be sent to the FDA per the CFR guidelines:
   - Non-serious adverse events will be submitted at time of the annual report.
   - Serious and unexpected adverse events related to the drug will be reported within 15 calendar days.
   - Unexpected fatal or life-threatening adverse events related to the drug will be reported within 7 calendar days.

   New animal findings that suggest significant risk in human subjects will be reported in 15 calendar days.
2. Telephoned IND Safety Reports – Fatal or life-threatening suspected adverse reactions

Reporting Adverse Events to Other External Entities

Adverse events will be reported to the DOD on a quarterly basis.

9.3 Withdrawal of Subjects due to Adverse Events

All potential systemic and ocular adverse events will be assessed carefully at follow-up visits in the study. The safety of subconjunctival and topical bevacizumab will be assessed through the collection and analysis of any systemic or ocular adverse events during the study based on subject self-reporting, physical and ophthalmic examination, and changes in vital sign (blood pressure). All participants will be asked

to return to clinic as standard for participants undergoing a penetrating keratoplasty. In addition, all standard of care post-operative medications are being maintained for the study. Unscheduled visits have also been built into the study protocol to ensure that participant's care is not limited by the investigational medication or study events. In the event of an unscheduled visit, participants can be seen by the investigator, fellow, or resident and imaging is not required.

Stopping criteria: development of non-healing epithelial defect of ≥ 2 x 2 mm in the graft lasting ≥2 weeks while on investigational treatment.

Emergency care is available at all participating institutions in their emergency wards, which are staffed by ophthalmologists 24 hours per day, 365 days per year. Additionally, cornea specialists are on call at all times and can be reached by cell phone or pager to attend to any problem that may arise for these participants. Emergency care will be billed to the study, participant or third party payer as appropriate.

Special precautions to be taken by the human subjects prior to and during participation in this study include: 45 day washout of anti-VEGF agents (intraocular or systemic), no change in topical corticosteroid regimen for 14 days prior to transplantation, 12 months or greater of no thromboembolic event, control of diabetes, and pregnancy prevention.

Participants will be dilated at the screening, week 16 and week 52 visits. It is not recommended that participants drive while their eyes are dilated and are encouraged to find alternate transportation. Following the surgical procedure, participants will not be allowed to leave their hospital without being accompanied by another adult.

All participants who experience an adverse event will be asked to complete all 52 weeks of the study, regardless of severity or if the participant has been asked to discontinue use of the study medication. Subjects will not be replaced if withdrawn.

The Severity of each adverse event will be defined by Mass. Eye and Ear criteria:

Adverse Event (AE): Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

AE Related to Research: There is a reasonable possibility that the adverse event, incident, experience or outcome may have been caused by the research procedures, e.g. blood draw, interview, survey, etc.

AE Unrelated to Research: Adverse events related to circumstances independent of the research, e.g. relating to the underlying disease, disorder, or condition of the subject.

Expected Adverse Event: Any adverse event that does not meet the definition of unexpected adverse event.

External Adverse Events (Safety Reports): In the context of multi-center studies, adverse events experienced by research participants enrolled at study sites that are not under MEEI HSC's jurisdiction.

Internal Adverse Events: Adverse events experienced by research participants enrolled at study sites that are under MEEI HSC's jurisdiction. In the context of a single-center study, all adverse events would be considered internal adverse events.

Non-Serious Adverse Event: Any adverse event that does not meet the definition of a serious adverse event.

Serious Adverse Event (SAE): Any event temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death
- Is life threatening
- Requires hospitalization/prolongation of hospitalization
- Results in congenital anomaly

- Results in persistent or significant disability/incapacity
- Required intervention to prevent permanent impairment/damage

Unexpected Adverse Event: Any adverse event occurring in one or more subjects participating in a research protocol, the nature, severity, or frequency of which is not consistent with either: (1) the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in the protocol-related documents; or (2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

10. STATISTICAL METHODS/DATA ANALYSIS

10.1 Primary endpoint(s) or outcome measure(s):

- **Systemic safety**: Incidence and severity of systemic adverse events during the study (based on physical examination, subject self-reporting, and changes in vital sign).
- **Ocular safety**: Incidence and severity of ocular adverse events during the study (based on ophthalmic examination and subject self-reporting).

10.2 Secondary endpoints or outcome measure(s)

- **Primary Endpoints**:
  - Twelve-month endothelial rejection rate
- **Secondary Endpoints**:
  - Time from surgery to occurrence of any rejection episode (endothelial, epithelial, subepithelial)
  - Time from surgery to overall graft failure (regardless of cause)
  - Incidence of delayed epithelial healing at Day 7
  - Endothelial cell density at Weeks 26 and 52
  - Central corneal thickness at Weeks 4,16, 26, and 52
  - Frequency of primary graft failure
  - Corneal NV Metrics at the Weeks 4,16, 26, and 52
    - Neovascular Area (NA), measuring the area of the corneal vessels themselves
    - Vessel Caliber (VC), measuring the mean diameter of the corneal vessels

- Invasion Area (IA), measuring the fraction of corneal area in which vessels are present

10.3 Sample Size Determination

We based sample size determination on rate of rejection observed in prior studies of high-risk corneal transplantation. In those studies, endothelial rejection episodes tend to occur in the range of about 60% of transplants [22]. We used rates ranging from 50 to 70 percent to compute the sample size (number of participants to enroll) for the study using the method described by Lachin and Foulkes (LF), as well as the Rubenstein, Gail and Santner approach (RGS). We specified the probability of a type 1 error equal to 0.05, study power equal to 80%, a follow-up period of 12 months, and 4% loss to follow-up. For practical purposes, we also took into consideration the time available for the study and the number of high-risk procedures done at each study site. Given these considerations, we estimated that if the treated group were half as likely to experience a rejection episode using the active treatment compared with the vehicle treatment (e.g. a rejection rate of 30% in the active group vs. 60% in the vehicle group), we wanted to be able to detect this difference. Calculations resulted in sample size estimates of between 65 and 124 participants to achieve this result. There were minor differences between the LF and RGS approaches (**Table 2**). Using the midrange estimate we aim to enroll 90 participants into this study.

