Mycotic Ulcer Treatment Trial (MUTT)

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Aravind Eye Hospital, Pondicherry, India
Aravind Eye Hospital, Coimbatore, India
Aravind Eye Hospital, Tirunelveli, India
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1. Introduction

We propose to conduct randomized, masked controlled trials to evaluate which topical agent, voriconazole or natamycin, is the superior treatment for fungal keratitis, and whether adding a systemic antifungal confers additional benefit. Historically, fungal keratitis has been endemic in warmer climates such as India and has been relatively uncommon in temperate regions of the United States. For example, in settings such as South India as many as 50% of infectious ulcers are fungal[1], while they made up approximately 8% of infectious ulcers seen at the Proctor Foundation at the University of California, San Francisco prior to 2005.[2] However, in 2006, there was an epidemic of keratitis due to *Fusarium* species in the U.S. and Asia, and there was heightened concern about how to best care for these patients.[3-5] Although the peak of the epidemic has subsided, the concern over the best way to care for fungal keratitis patients remains.

Fungal corneal ulcers tend to have poor outcomes with the commonly used treatments, natamycin and amphotericin B.[6] There has been only a single randomized trial of antifungal therapy for mycotic keratitis[7], and no new ocular antifungal medications have been approved by the FDA since the 1960s. The triazole voriconazole has recently become the treatment of choice for systemic diseases such as pulmonary aspergillosis.[8] *Aspergillus* species are also a common cause of fungal ulcers, and the use of topical ophthalmic preparations of voriconazole has been described in numerous case reports in the ophthalmic literature.[9-17] In addition, there have been reports of successful outcomes combining topical and oral voriconazole. However, there has been no systematic attempt to determine whether topical voriconazole is more or less effective clinically than the commercially available natamycin, or whether adding oral voriconazole to the topical treatment improves outcomes. Although there are suggestions *in vitro* and *in vivo* that particular fungi respond better to one agent or another, there is little data available for physicians to make an informed, evidence-based decision on choice of antifungal. Our preliminary studies indicate that the newer triazoles are more effective than natamcyin *in vitro* against filamentous fungi such as *Fusarium* and *Aspergillus* species, the most common causes of fungal keratitis worldwide. We also found that although most cornea specialists indicate that voriconazole would be their preferred treatment, their actual practice often differs because of the lack of evidence supporting the newer antifungals. *In vitro* results and case reports may be hypothesis-generating, but they are insufficient to answer the question of which drug should be used in fungal keratitis patients. A randomized clinical trial is needed to definitively determine which antifungal treatment is optimal for the treatment of fungal corneal ulcers.

In this study, we will enroll patients with fungal corneal ulcers at the Aravind Eye Hospital in South India and at the Proctor Foundation, UCSF. Patients will be enrolled in one of two trials: topical natamycin vs topical voriconazole (MUTT I) or topical voriconazole with oral placebo vs topical voriconazole with oral voriconazole (MUTT II). Patients will be randomized to receive either topical natamycin or topical voriconazole, or topical voriconazole with oral placebo or topical voriconazole with oral voriconazole. The large, standardized database generated will also be used to address secondary questions that will allow physicians to tailor their treatment of fungal ulcers in an evidence-based manner.

MUTT Pilot

The Mycotic Ulcer Treatment Trial Pilot study enrolled patients at Aravind Eye Hospital (Madurai and Pondicherry centers) from November 2007 until May 2008. The primary objective of the study was:

- To assess whether treatment of mycotic corneal ulcers with 1% topical voriconazole yields better visual acuity (best spectacle-corrected visual acuity) after three months than does standard treatment with 5% natamycin
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The secondary objectives of the study were:

- To assess the effect of repeat scraping of the epithelium
- To assess for a significant difference in adverse events, in particular corneal perforations
- If a difference is not found, to determine the feasibility and sample size of a larger trial

This study was a randomized, double-masked, placebo-controlled trial. 120 patients were enrolled into one of four arms: topical natamycin or topical 1% voriconazole with or without re-scraping of the epithelium at 1 and 2 weeks. The primary endpoint was best-spectacle corrected visual acuity at 3 months. Natamycin and voriconazole were donated by Alcon and Pfizer, respectively. Note that this study was designed to gather preliminary data to obtain NIH grant support as well as to refine the sample size for the main trials. Thus, it was not powered to detect a significant difference in efficacy.

For the primary outcome, 3-month best-spectacle corrected visual acuity, voriconazole was associated with a 0.066 logMAR decrease (2/3 line improvement in visual acuity) (95% CI -0.26 to +0.13, p=0.51) compared to natamycin. Scraping was associated with a 0.12 logMAR increase (1-line loss in vision) (95% CI -0.075 to +0.31, p=0.23) compared to not scraping.

A post-hoc subgroup analysis of 3-month BSCVA was performed in the group of patients with baseline visual acuity 20/40 to 20/800. In this subgroup, voriconazole was associated with a 0.199 logMAR decrease (2-line improvement in visual acuity) (95% CI -0.47 to +0.074, p=0.16) compared to natamycin. Scraping was associated with a 0.18 logMAR increase (1.8 lines worsening) (95% CI -0.098 to +0.48, p=0.21).

There were a total of 19 perforations during the three-month follow-up period. 9 perforations were in the natamycin arm, and 10 in the voriconazole arm (p>0.99). 6 were in the non-scraped arm and 13 in the scraped arm (p=0.13).

These results show a non-significant 2/3 line benefit in 3-month BSCVA with voriconazole. There is a trend towards voriconazole-treated patients having a 2-line benefit in 3-month BSCVA in the subgroup with baseline visual acuity of 20/40 to 20/800. There is also a trend towards repeat scraping being associated with worse outcomes. There appears to be no difference in perforations between the natamycin and voriconazole arms. Given the results of the pilot study, we have decided not to include repeat scraping in the main trial. In addition, we believe that we have the greatest chance of demonstrating a visual acuity change in the subgroup of patients with baseline visual acuity of 20/40 to 20/800 since their vision isn't too good to improve further and not too poor to have little hope of improved vision. This subgroup of patients will be enrolled in MUTT I (see subsequent description of trials).

Research Questions

Which topical agent, voriconazole or natamycin, is more effective in the treatment of filamentous fungal corneal ulcers?

Is the administration of oral voriconazole with topical voriconazole more effective than topical voriconazole alone in the treatment of filamentous fungal corneal ulcers?

1.1. Study Objectives

1.1.1. Primary Objectives

- To assess whether treatment of mycotic corneal ulcers with 1% topical voriconazole yields noninferior visual acuity outcomes (best spectacle-corrected visual acuity) after three months than does standard treatment consisting of the topical administration of 5% natamycin (specifically, the noninferiority threshold is 0.15 logMAR, discussed in the Statistical Analysis Plan).

- To assess whether topical treatment of mycotic corneal ulcers with 1% topical voriconazole and 5% topical natamycin together with oral administration of voriconazole yields a lower rate of perforation after three months than treatment with topical voriconazole (1%) and topical natamycin (5%) alone.

1.1.2. Secondary Objectives

- To determine whether patients treated with voriconazole instead of natamycin exhibit a difference in infiltrate/scar size at three weeks and at three months, as determined by slit lamp examination, and separately by photography. Similarly, we wish to compare infiltrate/scar size at three weeks and at three months between patients receiving both topical and oral voriconazole with those receiving only topical voriconazole.

- To determine whether patients treated with 1% topical voriconazole and 5% topical natamycin together with oral voriconazole yields better visual acuity (best spectacle-corrected visual acuity) after three weeks and three months than treatment with topical voriconazole (1%) and topical natamycin (5%) alone.

- To determine whether patients treated with voriconazole have a different re-epithelialization time when compared to patients treated with natamycin. Similarly, we wish to compare re-epithelialization time between patients receiving both topical and oral voriconazole with those receiving only topical voriconazole.

- To determine whether voriconazole yields better best spectacle-corrected visual acuity outcomes than natamycin for particular fungal pathogens, in particular, for *Fusarium* spp. It is possible that any potential benefits of voriconazole vs. natamycin may be limited to particular fungal pathogens. Similarly, we wish to determine whether or not oral voriconazole together with topical voriconazole yields better outcomes than only topical voriconazole for particular fungal pathogens.

- To assess whether clinical outcomes, i.e., (a) best spectacle-corrected visual acuity, (b) infiltrate/scar size, and (c) re-epithelialization time, are correlated with *in vitro* measures of fungal susceptibility to the medication (the minimum inhibitory concentration or MIC).

1.2. Study Outcomes

1.2.1. Primary Outcome

MUTT I: BSCVA measured in logMAR at 3 months after enrollment, correcting for enrollment BSCVA and treatment arm in a multiple linear regression model.

MUTT II: Comparison of rate of perforation between the treatment groups (i.e., topical voriconazole and natamycin, vs. oral voriconazole and topical voriconazole and natamycin).

1.2.2. Secondary Outcomes

i. BSCVA measured in logMAR at 3 weeks after enrollment, adjusting for enrollment BSCVA and treatment arm in a multiple linear regression model

ii. BSCVA in logMAR only in Indian sites, 3 weeks and 3 months after enrollment, adjusting for enrollment BSCVA and treatment arm in a multiple linear regression model

iii. BSCVA in logMAR at 3 months after enrollment, adjusting for enrollment BSCVA and treatment arm in a multiple linear regression model, for MUTT II

iv. Hard contact-lens corrected visual acuity measure in logMAR at 3 weeks and 3 months after enrollment

v. Size of infiltrate/scar at 3 weeks and 3 months after enrollment, using enrollment infiltrate/scar size as a covariate.

Infiltrate/scar size will be calculated as the geometric mean of two principal axes in mm measured from the slit lamp examination at enrollment, 3 weeks and 3 months and by digital camera image at enrollment, 3 weeks, and 3 months after enrollment. Infiltrate/scar size will be assessed at 3 weeks and 3 months following enrollment, adjusting for enrollment infiltrate/scar size and treatment arm in a multiple linear regression model

vi. Time to resolution of epithelial defect.

Resolution of epithelial defect is defined as less than ½ by ½ mm (specifically, the largest diameter of the epithelial defect is less than 0.5 mm), as it is difficult to distinguish a smaller defect from the small amount of fluorescein staining seen in a healed defect. Time of epithelialization will be defined as the midpoint between the last observed date with an epithelial defect and the date of the first visit with no epithelial defect.

vii. Number of perforations and other adverse events

The number of adverse events (which include a toxic reaction to the study medication) and serious adverse events (which includes perforations) will also be compared.

viii. Minimum Inhibitory Concentration of isolates

vii. Microbiological cure at 7 days.

1.3. Study Design

The proposed study is a mixed block randomized, double-masked, controlled trial to determine which antifungal treatment is more effective in the treatment of fungal corneal ulcers.

368 patients presenting to the Aravind Eye Hospitals (Madurai, Pondicherry, Coimbatore) in India, and to the F. I. Proctor Foundation, University of California, San Francisco will be randomized to MUTT I and 240 patients presenting to the Aravind Eye Hospitals (Madurai, Pondicherry, Coimbatore, and Tirunelveli) in India, to the F. I. Proctor Foundation, University of California, San Francisco, to the Lumbini Eye Institute, Nepal, and to the Bharatpur Eye Hospital, Nepal will be randomized to MUTT II. In MUTT I, patients will be randomized to receive 1) topical voriconazole or 2) topical natamycin, and in MUTT II 1) topical voriconazole and topical natamycin with oral voriconazole or 2) topical voriconazole and topical natamycin with oral placebo. They will be followed closely (every 3 days +/- 1 day) until re-epithelialization, and then rechecked at 3 weeks and 3 months after enrollment.

Stratified, block randomization of the patients will ensure that an equal number of patients are randomized to each study arm at each study site. The exact block size will only be known to the biostatistics team at Proctor. A randomization list will be set up by the biostatistician (Travis Porco). Details are provided in the Statistical Analysis Plan.

2. Background and Significance

Infectious keratitis is the leading cause of monocular blindness worldwide. In some settings, fungal ulcers can account for as many as 50% of all corneal ulcers.[18] Various centers have reported that an increasing proportion of infectious keratitis is caused by fungal infection.[4, 19,20, 21] Treatment of fungal keratitis is generally more difficult than that of bacterial ulcers, and visual impairment can be more severe.[6]

Epidemiology of fungal keratitis in the United States. More than 30,000 people develop keratitis each year in the United States.[22] In the developing world, keratitis can occur at a rate 10 to 70 times as high and represents a major cause of monocular blindness.[23-25] Fungal keratitis occurs mostly in warm, tropical regions. The Bascom Palmer Eye Institute in Miami reported that from 1969 through 1997, fungi were identified in 16% of all the corneal ulcers in which a microbe could be isolated[26], and that between 1977 and 1982, 24% of non-contact lens related corneal ulcers were associated with fungal pathogens.[27] The Proctor Foundation isolated fungi in 8% of corneal specimens received between 1976 and 1999.[2] By contrast, at the New York Eye and Ear Infirmary between 1987 and 2003, only 1.2% of 5083 cases of corneal ulceration grew fungus in culture.[28] Wills Eye Hospital in Philadelphia identified only 24 cases of culture proven fungal keratitis at their institution between 1991 and 1999.[29]

In tropical countries as many as 50% of corneal infections are fungal. Srinivasan and colleagues reported that 47% of patients presenting with corneal ulceration to Aravind Eye Hospital had pure fungal infections.[18] In the less tropical climate of Nepal, Upadhyay and colleagues found 6.7% pure fungal isolates and 10% mixed bacterial and fungal isolates presenting to Tribhuvan University Teaching Hospital in Kathmandu.[24] The incidence of microbial corneal ulceration in Nepal and India is 10 to 70 times greater than in the United States.[23-25]

Risk Factors. Unlike bacterial keratitis, which is strongly associated with contact lens use in the United States, fungal keratitis has more often been associated with other risk factors for corneal ulceration, particularly trauma.[30] The Bascom Palmer Eye Institute reported an 8-fold lower rate of fungal keratitis among contact lens associated corneal ulcers than among non-contact lens ulcers (3% vs. 24%).[27] HIV infection[31] and ocular surface disease have also been associated with fungal keratitis in some studies.[28] In the developing world, fungal keratitis cases are overwhelmingly associated with trauma. At Aravind Eye Hospital, 92% of patients presenting with fungal corneal ulcers have a previous history of eye trauma.[32] In another large study from South India, 54% of patients with fungal ulcers reported a history of ocular trauma.[33] Thus, the association of fungal keratitis with ocular trauma and hot, humid environments has been well established.

Recent epidemic of *Fusarium* keratitis associated with contact lens wear. A large outbreak of keratitis due to *Fusarium* species in 2006 has caused many long held assumptions about fungal keratitis to be re-examined. The epidemic was first reported in Asia[34] and later in the United States.[3, 5, 12, 21, 35-37] There was a strong association with contact lens wear and Bausch & Lomb ReNu with MoistureLoc contact lens solution. The cases have not been restricted to the Southern United States, but have occurred throughout the Northeastern U.S. and other regions.[35] The etiology of this outbreak has still not been fully elucidated, but reports suggest that it is not due to inoculation of the cornea with solutions contaminated with *Fusarium* species during manufacture. This is supported not only by the lack of contamination found in the manufacturing site, but also by the isolation of multiple species of *Fusarium* during the investigation of the outbreak.[37] Both of these findings suggest that there was not a single source of contamination, but rather that fungal inoculation of the contact lenses occurred in the community, perhaps because of inadequate or improper use of contact lens decontamination protocols. This outbreak is troubling because it occurred among populations (contact lens users) and regions (the Northeast) previously thought to be at low risk for fungal keratitis. Many of the patients reported had severe visual loss and approximately 34% required corneal transplantation.[37] Finally, while there has been a reduction

of cases of fungal keratitis since the withdrawal of MoistureLoc solution from the market, some centers continue to see higher rates of fungal keratitis among contact lens wearers than historical norms suggesting that this problem may persist even in the absence of MoistureLoc solution.[38] In addition, it became evident during the epidemic that although there are more therapeutic options available for treating patients with fungal corneal ulcers, the lack of information on the safety and efficacy of newer antifungals made optimal treatment of patients difficult.

Fungal keratitis is more severe than bacterial keratitis. The recent epidemic of keratitis due to *Fusarium* species is of particular concern, because treatment of fungal keratitis is more complicated and less successful than bacterial keratitis. A higher rate of corneal perforation and corneal grafting has been observed in patients with fungal keratitis compared with bacterial keratitis.[39] In a series of patients from Taiwan with keratitis due to *Fusarium solani*, 16% required therapeutic keratoplasty.[40] In a study from Aravind Eye Hospital 15% of patients with fungal keratitis perforated, and 31% were classified as treatment failures, despite aggressive treatment with available antifungal medications.[41] Furthermore, studies comparing results of therapeutic keratoplasty for microbial keratitis have generally reported worse results with fungal keratitis than bacterial keratitis.[42-44] Finally, care for fungal keratitis is expensive. A recent study from Australia comparing different types of microbial keratitis found that fungal keratitis costs 2 or 3 times as much to treat as a bacterial ulcer.[30]

The microbiology of fungal keratitis varies with climate. Mycotic keratitis can be caused by a variety of fungi. There appears to be a relationship between climate and the type of fungi associated with keratitis. Yeasts such as *Candida* species are the more common organism isolated from fungal ulcers occurring in temperate climates whereas filamentous fungi are more common in more tropical climates. Overall, because the vast majority of cases of fungal keratitis occur in tropical countries, *Fusarium* and *Aspergillus* species are the predominant corneal fungal pathogens. Dimorphic fungi, by contrast, are not a significant cause of fungal keratitis. In Table 1, we summarize the results of several reports describing the etiology of fungal keratitis in different regions.

In addition to causing most cases of fungal keratitis worldwide, filamentous fungi are regarded as more virulent corneal pathogens than yeast.[45, 46] A study comparing *F. solani* to *Candida albicans* in rabbit corneas observed that *F. solani* spread more rapidly to the anterior chamber and produced more inflammation than *C. albicans.*[47] Investigation of this issue in humans is challenging because corneal infections with yeast and filamentous fungi do not occur in large numbers and in equal proportion in the same clinical settings.

Table 1. Etiology of fungal keratitis in different studies and regions.