Table 2. Sample Size Calculation

Cumulative Rejection Rate among Control Group	Total Sample Size	
	LF	***RGS***
0.50	122	124
0.60	90	90
0.70	66	65

10.4 Analysis Population

Population analysis is not applicable in this study.

10.5 Effectiveness Analysis

The primary efficacy analysis will follow an intent-to-treat analysis strategy including all randomized eyes. The major goal of this study is to determine whether treatment with bevacizumab significantly reduces the occurrence of transplant endothelial rejections compared to a control regimens following corneal transplantation in high-risk eyes. The randomized design should provide for balanced distributions of baseline characteristics between the two treatment groups. Nonetheless, we will conduct an initial analysis to determine any chance imbalances that may have occurred. These analyses will form part of the routine monitoring of the trial and will be regularly reported to the DSMB. For continuous and ordinal variables (e.g. age), comparisons will be made using the Mann-Whitney Wilcoxon test based on ranks. For categorical variables (e.g. sex, race) comparisons will be made using Chi-square or Fisher exact tests as appropriate. In addition to inter-group (arm) analysis of different time points, the "area under the curve" (severity vs. time) will be evaluated for neovascular area (invasion area), length, and caliber between different areas. These analyses are intended for data monitoring and quality control, and not to determine which baseline covariates to include in efficacy analysis.

The primary endpoint of the trial is the time to first endothelial rejection. We will use a proportional hazards regression framework with robust variance estimation to test the effectiveness of bevacizumab in reducing the occurrence of transplant rejection over time following high-risk corneal transplantation. This analysis will stratify on graft/regraft and center as was done in the randomization scheme. We will obtain an estimate of the incidence rate ratio (relative risk) of rejection for eyes assigned to bevacizumab versus vehicle. This framework allows for the control of covariates, which may be unbalanced by chance despite the randomization of treatment

assignment. If Kaplan-Meier plots of event free survival by study time, or related plots of log (-log) (survival) indicate violations of the proportional hazards assumption, then weighted log-rank tests will be used according to strategies described by Peckova and Fleming. The clinical significance of any observed benefit associated with bevacizumab therapy will also be quantified by the number needed to treat. This measure has the advantage of quantifying the absolute, as compared to the relative treatment effect.

Any deviations from the previously described statistical plan will be described and justified in a protocol amendment and/or in the final report submitted to this application.

10.6 Safety Analysis

Interim Analysis for Treatment Monitoring: We have established an independent Data and Safety Monitoring Board (DSMB) to monitor progress and make recommendations regarding early termination of the study as outlined below. In some trials, motivated primarily by a desire to avoid putting participants at unnecessary risk or to detect as early as possible whether the effect of the intervention is so great that further data collection is unnecessary, the committee is aided by setting a statistical criterion or "stopping rule" that, when met by the accumulating data, indicates that the trial can or should be stopped early. While the frequency of meetings and approach to interim monitoring will be the choice of the DSMB, we will recommend to conduct one interim analysis at the midway point of the study. We recommend that this analysis should examine the primary study endpoint and, following the Peto approach, consider early termination of the study if the p-value at the interim analysis is <0.001. Although this is a stringent criterion, it is appropriate in low- to moderate-risk trials and balances the risk of overestimating treatment effects. In addition, the occurrence of any adverse events will be described and compared between treatment arms of the study. There is no explicit pre-specified harms-related hypothesis as the treatment has been studied and used systemically

and is well tolerated in this topical ocular preparation. If interim analyses show unexpected differences in benefits or toxicity, the study investigators and DSMB may consult with other independent advisors comprised of experts in the field of Ophthalmology and Cornea and External Diseases to consider all of the evidence and aid them in the decision process.

Secondary Analyses: In addition to the primary comparisons of bevacizumab versus vehicle, we plan to conduct a number of pre-specified analyses of secondary study endpoints. Analyses of secondary "time-to-occurrence" type endpoints (e.g. any graft failure, any graft rejection) will use the proportional hazards regression framework (as used for the primary outcome). Analyses of quantitative endpoints (e.g. endothelial cell density, corneal thickness) will be performed using the Mann-Whitney Wilcoxon test based on ranks for comparison of active versus vehicle groups.

11. DATA AND SAFETY MONITORING

11. 1 Site Training and Compliance

Site expectations for data entry, record keeping and regulatory documents are outlined in this protocol and are communicated verbally to each site investigator and coordinator either during monthly study coordinator conference calls and quarterly investigator calls or at the study annual meeting.

Additional information on monitoring can be found in Section 12. Clarification on training and study management can be found in Appendix 3.

11.2 Data and Safety Monitoring Plan

Research subject safety is a priority. Therefore, a Data and Safety Monitoring Plan to ensure the safety of participants and the validity and integrity of the data collected has been established for this project. This plan will be shared with each site's IRB.

As detailed previously, at each study visit the investigator will query and examine each participant regarding potential side effects and adverse reactions. The efficacy outcomes that will be monitored include endothelial rejection rate, occurrence of any rejection episode, overall graft failure, delayed epithelial healing, endothelial cell density, central corneal thickness, occurrence of primary graft failure and quantification of corneal neovascularization. The primary safety variable monitored will be the occurrence of adverse events. A Data and Safety Monitoring Plan will be in place which includes the following: The severity of each adverse event observed (ocular and systemic) will be rated from mild (awareness of sign or symptom, but easily tolerated) to severe (incapacitating with inability to work or do usual activity). The relationship of the event to the study medication will be assessed by the investigator as 'none', 'possible', 'probable', or 'definite'. Safety variables will be

evaluated at baseline and at all study visits. If adverse events occur, the first concern will be the safety of the study participants. Any serious adverse event occurring during the study period will be reported to all site PIs, all site IRBs, the FDA as outlined in the Study Management Plan and the designated Data and Safety Monitoring Board (DSMB) (see below).