Location	Years	*Fusarium* species	*Aspergillus* species	*Candida* species
Philadelphia, US[29]	1991-1999	25%	4%	46%
Miami, US[19]	1982-1992	62%	4%	13%
New York City, US[28]	1987-2003	10%	12%	67%
Hyderabad, India[33]	1991-2000	37%	31%	1%*
Tirunelveli, India[32]	1999-2002	43%	26%	0%

*Reported as "yeast"[33]

Medical therapy for fungal keratitis. Currently, classes of antifungal medications used for filamentary fungal keratitis include the polyenes, triazoles and echinocandins. Natamycin, a polyene, is the only topical ophthalmic antifungal medication approved by the United States FDA, and has long been

considered the drug of choice for filamentous fungal keratitis. However, for systemic diseases such as pulmonary aspergillosis, newer triazoles are now the recommended therapy.[8] Table 2 summarizes antifungals used topically to treat fungal keratitis.

Table 2. Antifungal medications used in the topical treatment of fungal keratitis

Polyene	Azole	Echinocandins
Binds irreversibly to ergosterol increasing cell wall permeability	Inhibits biosynthesis of a fungal ergosterol	Inhibits synthesis of beta (1,3)-D-glucan, a fungal cell wall component
Natamycin Amphotericin B	Itraconazole Econazole Voriconazole Posaconazole	Caspofungin

There are limited data comparing the efficacy of different proposed treatments for fungal keratitis. The only randomized clinical trial we are aware of comparing ocular antifungals was reported in 2003. 116 patients from Southern India with predominantly *Fusarium* and *Aspergillus* species fungal ulcers were randomized to treatment with either 2% econazole or 5% natamycin. No significant clinical differences were observed between the two treatment groups.[7] Another Indian study compared outcomes of two sequential series of 100 patients with fungal keratitis treated with either 1% itraconazole or 5% natamycin. *Fusarium*, *Aspergillus*, and *Curvularia* species were the most common isolates.[48] There was a better clinical response to natamycin in the patients with *Fusarium* species, but no difference was observed in the patients infected with other fungi. Outside of these studies, the literature regarding the treatment of fungal keratitis consists mostly of small clinical series and case reports.

There is evidence of the differential efficacy of natamycin compared to amphotericin in the treatment of corneal infection with filamentous fungi compared to yeast, even though they are both polyenes. Animal studies[49, 50] and clinical experience[51, 52] suggest that topical amphotericin is superior to natamycin in the treatment of *C.albicans.* On the other hand, many clinical series have established the effectiveness of natamycin in treating corneal infections with filamentous fungi.[7, 51-54] A meta-analysis of 138 patients from different published studies of keratitis due to *Fusarium* species showed a cure rate of 81% in patients treated with natamycin, but only 55% for amphotericin, 69% for ketoconazole, and 63% for keto/miconazole.[46] However, many of the patients with a so called "cure" are left with very poor vision. Natamycin is also efficacious against *Curvularia* species.[46] Another consideration in selection of an agent for the treatment of fungal keratitis is toxicity. Amphotericin is more toxic to the ocular surface than natamycin.[45] For this reason, the concentration used in ocular topical preparations of amphotericin has steadily declined since it was first used in 1959.[45, 55] Overall, natamycin is the most widely used topical antifungal agent because it is available in an ocular preparation, well tolerated by the ocular surface and active against the most common fungal keratitis pathogens, filamentous fungi.

Voriconazole is a new azole broad spectrum antifungal. Voriconazole is an antifungal, which like other azoles inhibits ergosterol synthesis via 14-alpha demethylase. It was FDA approved in 2002 for the treatment of invasive aspergillosis as well as salvage therapy for *Scedosporium* and *Fusarium* species. It has subsequently been approved for treatment of candidiasis and has become the treatment of choice for invasive filamentous fungi[14] and, with the exception of *C.glabrata*, it is more active against *Candida* species than amphotericin.[56] It has also been shown to be effective against fluconazole-resistant *Candida* species.[57]

In recent years there has been interest in the use of topical voriconazole on the cornea. A study of ocular fungal isolates from the Bascom Palmer Eye Institute in Miami demonstrated 100% *in vitro* susceptibility to voriconazole of *Fusarium, Aspergillus,* and *Candida* species.[58] None of the other antifungals evaluated

in this study were as broadly effective against these organisms. A 2007 study from India also reported voriconazole to have the broadest *in vitro* activity against corneal fungal isolates.[59] Given this activity against the major pathogens of fungal keratitis, it is a logical medication to consider as a first line therapy. There have been several clinical reports of successful treatment of keratitis caused by a variety of fungi with topical voriconazole, but no direct comparison with other antifungal strategies.[9, 11, 15, 60, 61] However, treatment failures have also been described in cases of fungal keratitis treated with 1% voriconazole even when the isolates showed low MICs to the drug.[62] Since there have been no randomized, masked studies comparing voriconazole with other antifungals, we lack the evidence necessary to determine the role of this promising medication in the treatment of fungal keratitis.

Unlike other antifungals such as amphotericin B, voriconazole is bioavailable after intravenous *and* oral dosing. According to a clinical pharmacy on line monograph "oral steady state plasma concentrations have ranged from 2.1 to 4.8 mg/L (peak) and 1.4 to 1.8 mg/L (trough). As noted earlier, combined topical and oral voriconazole produces levels of drug in the anterior chamber of the eye in excess of the MIC for most corneal fungal pathogens (Thiel 2007). There have been case reports showing successful treatment of fungal ulcers with combined topical and oral voriconazole, but this has not been sufficiently studied.

Voriconazole has excellent corneal penetration. The corneal epithelium poses a significant barrier to many potential ophthalmic antimicrobials. A recent report investigated the aqueous and plasma concentration of voriconazole in 5 patients with fungal keratitis receiving either 1% topical voriconazole alone or oral and topical voriconazole.[63] While the highest concentrations of voriconazole were detected in patients receiving both topical and oral voriconazole, even patients receiving topical therapy achieved anterior segment concentrations above the MIC of most *Candida* species.[63] The authors also note that this ability of voriconazole to achieve significant anterior chamber concentrations appeared to be independent of whether or not the epithelium was intact. They hypothesize that the relatively small molecular weight of the compound, 349 g/mol, as well as its lipophilic properties, favor penetration and retention in the cornea compared with other antifungals. By contrast natamycin (666 g/mol) and particularly amphotericin B (924 g/mol) are poorly absorbed by the cornea in the presence of intact epithelium.[64] Some cornea specialists advocate repeat epithelial debridement to enhance penetration of both natamycin and amphotericin, but the potential benefit from scraping has not been adequately studied. Because voriconazole likely dissociates from its hydrophilic carrier, sulfobutyl ether-beta-cyclodextrin, in the corneal stroma, the concentrations of voriconazole in the cornea are likely much higher than the aqueous chamber concentrations.[63] An earlier study in rabbit corneas infected with *P.lilacinus* keratitis detected the presence of voriconazole after topical administration in the cornea, vitreous and chorioretina of both infected and non-infected eyes.[10] Additionally, voriconazole associated corneal toxicity was not observed in non-infected eyes. Another recent report in humans undergoing cataract surgery demonstrated that voriconazole 1% eyedrops were well tolerated and penetrated into the human aqueous humor when administered at hourly or 6-hourly intervals.[65] The increased penetration of voriconazole compared to natamycin could be an advantage, particularly for corneal ulcers deep in the stroma. However, this theoretical advantage has not been shown to lead to better clinical outcomes than natamycin.

MIC and the treatment of fungal keratitis. The effectiveness of the antifungal agent is greatly affected by the substantially different concentrations of drug achieved locally.[66] In practice, the topical antifungal medications are given in different concentrations and this is not taken into account when comparing absolute MIC values. For example, natamycin is relatively well tolerated and commercially available in a 5% concentration, while amphotericin is typically prescribed at a concentration of only 0.15% due to toxicity.[66, 67] After correction for the available dose, natamycin has lower relative MICs than amphotericin for both *Fusarium* and *Aspergillus* species.[68] The major advantage of a triazole such as voriconazole over amphotericin is not so much lower MICs, but that the voriconazole can be given at a far higher concentration (typically ~6-fold higher). The adjusted MIC reveals that if penetration is not an issue, natamycin may be competitive with voriconazole for *Fusarium* species. As noted earlier, neither amphotericin nor natamycin penetrate well through an intact epithelium.[67]

Although susceptibility testing is used far more frequently for bacterial than fungal disease, fungal susceptibility testing is gaining credibility in the literature.[69] Bacterial studies have shown that antibiotic susceptibility results may be reasonably predictive of the therapeutic response rate in cases of bacterial keratitis. Wilhelmus *et al*. found that 91% of susceptible isolates from bacterial keratitis cases showed clinical improvement compared to 73% of resistant isolates.[70] MIC was significantly correlated with final infiltrate-scar size in our SCUT pilot trial on bacterial corneal ulcers.[71] In the non-ophthalmic literature, antifungal susceptibility testing has been found to be feasible and predictive of clinical outcomes: from a wide range of studies, infections due to susceptible isolates respond to therapy approximately 90% of the time, while infections due to resistant isolates respond 60% of the time.[69] In the ophthalmic literature, little has been done to investigate this question for fungal infections.

Relative efficacy of natamycin and voriconazole for the treatment of fungal keratitis. Given the recent epidemic in the developed world and the endemic state in the developing world, there is a critical need to provide ophthalmologists with the evidence necessary to best treat fungal corneal infections. Even with the epidemic subsiding, the issue regarding how to best treat fungal keratitis patients remains. There is a lack of evidence in the literature to guide optimal treatment. Currently, some clinicians go to great effort and cost to treat their patients with voriconazole without any certainty that it is safer or more effective than natamycin. We believe that a comparison of natamycin to voriconazole is a critical issue. Natamycin, a polyene, is well tolerated by the ocular surface and the only antifungal agent commercially available in an ophthalmic preparation. However, natamycin has limited efficacy against *Candida* species, a frequent corneal pathogen in temperate regions of the U.S. By contrast, voriconazole, an azole, is effective against both filamentous fungi and *Candida* species. Its small molecular weight and lipophilic characteristics enhances its penetration of the corneal epithelium. Despite these potential advantages, topical voriconazole must be prepared by a compounding pharmacist, and thus is not in wide use as an ophthalmic medication. MUTT I will help determine if substantial advantages over natamycin warrant its use. MUTT II will assess whether adding oral voriconazole to topical voriconazole confers additional benefit in terms of reduced perforations and improved clinical outcomes in patients presenting with severe ulcers. Both studies will also provide a large, standardized, prospectively collected database linked to a library of fungal isolates, which will allow future studies of fungal ulcer treatments.

3. Organization and Policies

3.1. Study Organization

3.1.1. Executive Committee

The Executive Committee will be co-chaired by Drs. Acharya and Lietman at the Proctor Foundation. This committee will act as the administrative and executive arm of the clinical trial and will meet once a month to provide overall oversight for the study and make decisions on day-to-day operation issues as described in the following:

- Monitor study progress and data collection process
- Discuss any quality control issues that have arisen in the Clinical Coordinating Center (CCC) and Data Coordinating Center (DCC)
- Evaluate and adopt changes in study procedures as necessary
- Communicate with and implement recommendations from the Data and Safety Monitoring Committee
- Make executive decisions on the allocation of resources
- Establish policies on publications and authorship
- Approve and oversee ancillary studies

3.1.2. Clinical Coordinating Center (CCC)

Nisha Acharya, MD MS from the Proctor Foundation will be responsible for directing the Clinical Coordinating Center (CCC). The role of the CCC includes oversight and coordination of the implementation of the trial at Aravind and at Proctor. Specifically, this includes functions such as maintaining an up-to-date manual of operations, obtaining human research approvals from Institutional Review Boards, conducting training and certification of all personnel (physicians, refractionists, photographers, etc.), supervising preparation and dispensing of study medication, ensuring proper masking and monitoring protocol adherence and recruitment.

The CCC will monitor the recruitment progress with weekly reports from each study sites. It will also organize periodic site visits (at least 2 times a year) to conduct chart reviews, training and certification of study personnel. Under the direction of the CCC, Dr. Salena Lee, the senior optometrist at the Proctor Foundation, will be responsible for training/certifying study refractionists and monitoring refraction protocol. Vicky Cevallos, the microbiologist at the Proctor Foundation, will train/certify lab personnel and conduct quality control in microbiology. The CCC will direct the Imaging Committee at Dartmouth College to train/certify study photographers and clinical graders and monitor the quality of clinical photography for the trial.

3.1.3. Data Coordinating Center (DCC)

Thomas Lietman, MD from the Proctor Foundation will be responsible for directing the Data Coordinating Center (DCC), which is responsible for supervising data collection, data management, data quality control, data analysis, event adjudication, and training and certification of study site staff in the data management systems. The DCC will be responsible for coordinating and supporting the activities of the Data Safety Monitoring Committee, including preparing interim and final data reports and organizing meetings with the Data Analysis Committee. The DCC will also be responsible for the dissemination of datasets for use by the Data Analysis Committee and other investigators.

Dr. Lietman will chair weekly meetings of DCC personnel, which include the biostatistician, Dr. Travis Porco, and the statistical programmer, Ms. Kathryn Ray, to monitor the progress and quality of data entry/management and address any issues. The DCC will be in close contact with the data analyst, Mr. Saravanan in India, for quality assurance of data entry.

3.1.4. Data and Safety Monitoring Committee

The Data Safety and Monitoring Committee (DSMC) will consist of independent experts in bioethics, biostatistics, epidemiology, ophthalmology, and international health care. It will also include members from both the United States and India.

The Committee will be empanelled prior to the beginning of the study. The committee will meet in person at least once per year, and will convene quarterly teleconferences for progress reporting. Ad hoc meetings as needed may also be convened. All study protocols will be subject to review and approval by Institutional Review Boards at the UCSF and Aravind, and by the DSMC. Committee members will monitor any severe or unexpected trend that threatens the safety of the patients. Stopping guidelines will be agreed upon prior to the start of the study, and the DSMC will be authorized to end the study if they deem necessary. Details are provided in the Statistical Analysis Plan.

3.1.5. Data Analysis Committee

Travis Porco, PhD, MPH will direct the Data Analysis Committee (DAC). The primary functions of the DAC include designing a Statistical Analysis Plan, preparing and distributing randomization lists, performing data analysis, and coordinating publications and presentations. The Committee is responsible for obtaining data from the Data Coordinating Center, performing unmasked data analysis and for preparing reports for the Data and Safety Monitoring Committee, and at the conclusion of the study, working with the Principal Investigators to analyze and publish the findings from the study.

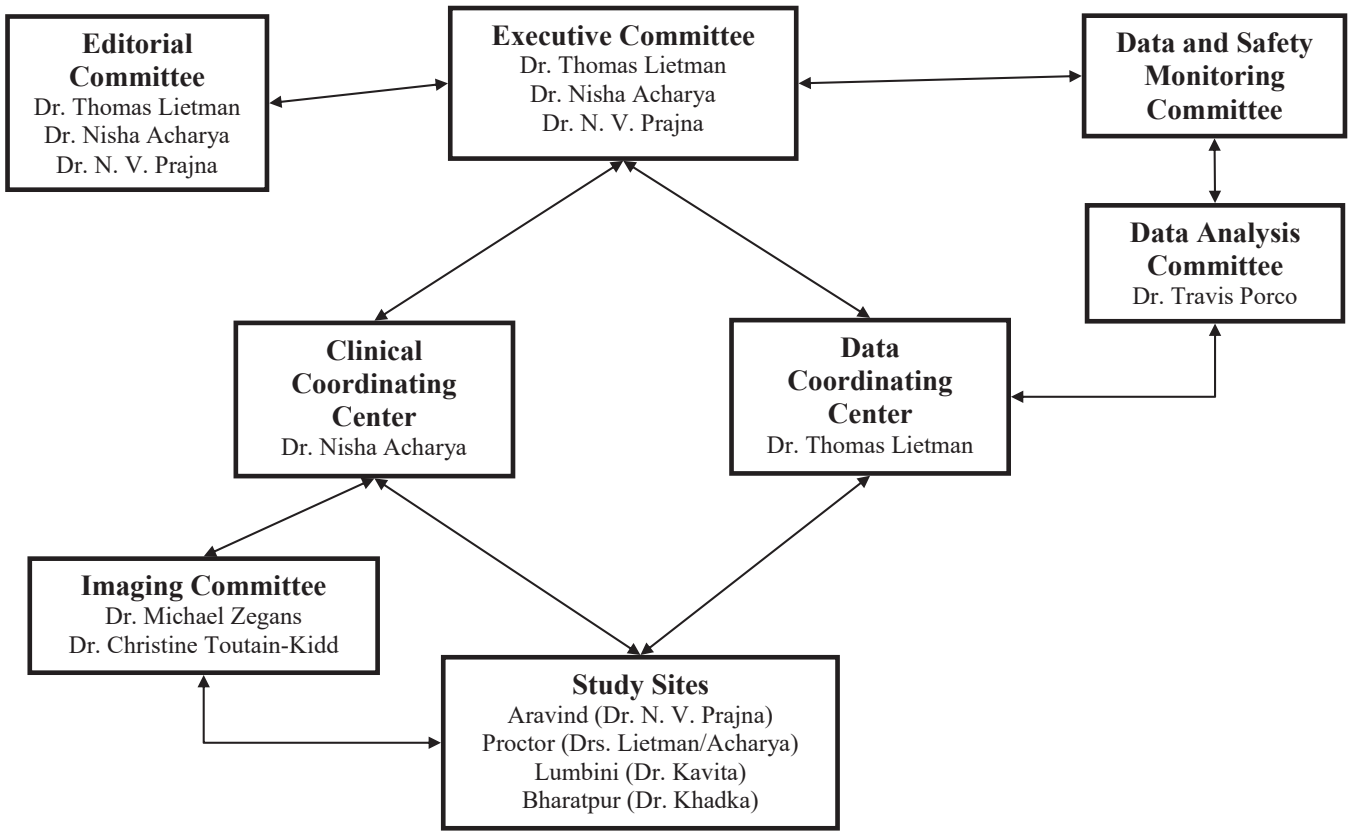
3.1.6. Imaging Committee

Michael E. Zegans, MD and Christine Toutain-Kidd, PhD will co-direct the Imaging Committee. The Imaging Committee will be responsible for implementing and monitoring clinical photography and for training photographers and graders to evaluate and archive the images. It also will serve as a resource for the image needs of study publications and presentations.

3.1.7. Editorial Committee

The Editorial Committee will be composed of the Principal Investigators. This committee has the responsibility to assist in the preparation of the primary study results and to review secondary manuscripts produced by the study investigators. Drs. Thomas Lietman, Nisha Acharya and N.V. Prajna have the responsibility to ensure the completion of the primary manuscript in a timely manner and direct its submission for publication.

MUTT Study Organization Chart



3.2. Collaborating Institutions

Aravind Eye Hospital, Madurai

Collaborating with the Clinical Coordinating Center at the Proctor Foundation, Dr. N. V. Prajna, Chief Medical Officer at Aravind Eye Hospital in Madurai, will be responsible for implementing the clinical content of the study in India. He will be assisted by a study coordinator, a microbiologist, ophthalmic assistants, 2 corneal fellows, 1 general ophthalmologist, refractionists and a data analyst.

Aurolab at Aravind Eye Hospital in Madurai will be responsible for preparing and distributing study medication and executing masked randomization.

The microbiology lab in Madurai will serve as a bank for all specimens collected at all Indian sites before being transported and archived at Dartmouth. It will also serve as a reference lab that will train microbiologists at other study sites in India and that will assist with any difficult microbiological identification.

Aravind Eye Hospital, Pondicherry

Dr. T. Krishnan will be the lead investigator and Dr. Rabindranath Reddy will be the co-investigator for this study in Pondicherry. Administration for the Pondicherry site will be done through Aravind Eye Hospital, Madurai, under the direction of Dr. N. V. Prajna.

Aravind Eye Hospital, Coimbatore

Dr. Revathi Rajaraman will be the lead investigator for this study in Coimbatore. Administration for the Coimbatore site will be done through Aravind Eye Hospital, Madurai, under the direction of Dr. N. V. Prajna.

Aravind Eye Hospital, Tirunelveli

Dr. Meenakshi Ravindran will be the lead investigator for this study in Tirunelveli. Administration for the Tirunelveli site will be done through Aravind Eye Hospital, Madurai, under the direction of Dr. N. V. Prajna.

Francis I. Proctor Foundation

Proctor Foundation will be the main coordinating center for the study.

Dr. Thomas Lietman and Dr. Nisha Acharya, the principal investigators of the study, will be assisted at the Proctor Foundation by co-investigators, a study coordinator, a microbiologist, a statistical programmer, and a statistician in the Department of Epidemiology and Biostatistics at UCSF. Their respective duties for the DCC and CCC are discussed in 3.1.2 and 3.1.3.

Dartmouth Medical School

Dr. Michael Zegans will be a co-investigator for the study. He will be assisted by Christine Toutain-Kidd, PhD, in overseeing the acquisition, analysis and archiving of digital images of the corneal ulcers for the trial. Dartmouth will also serve as the archiving center for all microbiological samples collected for the study.

Lumbini Eye Institute

Drs. Sushila Patel and Bel Thapa will be the lead investigators for this study at the Lumbini Eye Institute, Shree Rana Ambika Shah Eye Hospital in Lumbini, Nepal.

Bharatpur Eye Hospital

Dr. Byanju Raghunandan will be the lead investigator for this study in Bharatpur, Nepal.

3.3. Duties and Responsibilities of Staff

3.3.1. Study Coordinator

- Ensure the execution of the study as per the protocol
- Arrange training of the lab technicians, ophthalmic assistants, and refractionists
- Coordinate with the collaborating centers
- Prepare weekly reports regarding recruitment and follow up progress
- Handle correspondence between centers
- Send reminders to study subjects for follow-up visits
- Make sure that appropriate patients are screened and enrolled in to the study (including obtaining appropriate assent/consent)
- Assist microbiologist, ophthalmic assistants, and refractionists for conformity to study procedures
- Verify data forms for completion and collect missing information
- Transfer study forms for prompt data entry
- Maintain stock of and dispense study medications
- Maintain IRB approval and renewals

3.3.2. Ophthalmologist

- Assume responsibility for the study in the absence of the Principal Investigator
- Responsible for enrolling study subjects
- Provide information for completing the clinical examination forms
- Obtain written consent from the subjects with the help of study coordinator
- Initiate study medication as per the randomization
- Responsible for the care of the patient throughout the course of the ulcer
- Treat patients with adverse events

3.3.3. Microbiologist

- Train and supervise laboratory assistants for all lab procedures concerning the study
- Perform microbiological tests and/or cultures to confirm the organism
- Fill out the Microbiology Report form
- Make sure all equipment is calibrated and maintained
- Maintain stock of laboratory reagents and supplies
- Follow standard laboratory procedures for the tests
- Maintain quality control measures as per approved standards

3.3.4. Data Analyst

- Develop data entry programs specific to the study forms under the supervision of DCC
- Monitor the flow of forms from the coordinator

- Supervise data entry operators for any errors or omissions
- Develop consistency checks
- Communicate with study coordinators and data entry operators to correct any mistakes in study forms and data entry
- Transfer data as and when requested by the DCC
- Back up all data appropriately

3.3.5. Data Entry Operator

- Enter all data from study forms when submitted by the study coordinator
- Verify inconsistencies in the forms and send them for correction to the study coordinator
- Perform double entry to ensure accurate recording

3.3.6. Biostatistician at Proctor

- Review data for quality control purposes
- Prepare Statistical Analysis Plan
- Prepare reports for DSMC
- Lock database at completion of study after database is cleaned
- Conduct both interim and final data analyses
- Prepare randomization list and distribute the list to a pharmacist and non-masked, senior physicians at the study sites.
- Prepare final database and codebooks for archives (at closeout of the study)
- Assist in preparation of dissemination of results, including publications

3.3.7. Ophthalmic Assistants

- Obtain patient information
- Measure preliminary vision
- Assist ophthalmologist in the clinical examination/enrollment of the subject
- Counsel and motivate patients to return for scheduled follow-up visits

3.3.8. Refractionists

- Record spectacle-corrected, best-corrected, and hard-contact lens over-refraction visual acuity
- Enter visual acuity on the study form

3.3.9. Ophthalmic Photographer

- Take clinical photographs for corneal ulcer cases enrolled per study protocol
- Ensure the timely transfer of photographs to the imaging center at Dartmouth

3.3.10. Ward Nurses (at Aravind Eye Hospitals)

- Provide basic care to study subjects being treated as inpatients
- Administer medication to the study subjects and record treatment compliance
- Request fresh study medications from the study pharmacy regularly

3.4. Policy Matters

3.4.1. Protocol Revisions during the Trial

Any changes to the protocol made during the course of the study must be approved by the Executive Committee and will be incorporated in the revised protocol and the MOP as Addenda. The changes should be submitted and approved by the IRB of all collaborating centers and by the DSMC.

3.5. Presentations and Publications

3.5.1. MUTT Authorship Policy

Papers Presenting Primary Results

- All papers will incorporate the standards documented in the CONSORT guidelines.
- Acknowledgements will include: Grant source(s) or NEI grant(s)
- Centers will be listed in order:
  The Aravind Eye Hospitals, Proctor Foundation, Dartmouth School of Medicine, Lumbini Eye Institute, Bharatpur Eye Hospital, Coordinating Center PIs and staff, and Executive Committee members
- DSMC members will approve the manuscript prior to submission and will be acknowledged in the manuscript.
- The first author of the primary manuscript will be from Aravind Eye Hospital.

Papers for Secondary Analyses or Ancillary Studies

- All papers will incorporate the standards documented in the CONSORT guidelines.
- Editorial committee as identified above
- For secondary analyses, authorship list will end with: “for the Mycotic Ulcer Treatment Trial” (see MUTT Group Authorship List)

Review of Manuscripts

No paper may be submitted without the approval of the Editorial Committee and the DSMC.

4. Patient Flow

4.1. Study Timeline

The target time period for completing the study is 4 years. 368 patients are targeted for enrollment in MUTT I and 240 in MUTT II, and it is expected that it will take approximately 3 years to enroll this number. Six months will be required for study set-up including assembling the DSMC, and 6 months will be needed to complete follow-up after enrollment is completed. The final six months of the study will be utilized to conduct the final analyses and to publish the results.

Enrollment Timeline and Goal

Date	**Planned Activity**			
June 2009	IND Approval			
July 2009	Finalize MOP, Indian State Department Approval and 1st meeting of DSMC			
August 2009	Final pre-study training and certification of graders, refractionists and photographers Aravind IRB approval, UCSF IRB approval			
February 2010	First enrollment			
October 2009 to April 2013 (MUTT I) or to January 2015 (MUTT II)	Enrollment goals by center			
	AECS* (all sites)	Proctor	Lumbini	Bharatpur
MUTT I	363	5	0	0
MUTT II	160	5	50	25
January 2015	Last Enrollment			
May 2015	Last 3-month follow-up visit			
Until January 2016	Analyze, publish, and disseminate results			

*AECS=Aravind Eye Care System

"Enrollment" is usually the first visit at the study institution where the patient meets the criteria. Note that typically patients are enrolled on the day of presentation, after all inclusion criteria, including

positive fungal smear (KOH wet mount, Giemsa or Gram stain) and negative Gram stain for bacteria, have been met. This is study Day 1.

4.2. Procedure Flowchart

Time point	Procedures
Enrollment	1. Patient history and visual acuity (uncorrected, pinhole or if needed, best spectacle-corrected) 2. Ophthalmic slit-lamp examination 3. Specimen collection by corneal scraping and microbiology testing of the specimen 4. Checking if the patient meets the inclusion and exclusion criteria 5. Written informed consent for enrollment in the trial 6. Enrollment and randomization to one of the treatment arms 7. Digital photography 8. Treatment (voriconazole or natamycin eye drops): continued for at least 3 weeks and then at the physician's discretion 9. Liver function test
1 day after Enrollment (or 1 day after scraping if Enrollment happens 1 day after presentation)	1. Measurement of best spectacle corrected visual acuity (baseline measurements). Note this is defined as the "enrollment" BSCVA.
Every 3 days (+/-1day) until corneal re-epithelialization	1. Slit-lamp examination *(after cleaning the patients' eyes)* 2. Replacement of the old medication bottles with the new bottles every 1 week +/- 2 days 3. Assessing patient compliance with treatment 4. Checking for and reporting any adverse events
6 days (±1 day) after Enrollment	1. Re-culture cornea
At re-epithelialization	1. Slit-lamp examination *(after cleaning the patients' eyes)* 2. Checking for and reporting any adverse events
2 weeks from Enrollment	1. Liver function test and voriconazole levels
3 weeks from Enrollment	1. Measurement of best spectacle-corrected visual acuity *(after cleaning the patients' eyes)* 2. Digital photography 3. Slit-lamp examination 4. Determine whether the medication should be continued per the

	physician's discretion 5. Assessing patient compliance with the treatment 6. Checking for and reporting any adverse events
3 months from Enrollment	1) Measurement of best spectacle-corrected visual acuity 2) Measurement of hard contact lens corrected visual acuity 3) Digital photography 4) Slit-lamp examination 5) Checking for and reporting any adverse events

4.3. Eligibility Requirements

4.3.1.1 Inclusion Criteria:

MUTT I

- Presence of a corneal ulcer at presentation
- Evidence of filamentous fungus on smear (KOH wet mount, Giemsa, or Gram stain)
- Visual acuity between 6/12 (20/40, 0.3 logMAR) and 6/120 (20/400, 1.3 logMAR), inclusive
- The patient must be able to verbalize a basic understanding of the study after it is explained to the patient, as determined by physician examiner. This understanding must include a commitment to return for follow-up visits.
- Willingness to be treated as an inpatient or to be treated as an outpatient and return every 3 days +/- 1 day until re-epithelialization and every week to receive fresh medication for 3 weeks
- Appropriate consent

MUTT II

- Presence of a corneal ulcer at presentation
- Evidence of filamentous fungus on smear (KOH wet mount, Giemsa, or Gram stain)
- Visual acuity worse than 6/120 (20/400,1.3 logMAR)
- The patient must be able to verbalize a basic understanding of the study after it is explained to the patient, as determined by physician examiner. This understanding must include a commitment to return for follow-up visits.
- Willingness to be treated as an inpatient or to be treated as an outpatient and return every 3 days +/- 1 day until re-epithelialization and every week to receive fresh medication for 3 weeks
- Appropriate consent

4.3.2. Exclusion Criteria

MUTT I

- Impending perforation
- Evidence of bacteria on Gram stain at the time of enrollment (see Section)
- Evidence of acanthamoeba by stain
- Evidence of herpetic keratitis by history or exam
- Corneal scar not easily distinguishable from current ulcer
- Age less than 16 years (before 16th birthday)
- Bilateral ulcers
- Previous penetrating keratoplasty in the affected eye
- Pregnancy (by history or urine test) or breast-feeding (by history)
- Acuity worse than 6/60 (20/200) in the fellow eye (note that any acuity, uncorrected, corrected, pinhole, or BSCVA 6/60 or better qualifies for enrollment)
- Acuity worse than 6/120 (20/400) or better than 6/12 (20/40) in the study eye (note that any acuity, uncorrected, corrected, pinhole, or BSCVA can be used for enrollment)
- Known allergy to study medications (antifungal or preservative)
- No light perception in the affected eye
- Not willing to participate

MUTT II

- Evidence of bacteria on Gram stain at the time of enrollment (see Section)
- Evidence of acanthamoeba by stain
- Evidence of herpetic keratitis by history or exam
- Corneal scar not easily distinguishable from current ulcer
- Age less than 16 years (before 16th birthday)
- Bilateral ulcers
- Previous penetrating keratoplasty in the affected eye
- Pregnancy (by history or urine test) or breast-feeding (by history)
- Known liver disease, including hepatitis or cirrhosis (Child-Pugh A-C)
- Liver function tests >2 times the upper limit of normal at baseline
- Acuity worse than 6/60 (20/200) in the fellow eye (note that any acuity, uncorrected, corrected, pinhole, or BSCVA 6/60 or better qualifies for enrollment)
- Known allergy to study medications (antifungal or preservative)

- Currently on contraindicated medication (see appendix for full list)
- No light perception in the affected eye
- Not willing to participate

4.3.3. Exclusion of Bacterial Keratitis

We will attempt to exclude bacterial ulcers from the study population, since a mixed infection requires additional anti-microbial treatment. Specifically, Gram stain at enrollment (and the bacterial culture if available) must be negative for signs of bacteria.

Bacterial cultures will be kept for 1 week from collection. If they show positive bacterial growth after enrollment, the appropriate anti-bacterial will be added and the patient will remain in the study. If the treating physician suspects bacterial infection on a subsequent exam, they may perform repeat cultures.

4.4. Enrollment

4.4.1. Eligibility Evaluation

No single clinical sign can identify a fungal ulcer. All ulcers will be carefully examined at the enrollment visit to identify any of the classical signs of keratomycoses, as described by Kaufman[72]. Although these are not enrollment criteria, they will be recorded out of interest and for secondary analysis:

1. Feathery edges: branching lines in the corneal stroma radiating from, and extending beyond, the margin of the ulcer. Usually precede the appearance of satellite lesions.
2. Satellite lesions
3. Elevated lesions: the entire lesion, or large areas of it, may be elevated well above the surrounding cornea, and may have a rough, granular surface.
4. Hypopyon: due to severity of the reaction. Usually sterile. Often waxing and waning.
5. Endothelial plaque: Dense white endothelial plaque.
6. Corneal Ring: White ring in the mid-periphery of the cornea (dense aggregate of polymorphs, eosinophils and plasma cells).

At enrollment (Day 1), all patients will receive a corneal scraping (section 6.2) for a KOH wet mount for fungus and Gram stain for bacteria. If KOH is not available, Giemsa or Gram stain will be allowed to evaluate for fungal elements. Only corneal ulcers with a positive smear for filamentous fungus on KOH wet mount, Giemsa or Grain stain and with a negative Gram stain for bacteria will be entered into the trial.

Ulcers known at enrollment to be mixed on the first smear will be excluded. A recent survey of microbial keratitis in South India revealed that only 3.4% of cases were of mixed bacterial and fungal growth[18]. Also, it is known that 95% of fungal ulcers can be identified with KOH smear alone[27], and the vast majority of the remaining fungal keratitis pathogens can be identified successfully in culture within 36-48 hours[73].

Once the patient is diagnosed as having a fungal corneal ulcer proven by a fungal smear (e.g. KOH preparation, Giemsa and/or Gram stain) without evidence of bacterial co-infection on Gram stain and fulfills all the inclusion/exclusion criteria, he/she becomes an eligible subject for enrollment in the study.

4.4.2. Consent and Randomization

Consent and assignment of randomization takes place after all inclusion criteria, including positive fungal stain and negative gram stain for bacteria have been met. The ophthalmologist and/or the study coordinator obtain written consent from the subject after explaining the nature of the study, randomization, and the potential benefits and risks of participating in the study. The subject is assured that participation in this study is voluntary and he/she can withdraw at any time if he/she feels uncomfortable. After obtaining the written consent, the patient is assigned the next identification number from the randomization list.

As a part of the consent process, it should be clearly determined if a patient can either stay in the hospital for 3 weeks or he/she is committed to come back to the hospital every 3 days (+/- 1 day) for 3 weeks and treated as an outpatient (see Section 4.4.3)

At enrollment before treatment begins, all participants will receive a liver function test.

4.4.3. Hospitalization

The standard of care at the Aravind Eye Hospitals is to hospitalize all fungal corneal ulcers. For this study, all willing patients will be treated as inpatients until they are re-epithelialized or until 3 weeks from enrollment, whichever comes first.

During the treatment as inpatients, the study participants will be examined per the Study Forms Completion Schedule, Appendix A, Form A.1, to monitor re-epithelialization and stromal infiltrate/scar size. The ophthalmologist records these findings on the Clinical Examination Form, Appendix A, Form A.3, after each examination.

Study patients at Aravind are discharged once re-epithelialization has occurred. If a patient has healed within 3 weeks from enrollment, the patient can be discharged and come back every 7 days for fresh medication until the end of 3 weeks. Note the medication schedule for the study drug should continue regardless of re-epithelialization.