At each of the visits throughout the study, the investigator will begin querying for adverse events by asking each participant a general, non-directed question such as "How have you been feeling since the last visit?" Directed questioning and examination will then be done as appropriate. The investigator will ask questions to subjects at each visit to determine if they have had any changes to the use of concomitant medications since the previous visit. A comprehensive eye examination including best-corrected visual acuity, measuring intraocular pressure, evaluation of the condition of the lid/lashes, conjunctiva, cornea, anterior chamber, iris/pupil, lens, vitreous, macula and optic nerve will be performed. Any changes in the study eye from the baseline visit will be recorded.

The DOD will be informed of any actions taken by the IRB as a result of its continuing review. This study holds an IND from the FDA. Therefore, individual adverse event reports will be submitted to the DOD, FDA and IRBs in accordance with regulations. The research staff at MEEI, the primary site of the protocol, will serve as the central reporting entity. They will be responsible for preparing summary reports of adverse events and distributing the information to all participating sites. Detailed information on adverse event and unanticipated problem reporting is included in the Study Management Plan.

Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) has been formed that will review the data and any reported adverse events on a periodic basis. This group is comprised of researchers and physicians who are not associated with the trial. The members include Joseph Rizzo, MD, who will serve as the DSMB Chair, Lou Pasquale, MD, Debra

Schaumberg, OD, ScD, MPH and Reverend Stephen Ayres, PhD, Episcopal Diocese of Boston.

Joseph Rizzo, M.D. serves as Director of the Neuro-Ophthalmology Service at Massachusetts Eye and Ear Infirmary. He is board certified in psychiatry and neurology as well as ophthalmology and holds the position of David G. Cogan Professor of Ophthalmology in the Field of Neuro-Ophthalmology at Harvard Medical School. Dr. Rizzo has conducted clinical research for over 25 years.

Louis Pasquale, M.D. serves as Director of the Glaucoma Service as well as the Ophthalmology Telemedicine Program at Massachusetts Eye and Ear Infirmary. He is board certified in ophthalmology and holds the position of Associate Professor of Ophthalmology at Harvard Medical School. Dr. Pasquale has been involved in clinical research for over 25 years.

Debra Schaumberg, Sc.D., O.D. is Adjunct Professor of Epidemiology at Harvard School of Public Health. She also holds the position of Associate Professor of Ophthalmology at Massachusetts Eye and Ear Infirmary. Dr. Schaumberg has been involved in research for over 20 years.

Reverend Stephen Ayres is Vicar of Christ (Old North) Church in Boston, Massachusetts. He has served as a community representative on the Schepens Eye Research Institute's IRB since 2003.

Research Monitor

The Research Monitor, Joseph Rizzo, MD, is responsible to oversee the safety of the research and report observations/findings to the MEEI IRB. The Research Monitor will review all unanticipated problems involving risk to volunteers or others associated with the protocol and provide an unbiased written report of the event to the MEEI IRB. The Research Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The Research Monitor shall have authority to stop the research protocol in progress, remove individual human subjects from the study, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can

assess the monitor's report. The Research Monitor is responsible for promptly reporting their observations and findings to the IRB.

Since this is a multi-center study participants will be drawn from the surrounding areas of each participating site. The DSMB plan will be incorporated in site IRB applications. The DSMB will ensure the safety of participants and the validity and integrity of the data collected. The Statement of Work highlights the timing of the DSMB meetings throughout the course of the study. A quarterly update will also be e-mailed to all sites, the DOD, trial statistician and DSMB. The update will include an overview of adverse events, an enrollment update, change in any personnel, reports from the Data Safety Monitoring Board.

All investigators and key personnel will be invited to attend annually a study meeting, which will be held at ARVO. The meeting will allow for all investigators to discuss progress of the study, issues that have arisen within the past year, feedback from the Data Safety Monitoring Board and adverse events.

12. DATA HANDLING, RECORD-KEEPING AND MONITORING

12.1 Data Recording, Record-Keeping and Monitoring

A Case Report Form (CRF) will be completed for each subject enrolled into the clinical study. The Sponsor-Investigator will review, approve and sign/date each completed CRF; the Sponsor-Investigator's signature serving as attestation of the Sponsor-Investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic. In this study, the CRF will be digitally represented, in the form of a 21 CFR Part 11-compliant online data capture program called StudyTrax.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in Source Documents. StudyTrax will serve as the Source Data in addition to the Case Report Forms.

Case Report Forms (CRF) will be completed at each participant visit on StudyTrax, a software designed to capture study related data. Study Staff at New York Presbyterian Hospital (NYPH) and Bascom Palmer Eye Institute (BPEI) will enter all study data from participant visits directly into StudyTrax. Treatment randomization codes will be documented in StudyTrax for each participant. StudyTrax will be designed with certain limits, ensuring that all required information is entered. Limits in specific data fields can be set to minimize typographical errors. Were StudyTrax not available, paper CRFs will be sent over to MEEI via secure e-mail or via fax, and the data will be uploaded to StudyTrax by MEEI study staff.

The Study Coordinator (SC) at MEEI will review each CRF from MEEI, BPEI and NYPH to ensure that all required study assessments have been completed. If data is missing, the coordinator will query the site to ensure completeness. Following this, the MEEI Clinical Research Fellow assigned to this study will review the documents for safety.

The Clinical Research Fellow has in depth knowledge of the study protocol, procedures and investigational medication. If there are any safety concerns or uncertainty the Fellow will review the documents with Dr. Dana at the time the event was discovered or reported. Dr. Dana, who is responsible for overall monitoring of the study, will review all CRFs and reports at the following visits per participant: screening, and weeks 4, 26, 52. The efficacy outcomes that will be monitored include endothelial rejection rate, occurrence of any rejection episode, overall graft failure, delayed epithelial healing, endothelial cell density, central corneal thickness, occurrence of primary graft failure and quantification of corneal neovascularization. Participants will be monitored continuously for systemic and ocular safety during their ophthalmic examinations. If there is an adverse event or safety issue, the guidelines above will be followed.