If a patient refuses to be treated as an inpatient but shows interest in participating in the study, the patient will be enrolled and treated at an outpatient. Prior to consent and enrollment, it will be clearly communicated with the patient that he/she will need to come back to the hospital every 3 days +/- 1 day until re-epithelialization.

If re-epithelialization takes longer than 3 weeks, the patient may be discharged at 3 weeks and continue to be treated as an outpatient at the physician's discretion.

4.4.4. Treatment

Once the patient has been randomized to a treatment arm on the day of enrollment (Day 1), they will start to receive the study medication per the timeline in Medication Dispensing Schedule (Section 5.3) until 3 weeks from enrollment. After 3 weeks, anti-fungal drops may be continued if deemed necessary by the treating physician.

Topical medication will be kept preferably in a refrigerator or in a cool, dark place and changed every 1 week +/- 2 days.

The following changes in the medication should be recorded in Medication Change Form (Appendix A, Form A.10):

- Stopping Study Medication
- Change of topical antifungal medication (after stopping study medication)
- Addition of topical antifungal medication
- Addition of systemic antifungal medication
- Addition of antibacterial medication
- Addition of other topical or systemic medication

4.5. Follow-up Visits

After re-epithelialization, the patient will be followed up at 3 weeks and at 3 months from enrollment.

The patient will be advised not to take any additional topical ocular medications during the follow-up period of 3 months, and to contact the study coordinator at their study center if such a medication is advised by medical personnel.

4.5.1. Scheduling Visits

Follow-up visits will be scheduled for the patient. The follow-up dates are calculated from enrollment. Also, the signs and symptoms of worsening of the ulcer will be explained to the patient. The patient will be instructed to return to the study center immediately if any of these occur.

4.5.2. Follow-up Reminders

Subjects will be reminded of follow-up visits by letters or phone calls. Subjects may be contacted directly by field workers if there is any concern that phone calls or a letter would not be adequate. It may be appropriate to send a field worker to the house of the individual, particularly if phone service is not available. A minimum of two reminders will be made to motivate the subjects to return for follow-up visits.

Subjects are allowed a time period to qualify as a 3-week follow-up (2.5 weeks to 5 weeks), and for the 3-month follow-up (2.5 months to 5 months).

If the patient fails to come for a follow-up visit, effort should be made to make the patient aware that it is still important to return for future visits.

4.5.3. Treatment Compliance

For inpatients, the administration of study medication will be performed and recorded by the ward nurses (Form A.10, In-patient compliance form).

For outpatients, compliance to study medication will be monitored by asking them how many doses they may have missed (Form A.10, Out-patient compliance form). Subjects will be asked to return their used drop bottles when they come back to get new bottles of medication (every 1 week +/- 2 days for 3 weeks) and during the follow-up visits, if applicable. During these visits, they will be specifically asked if anybody else used the bottles of study medication.

4.6. Adverse Events and Patient Dropout

4.6.1. Adverse Outcomes

Adverse Outcomes include Adverse Events, both Non-serious and Serious, details of which may be found in Appendix A, on Forms A.13 (Adverse Events) and Serious Adverse Events.

In case of an Adverse Event (AE), the Adverse Event Form, A.13, is to be completed and signed by the investigator. Any significant study drug-related adverse events will be reported by the DSMC to the Drug Controller of India and the FDA, as appropriate.

In case of a Serious Adverse Event (SAE), the Serious Adverse Event Narrative Form will be completed by the Investigator and submitted to the Medical Monitor, Dr. Stephen McLeod, within 24 hours of the SAE. Information recorded on this form will include the nature of the event, date of onset, date of resolution, date of notification to Medical Monitor, and action taken. The form will be reviewed, signed and forwarded to the DSMC by the Medical Monitor.

Any elective procedure or surgery that does not qualify as an adverse event should be recorded in the Medication Change Form (Appendix A, Form A.12).

4.6.2. Patient Death

Any patient death that occurs during the study will be reported by the study center's Study Coordinator to the Medical Monitor within 24 hours. A copy of the death certificate and hospital discharge summary (if available) will be maintained by the Data Coordinating Center. The study arm of any deceased patient will be made available to the DSMC by the UCSF statistician if appropriate.

4.6.3. Criteria for Stopping the Study Drug

If the study eye develops any of the serious adverse outcomes, the study medication (antifungal) may be discontinued if it is felt to be responsible for the adverse event, and the patient may be treated at the discretion of the study investigators without breaking the randomization code. Even after the study medication is stopped, patients will not be withdrawn from the study. They will continue with scheduled follow-up examinations.

The stopping of study medication and any consequent change or addition of medication should be recorded in the Medication Change Form, Appendix A, Form A.12.

4.6.4. Criteria for Drop-out from the Study

Subjects will be considered to have dropped out from the study only if they declare that they are no longer interested in further participation or are deceased. It should be noted that missing a visit does NOT mean a patient has dropped out of the study. For example, if they miss the 3-week visit, they are still requested to come in at 3 months. Also, an adverse event or discontinuation of study medication does NOT mean a patient has dropped out of the study. They should continue to be followed in the same manner. Positive bacterial culture after enrollment does not satisfy the criteria for drop out.

In the case of patient drop-out, the Patient Dropout Form should be completed.

5. Study Medication

5.1. Contents of Study Medication

Natamycin and voriconazole will be acquired from the US manufacturers, Alcon and Pfizer, respectively.

Topical voriconazole (VFEND® I.V., Pfizer, New York, NY) will be prepared as a 1% solution. A vial of VFEND® I.V. containing 200 mg of voriconazole will be reconstituted with 18mL of sterile water for injection preserved with 0.01% benzalkonium chloride and QS to 20mL under aseptic conditions. The resulting 1% voriconazole solution will be aliquoted to opaque glass containers, which will be sealed air-tight (3mL/container).

Natamycin (Natacyn®, Alcon, Fort Worth, TX) will be repackaged in the identical container (3 mL/container) as the one used for voriconazole to ensure the masking of study medication.

200 mg oral voriconazole (VFEND® Tablet, Pfizer, New York, NY) will be used as a clinical supply with matching placebo tablets.

Study medications must be refrigerated until use and discarded after 14 days from reconstitution and/or repackaging per USP 797.

Sample preparations of natamycin and voriconazole will be assayed for activity at enrollment, and brought back from India 1/3 of the way through the study and at the conclusion of the study.

All medications used in the study are prepared according to the FDA Good Manufacturing Practice Guidelines.

5.2. Medication Packaging and Labeling

All of the eye drop bottles will have identical labeling and color and will be opaque to mask the patients and study personnel.

The side of each dropped bottle will be covered by the opaque black electrical tape. Extra caution will be taken to cover the narrowing neck of the bottle and a few millimeter of the bottom as much as possible (the electrical tape is stretchable and can accommodate uneven surface). The top of the dropper nozzle and the bottom of the bottle are covered by opaque round stickers (the diameter ~19mm). Then the warning label is affixed. Before distribution to a patient, a label with patient random ID will be affixed to the side of the bottle.

Oral medications (voriconazole and placebo) will have the same physical appearance.

5.3. Medication Dispensing Schedule

Antifungal treatment will begin in all patients at the time of enrollment, after Gram stain, KOH/ Giemsa stain, cultures, slit lamp examination and baseline photography have been performed.

If eligible for entry into MUTT I, the patient is masked and randomized to receive voriconazole or natamycin. One drop of the randomized medication will be applied to the affected eye every one hour while awake for 1 week, and then every 2 hours while awake until 3 weeks after enrollment.

Further continuation of antifungal treatment will then be at the discretion of the physician. For all the arms, patients will be discouraged from using any topical drops not prescribed by the study physician.

All antifungal medications will be kept preferably in refrigeration or in a dark, cool place. Topical medications will be switched with fresh bottles every 7 days +/- 2 days.

Study participants who meet eligibility criteria for the MUTT II will be randomly assigned to one of two oral treatment groups:

- Oral voriconazole twice daily for 3 weeks or until discontinued because of toxicity

or

- Matching placebo twice daily for 3 weeks or until discontinued because of toxicity

The oral arm will be dosed according to the following weight-based schedule:

Weight (kg)	Loading (mg) q 12 x 24 hrs	Maintenance (mg) q 12 hrs x 20 days
<40	**200**	**100**
40 to 50	300	150
>50	400	200

Patients randomized to the placebo arm will receive weight-based dosing placebos that are identical to the weight-based dosing voriconazole tablets.

- Drug will be given 1 hour before or after meals.
- Patients will be instructed to avoid grapefruit or its juice because of its ability to interact with the cytochrome P450 system.
- Patients will be instructed to avoid alcohol consumption while on the medication.

To ensure the safety of the patients, liver function tests will be performed at baseline and at 3 weeks from enrollment. The serum at the 3-week follow-up will be also used to measure the concentration of voriconazole available in the bloodstream.

Metabolism of voriconazole occurs in the liver via the cytochrome P450 enzymes 2C19, 2C9 and 3A4. It is felt that the CYP2C19 is a major contributor to voriconazole metabolism, explaining about 49% of single-dose variability in one study[74]. Review of literature on genetic polymorphisms in the CYP2C19 enzyme Southern India shows a single published study[75]. Interestingly, though the rate of CYP2C9 was in between the rate for Caucasian and Chinese populations, the frequency of 12.6% for CYP2C19 genotype (predicting poor metabolzers) is higher than other major populations studied to date. This suggests the possibility that the population in this study may have a relatively high number of subjects who metabolize voriconazole more slowly as compared with other populations previously studied. The clinical significance of these genotypic differences is unclear; one study reported no relationship between this polymorphism and hepatotoxicity, but another suggests a possible association[76, 77]. There are no current guidelines for altering doses in different populations due to genetic polymorphism frequency. Nevertheless, due to highly variable voriconazole metabolism in all treated patients, most experts will follow drug levels to minimize toxicity and maximize efficacy in patients receiving longer courses of voriconazole for serious infections. The duration of treatment in this trial is not long enough to warrant therapeutic drug monitoring; however, patients will be closely monitored for clinical toxicity. Voriconazole will be stopped and levels will be obtained in the event of any concern for toxicity. Trough levels will also be obtained at the end of three weeks.

In India, most patients will receive all medication use prior to re-epithelialization as an inpatient and the medications will be administered by the ward nurses. The drug administration will be recorded by ward nurses as indicated on the In-Patient Compliance Form, found in Appendix A, Forms A.10.

If a patient is being treated as an outpatient, he/she will be instructed to keep all topical antifungal medications in a refrigerator or in a dark, cool place and replace it with a new bottle every 7 days +/- 2 days until 3 weeks from enrollment. Note that patients are required to be seen every 3 days +/- 1 days from enrollment until re-epithelialization. The patients will also be instructed that they alone are to receive the study eye drops, and that the study medication may actually harm eyes with other conditions. Patients receiving oral medication will be given 7 days' worth of medication at a time, and will be required to return for new medication every 7 days for 3 weeks. When a patient comes back to the clinic to get fresh medication, he/she will be asked how many doses he/she may have missed, which will be recorded on the Out-Patient Compliance Form found in Appendix A, Forms A.10.

5.4. Adjunctive Therapies

Use of the following medications will be allowed but is not expected to influence the trial's results.

- Cycloplegia: Patients may receive homatropine hydrochloride 2-5%, or a related mydriatic, at the discretion of the physician.
- Hypotensive agents: If at any time during the trial interval, intraocular pressure becomes elevated to greater than 25 mm Hg and the physician feels a hypotensive agent is justified, betaxolol hydrochloride 0.5% (Betoptic) solution will be added, to be used one drop twice daily to the affected eye. If additional therapy is needed or if the patient cannot tolerate Betoptic, other topical or oral medications such as acetazolamide 500 mg twice daily will be added.
- Pain medication: Oral acetaminophen (1g every six hours) may be used as needed for pain control. Additional medications to treat pain may be used according to the physician's discretion.

Adjunctive medicines will be purchased from the US manufacturer, or produced by the Aravind Eye Hospitals Pharmacy, an accredited pharmacy that makes all the topical medications used by the five Aravind Eye Hospitals in South India.

5.5. Side Effects and Drug Interaction

The most frequently reported adverse events in the therapeutic trials of systemic voriconazole were visual disturbances, fever, rash, vomiting, nausea, diarrhea, headache, sepsis, peripheral edema, abdominal pain, and respiratory disorder. Thirty to 40% of patients receiving systemic voriconazole experience photopsias, photophobia or color vision changes. These effects are dose dependent and often resolve with ongoing dosing. These side effects consistently resolve within 14 days of discontinuation of the medication and no long term visual disturbances have been reported (Walsh NEJM 2002). If a patient develops visual changes while on the oral medication the patient will be encouraged to stay on the treatment unless the visual changes are not tolerable. The treatment-related adverse events which most often led to discontinuation of voriconazole therapy were elevated liver function tests, rash, and visual disturbances.

One case of conjunctival chemosis and hyperemia, thought to be allergic in nature, has been reported with the use of natamycin. Ophthalmic natamycin suspension has been used for the treatment of fungal keratitis for many years and no other frequent adverse events are known.

5.6. Management of Medication Toxicities for MUTT II

Introduction:
It is important to note that many of the original clinical trials of voriconazole were performed in medically ill patients who were commonly treated with iv voriconazole for months. Our patient population will likely have a lower rate of medical co-morbidities, will not be receiving iv therapy and will only be treated for 3 weeks. Thus we anticipate that the toxicity rates discussed below may represent an over-estimation of what our patients will experience. However, the effect of differential metabolism due to genetic differences in CYP enzymes is unknown.

Visual changes:
30 to 40% of patients receiving systemic voriconazole experience photopsias, photophobia or color vision changes. These effects are dose dependent and often resolve with ongoing therapy. These side effects consistently resolve within 14 days of discontinuation of the medication and no long term visual disturbances have been reported[78].

- If a patient develops visual changes while on the oral medication, the treating physician will monitor the patient. As in prior trials, the medication will be discontinued at the discretion of the treating physician. The decision to stop the medication will be based on the significance of the side effects, the tolerability and/or progression or regression over time. Each patient typically has differing effects and tolerability of the effects; thus, no standard protocol will be formulated.

Headache:
3-4 % of the patients treated with voriconazole develop headache. Headaches will be treated symptomatically and medication will be continued unless the headaches become so severe as to necessitate discontinuation.

Dermatologic Reactions:
Reported in up to 6% of patients treated with voriconazole. These are generally mild. If patients develop a rash, they should be monitored closely and consideration given to discontinuation of VFEND. It is recommended that patients avoid strong, direct sunlight during VFEND therapy as photosensitivity reactions have been reported.

Anaphylactoid Reactions:
Rare. Associated with i.v. infusion and unlikely to be an issue with oral therapy.

Renal Failure:
Rare complication of voriconazole. This has been associated only with the i.v. formulation of the drug and *not* the oral formation (it is due to a component of the i.v preparation). Patients with clinical indications of renal failure will be evaluated by an internist, medication will be given unless there is concern by the internist.

Liver Failure:
10-20% of patients taking voriconazole have been reported to have elevation of hepatic enzymes and/or bilirubin, as with other azoles. This effect is generally dose dependent and reversible with discontinuation of therapy. Hepatic failure is rare. As noted above we will screen by history and liver function tests to exclude patients with significant pre-existing liver function abnormalities. Liver function testing will also occur at the conclusion of therapy and with concern for toxicity. Patients developing jaundice or other clinical signs of liver dysfunction will be evaluated by an internist and if significant liver dysfunction is confirmed voriconazole will be discontinued.

Dizziness: 1-3% of patients. Not anticipated to be severe. Discontinuation is at the discretion of the treating physician depending on the clinical severity of the problem.

5.7. Monitoring of Medication Toxicities for MUTT II

5.7.1. Clinical monitoring

Patients will be monitored clinically by the MUTT clinicians. Any concern for medication toxicity will be referred to the internist.

5.7.2. Laboratory monitoring

As noted above, labs will be monitored at baseline and completion of the three weeks of medication. For any clinical concern, additional labs will be ordered at the discretion of the internist.

5.7.3. Notification of MUTT researchers/masking

MUTT clinicians will be masked to the laboratory results unless clinically necessary. Upon completion of the three weeks trial, if the MUTT researchers elect to prescribe oral voriconazole therapy, they will contact the internist to get authorization to begin oral voriconazole. The internist will continue to follow any patient that is prescribed oral voriconazole.

The MUTT study medical monitor will be informed of any concern for toxicity and will report to the DSMC as per protocol for Adverse Event reporting. An adverse event form will be completed by the treating physician.

5.8. Safety Monitoring

Patients will review their medication tolerance at each clinic visit. A member of the investigation staff will be on call at all times to address any concerns regarding medications or other issues. A Data Safety Monitoring Board (DSMB) will meet regularly to review medication tolerance, efficacy, safety and toxicities.

5.9. Adverse Event (AER) Reporting

The PI and co-investigators will monitor the progress of the trial. Monitoring will also consider factors external to the study when interpreting the data, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.

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Investigators are required to report serious adverse events to the sponsor, IRB's and any other relevant body. Investigators are required to notify the Institutional Review Boards depending on the requirements of each reviewing institution if a patient has an adverse event or serious adverse event. The FDA will also be informed of any relevant adverse events.

Reporting requirements and procedures depend upon: (1) whether agents are suspected of causing the adverse event, (2) whether the possibility of such an adverse event was reported in the protocol, consent form, or manufacturer's literature (expected or unexpected adverse event), (3) the severity or grade of the adverse event, (4) the phase of the study and attribution (the determination of whether an adverse event is related to a medical treatment or procedure). All reactions in a "reportable" category must be reported.

There are reported drug interactions with systemic voriconazole, many due to CYP450 induction. Therefore, voriconazole should be avoided or is contraindicated when taking rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine. Monitoring for adverse events is recommended when taking oral contraceptives. Patients on sulfonylurea oral hypoglycemics will have their blood glucose monitored closely and dosing adjustment if necessary.

Drug Interactions with oral voriconazole:

- Rifampin
- Carbamazepine
- Long acting barbiturates
- Terfenadine
- Astemizole
- Cisapride
- Pimozide
- Quinidine
- Sirolimus
- Rifabutin
- Ergot alkaloids (egotamine, dihydroergotamine)
- Phenytoin
- HIV Protease Inhibitors
- NNRTIs
- Cyclosporine
- Tacrolimus
- Phenytoin
- Warfarin
- Omeprazole
- Benzodiazepines
- Statins
- Calcium Channel Blockers
- Vinca alkaloids

There is no reported drug interaction of significance with the ophthalmic natamycin suspension.

5.10. Study Medication Accountability

An inventory of the medications along with the date of expiration will be maintained by the Study Coordinator at all study centers. Overall management and ordering of study medication will be supervised by the Clinical Coordinating Center. All the study medications will be transported and stored according to manufacturer's guidelines. In the clinic, it will be kept separately from other medications.

6. Examination and Procedures

At the enrollment and follow-up visits, the following procedures will be performed as described in the Procedure Flowchart (Section 4.2) and the results from each procedure will be recorded as described in the Study Forms Completion Schedule (Appendix A, Form A.1).

6.1. History

At the enrollment visit (Day 1), the examining ophthalmologist will obtain the demographic details (such as name, age, sex, occupation and address) and the relevant history from the patient, and record it in the Patient History Form (see Appendix A, Form A.2). In addition to the demographic information, a clinical history will be taken to elucidate risk factors for corneal ulceration.

6.2. Specimen Collection for Microbiological Tests

After patient history and slit lamp examination at the enrollment visit (Day 1), corneal scraping is performed to determine the eligibility of the patient (positive fungal stain and negative bacterial stain, see Section 7.1). Corneal scraping is recommended as standard of care for all corneal ulcer patients at the Aravind Eye Hospitals. There is a small risk to patients of inducing a perforation, worsening an epithelial defect, or contaminating an ulcer, and patients are duly informed of these risks prior to the taking of the corneal scrape.

Corneal scraping will be obtained as follows:

- A drop of topical anesthetic (0.5% tetracaine or 4% lidocaine) is administered to the eye to be examined.
- Aseptic technique is used to obtain each corneal scrape. A flame-sterilized Kimura spatula is used, with the aid of slit lamp magnification, to obtain a scrape from the leading edge and base of the corneal ulcer. The Kimura spatula is again flame sterilized between the takings of each sample.
- Two scrapings are smeared directly on to two separate glass microbiology slides for Gram stain and for KOH wet mount (if necessary, Giemsa or Gram Stain can be used to identify fungal elements as well) (see Section 7.1.2).
- Three further scrapings are taken and directly inoculated on to sheep's blood agar, chocolate agar, potato dextrose agar or Sabouraud's agar for bacterial and fungal culture (see Section 7.2).

6.3. Refraction and Visual Acuity Procedure

6.3.1. Refraction Procedure

Beginning Approximate Refraction

The beginning approximate refraction should be obtained by performing retinoscopy or auto-refraction. One of these measurements is used as the beginning approximate refraction at each visit.

Refraction may be initially performed at distances different than four meters (i.e. using a phoropter in a common refraction lane). However, if this is done, the **spherical power refinement step** must be repeated with lenses in place after the subject has been **positioned at four meters** for visual acuity testing.

The examiner will use Chart R for manifest refraction. Each eye is refracted at four meters unless the visual acuity measured at this distance on chart R is worse than 20/200 (defined as missing 2 or more letters on the top line, the 20/200 or 6/60 line). This subject must then be moved to a distance of one meter from the study visual acuity chart, and refraction must be performed and recorded at this distance. Use of Visual Acuity Form R is optional but may be helpful in keeping track of patient's acuity level.

Subjects who arrive for examination wearing contact lenses are asked to remove the lenses prior to refraction. The subject must wait for 30 minutes after removing the contact lenses before refraction can be performed.

Manifest Refraction

In general, instructions are to 'push plus' and to add minus diopter corrections only if there is a demonstrated increase in visual acuity, i.e., the patient is able to read more letters. The steps are as follows:

- Seat the subject 4 meters in front of the refraction chart;
- Place and adjust the trial frame on the subject's face so that the lens cells are parallel to the anterior plane of the orbit;
- Adjust the pupillary distance of the trial frame to make sure that the lenses position in front of the centers of the pupils;
- Adjust the lens cells for the proper distance from the cornea;
- Occlude the eye not being refracted;
- Insert spherical lens correction into the compartment closest to the eye;
- Place cylindrical lens correction in the compartment in the front of the frame;
- Set cylindrical lens to appropriate axis setting

The following example demonstrates the steps used for a patient with visual acuity of 20/80 or 6/24 or better. **Please refer to the table below to make appropriate lens changes when working with patients with worse acuity.**

Vision with Best Correction	**Sphere**		**Cylinder Use correct JCC as below**			**Sphere Refinement**	
	Power	Increment	Axis	Power	Increment	Power	Increment
6/3-6/24	+0.50	+0.50	0.50	0.25	+0.25	+0.37	+0.25
20/10-20/80	-0.37	-0.25			-0.25	-0.37	-0.25
(4 meters)	+0.50	+0.50				+0.37	+0.25
6/30-6/60	+1.00	+1.00	1.00	1.00	+1.00	+0.50	+0.50
20/100-20/200	-1.00	-1.00			-1.00	-0.50	-0.50
(4 meters)	+1.00	+1.00				+0.50	+0.50
<6/60	+2.00	+2.00	1.00	1.00	+1.00	+1.00	+1.00
(<20/200)	-2.00	-2.00			-1.00	-1.00	-1.00
(1 meter)	+2.00	+2.00				+1.00	+1.00

Determine Sphere Power

- Ask patient to identify the orientation of the tumbling "E"s on the smallest possible line that he/she can see on the Chart R;
- With the subject looking at the smallest line legible on the visual acuity chart, place a +0.50 spherical lens in front of the eye being tested. Ask the subject, "Is it better, worse, or no change?"
- If the subject responds that vision is made better or is the same, replace the spherical lens with one that is +0.50 more plus;
- Continue checking to see if the subject will accept more plus by repeating the step above;
- When an additional +0.50 lens makes the subject's vision worse, remove the +0.50 lens, then hold a -0.37 spherical lens over the tested eye;
- If this lens improves the subject's vision, even by one letter, replace the spherical lens by one that is -0.25 more minus.
- If the -0.37 spherical lens does not allow the patient to read more letters or if it makes the vision worse, move on to cylindrical testing.

Determine and refine cylinder axis (The following descriptions are for plus cylinders. Adjust accordingly for minus cylinder refraction.)

- Ask the subject to look at a letter on a line of Chart R which is one line larger than the smallest line he/she could read;
- **If no cylinder** is present in the beginning approximate refraction, place the +/- 0.50 diopter cross-cylinder with the positive axis first at 90º, then at 180º, then at 45º and 135º;
- Ask the patient to determine if vision is better with or without the placement of the +/- 0.50 diopter cross-cylinder at each of the four major meridians;
- If the subject prefers none of the four positions, no further testing is required;
- If the subject states that the vision is improved at any one of these four axis positions, place a + 0.50 cylindrical lens in the trial frame at the preferred axis and proceed to refine the axis;
- **If cylinder is present** in the beginning approximate refraction or if you have added +0.50 cylinder power in the above step, position the +/- 0.50 diopter cross-cylinder first with the positive axis 45º to the right of the cylinder axis (position one), and secondly with the positive axis at 45º to the left of the cylinder axis (position two). Ask the subject which position improves the vision (position one or position two?);
- If the subject responds that neither position is better and if this was the first test of axis position, move the axis of the cylinder in the trial frame 15º to the right or left and repeat;
- If the subject prefers one position to the other, rotate the cylinder toward the preferred positive axis of the cross-cylinder in the step sizes recommended below and repeat. (If the subject states that one position of the cross-cylinder is no better than the other position, proceed to refining cylinder power).

AXIS STEP SIZES FOR REFINEMENT OF CYLINDER

Cylinder Power	Axis Step Sizes
< 1.00 D	10°
1.00 to < 2.00 D	5°
2.00 to < 3.00 D	3°
3.00 to < 5.00 D	2°
5.00 to < 8.00 D	1°

Refine cylinder power as follows:

- Ask the subject to look at the lowest line on the visual acuity chart which can be read;
- Align the ± 0.25 diopter cross-cylinder first with the positive axis and then with the negative axis coincident with the cylinder axis. Ask the subject which is better;
- If the subject prefers the negative axis coincident with the cylinder axis, decrease the power of the cylinder in the trial frame by 0.25 diopter;
- If a subject indicates a change that would introduce negative cylinder power, remove all cylinder power and continue testing for positive cylinder power at an axis 90º away from the previous axis;
- If the subject prefers the positive axis coincident with the cylinder axis, increase the power of the trial frame by +0.25 diopter and retest;
- Beginning with cylinders of 1.00 diopter or more, for each 0.5 diopter change in cylinder power, adjust the sphere by 0.25 diopters of the opposite power. This is to maintain spherical equivalent.

End the refraction by challenging with +0.37 and -0.37 spherical lenses and adjust the sphere until the subject responds that additional positive sphere makes the vision worse. Remember always to end the refraction by checking with plus power.

Remember to refer to the table on page 38 to ensure that you are refracting with trial lenses appropriate to patient's level of acuity. Remember to check visual acuity as lenses are added. If visual acuity improves significantly, lenses appropriate to the level of acuity need to be used.

- **If refraction cannot be performed at 4 meters**, defined as missing 2 or more letters on the largest line, then the subject should be moved to 1 meter and the above refraction sequence followed. **At 1 meter, a + 0.75 spherical lens is added** to the beginning approximate refraction to adjust for the accommodative difference between 4 and 1 meter.

- **Record the lens correction** obtained in this refraction for the right eye on the appropriate examination forms in the section for visual acuity measurements (Appendix A, Form A.7).

Hard Contact Lens Over refraction

In our study, visual acuity will be measured with hard contact lens refraction at the 3-month study visit. The procedure for hard contact lens refraction is described below.

Corneal curvature measurements will be obtained using either a manual keratometer (B&L type) or auto-keratometer (Topcon KR 8800). The mean of the keratometer readings in the two major meridians will be calculated. If using an auto-keratometer, pre-calculated average may be used.

A rigid lens with a base curve corresponding to this average measurement will be selected from a set of trial rigid lenses provided by the study center/Proctor group.

One drop of anesthetic (0.5% proparacaine or 4% Lidocaine) will be instilled into the study eye. This selected lens will then be inserted on the study eye along with a drop of conditioning solution (Boston Simplus or AMO Total Care or equivalent conditioning solution). The contact lens will be allowed to settle for a period of three to five minutes.

Slit lamp examination will subsequently be performed to ensure that the contact lens covers the entire diameter of the pupil and that the lens surface is wetting properly. A penlight may be used if a slit lamp is not readily available.

- If the lens is noted to be decentered, sodium fluorescein may be instilled to assess whether the lens is too flat or too steep. The initial lens should then be removed and a lens with a 1.00 diopter change in base curve should be used. These steps should be repeated using 1.00 diopter step changes in base curve until full pupil coverage is achieved.
- If the lens surface is noted to be not wetting properly, the lens should be removed and cleaned thoroughly and reinserted for evaluation as described above.

If the lens has been determined to have a proper fit, retinoscopy must be performed over the contact lens. The resultant spherical or sphero-cylindrical retinoscopy result will be placed in a trial frame. Trial frame refraction will be performed according to the guidelines described at the beginning of this section. Visual acuity will be measured following refraction according to the guidelines outlined below.

6.3.2. Visual Acuity Procedure

Visual acuity measurement is necessary for our primary and secondary outcomes in this trial. Therefore, we have created a standardized method that aims to minimize any bias from either the refractionist or the patient. This has been adapted from the Visual Acuity protocols from the Age Related Eye Disease Study (AREDS 1999)[79], and the Aravind Eye Hospital's Practical Guide to Refraction[80] and is the same method we are currently using in the NEI funded Steroids for Corneal Ulcers Trial.

Visual Acuity Charts and Illumination

Visual Acuity Charts

The following equipment is used: a set of three charts, which are modified Bailey-Lovie charts, Charts S, A and R (Precision Vision Chart 2305, 2305a and 2305b, respectively). Each chart consists of 14 lines of high-contrast "tumbling E's". There is a geometric progression of letter size (and, thus, an arithmetic progression of the logarithm of minimum angle of resolution) from line to line.

Chart S is for measuring aided visual acuity of the study eye at all visits; Chart A is to be used for measuring visual acuity of the fellow eye at the enrollment visit and hard contact lens acuity at the 3-month visit. Chart R will be used for obtaining all manifest subjective refractions.

Visual Acuity Boxes

The dimensions of the light box are 24 and 3/4 inches (62.9 cm) by 25 and 3/4 inches (65.4 cm) by 7 inches (17.8 cm). The box can be mounted on a wall or on a cylindrical stand. The stand is mounted on a five-pronged wheelbase, with each prong about 14 inches (35.6 cm) long; two of the five wheels are lockable. When the box is mounted on the stand, its height can be varied. The light box should be mounted at a height such that the top of the third row of letters (0.8 logMAR, 45 letters, 20/125 Snellen) is 49 +/- 2 inches (124.5 +/-5.1 cm) from the floor. The rear of the box provides storage space for the two charts not being used.

Illumination

The overhead room lights should be turned **off** during the visual acuity test. The box itself provides sufficient illumination for the examiner to record the test results. Additional light can have an adverse effect such as glare. With the box light off, not more than 15 foot-candles of light should fall on the center of the chart. The visual acuity light box is equipped with two **20-watt** fluorescent tubes. Because the illumination of fluorescent tubes diminishes by 5 percent during the first 100 hours and by another 5 percent during the next 2,000 hours, new tubes should be kept "on" for about 4 days (approximately 96 hours, does not have to be continuous) before use. All tubes should be replaced once a year.

Each tube is partially covered by a 14-inch (35.6 cm) fenestrated sleeve, open in the back, which serves as a baffle to reduce illumination. Each sleeve should be centered on the tube such that an equal length of tube (about 4 and 3/16 inches or 10.6 cm) is left uncovered to the right and left of the sleeve.

4- and 1-Meter Visual Acuity Lanes

A distance of 4 meters (13 feet and 1.5 inches, or 157.5 inches) is required between the subject's eyes and the visual acuity chart for the 4-meter test, and a distance of 1 meter (39 and 3/8 inches) is required for the 1-meter test. The room for visual acuity testing must have, in addition to the 4-meter lane, space for the visual acuity box (and possibly a stand) and space for the subject.

- Wall-mounted box: In addition to the 4-meter lane, 7 inches (17.8 cm) must be allowed for the depth of the box plus space for the subject to sit or stand.

- Stand-mounted box: In addition to the 4-meter lane, 13 inches (33 cm) must be allowed for two of the stand's casters to touch the rear wall (or a line marked on the floor when there is no wall) plus space for the subject to sit or stand.

Marking the Distance

4 Meters

1. If the chair and visual acuity box are permanently affixed, distance measurements need to be made only once and no floor marks are needed to ensure the correct distance.

2. If the box is mounted on the wall but the subject's chair is not permanently affixed, the 4-meter distance of the subject's eye from the chart must be marked clearly and permanently.

3. If the box is mounted on a movable stand, the 4-meter distance must be marked clearly and permanently. The location and orientation of the box must be rechecked each time a new chart is put in place or the box is touched. When the stand touches the rear wall of the room, two of the five casters should touch the wall.

1 Meter

The 1-meter distance is measured from the eye of the subject, who is seated comfortably in a chair with his or her back firmly placed against the chair's back, to the center of the second (for testing the left eye) or fourth letter (for testing the right eye) of the third line of the chart. A stick, one meter long, should be used to confirm the distance for each subject.

Visual Acuity Procedure

1. Visual acuity will be measured several times throughout the course of this study.
2. It is very important that the examiner is not aware of patient's clinical records, in order to minimize bias. Therefore, the visual acuity examiner will not have access to the patient's previous clinical examination or treatment results.
3. Visual acuity is tested separately for each eye (one eye at a time). The patient's untested eye will be completely covered with a patch, to block out all light from entering this eye. The examiner must constantly ensure that this eye remains occluded at all times.
4. The patient is instructed to read each letter on the chart starting with the largest line, at the 4-meter distance.
5. The patient will be asked to state the orientation of each letter and show the orientation of the tumbling "E" with the hand that they are not using to occlude the untested eye. If the patient cannot identify the orientation of a letter, they are encouraged to guess. Only one response is allowed per letter.
6. The examiner is NOT allowed to inform the patient of an incorrect response.
7. The examiner must ensure that the patient does not squint (creating a pin-hole effect) or lean forward (reducing the distance to the chart).
8. Once a patient has given a response for a letter and has moved on to provide a response for the next letter, any corrections of previous response will not be accepted.
9. If the subject gives two possible responses for a letter, tell the patient to commit to one answer. The examiner CANNOT, at any time, give the patient any indication as to whether a response is correct or incorrect.
10. If 3 or less letters are identified correctly on any row from Row 3 or below, STOP testing on that row.
11. In the case that the patient reads less than 10 letters at 4 meters, either the patient or the chart will be moved so that there is a distance between the two of 1 meter.
12. Visual acuity will then be retested at this 1 meter distance.
13. If less than 10 letters are read at 1 meter, then the examiner must proceed to Low Vision Testing, starting with Count Fingers testing. If the patient does not adequately Count Fingers (see below), proceed to Hand Motion. If the patient does not adequately recognize Hand Motion (see below), then proceed to Light Perception.

Testing of Count Fingers Vision

In testing for count fingers vision, the examiner's hand presenting 1, 2, or 5 fingers is held steady at a distance of ½ meter directly in front of the eye being examined. The fellow eye is completely occluded with a patch. Refractive correction should not be used. A light should be shown directly on the hand from behind the subject and room lights should be turned on. The examiner's fingers should be presented in random order and repeated 5 times. Eccentric viewing should be encouraged. If the subject correctly identifies three of the five presentations, then count fingers visions is noted. If not, then the subject must be tested for hand motion vision.

Testing of Hand Motion Vision

The examiner's hand with all fingers spread out should be extended ½ meter directly in front of the eye being examined. The fellow eye should be occluded with a patch. Refractive correction should not be used. A light should be shone directly on the hand from behind the subject and room lights should be turned on. The examiner's hand should be moved in an up-and-down direction (vertically) or in a side-to-side direction (horizontally) at a constant speed of approximately one back and forth presentation per second. The subject is instructed that the examiner's hand will be presented and they will have to respond to the question: "What am I

doing with my hand?". This should be repeated five times. Four out of five correct responses indicate that hand motions visions is present. If the subject does not correctly identify four out of five, then you must test for light perception.