Each site is responsible for verifying the data collected into StudyTrax. A monitoring log will be kept to verify the PI review of the data collected during each study visit.

Investigators, fellows, study coordinators, and technicians will have access to participant data through StudyTrax. Study staff at Bascom Palmer and Cornell will only be able to view and edit their own participant data. The PI, fellow, and research coordinator at MEEI will have access to participant information and data at Bascom Palmer and Cornell as to follow the data safety monitoring plan.

Confidentiality

Identifying health information will be collected from each participant only with their permission (obtained in the Informed Consent and HIPAA). Solely information needed for the research project will be collected. All health information will be protected and kept confidential to preserve the confidentiality of all participants in accordance with HIPAA regulations. All subjects will be given a HIPAA authorization to sign acknowledging their understanding of their private health information rights. Protected health information with participant identifiers will only be accessible to research personnel who require it to ensure safety of the participants and integrity of

the data. The source documents with identifiable health information will only be shared amongst members of the research team involved in the subject's care. All other research personnel will be given data with the participants study identification number only.

All paper study records will be kept locked allowing only research staff access. Electronic records will be stored on computers that are password protected and equipped with anti-virus software within each institution. The Electronic Data Capturing system, StudyTrax, is also password protected and access is controlled by one administrator at Mass. Eye and Ear. Each site will only have access to their own records, except for the primary enrolling site, Mass. Eye and Ear, whose study coordinator and clinical research manager will have access to all site information.

Slit lamp photographs of participants will be shared through StudyTrax, which is supervised by Mass. Eye and Ear. The University of Miami and Weill Cornell Medical College enrolling sites will be given privileges to upload photographs to be attached with corresponding patient visits.

All study records are subject to review by representatives of the USAMRMC.
The data from this project may be used in publications or manuscripts written by the research team.

Data Transmission

Data will be entered at the time of the study visit by the investigators or study staff into StudyTrax. StudyTrax allows research teams to create unique case report forms (CRFs), determine levels of user access for increased security, extract data into SAS, SPSS or excel spreadsheets, query data, create audit trails, and track adverse events and participant's medications. The system is HIPAA and 21 CFR Part 11 compliant. The central enrolling site will be able to access the information from each visit on StudyTrax and will check to ensure that all assessments are done as described in the

protocol. If any information is noted to be missing, the study coordinator from MEEI will contact the site coordinator and gather the missing information. The MEEI will check all entries and immediately notify the sites of any errors, so that they can be addressed in a timely fashion.

Data Security

All data will be entered into StudyTrax. StudyTrax is a leading provider of electronic data capturing and is designed to meet the needs of academic clinical research centers. StudyTrax is a web-based system hosted by Partners HealthCare that can be used by all study sites with a secure, unique username and password.

Data Extraction

All participant information and study data can be extracted from the website at any given time for any of the study parameters. This functionality can greatly assist in an interim analysis or assessment of study adverse events. Data extraction will occur at a minimum of every three months and be sent to the Statistician for independent trial monitoring. This will assist in making sure that enrollment targets are being met, there are no detected errors or concerns with randomization.

Disposition of Data

Electronic case report forms will be stored in the StudyTrax system. Following the data lock at the end of the study, the information collected in the case report forms will be exported to excel spreadsheet. The key to the study participant identifications will be held in a separate electronic file. The de-identified excel spreadsheets of participant data will be saved for two years from the termination of the study. All files will be backed up on a secure server during this time. Following data export, the StudyTrax account will be closed for all sites. Hard copies of documents (regulatory documents, consent forms and other study documentation) will be maintained at each site in a secure, locked space.

Sharing Study Results

The decision to share study assessments and results completed while participating in the study with the participant and/or their other physicians will be evaluated on a case-by-case basis. The decision will ultimately be made by the investigator and/or participant.

Sharing of Corneal Photographs

Individual sites are required to upload slit lamp photos to StudyTrax from visits where those images are required.

Regulatory Documents

Monitoring of the electronic regulatory documents will be conducted monthly. Queries will be sent to each site following the review to address any outstanding items. All queries and subsequent resolutions will be documented and stored in the MEEI regulatory binder.

Routine Reporting

The MEEI will provide quarterly reports to all sites, including a Performance Report and a Quality Control Report. The Performance Report will include enrollment and randomization rates, reasons for randomization ineligibility; number of completed follow-up visits, number of participants not taking their study drug, and reasons for discontinuation. A Quality Control assessment will also be provided and will detail any ID and acrostic errors, inappropriate randomizations, missing data broken down by form, reasons for missed visits, number of visits taking place outside of the designated time windows, etc. Finally, the Data and Safety Monitoring Committee will be provided an analysis of treatment safety and efficacy in addition to the routine progress and quality control reports. The routine reporting and ongoing QC checks described will help ensure maximal data integrity and timely completion of study as forecast.

The Principal Investigator will comply with the following Department of Defense reporting requirements:

(1) Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects will be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc), significant change in study design (i.e. would prompt additional scientific review) or a change that could potentially increase risks to subjects.

(2) Any changes of the IRB used to review and approve the research will be promptly reported to the USAMRMC ORP HRPO.

(3) All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

(4) Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the Institutional Review Board (IRB), the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

(5) A copy of the continuing review approval notification by the IRB of Record will be submitted to the HRPO as soon as possible after receipt. A copy of the continuing review report approved by the IRB will also be provided. Please note that the HRPO also conducts random audits at the time of continuing review. Additional information and documentation may be requested at that time.

(6) The final study report, including any acknowledgement documentation and supporting documents, will be submitted to the HRPO when available.

(7) The knowledge of any pending compliance inspection/visit by the FDA, DHHS Office of Human Research Protections (OHRP), or other government agency concerning this research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any regulatory agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements, must be promptly reported to the HRPO.

The USAMRMC ORP HRPO conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

13. STUDY DISCONTINUATION CRITERIA

13.1 Discontinuation of Individual Research Subjects

Subjects have a right to withdraw from the study at any time. The subject may be withdrawn from the study for any reasons; such as if it is deemed in the best interest of the subject, due to concurrent illness, adverse events, or worsening condition. The primary investigator may request the withdrawal of a subject because of protocol violations, administrative reasons, or any other valid and ethical reasons.

Stopping Criterion: development of non-healing epithelial defect of ≥ 2 x 2 mm in the graft lasting ≥2 weeks while on investigational treatment.

Early Termination Assessments: Subjects who voluntarily withdraw from the study prior to completion will be asked to return for an early termination evaluation in 7 days (±3 days) for monitoring of all adverse events. The schedule of assessments for early termination is the same as that for the final visit (Week 52).

Withdrawal of subjects due to non-compliance/adherence: The decision to withdraw a participant due to non-compliance or adherence to study medications, standard-of-care medications, or study visits will be made by the principal investigator, Dr. Reza Dana.

Withdrawal of subjects due to adverse events: Subjects who experience adverse events will not be required to withdraw from the study. Rather, subjects will be followed throughout the 52 week period, barring voluntary participant withdrawal. Voluntary withdrawal effectively stops the collection of study data for the participant and only previous study visits can be analyzed.

Replacement of Withdrawn Study Participants: Participants who have voluntarily withdrawn or have been withdrawn by the PI will not be replaced.

13.2 Sponsor-Investigator Discontinuation of the Clinical Research Study

Trial Suspension and Termination: If Dr. Dana and/or the DSMB determines that the trial needs to be terminated or temporarily suspended, the investigators, FDA, DOD, and HSC will be notified. Dr. Dana, research staff and the HSC will determine the best method to notify participants of study termination or suspension to ensure participant safety, confidentiality and data integrity. The investigators will notify all participants of the termination or suspension and reasoning. Outstanding data from outside MEEI will be collected for analysis and reporting purposes.

If the trial is terminated, active participants will be asked to return to MEEI for routine follow-up, yet data will not be recorded for study purposes.

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REFERENCES CITED

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15. APPENDICES

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APPENDIX 1: OUTLINE OF STUDY VISITS AND PROCEDURES

Outline of Study Visits and Procedures		
STUDY VISIT	STANDARD OF CARE PROCEDURES	NON-STANDARD OF CARE PROCEDURES
Screening Visit (Baseline)	Record current ocular and systemic medications	Review of eligibility criteria
	Record significant medical/surgical history in the past 5 years	Written informed consent
	Review of systems	Measurement of systolic and diastolic blood pressures
	Record demographic data, including date of birth, sex, and race/ethnicity	Urine pregnancy test for women of childbearing potential, if appropriate
	Slit-lamp examination (OU)	Corneal photography (Study eye)
	Best spectacle-corrected visual acuity (BSCVA) manifest refraction (OU)	
	Intraocular pressure (OU)	
	Dilated funduscopy (OU)	
	Pachymetry (OU)	
Day 0	Penetrating keratoplasty	Subconjunctival injection of bevacizumab or saline
Post-Op Day 1	Best spectacle-corrected visual acuity (BSCVA) (Study eye)	Blood Pressure
	Slit-lamp examination (Study eye)	Start study eye drop 4 times per day for 4 weeks
	Intraocular pressure (Study eye)	
	Review of systems and ocular and systemic medications	
	Start topical prednisolone acetate 1% 6 times per day, continue for 2 weeks	
	Start topical antibiotic 4 times per day until graft epithelialization or continue as necessary	
Post-Op Day 7	Best spectacle-corrected visual acuity (BSCVA) (Study eye)	Blood Pressure
	Review of systems and ocular and systemic medications	Study medication compliance check
	Slit-lamp examination (Study eye)	Adverse event query

	Intraocular pressure (Study eye)	
	Continue prednisolone acetate 1% 6 times per day for one more week. At the beginning of post-op week 3, reduce to 4 times per day. Continue this regimen for 6 weeks (post-op week 8)	
Post-Op Week 4	Slit-lamp examination (OU)	Blood Pressure
	Review of systems and ocular and systemic medications	Study medication compliance check
	Pachymetry (OU)	Adverse event query
	Intraocular pressure (OU)	Stop study eye drop and return of all unused bottles
	Best spectacle-corrected visual acuity (BSCVA) (OU)	Corneal Photography (Study eye)
	Continue prednisolone acetate 4 times per day	
Post-Op Week 8	Best spectacle-corrected visual acuity (BSCVA) (OU)	Adverse event query
	Slit-lamp examination (OU)	Blood Pressure
	Intraocular pressure (OU)	
	Review of systems and ocular and systemic medications	
	Taper prednisolone acetate to 3 times per day. Continue regimen for 4 weeks (post-op week 12). At post-op week 13 begin prednisolone 2 times per day for 6 months (post-op month 9).	
Post-Op Week 16	Best spectacle-corrected visual acuity (BSCVA) (OU)	
	Slit-lamp examination (OU)	Corneal photography (Study eye)
	Intraocular pressure (OU)	Adverse event query
	Review of systems and ocular and systemic medications	
	Dilated Funduscopy (Study eye)	
	Pachymetry (OU)	
	Continue prednisolone acetate 2 times per day until post-op month 9/week 39	

Post-Op Week 26	Best spectacle-corrected visual	Adverse event query

	acuity (BSCVA) (OU)	
	Slit-lamp examination (OU)	Corneal photography (Study eye)
	Intraocular pressure (OU)	Confocal/specular microscopy (Study eye)
	Review of systems and ocular and systemic medications	
	Pachymetry (OU)	
	Continue prednisolone acetate 2 times per day until post-op month 9/week 39	
Post-Op Week 39	Best spectacle-corrected visual acuity (BSCVA) (OU)	Adverse event query
	Slit-lamp examination (OU)	
	Intraocular pressure (OU)	
	Review of systems and ocular and systemic medications	
	Taper prednisolone acetate to 1 time per day. Continue indefinitely unless instructed otherwise by the investigator.	
Post-Op Week 52	Best spectacle-corrected visual acuity (BSCVA) manifest refraction (OU)	Adverse event query
	Slit-lamp examination (OU)	Confocal/specular microscopy (Study eye)
	Intraocular pressure (OU)	Corneal photography (Study eye)
	Review of systems and ocular and systemic medications	
	Pachymetry (OU)	
	Dilated funduscopy (Study eye)	