Testing for Light Perception

The indirect ophthalmoscope is used as the light source for testing light perception. Room lights should be off. The opposite eye must be completely patched. No correction should be used. From a distance of ½ meter with the light source turned up to maximum intensity, the light from the indirect ophthalmoscope is directed into the subject's eye four times. The subject is asked to respond when the light is "on". Light Perception is recorded if the examiner is convinced the subject sees the light. Otherwise, the vision should be recorded as "No Light Perception".

Scoring Best Corrected Visual Acuity

On the Visual Acuity Forms, the examiner will circle all letters read correctly. Letters read incorrectly or not read at all will be left unmarked. At the end of each row of letters, the examiner will write down the total number of letters read correctly. Each box/underline will be filled out. If visual acuity was not tested at 1 meter, the examiner will indicate this on the form.

After each measurement of visual acuity, the biostatistician will calculate the score for the visit. The visual acuity score is defined as follows:

- If 10 or more letters are read correctly at 4 meters, the 4 meter visual acuity score is equal to the number of letters read correctly at 4 meters plus 30.
- If 3 or fewer letters of the largest line are read correctly at 4 meters, the visual acuity score is equal to the number of letters read correctly at 1 meter.
- If 3 or fewer letters are read at 1 meter, then low vision testing must be performed. (This result, count fingers, hand motion, light perception, or no light perception, will be used for the analysis by converting into a logMAR score according to Visual Acuity Calculation Table).

The highest attainable 4-meter visual acuity score is 100.

The Snellen equivalent, defined as the Snellen ratio corresponding to the most difficult line for which the subject read at least 4 of 5 letters correctly, will also be entered by the examiner on the 4-meter Visual Acuity Form A.7.

The data entry staff will enter the number correct on each row at 4 meters, and if applicable, 1 meter and low vision testing results (They will not enter which letters the examiner circled, i.e., which letters the patient identified correctly.). The biostatistician will calculate the visual acuity score and double check the data entered by the examiner and data entry staff by reviewing the original forms.

6.3.3. Visual Acuity Training and Certification

The goal of the certification process is to standardize methodology for refraction and visual acuity measurement. All visual function examinations must be performed by study-certified technicians. The principal investigator at each site is responsible for ensuring that the appropriate personnel are identified, trained and certified. A qualified visual function examiner from the Clinical Coordinating Center (Proctor Foundation) will certify the rooms and technicians at each site.

Technicians are expected to perform the refraction and visual acuity tests on at least one and possibly more non-study subjects according to protocol requirements. The examiner will determine whether or not the candidate executes the study protocol accurately for each procedure. A checklist (Visual Acuity/Refraction Certification Form, Appendix B, Form B.1) containing required procedures will be used to facilitate this process.

Room certification will be performed and recorded (Room Certification Form, Appendix B, Form B.2) to ensure that all study rooms meet illumination, equipment, and distance requirements. Logs should be kept to document dates of light box bulb replacement.

Certification is valid for a period 12 months (± 1 month) from the date of certification. The process should begin as soon as possible, as technicians must be certified before the first study subject is seen. **A minimum of two certified technicians** are required at each site.

The visual acuity measuring schedule may be found on the Study Forms Completion Schedule, Appendix A, Form 0.

6.3.4. Inter-observer Variation of Visual Acuity Measurements

Every 10^{th} patient at each center will be selected for monitoring inter-observer agreement on visual acuity measurements (i.e. patient 5, 15, 25, ...). For each selected patient, coordinators will arrange for two certified refractionists to measure visual acuity at enrollment and at 3-months independently and in a masked manner. We will compute the intraclass correlation coefficient (using 2-way ANOVA with random effects for patient and grader, to partition subject-level and grader-level variation, separately for the enrollment and 3-month visits).

VISUAL ACUITY CALCULATION TABLE

Snellen	LogMAR	Letters
NLP	2.0*	na
LP	1.9*	na
HM	1.8*	na
CF	1.7*	na
20 /800	1.6	5
20 /640	1.5	10
20 /500	1.4	15
20 /400	1.3	20
20 /320	1.2	25
20 /250	1.1	30
20 /200	1.0	35
20 /160	0.9	40
20 /125	0.8	45
20 /100	0.7	50
20 /80	0.6	55
20 /63	0.5	60
20 /50	0.4	65
20 /40	0.3	70
20 /32	0.2	75
20 /25	0.1	80
20 /20	0.0	85
20 /16	-0.1	90
20 /12.5	-0.2	95
20 /10	-0.3	100

Note: For values < 4, patients will be assigned the corresponding logMAR score for CF, HM, etc. For letter values ≥ 4, letters will be converted to logMAR for analysis using the formula 1.7-(letters correct)/50.

*As in Herpetic Eye Disease Study (HEDS)

6.4. Slit Lamp Examination

Slit lamp biomicroscopy will be performed by the examining ophthalmologist, using a Haag-Streit 900 slit lamp biomicroscope. Several of our secondary outcome measures are slit lamp-based observations: size of infiltrate/scar pre- and post-treatment, time until re-epithelialization, clinical signs of treatment failure (e.g. corneal perforation). To ensure accuracy of these observations, slit lamp examination will be standardized according to the protocol shown below. This protocol has been adapted from the Herpetic Eye Disease Study (HEDS) Manual of Operations and from the Steroids for Corneal Ulcer Trial (SCUT) Manual of Operations.

The examining ophthalmologist will record the following:

- Size of central epithelial defect in the affected eye
- Size of underlying stromal infiltrate/scar
- Depth of stromal infiltrate/scar
- Size of a hypopyon, if present
- Intraocular pressure (optional)

At enrollment visit, slit lamp examination also includes the clinical evidence of fungal ulcer (Appendix A, Form A.3).

At enrollment and the 3 month visit, dilated fundus examination will be performed with the slit-lamp and a 90 D lens.

Slit lamp examination will be performed as follows:

1. Turn the slit lamp on, with the transformer switch at 6.0 volts (position 3).
2. Examine the cornea in the standard way, using the 10X oculars, and the 1X and 1.6X magnification settings.
3. The size of epithelial defect and infiltrate/scar is documented (see Section 6.4.1). The depth of infiltrate/scar will be also recorded. Then the shape of the corneal epithelial defect and infiltrate/scar is drawn in the Clinical Examination Form (Appendix A, Form A.3).
4. Presence of a hypopyon will be recorded by measuring its greatest depth vertically from its limbal margin inferiorly, to its greatest height superiorly – the measurement of hypopyon should be rounded to the nearest 0.5mm.

6.4.1. Slit Lamp Epithelial Defect and Infiltrate/Scar Measurements

Measurements of the size of the corneal epithelial defect and the infiltrate/scar will be taken using both standard slit lamp examination, as well as photography combined with image analysis software (see Section 8). The slit lamp method of size estimation is described below:

1. The corneal epithelial defect is stained using fluorescein.
2. Set the axis of the illuminating arm parallel to 0^{o}, then fully tighten the locking knob to hold the arm in this position.
3. Fully open the light filtration knob, at the top of the illuminating arm.
4. To commence measurement of the epithelial defect/infiltrate/scar, set the slit beam height to the maximum (8.0mm), and the slit beam width to 1.0mm. The longest linear diameter of the epithelial defect is then measured by adjusting the height of the slit until it matches the inner borders of the fluorescein stained epithelial defect, or infiltrate/scar margin. This length is recorded on the ulcer drawing in the study record.

5. The slit illumination housing is then rotated by 90° so that the slit beam is now perpendicular to the length diameter just measured. The slit beam is moved along the long axis of the infiltrate/scar to the widest point. The slit height is again adjusted as in step 4, and the maximum perpendicular width of the epithelial defect/infiltrate/scar is measured.
6. The estimated area of the epithelial defect/infiltrate/scar is calculated by multiplying the longest diameter and the perpendicular width.
7. All measurements are read to the nearest 0.1mm (i.e. the limit of resolution of the Haag-Streit 900 slit lamp).

This method of estimation of corneal epithelial defect area using a slit lamp has previously been compared with digital photography plus image analysis. The results are very favorable, showing a high level of correlation between data obtained from the same patients using each of these methods in turn[81].

6.4.2. Intraocular Pressure (IOP) Measurement

At all visits, including re-epithelialization and at subsequent visits, IOP measurement will be done if needed. It will be at the discretion of the physician which method to use for the IOP measurement. The different methods that can be used are Goldmann applanation tonometry, tonopen, non-contact tonometry (e.g., Pulse-air method), digital palpation etc.

6.4.3. Diagnosis of Perforation

An important Adverse Event is corneal perforation. We define this as a corneal ulcer which extends through the full thickness of the cornea, extending into the anterior chamber. On slit lamp examination, the following signs are indicative of perforation:

- Flat anterior chamber
- Focal iris – corneal adhesion (in particular, iris plugging a corneal hole)
- IOP $\leq$ 4 mmHg
- Positive Seidel's test: a drop of high concentration fluorescein dye placed at the site of perforation is caused to fluoresce by the trail of leaking aqueous from the anterior chamber.

6.4.4. Certification of Clinical Grading

Slit lamp examination is an important portion of the study, particularly since infiltrate/scar size is a secondary outcome. Measurement of infiltrate/scar size is typically done differently by different physicians, so standardization will be necessary. All study physician examiners will be certified by the Clinical Coordinating Center at Proctor. Certification will be granted after site personnel successfully demonstrate their knowledge and proficiency. The certification will last 12 months.

Training for certification will include an interactive slide presentation (approximately 1 hour and 15 minutes). This will be followed by a test of 10 photographs, and examination of a live patient. Trainees are only certified if they demonstrate an understanding of several key points from the slide presentation (checklist filled out by examiners), grade a test set of 10 photographs adequately (with dimensions within 1mm of the gold standard examiner), and grade a live patient adequately (checklist filled out by examiners). The result of certification will be recorded in the Clinical Exam Certification Form is in Appendix B, Form B.3

6.4.5. Inter-observer Variation of Clinical Grading Measurements

Every 10th patient at a center will be selected for monitoring the inter-observer agreement on clinical grading (i.e. patient 5, 15, 25, ...). For each selected patient, coordinators will arrange for two certified examiners to measure infiltrate/scar at enrollment and at 3-months independently and in the masked manner. We will compute the intraclass correlation coefficient (using 2-way ANOVA with random effects for patient and grader, to partition subject-level and grader-level variation, separately for the enrollment and 3-month visits).

6.5. Masking

The masking of the medication each patient receives is particularly crucial in this trial since natamycin is a suspension and leaves a white residue whereas the ophthalmic preparation of voriconazole is a clear solution. Best efforts will be made to mask patients and all study personnel including clinical examiners, refractionists, and photo graders to maintain patient compliance and to prevent any bias in study outcomes.

The pharmacy:
To mask study coordinators and study personnel involved in study outcome evaluation, the preparation, distribution and dispensing of study medication will be compartmentalized. The independent pharmacy (Aurolab for Indian sites and Leiter Pharmacy for Proctor) will be responsible for preparing and distributing study medications based on the randomization list. The pharmacy will be instructed not to disclose the treatment assignment to any study personnel under any circumstances (in rare occasion of a medical emergency which warrants breaking the code, the physician will contact senior personnel, Dr. P. Namperumalsamy at Aravind or Dr. Todd Margolis at Proctor who have the back-up copies of the randomization list). The pharmacy will prepare the study medications in identical types of bottles that are opaque and distribute them directly to ward nurses/ophthalmic assistants. The oral medications will also appear identical.

The ward nurses/ophthalmic assistants:
For study subjects being treated as inpatients, ward nurses or ophthalmic assistants will dispense the medication to patients. They will be also responsible for requesting study medication directly from the pharmacy by providing the patient IDs. Ward nurses/ophthalmic assistants are independent from other study personnel and will not have any direct contact with any study personnel involved in study outcome evaluation such as clinical examiners, refractionists, and photography graders. All ward facilities are physically separated from study coordinator office, clinical examination rooms, refraction rooms, and photography room. It will be particularly difficult to mask ward nurses/ophthalmic assistants since they are responsible for dispensing the medication and can see the opacity of the medication being applied. They will not be intentionally given the information on which medication each patient is randomized to receive and they will be specifically instructed not to discuss the appearance of the medication with patients or any study personnel.

The patients:
Patients will not be told which medication they will receive in the trial. Masking of the patients will be done by dispensing the topical medications in the same type of bottles and by dispensing a placebo pill that looks identical to the voriconazole pill. The bottles will be opaque (to mask whether the medication is clear voriconazole solution or opalescent natamycin suspension), and will have the same color, size and appearance. In spite of great efforts to mask patients, they may notice the residue of natamycin on their eyes or lack thereof. If a study participant is treated as an outpatient and is responsible for dispensing the study medication herself/himself, the appearance of the medication may be more obvious. Patients will be specifically instructed not to discuss the

appearance of the medication they receive with any study personnel to prevent any accidental unmasking of study personnel.

The refractionists:
The refractionists checking the uncorrected and corrected visual acuity will be masked. They will not have an access to any patient records or the randomization assignment. They will be instructed not to ask the patients or other study personnel about the medication each patient receives. Before a patient is examined for visual acuity by a refractionist, a ward nurse/ophthalmic assistant will clean the patient's eyes regardless of the medication he/she is receiving with saline solution.

The clinicians and photo evaluators:
Natamycin is a suspension and it leaves precipitates on the cornea, which can be seen by the clinicians during the slit-lamp examination or by the photo evaluator on the photography. Although it is difficult to mask the clinicians and the examiners who evaluate the photos when the patient is on the medication, every attempt will be made to mask them. Clinical examiners and photo evaluators will not be given the information on medication assignment or will not have an access to the randomization list. Before the slit-lamp examination and the photography, a ward nurse/ophthalmic assistant will clean the eyes of each patient regardless of the medication he/she is receiving. Even if the clinician/ photo evaluator notices the precipitates, he/she will not record it or discuss it with other study personnel. At the 3-month examination, the patients will likely be no longer on study medication and therefore will not have precipitates. Thus the primary and secondary outcomes at 3 months will not be affected by this possible unmasking during the earlier visits.

6.6. Laboratory Monitoring

At baseline before treatment and at 3 weeks, we will perform liver function tests on all study participants. Serum will be drawn from participants and sent to the clinical lab for LFTs (AST, ALT). At 3 weeks, serum will also be used to estimate the voriconazole blood level. If LFTs are out of normal range at baseline, oral voriconazole will not be started and LFTs will be re-checked weekly until they normalize. If they normalize, oral medication can be started. If LFTs are out of normal range at 3 weeks, they will be re-checked weekly until they normalize.

6.6.1. Baseline tests prior to inclusion in oral voriconazole arm of study: AST, ALT, bilirubin (total), INR, and serum albumin.
6.6.2. Testing at conclusion of oral (placebo or voriconazole) treatment (3 weeks): AST, ALT, bilirubin (total) and voriconazole trough
6.6.3. Testing at the time of early discontinuation of oral treatment (e.g for toxicity): AST, ALT, bilirubin (total), and voriconazole trough, plus any testing deemed necessary by the evaluating clinicians
6.6.4. Voriconazole trough testing
6.6.4.1. Sample collection
6.6.4.1.1. Timing of collection: Trough sample will be collected near the time of usual dosing for the patient (optimally within an hour of when the patient takes a dose).
6.6.4.1.2. Collection procedure
Will depend on whether serum or plasma used – depending on the lab chosen
2.6.5. Lab values will be considered abnormal if the fall outside of the range of normal based on the standards of the clinical laboratory at which they are obtained.

7. Microbiology Laboratory Procedures

7.1. Microbiology Inclusion Criteria

The following microbiology criteria need to be fulfilled for the patient to be included in the study:

- Evidence of fungi on 10% KOH wet mount (or on Giemsa or Gram stain)
- No evidence of bacteria on Gram stain

7.1.1. Specimen Collection for Microbiological Tests

Corneal scraping will be obtained as follows:

- A drop of topical anesthetic (0.5% tetracaine or 4% lidocaine) is administered to the eye to be examined.
- Aseptic technique is used to obtain each corneal scrape. A flame-sterilized Kimura spatula is used, with the aid of slit lamp magnification, to obtain a scrape from the leading edge and base of the corneal ulcer. The Kimura spatula is again flame sterilized between the takings of each sample.
- Two scrapings are smeared directly on to two separate glass microbiology slides for Gram stain and for KOH wet mount (if necessary, Giemsa or Gram Stain can be used to identify fungal elements as well) (see Section 7.1.2).
- Three further scrapings are taken and directly inoculated on to sheep's blood agar, chocolate agar, potato dextrose agar or Sabouraud's agar for bacterial and fungal culture (see Section 7.2).

7.1.2.Smear for KOH wet mount and Gram and Giemsa stains

Corneal material that has been collected with a sterile blade or platinum spatula is placed on two clean glass slides. One slide will be used for each of the following preparations.

10% potassium hydroxide (KOH) wet mount

This is a rapid method for observing fungal elements in a temporary preparation.

- A drop of 10% KOH is added to corneal material, the slide is covered with a glass cover slip and allowed to stand for 10 minutes. Host cells are partially digested by the alkali leaving the polysaccharide-containing fungal cell walls. Slide is examined with a microscope under low power and reduced light. Preparation is considered positive when fungal elements are seen.

Gram Stain

This stain is recommended for the differential staining of bacteria in primary specimens and from culture. Fungal elements can also be detected with this stain.

- Slide is air dried then fixed with heat or methanol. Gram stain reagents are applied following standard protocol and examined microscopically under an oil immersion lens at 1000x for white cells, bacteria, and other structures. Gram positive bacteria stain purple and gram negative bacteria stain pink. Fungal hyphae have variable staining characteristics and are usually seen as negative outlines. Interpretation of smear should take into account that bacteria comprising the normal flora of the conjunctiva and tear film may be detected in small numbers.

Giemsa Stain

This stain is for general differentiation of inflammatory cells, epithelial cell morphology and also for identification of bacterial, chlamydial, and fungal organisms.

- Smear is air dried then fixed with methanol and again dried. It is then covered with diluted Giemsa stain for 1 hour. The slide is then rinsed rapidly in 95% ethyl alcohol to remove excess dye, dried, and examined under an oil immersion lens at 1000x. All bacteria and fungi appear dark blue in color.