APPENDIX 2: RECRUITMENT SCRIPT

If no definite reasons to exclude the participant are found during the pre-screen review (as described in Section III of the Protocol), the participant will be approached by their physician (investigator) in the following manner:

Study Coordinator/Research Fellow (After receiving word from the investigator that a participant is interested): "Hello Mr./Mrs./Ms. _____. I am _____, a study coordinator/research fellow that works with Dr._____. Dr. _____ asked me to speak with you about a research study because you are considering/have been scheduled for a corneal transplant and are considered to be at high-risk for a graft rejection. The study Dr. _____ mentioned is looking at the safety and effectiveness of an investigational drug, bevacizumab, also known as Avastin. This study is looking at whether this investigational drug helps reduce the risk of graft rejection after surgery.

If you agree to participate, you will receive one of two study treatments: Avastin, an investigational drug or a placebo. A placebo is a treatment with no active ingredient. Although Avastin is currently approved by the FDA to treat certain cancers, it is not FDA approved for how it will be used in this study. During your surgery, your surgeon would either inject into your conjunctiva a dose of Avastin, if you are assigned to the active study treatment group, or an injection of saline, if you are assigned to the placebo group. Following surgery you would be given eye drops, either containing Avastin, the active study treatment, or Refresh Liquigel, an artificial tear for the placebo study treatment, to use four times a day for four weeks.

We will follow you closely after your surgery to see if you have any side effects related to the study medication. We will do other assessments throughout the study to also see if you have any signs of rejection.

Our pharmacy will randomly assign you a study treatment. They will give you the study medication in plain eye dropper bottles so neither you, Dr. _____, or I will know which eye drop you are taking. You will be assigned to either the active study treatment group or the placebo group. Depending on the group assigned you will receive either an Avastin injection at surgery and Avastin eye drops or a saline injection at surgery and Refresh Liquigel eye drops. In addition to the study medication, you would receive all standard treatments and medications during your surgery and post-operatively.

Overall, there are ten study visits: a screening appointment prior to your surgery, your surgery, and then 8 visits following your surgery. The screening visit is the first visit of the study and will only occur if you want to participate in the study. The other visits are considered to be standard of care, examinations that you would have done even if you didn't participate in this study. The last of these visits will occur about one year after your surgery. During these follow-up visits, three extra assessments will be done at some of the visits that are considered non-standard of care (meaning the assessments are being performed only for study purposes). The first study procedure is blood pressure measurements to ensure that your blood pressure remains stable. In people who have taken the study medication for systemic use (that is to treat their whole body), an increase of blood pressure has been seen and is considered to be a potential side effect of the medication. Therefore, for this study, we will track your blood pressure while you are taking the study medication. We will also ask you to have confocal/specular microscopy done. Confocal/specular microscopy is a non-invasive test which allows us to look at the different layers of your cornea in more detail than we are able to during your regular examination. Finally, we will also take pictures of

your eye with a slit-lamp camera to document the amount of blood vessels we can see on your cornea. The additional study procedures will take between 10 – 20 minutes. This time will be in addition to the normal time of your follow-up visits. The total time of each of the follow-up visits will be between 1 and 2 hours.

Here is a copy of the study consent form, which we would like you to review before participating in this study (as Study Personnel review consent form they mention key points in each section and stop to ask questions before moving forward to the next section):

- *In the first few pages, you will find that the consent form explains why we are doing this study, how we think it might help you, and the procedures conducted as part of the study.*
- *In the following section, you will see that risks of the study medications and the imaging procedures are explained. The treatments used in this study are FDA-approved for other uses, and therefore, considered to be investigational for this purpose. The confocal/specular microscopy and photography are procedures routinely performed by trained professionals at Mass Eye and Ear.*
- *Next you will see the potential benefits and alternative treatments are explained to you.*
- *Then you will see the explanations about participant confidentiality, your right to withdraw from the study, right to ask questions, costs and compensations, and what happens if there are any injuries while you are in the study. You have the right to withdraw at any point or ask any questions. The screening visit, study medication, and imaging done for research purposes only are all provided at no cost to you. Your insurance will not be billed for study related procedures.*
- *In the following pages, you will see the detailed list of study procedures for each of your ten visits. If you are not clear about any of these, do not hesitate to ask what they are or entail.*
- *Lastly, before you enroll in the study, we will have you sign the last page.*

You may take this consent form with you to read over. It is sometimes helpful to review it with family or friends and look up the different medications online. After reviewing it, please call or speak with me if you have any other questions. I can also help you schedule your study appointments with Dr. _____. Here is my card with my office number and e-mail address."

APPENDIX 3: STUDY MANAGEMENT PLAN

Investigator and Personnel Training

To ensure that all staff and investigators are adequately trained on the protocol procedures, assessments and regulatory requirements for this clinical trial the following will take place:

1. The Clinical Research Manager and Principal Investigator at Mass. Eye and Ear will review all of the protocol procedures and regulatory requirements with each Site Principal Investigator prior to the start of the enrollment phase of the study.
2. Each Site Principal Investigator will outline the roles and responsibilities of each personnel involved in the study. Copies of these documents will be held in the regulatory binder at MEEI.
3. The Site Principal Investigator will be responsible for training their site personnel. Prior to enrollment opening at each site, the Principal Investigator will document review and understanding of the protocol and responsibilities of all parties involved. This documentation will be held in the MEEI regulatory binder.
4. New study personnel can be included into the study at any time. Prior to study involvement documentation of their roles and responsibilities and understanding of the protocol will be documented.
5. Slit lamp photographs taken within the guidelines outlined in the protocol from BPEI and NYPH will be sent to MEEI prior to the start of enrollment. The photographs will be analyzed to ensure that equipment is functional and staff are adequately trained in the imaging techniques required for this study.
6. The sample photograph will be uploaded through MyFiles, a data sharing website that will be used throughout the study. This will ensure that staff are

able to access the program and are comfortable using it prior to the start of the study.

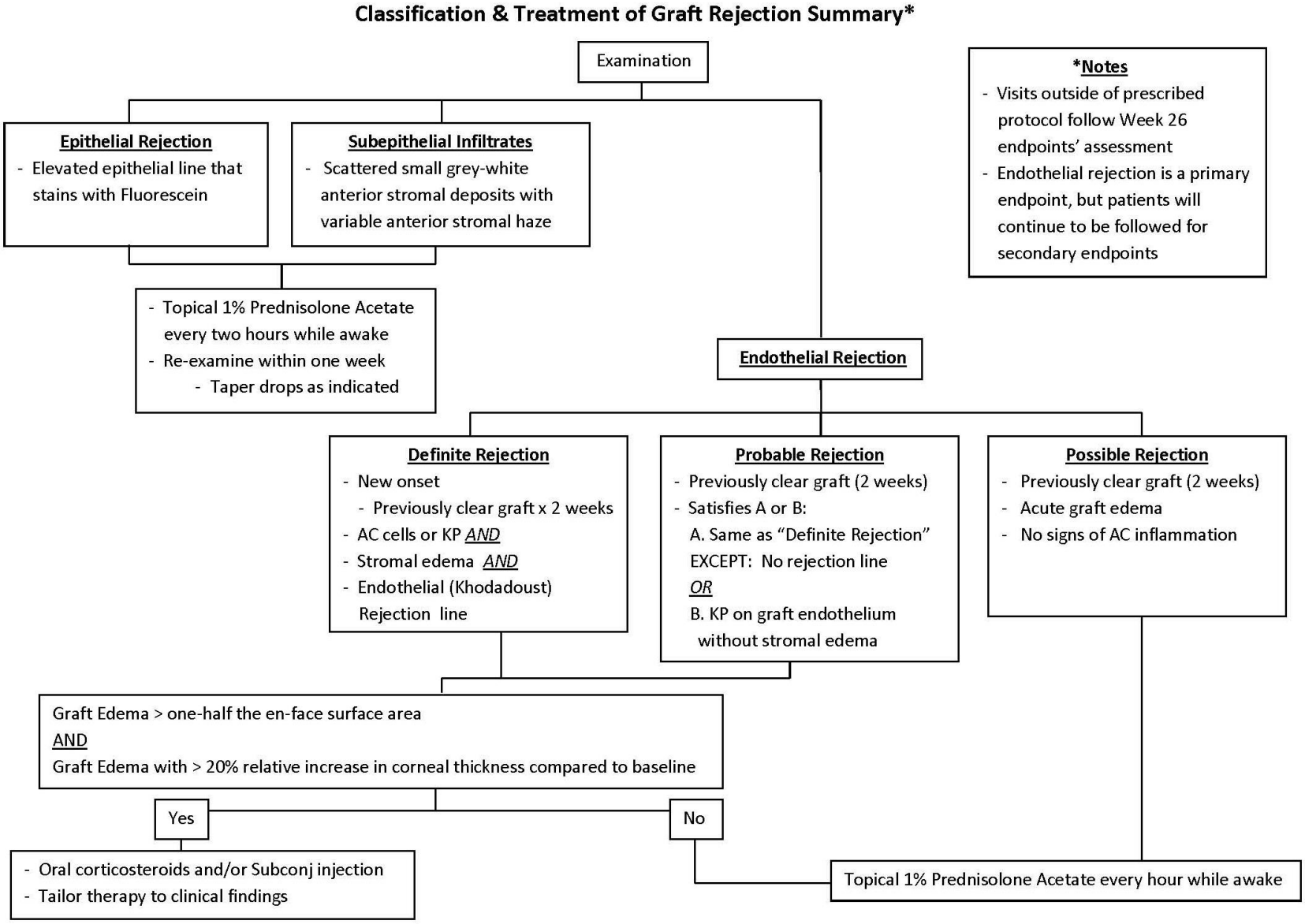
7. In the event that a site does not feel comfortable with the outlined procedures and data collection methods, the MEEI Principal Investigator, Dr. Dana, or the Clinical Research Manager will perform a site initiation visit.
8. A quarterly update will also be provided to all sites, trial statistician and DSMB. The update will include an overview of adverse events, an enrollment update, change in any personnel, reports from the Data Safety Monitoring Board.
9. All coordinators will participate in a monthly phone call to address any questions or concerns from any site, provide additional clarification, training or education on the protocol and study-related procedures.
10. All investigators will participate in a quarterly phone call to address any questions or concerns from any site, provide additional clarification, training or education on the protocol and study-related procedures.
11. All investigators and key personnel will be invited to attend annually a study meeting, which will be held at ARVO. The meeting will allow for all investigators to discuss progress of the study, issues that have arisen within the past year, feedback from the Data Safety Monitoring Board and adverse events.

Regulatory Documentation

NYPH and BPEI will be required to submit the following documents to MEEI prior to the initiation of the study on their site: a signed FDA form 1572, current CV of all investigators and all formal communication between the site IRBs overseeing this study at NYPH and BPEI, including stamped consent forms, approved protocol and approval notice. These documents will be kept on file with the IND and other regulatory study documents, including information and approvals by IRBs.

These procedures are set in place to allow Dr. Dana the ability to monitor the study and ensure that the risk to participants does not increase throughout the course of the study.

APPENDIX 4: CLASSIFICATION AND TREATMENT OF GRAFT REJECTION



APPENDIX 5: CORNEAL PHOTOGRAPHY PROTOCOL

The instructions below serve as a guideline for all sites when performing the corneal photography. Actual photography procedures may vary from site to site.