7.2. Cultures for Fungi and Bacteria

Specimens will be collected for bacterial and fungal cultures on the enrollment day (Day 1). The patients who are negative for bacteria on Gram stain and enrolled due to a positive fungal stain but later grow bacterial colonies on culture will NOT be dropped from the study. Appropriate antibacterial medication will be added and the patient will continue to be followed in the study.

7.2.1. Fungal Cultures

Incubation of fungal cultures will be in a 25^{o}C incubator or at room temperature. All fungal cultures will be read daily, with a formal result being reported at 7 days. Identification of any fungus grown will be done using gross and microscopic characteristics, as described in Proctor's previous study in conjunction with Aravind's microbiology laboratory[18].

Criteria for a positive fungal culture follow the standard definition[82]:

- Growth on any two media, or
- Moderate to heavy growth on one medium

Routine quality control measures should be in place to ensure standardization of all fungal media and techniques (see Section 7.7 and Section 12.1).

7.2.2. Bacterial Cultures

Bacterial cultures will be done as described in the *Manual of Clinical Microbiology* by the American Society for Microbiology (7th Edition)[83].

Incubation of all bacterial cultures will be at 37^{o}C with 5% CO_2, with controlled humidity. Cultures are considered negative if there is no growth after 7 days.

Bacterial cultures are considered positive if:

- Growth of same organism on two or more solid media
- Growth on a least one solid medium, at the site of inoculation, plus identification of the organism of appropriate morphology and staining characteristic on gram stained corneal smears.
- For the organisms *S. epidermidis* and diphtheroids, culture will be considered positive, only if moderate growth is seen on at least two solid media, plus identification of the organism of appropriate morphology and staining characteristics on gram stained corneal smears (in order to rule out possible contamination of normal flora from the lids)

Routine quality control measures should be in place in Aravind's Microbiology Laboratory to ensure standardization of all media and techniques (see Section 7.6 and Section 12.1).

7.3. Fungal Susceptibility Testing

Fungal susceptibility is determined according to methods outlined in Clinical and Laboratory Standards Institute documents M38-A and M27-A2[84, 85].

The inoculum is prepared by overlaying mature slants with sterile distilled water and gently scraping the surface with a wooden applicator stick. The suspension is permitted to sit for 5 minutes to allow large particles to settle out. The suspension is then adjusted spectrophotometrically to the correct optical density for each species as outlined in M38-A, providing an inoculum concentration of 2.5–5 x 10^4 conidia/mL, which is verified by colony count (plate 0.01mL of 1:100 dilution of the adjusted inoculum on Sabouraud agar, incubate it at 28°C to 30°C and observe daily for the presence of fungal colonies).

Broth macrodilutions is created by adding 0.1 mL of 10x concentrated drug being test to 0.9 mL of inoculum. The growth control receives 0.1 mL of 10-fold of the drug diluent without antifungal agent and is inoculated with 0.9 mL of the inoculum. For the test medium, RPMI-1640 medium (with glutamine, without bicarbonate, and with phenol red as a pH indicator) will be used voriconazole and antibiotic medium 3 for natamycin[84-88]. Tests are incubated at 35°C without agitation until growth is visible in the drug-free control tube.

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antifungal agent being tested that exhibited a 100% visual reduction in turbidity when compared with the control tube for natamycin at 48 hours[85]. An 80% reduction in turbidity is considered the end point for the voriconazole[85].

7.4. Laboratory Results Reporting

All microbiology results will be reported on the Microbiology Form (see Appendix A, Form 0) as soon as the formal result becomes available. This is typically at 24-48 hours for bacterial cultures, and 36-48 hours for fungal cultures (final formal result at 7 days). The stain results should be reported immediately.

A separate microbiology report form will be filled out each time a culture is performed.

7.5. Repeat Cultures

Cultures will be performed on all patients at enrollment. Repeat smear and cultures will be performed at 7 days post-enrollment. If the study physician feels that repeat cultures are necessary for treatment because the ulcer is worsening or because new signs have emerged, they may re-culture at any time. A second microbiology form will be filled out and become part of the study record.

Repeat cultures will be collected by the same protocol as the enrollment culture, as follows:

- A drop of topical anesthetic (0.5% tetracaine or 4% lidocaine) is administered to the eye to be examined.
- Aseptic technique is used to obtain each corneal scrape. A flame-sterilized Kimura spatula is used, with the aid of slit lamp magnification, to obtain a scrape from the leading edge and base of the corneal ulcer. The Kimura spatula is again flame sterilized between the takings of each sample.
- Two scrapings are smeared directly on to two separate glass microbiology slides for Gram stain and for KOH wet mount (if necessary, Giemsa or Gram Stain can be used to identify fungal elements as well) (see Section 7.1.2).

- Three further scrapings are taken and directly inoculated on to sheep's blood agar, chocolate agar, potato dextrose agar or Sabouraud's agar for bacterial and fungal culture (see Section 7.2).

7.6. Sample Transportation and Storage

All microbiological samples generated from the study will be transported and archived at Dartmouth.

The isolate samples will be packaged and shipped as UN 3373 Biological Substance Category B according to the International Air Transport Association (IATA) Dangerous Goods Regulations (DGR). All fungal isolates should be transported on agar slants at room temperature.

For a short-term storage, the isolates will be stored as water stocks:

1. Label sterile vial with specimen number
2. Fill vial with approximately 3-4 ml of sterile distilled water.
3. Using a sterile applicator stick, remove a portion of the isolate and place in water.
4. Secure lid with parafilm
5. Store water in labeled boxes at room temperature.
6. When recovery is desired, use a swab to obtain specimen from water.
7. Apply swab to fresh agar surface.
8. Incubate until sufficient growth is noted.

For a long-term archiving for the samples, the isolated will be stored as freezer stocks:

1. Label appropriate medium with specimen number
2. Using a sterile applicator stick, remove a portion of the isolate and inoculate agar
3. Secure lid with parafilm.
4. Allow to grow at room until significant growth is noted (at least one week)
5. Transfer slants with mature colonies to -70° C. Store indefinitely.
6. When recovery is desired, remove frozen vial from freezer.
7. Using a sterile applicator stick, scrape surface to remove ice crystals
8. Apply crystals to fresh agar surface.
9. Incubate until sufficient growth is noted.

7.7. Laboratory Quality Control Measures

Procedures
All laboratories must have detailed written instructions for all procedures and quality control.

Quality control
Internal quality control measures should be in place to regularly examine media, biochemicals, stains, and temperatures (including incubators, refrigerators, etc.) to ensure standardization.

- 10% KOH: Reagent must be tested prior to use to confirm that clinical material dissolves.
- Gram Stain: Stain must be tested daily and when a new lot is used.
- Each new batch of user prepared media must be tested for sterility and performance.
- All reagents must be tested for performance according to manufacturer instructions.
- Function checks of all equipment must be documented.
- QC results must be documented on QC forms.
- All out of control results must be documented on QC forms and corrective action must be taken.

8. Photographic Protocol

Corneal status will be documented using digital photography at enrollment, 3 weeks, and at 3 months. The images will be captured using standardized protocols by study certified ophthalmic photographers. The original electronic images will then be transferred to the MUTT Study imaging center (Dartmouth Medical School) via the Internet. Independent, masked observers will determine the area of the corneal scar using "Optscore" software, created specifically to study pictures of cornea. They will also grade the density and vascularization of the lesion, inflammation of the conjunctiva, presence of a hypopyon, and evaluate the quality of the images. All images and evaluations will be archived.

8.1. Imaging Equipment

Digital photographs of each study cornea will be captured using a Nikon D series digital SLR camera with a 105mm f/2.8D AF Micro Nikkor Autofocus Lens and a modified SB29s electronic flash or equivalent.

8.2. Imaging Personnel

Aravind photographers will be trained in MUTT photographic and computerized image transfer protocols and certified after the demonstration of appropriate skills by the Imaging Committee under the supervision of Clinical Coordinating Center. Each photographer will attend group and individualized training sessions, generate 2 high quality sets of digital corneal images using the study photographic protocol, and successfully transfer their sample images to the Imaging Center.

8.3. Imaging Schedule

Patients will be photographed at enrollment, 3-week, and at 3-month follow-up visits.

8.4. Imaging Procedure

At enrollment, photography will be done after slit lamp examination/scraping but before the study medication is initiated on the day of enrollment (Day 1). If photography cannot be done as scheduled (i.e. a patient enrolled late in the evening or late on Saturday), the attempt should be made to take study photographs at the earliest possible time when study photographers are available (i.e. the following morning or early on the following Monday). Follow-up photography will be done before staining of the cornea with fluorescein for slit-lamp examination on the day of the follow-up visits.

The first picture of each session will be taken at the 1:1.6 magnification with the patient identification number written on the index card and held under the patient's lower lid of the affected eye. The patient will be escorted to the Photography Department with patient number transcribed onto index card (the card can be cut to size for convenience). An assistant will hold the upper and lower lids and the number card. The patient may be light sensitive, particularly at the enrollment visit. If this is the case, the patient may be made more comfortable by photographing using a flashlight with the room lights off or turning off the overhead fan. For the enrollment photography session, topical anesthetic will be applied to the infected eye before any picture is taken in order to reduce the pain for the patient.

All of the other photos will be taken at the 1:1 magnification on the lens since these photos are important for measurement purposes. Every image should be focused by setting the magnification on the lens and moving toward and away from the patient until the focusing light reflecting off the cornea is sharp. At the magnification 1:1, the focus should be obtained by being at about 10 cm from

the patient's eye. The photographers should continue to image until they are comfortable that sharp, well-centered photographs have been obtained. At least four clinical photographs will be required at each patient photography session.

It is important that the complete circumference of the cornea (limbus) be seen in all visit photographs. It is also important that the patient gaze be directed to the center of the lens in all photographs: the cornea should be round and well centered.

The camera's ISO sensitivity will be 200. Image settings will be '.jpg', fine, large, and white balance will be set on 'Auto'.

Images will be captured to the camera's removable SD card, downloaded to the Aravind Photograph computers while the patient is still in room. The same procedure will be followed at the other sites. Images will be copied to folder named "MUTT.Images". Images will be renamed per the File Naming Conventions (section 8.5).

8.5. File Naming Conventions

The image files will be designated with 1) the patient number and 2) the timing of visit 'E' (enrollment), '3W' (3-week follow-up), '3M' (3-month follow-up) and 3) image number of the series. An image renamed to M101U_E1.jpg will be the first picture for the enrollment series for patient identification number M101U. An image renamed to M101U_3W1.jpg will be the first image of the 3 weeks images series. An image renamed to M101U_3M2.jpg will be the second image of the 3 months images series.

Patient names or any other identifying information such as birthday will not appear within any of the image files.

At each measurement session, data measurement file will be named using MUTT naming conventions which specify measurement date and grader.

8.6. Image Transfer

Images will be uploaded daily to www.mediamax.com into folder "File Manager".

Basic procedure for uploading images:

1. Log onto: www.mediamax.com
   Username: MUTTstudy
   Password: fungus
2. Go to "File manager" folder
3. Click on brown "Upload" button
4. Click "Browse" to navigate to image for uploading
5. Upload all images for day
6. Make sure all images are properly uploaded with accurate names/labels
7. Log out of mediamax.com

Using current Audio Visual computers, it will take about 1 minute of upload time per patient.

Upon completion, an email will be sent to Reading Center RE: Photographs Today's date describing the transfer (example: "A total of 3 files from 3 different patients were uploaded to Mediamax"). The images should be transferred daily at the end of working day after having seen study patients.

8.7. Image and Data Storage

All electronic images received will be catalogued at the Imaging Center. Two copies of all electronic images will be archived on a regular basis onto a hard drive and CD/DVD by Dartmouth Hitchcock Medical Center.

All photographic measurement data will be recorded in the MUTT Measurement Spreadsheet in Excel.

8.8. Photographic Evaluation

Grading will include evaluating progression of the disease process as well as a judgment regarding the quality of images. Independent, masked observers will review each set of photographs, making all measurements while viewing the images in "Optscore", our proprietary photo-grading software for assessing corneal ulcers that utilizes template matching.

8.8.1. Training and Certification of Masked Observers

The observers will be first or second year medical students at Dartmouth Medical School.

The observers will have taken a thorough training on photographic evaluation for this trial. The observers will first learn about various clinical features related to fungal keratitis that will be evaluated on the photography and how these are evaluated. Then using Optscore, they will train with a sample of pictures of corneal ulcers to familiarize themselves with the interface of Optscore software and practice photographic evaluation and measurement for this trial. At this stage, the trainees will practice grading at least 100 individual pictures of corneal ulcers, 15% of which are repeated randomly in order to assess the intra-grader variability.

Next, the trainees will undergo certification grading with a series of 50 photos. Their evaluations and measurements will be compared to a gold standard established by the head of
The Imaging Center (Michael Zegans, MD), using an ICC (intra-class correlation coefficient). If the trainees achieve an intraclass correlation coefficient greater than 0.9, they will be certified as an independent, masked observers. If not, they will go through the full training again, until their results are satisfactory. The observers will be periodically re-evaluated to maintain consistent grading and re-certified annually. If several weeks pass between each MUTT photograph evaluation session, the observers will be retrained and recertified.

8.8.2. Grading

Each photograph will be presented in the random order in the masked manner. No information on the patient, the time point of the follow up visit, or any other clinical information that may bias grading of the photography will be provided to the graders. To monitor grader variability, each photograph will be presented to at least two graders and a random sample of photographs will be repeated to a grader twice (flipped horizontally, presented in the random order with other photographs) (see Section 8.8.3).

Each set of patient photographs will be graded for (1) the location of corneal infiltrate or scar, (2) the size of corneal infiltrate or scar, (3) the density of infiltrate or scar, (4) the presence of corneal neovascularization, (5) the presence of conjunctival inflammation, (6) the presence of hypopyon, and (7) the presence of posterior synechia.

a. Location of Corneal Infiltrate/Scar:

The clinical images will be overlaid with corneal grading grid which indicates center 4 mm and 12 mm of the cornea. The area within the center 4 mm is considered central and the area between the center 4 mm and 12 mm is considered periphery.

The location of corneal infiltrate/scar will be evaluated using the following categories:

Zone 1: Entirely in periphery
Zone 2: Overlaps central 4mm circle and periphery without filling center
Zone 3: Entirely in central 4mm circle
Zone 4: Completely fills central 4mm circle and extends into periphery

b. Size of Corneal Infiltrate/Scar:

The size of corneal infiltrate/scar will be determined using measurement tools contained in our evaluation software. The circumference of the corneal infiltrate/scar will be traced using a computer mouse and the resulting area in mm^2 will be automatically calculated and entered into the MUTT measurements database.

c. Density of Corneal Infiltrate/Scar:

In each set of patient photographs, the density of corneal infiltrate/scar will be graded in the worst 50% of the lesion. The following categories will be used:

1 **Mild**: Iris detail easily visible or only slightly hazy in greater than 50% of the involved corneal area
2 **Moderate**: Iris detail visible but partially obscured in greater than 50% of the involved corneal area
3 **Complete**: Iris detail obscured in greater than 50% of the involved corneal area

Each set of patient photographs will also be graded for presence or absence of perforation.

d. Corneal Neovascularization:

Each set of patient photographs will be graded for the presence or absence of corneal blood vessels. Any corneal image with corneal blood vessels extending 1mm beyond the limbus, or 1/10 of the corneal diameter, will be noted as having corneal neovascularization. The location of corneal neovascularization will be evaluated as raw clock hours (0-12 clock hours) (i.e. if the blood vessels are extending 2 mm beyond the limbus at the bottom of cornea, it is at 6 o'clock).

e. Conjunctival Inflammation:

Each set of patient photographs will be graded for presence or absence of inflammation of the conjunctiva. If conjunctival inflammation is present, the location of inflammation will be evaluated as raw clock hours.

f. Presence of Hypopyon:

Hypopyon when present will be measured as height (the distance between the top of the hypopyon and the lowest point in the arc of the hypopyon) in mm. When absent, the record will be "0". The presence of hemorrhagic hypopyon will be also observed.

g. Presence of Posterior Synechiae

Posterior synechiae when present will be recorded as present "1" or absent "0".

8.8.3. Grader Variability

The observer variability for photographic grading will be evaluated throughout the study.

To monitor the intra-grade agreement, 10% of all study photographs will be randomly chosen and repeated twice for grading by each grader. The repeated photographs will be flipped horizontally and presented in a random order to prevent any bias from learning. The repeated grading of photographs will be compared using the ICC. If the ICC falls below 0.9 for a grader, the grader will go through re-training and re-certification as described in section 8.8.1.

To measure the inter-grader agreement, a random sample of photographs will be presented to multiple graders. All graders will be masked to other observers' grading. If the ICC falls below 0.9, standardization training of all graders will be conducted.

8.8.4. Monitoring Photographic Quality

A four point quality scale (Excellent = 4, Good = 3, Fair = 2, Unusable = 1) will be used to grade each image set for quality of exposure, focus, and field definition. Additional training will be made available to the study photographers, should imaging quality not meet defined standards (the average quality scale below 2 over the period of 3 months).

9. Protection of Human Subjects

9.1. Informed Consent

The ophthalmologist/ophthalmic assistant reads out the informed consent form in the local language to every eligible subject individually before enrollment. A signature is obtained from the eligible patients willing to participate in the study on the Patient Consent Form (Appendix A, Form A.7). In India, the consent forms will be translated in Tamil. The translated consent form will be back-translated into English by an impartial translator to ensure that the content of the form is accurate.

9.2. Adequacy of Protection Against Risks

There are several layers of procedures to help minimize study-associated risk. The risk will be minimized by admission of individuals as inpatients until the ulcer has re-epithelialized, and by frequent examinations by an ophthalmologist. Should the condition of the ulcer deteriorate, the study medication will be discontinued in that individual if the physician caring for the patient deems this is in the patient's best interest. In the event of any adverse effects related to the study, it will immediately report to the Medical Monitor and the DSMC and appropriate medical care (including emergency surgical services) will be provided.

9.3. Inclusion of Female Subjects, Pregnant and Breast-feeding Women, and Children

Sex will not be used as a criterion for inclusion/exclusion. A previous survey at Aravind suggests that approximately 39% of presenting ulcers will be in women[1].