1. Adjust the backlight at mid to low level
2. Slit lamp beam will be on at 6V, fully open, through diffuser
3. Position the participant's eye so the cornea is facing completely straight. Position the cornea to be in the center of the photo, and ensure that the participant's forehead is always pressed against the plastic band and the chin resting on the chinrest.
4. Adjust the position of the light sources to remove the glare from the areas with vessels, while still keeping the cornea as illuminated as possible. This can be accomplished by physically moving the light sources, or by loosening and adjusting the position of the slit beam.

- If vessels cover the entire cornea, position the glare in the center of the cornea (if no vessels exist centrally). If vessels intrude into the center, position the glare outside of the corner by loosening the slit beam and positioning the reflex in the limbus or conjunctiva.

- Always keep the position of the glare constant throughout photos from all visits

- Print out a screenshot of the baseline photo so that the exposure and glare position can be replicated at future visits

5. Keep the aperture at 1 and exposure at 80, however, exposure can be adjusted slightly if photo is especially dark or reflective.

- Vary exposure as needed to create an ideal lighting for visualizing the vessels.

- Record exposure used for each set of photos so that this can be replicated in the following visits.

6. With a dark iris where the vessels cannot be visualized well, use retroillumination if all other attempts at illuminating the corneal vessels fail.
7. Ensure that all blood vessels around the cornea are focused when taking the photo.
8. If imaging is impaired due to lid positioning, use a lid speculum to aid in keeping lids open.

Avastin Photo Labeling Protocol

Within each patient's appropriate visit in StudyTrax, label each photo with the following information:

a. Participant ID number
b. Eye photographed
c. visit number – screening (w0), week 4 (w4), week 8 (w8), etc.
d. photo number – p1, p2, p3…

For example, MEEI012_OD_w0_p1 would be the name of the first photo taken of the right eye at the screening visit for participant MEEI012. Photos should be exported as jpeg images. Images will then be analyzed in a masked fashion by investigators.

APPENDIX 6: SCHEDULE OF EVENTS

Table 1: Schedule of Events and Procedures										
Visit	1	2	3	4	5	6	7	8	9	10
Time period	**Screening Visit**	**Day 0** 90 days from screening	**Day 1** ±0 days	**Day 7** ±6 days	**Week 4** ±10 days	**Week 8** ±2 weeks	**Week 16** ±2 weeks	**Week 26** ±2 weeks	**Week 39** ±2 weeks	**Week 52** ±2 weeks
Obtain Informed Consent	X	SURGERY DAY (subconjunctival injection of 2.5 mg/0.1 ml of bevacizumab (vs. vehicle)								
Inclusion/ exclusion criteria	X									
Medical and Ophthalmic History	X		X	X	X	X	X	X	X	X
Pregnancy test[3]	X									
Blood Pressure	X		X	X	X	X				
BCVA	X[2]		X[1]	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Biomicroscopy	X[2]		X[1]	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Intraocular Pressure	X[2]		X[1]	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Pachymetry	X[2]				X[2]		X[2]	X[2]		X[2]
Dilated Funduscopy	X[2]						X[1]			X[1]
Study Rx			Start		Stop					
Compliance Check				X	X					
Adverse Event Query			X	X	X	X	X	X	X	X
Confocal/specular microscopy								X[1]		X[1]
Corneal Photography	X[1]				X[1]		X[1]	X[1]		X[1]

[1] = Only the Study Eye Required
[2] = Both Eyes Required
[3] = If the pregnancy test performed at the screening visit is greater than 30 days from Day 0, an additional pregnancy test will be performed within 30 days of a participant's surgery date.

Participants will be instructed to strictly follow the study visit schedule. This study will not include blood sampling for serum levels of bevacizumab or any pharmacokinetic measures.

APPENDIX 7: ACRONYMS

AMD	Age-related Macular Degeneration
ARVO	The Association for Research in Vision and Ophthalmology
CA	California
CDMRP	Congressionally Directed Medical Research Programs
CFR	Code of Federal Regulations
CRF	Case Report Forms
BCVA	Best Corrected Visual Acuity
BPEI	Bascom Palmer Eye Institute
BSCVA	Best Spectacle-Corrected Visual Acuity
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
DOD	Department of Defense
e.g.	Exempli gratia
FB	Foreign Body
FDA	Food and Drug Administration
FL	Florida
GS	Guanosine Synthetase
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
Hx	History
IA	Invasion Area
ICF	Informed Consent Form
ID	Identification
IND	Investigational New Drug
IRB	Institutional Review Board (Human Studies Committee)
LF	Lachin and Foulkes
MA	Massachusetts
MD	Doctor of Medicine

MEEI	Massachusetts Eye and Ear Infirmary
mg	Milligrams
ml	Milliliters
mm	Millimeters
NaCl	Sodium Chloride
NYPH	New York Presbyterian Hospital
NIH	National Institutes of Health
NA	Neovascular Area
NV	Neovascularization
NY	New York
OD	Right eye
OS	Left eye
OU	Both eyes
PH	Pinhole
PhD	Doctor of Philosophy
PI	Principal Investigator
PK	Penetrating Keratoplasty
Post-Op	Post-operative
QC	Quality Control
RGS	Rubenstein, Gail and Santner
Rx	Prescription
SAS	Statistical Analysis System
SPSS	originally, Statistical Package for the Social Sciences
UCVA	Uncorrected Visual Acuity
U. Miami	University of Miami
US	United States
VC	Vessel Caliber
Vs	versus
VEGF	Vascular Endothelial Growth Factor
Wk	Week
WWI	World War I
WWII	World War II

SYMBOLS

≥	Greater than or equal to
≤	Less than or equal to
>	Greater than
<	Less than
±	Plus or minus
°C	Degrees is Celsius
°F	Degrees in Fahrenheit
@	at
%	percent
®	registered sign