Pregnant and breast-feeding (lactating) women will be excluded from the trial due to the unknown risk of the antifungals to the fetus and infants. Urine pregnancy testing will be performed on all female patients being screened for the study.

All patients aged 16 and over will be included in the study. Hard contact lens refraction is an important outcome in this study but contact lens fitting is difficult in children under the age of 16 years. We, therefore, have excluded children under 16 years of age. Previous surveys at Aravind and Proctor suggest that less than 5% of presenting ulcers will be in children less than 16 years old[1]. An assent will be obtained from a parent/guardian of any children <18 years of age entering the study.

9.4. Inclusion of Minorities

We anticipate that all Aravind patients will be native South Indians, an ethnic group often reported as "South Asian". Proctor patients are most commonly from the following ethnic groups: White (non-Hispanic), Hispanic, African-American, and Asian. Patients presenting to the Lumbini Eye Institute will be from both India and Nepal and Bharatpur Eye Hospital patients will all be Nepali. Note that there will be no ethnic restrictions on enrollment.

9.5. Compensation to the Subjects

There is no cost to the subject. There is no reimbursement for overall participation in this study. Travel costs can be reimbursed for Indian subjects.

10. Data Management

This section discusses how the data will be collected from the different Study Centers, entered into the computer database and stored, and transferred to the Data Analysis Committee under the supervision of the Data Coordinating Center.

10.1. Data Collection Forms

The data collection forms are derived from the Steroids for Corneal Ulcers Trial and for the Mycotic Ulcer Treatment Trial pilot study. These forms have been extensively field-tested by UCSF and Aravind investigators.

Study personnel at each site will be given specific training for each form for which they will be responsible. Simulated patients will be used in each training session. Paper forms for each patient will be completed by investigational personnel at each study site. Questions which arise during the use of the forms will be communicated with the Data Coordinating Center at Proctor, and the results and answers will be communicated to all sites. Teleconferences and site visits will be held periodically for all users of the forms to review procedures and address questions.

10.2. Data Review

Before data entry, the forms will be reviewed and cross-checked for consistency and completeness by the Study Coordinator. If the forms are not filled out completely, the Study Coordinator will contact the person responsible for completing the form to provide the missing data, or clarify any inconsistent data. The Study Coordinator is the only person who is authorized to add missing data or make any changes to the study forms. All changes should be made with a red ink pen, and then signed and dated.

10.3. Data Entry

Once the study forms are reviewed and approved by the Study Coordinator, the Data Entry Operators will enter the data. Each Aravind site will have access to 3 or more workstations, networked – with at least one dedicated to data entry. All data will be entered into a Microsoft Access database that has been designed for this study within two working days of collection, in order to prevent the accumulation of un-entered forms. The database program contains an entry module for each form, prompting the user to enter the data in the same order as the form and clearly indicating each question. All data will be double-entered by independent Data Entry Operators at each center for ensure the accuracy.

For Indian centers, data will be entered and the study forms will be kept by each center where the patients are enrolled. For patients enrolled at the Proctor Foundation, Lumbini Eye Institute, or the Bharatpur Eye Hospital the study forms will be sent to Madurai via emails for data entry and the original study forms will be kept at Proctor Foundation.

10.3.1. Data Entry Errors

The Data Analyst will periodically compare double-entered study data using an automated program. Wherever there is a mismatch, an error file is generated with relevant data such as the form, field name, and data for which discrepancy is found. The Data Analyst will then contact the Data Entry Operators to verify the forms and re-enter the data.

10.3.2. Data Consistency and Validity

Through range checks, the data entry software ensures to a large extent that there are no inconsistencies or invalid data. However, the data (now free of entry errors) will be checked for consistency and validity again. The software has been developed for checking the consistency of data. The database program will check for the following errors: (1) improper entry of the patient ID based on the checksum, (2) data fields that are out of range, (3) inconsistent or illogical entries, (4) incompleteness, and (5) numerical values that are far outside the range of those previously entered. The software will create an error file with relevant data such as the form identification, field names and the data. The Data Analyst will then contact the Study Coordinator to consult the forms, resolve the inconsistency and have the Data Entry Operators enter the correct data. The corrected data will then be electronically merged with the full database.

10.4. Data Transfer and Analysis

At the end of each week, a physical network connection will be made to each computer. Under the supervision of the Data Coordinating Center, the Data Analyst will review the database from all sites for entry error and consistency. Once the data quality is checked, the data will be encrypted, and transmitted by secure FTP to the Data Coordinating Center at Proctor. At the Data Coordinating Center, the database manager will merge the data sets into the central SQL database, and conduct checks for completeness and validity. Questions which arise will be communicated by secure encrypted email to the study coordinator for each site for real-time resolution.

Then the Data Coordinating Center will transfer the database to the Data Analysis Committee for analysis.

10.5. Data Analysis

The Data Analysis Committee will be responsible for analyzing the data transferred from the Data Coordinating Center. It will merge the unmasked data with the randomization list, perform statistical analysis and prepare reports, if needed.

Data sets for analysis will be produced by the statistical programmer, Ms. Kathryn Ray. Each will be a Microsoft Excel® worksheet containing a single header line whose variable names match the Access database. Each analysis set will be in the form of a rectangular table in which each column corresponds to a single variable and each row to an observation. All missing values will be coded explicitly using the string NA (as used in the R software). Codes for categorical variables (such as 1 for male, and 2 for female) will be avoided in favor of self-documenting character strings (such as Male, Female) whenever possible. Automated checks will be made to ensure consistency and that each variable in the analysis set has in-range values (protecting against negative ages, spelling errors in categorical factors, and similar errors).

A detailed codebook will be prepared, containing for each variable, (a) the form from which the variable derived, (b) the text of the question, (c) all possible values for the variable, and (d) summary statistics for the variable. Note that all codes and character strings that represent categorical factors will be clearly defined in the codebook. Units for each continuous variable (e.g. mm vs. mm2, logMAR, etc.) will be unambiguously indicated for each variable. Each release of the analysis set will be accompanied by the corresponding version of the codebook. Version numbering with dates will be strictly observed. Standard report-generation software included with the R statistical and data analysis package will be used to ensure consistency of the codebook and analysis set at all times.

Data monitoring reports will be prepared based on analysis data sets. These will be prepared using report-generation software. Monitoring reports will include (a) recruitment reports for each center, (b) compliance reports, (c) retention reports, and (d) data quality reports. These will be reviewed by the Data Coordinating Center on a monthly basis, and communicated to the study sites on a monthly basis.

10.6. Data Storage and Security

Paper forms will be maintained in locked file cabinets in locked rooms only accessible to senior research staff.

All electronic storage, both at the Aravind sites and at Proctor and Dartmouth, will be subject to standard security procedures in compliance with established enterprise information security standards. Each Aravind computer will be hardware-firewalled and will not be accessible outside the Local Area Network. AES hard-disk encryption will be used for each machine, and the machine will not be accessible without a network account and password. Only one individual at each site will have password access to the machine; new accounts may be provided by the local network administrator only with the approval of the Data Coordinating Center. Accounts will be immediately deactivated for data entry or other personnel who leave the study. The computer used for data entry and storage will be kept in a physically locked room, and study personnel will be able to access this machine. At each center, the data will be backed up weekly on the server and monthly on a CD/DVD, which will be kept at a safe, locked cabinet.

The database transferred to the Data Coordinating Center will be stored on a SQL server located at 95 Kirkham Street, San Francisco, CA and three sets of backup copies on CD/DVD will be kept. The server is hardware-firewalled and also uses hard-disk encryption; it is inaccessible outside the Proctor network and cannot be physically accessed by anyone other than the network administrator. Other visitors must be accompanied by an information security professional and the visit will be logged. All data will be protected with password and the computer/server on which the data is stored and the backup copies on CD/DVD will be located in a lock-secured facility. Each back-up file will be archived offsite. In the event of disruption due to unforeseen circumstances, all materials needed to continue will be available from the offsite archive.

11. Statistical Analyses

11.1. Calculation of Sample Size and Power Analysis

For details on sample size calculation and power analysis, please see the Statistical Analysis Plan (SAP).

11.2. Data Analysis

For details on data analysis, please see the Statistical Analysis Plan (SAP).

12. Quality Control

12.1. Laboratory Quality Control Measures

The Aravind Microbiology laboratory has been awarded accreditation from the National Accreditation Board for Testing and Calibration Laboratories – NABL – which is part of the Department of Science and Technology in the Ministry of Science of Technology of the Government of India. The accreditation is for ISO-17025. For more information on quality control procedures, see Section 7.6.

12.2. Medication Storage and Expiry

Study medications will be stored in a standard refrigerator dedicated for this study in the pharmacy prior to use. The expiration dates on the medication will be regularly monitored and all expired study medicine will be discarded appropriately.

12.3. Periodic Reports

The Study Coordinators will send weekly reports on the number of eligible patients screened, the number of patients enrolled, the number of patients who have come back for follow-up visits to the Executive Committee and the investigators. This will be used to monitor the enrollment/follow-up progress and any protocol violation in enrollment.

The study data will be periodically transferred to the Data Coordinating Center to monitor the quality of data management. With the help of the Data Coordinating Center and the Data Analysis Committee, the Clinical Coordinating Center will evaluate the quality of study activities (clinical examination, treatment compliance, refraction, etc.).

When a site visit or training/certification is conducted at a study site, a report will be prepared and sent to the Executive Committee.

The minutes of the DSMC meeting will be circulated electronically among the investigators and the members of the DSMC after each meeting.

12.4. Data Management, Security and Quality Assurance

See Section 10.

12.5. Monitoring Compliance

For in-patients, the directly observed administration of study medication by ward nurses/ophthalmic assistant will be recorded at the time of administration.

For out-patients, the ophthalmologist/ophthalmic assistant will collect self-reported details of the number of doses missed by the patient during the treatment. At each visit, the patient will be asked if somebody else used the bottles of study medication and this should be recorded on the form.

12.6. Certification

For training and certification process, see Section 6.3.3 (Visual Acuity Training and Certification), Section 6.4.4 (Certification of Clinical Grading), and Section 8.8.1 (Training and Certification of Masked Observers).

13. Cost-Effectiveness Analysis

A cost-effectiveness analysis (CEA) will be conducted alongside the trial. We will conduct similar analyses for the trial comparing topical voriconazole and natamycin, and for the trial comparing oral voriconazole to placebo. We will calculate the incremental cost-effectiveness of these treatments in separate analyses for India and for the United States. In both analyses, the primary outcome will be the cost per quality-adjusted life year (QALY) for each of the treatment arms.

13.1. CEA for India

We will conduct a trial-based CEA using only the data collected from Aravind Eye Hospital in India. This analysis will compare the cost per QALYs in both of the clinical trials at three months. Calculation of QALYs requires health utility values, which are estimates of quality of life. There are currently no health utility estimates for infectious keratitis, so we will be estimating these as part of this study. Specifically, we will calculate utility values from all study subjects using three separate methods. First, we will use visual acuity at baseline, three weeks, and three months, and convert this to a utility value using previously-published formulas[89]. For this method, disutility values of serious adverse events and transaminitis will be estimated, also using previously published values. Second, we will administer the EQ-5D questionnaire in the interviewer format, also at baseline, three weeks, and three months. This instrument has been validated in the Tamil language[90]. The results of this questionnaire will be converted to utility values using previously published studies[91]. Third, we will use the time tradeoff technique to measure utility values directly, also at baseline, three weeks, and three months. This technique is widely used for generating utility values, and consists of asking a person how much time they would give up at the end of their life, in exchange for having perfect health until death[92]. We will confirm convergent validity of these utility values by comparing the estimated utility values from each of the three methods described above, as well as the IND-VFQ33, which is a visual function questionnaire that has been validated in India[93]. The IND-VFQ33 will also be performed at baseline, three weeks, and there months. Once we have calculated utility values, we will use standard methodology to calculate the QALYs gained from each intervention. Costs will be estimated from the societal viewpoint, and will include the costs of medications, laboratory evaluations, number of clinic visits, number and length of hospitalization, and the opportunity cost of illness (days of work missed due to illness). Our primary outcome will be the cost per QALY for each treatment arm, which will be expressed as incremental cost-effectiveness.

13.2. CEA for USA

The costs of treatment and practice patterns are different in India and the United States. Therefore, cost-effectiveness will be calculated separately. The CEA for the United States will be modeled using decision analysis. The decision tree will be completed using data on the complication rates and final visual acuity from both clinical trials, using data from India and the United States. We will use the visual acuity efficacy and rates of adverse events from each of the clinical trials to calculate health utility values and QALYs. Although we will probably not have enough patients in the United States to accurately estimate utility values for this population, we will nonetheless perform the EQ-5D, as well as the NEI-VFQ25, which is a validated visual quality of life questionnaire[94]. These questionnaires will be performed at baseline, three weeks, and three months. Even if the sample size is small, we may be able to use the results from these questionnaires to assess consistency of utility values between India and the United States, and for hypothesis testing. In addition to the cost of medications in the United States, we will estimate the costs for clinical care by using data on reimbursements for clinic visits, hospitalizations, and laboratory evaluations. The primary outcome will be costs per QALY, expressed as an incremental cost-effectiveness.

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Appendix A: Patient Forms

Appendix A contains initial versions of all the forms that are to be used in the study, as well as charts and diagrams. Future revisions will be stored in separate documents.

A.1. Study Forms Completion Schedule

The study forms completion schedule will be used to keep track of which forms should be completed at which time point of the study. By the end of the study, each participating patient's file will have record of all the necessary study forms.

A.2. Patient History Form

The Patient History Form records the demographic information of the patient upon enrollment.

The form should be filled in by the ophthalmologist, and signed upon its completion. The medical record number from the hospital file is also noted on the form. A Study Patient ID is given once eligibility is determined and consent is obtained.

For females:

- Collect details of lactation, potential pregnancy, and perform and record the pregnancy test result

Under Risk Factors:

- Collect details of the object of injury and other risk factors diagnosed and record them
- Collect details of the recent medications used by the patient
- Perform Gram stain and KOH wet mount (Giemsa stain can be done as well) and record the results

A.3. Clinical Examination Form

This form is to be filled in by the ophthalmologist and should be signed upon its completion.

Record the medical record number from the hospital file and the Study patient ID which is assigned after eligibility is determined and consent is obtained.

After filling in the basic information about the patient:

- Record the study time point
- Mark the area of the ulcer in the affected eye in the diagram provided in the form
- Measure the size of the epithelial defect and stromal infiltrate/scar
- Mark the infiltrate depth based on the depth categories
- Measure the height of hypopyon and record it based on the categories provided
- Check off which ulcer characteristics are present at enrollment

The details of the patient's fundus and lens examination should be recorded at enrollment and at 3 months. If the examination of fundus and lens cannot be done at enrollment because the patient is photophobic, it can be repeated again in a few days.

A.4. Inclusion/Exclusion Criteria Form

This form is to be filled in by the investigator who is assisted by the study coordinator and should be signed upon its completion.

After filling in the patient identification details, check the listed inclusion criteria on the form. If the patient meets all inclusion criteria and none of the exclusion criteria are met, and informed consent is obtained, a study patient ID number based on the sequence of entry into the study will be assigned (the randomization and study ID lists are generated by Travis Porco, Bio-statistician for the Proctor Foundation).

A.5. Patient Consent Forms

This form is to be read out to the patient by the ophthalmologist/study coordinator/ophthalmic assistant.

Once the patient is determined eligible for the study, fill in the patient identification details and explain the contents of the patient consent form and obtain the signature of the patient for permission to enroll in the study. For participants who are 16 to 18 years of age, a minor assent form is provided, to be signed by both the minor and one of the parents or guardians. A copy of the signed consent form should be provided to the patients.

A.6. Microbiology Form

This form is to be filled in by the microbiologist and should be signed upon its completion.

After filling in the patient identification details, record the stain results for fungus and bacteria. When available, mark the organisms found in the culture of the patient sample, obtained from the microbiology laboratory.

A.7. Visual Acuity Form

After filling in the patient identification details, the examiner will record the correctly and incorrectly identified letters on the Visual Acuity form. As we are using two visual acuity charts (Precision Vision Cat. No. 2305 & 2305a), we have two Visual Acuity Forms to reflect the slightly different orientation of the letters in each chart. Note: a third eye chart is available for refraction only (Precision Vision Cat. No. 2305b).

The Visual Acuity form should be filled out at each of the following visits: Enrollment, 3-week, and 3-month follow-ups. This form must be signed by the examiner once completed.

A.8. Follow-up Compliance Forms

This form is to be filled out by the ward nurses or ophthalmic assistants who are responsible for dispensing the study medication to the inpatients. For outpatients, the form will be filled out by the study coordinator or ophthalmic assistants.

For in-patients, the directly observed administration of study medication by ward nurses/ophthalmic assistants will be recorded at the time of administration.

For out-patients, the study coordinator/ophthalmic assistant should collect self-reported details of the number of doses missed by the patient during the 3 weeks after discharge. At each visit, the patient will be asked if somebody else used the bottles of study medication and this should be recorded on the form.

A.9. Follow-up Reminder Letter

The follow-up reminder letter will remind patients of the date and place of the upcoming follow-up visits, and in addition, it will advise the patient on what to do in case the patient would be unable to make the scheduled visit. This letter will be specific to each center.

A.10. Medication Change Form

If any medication is changed from or added to the study medication, this form should be filled out and signed by the investigator.

Any elective surgery or procedure that does not qualify as an adverse event can be recorded on this form as well.

A.11. Adverse Event Form

In the case of an Adverse Event, this form is to be filled in by the investigator, and should be signed upon its completion. In case of an Serious Adverse Event, a Serious Adverse Event Narrative Form should be filled out in conjunction and sent to the Medical Monitor within 24 hours of awareness.

A.12. Serious Adverse Event Narrative Form

In case of a serious adverse event, this form will be filled out by the Investigator and submitted to the Medical Monitor, Dr. Stephen McLeod, within 24 hours of the awareness of the SAE. Information recorded on this form will include the nature of the event, date of onset, date of resolution, date of notification to Medical Monitor, and action taken. The form will be reviewed and signed by the Medical Monitor and then sent to the DSMC.

A.13. Patient Dropout Form

This form is to be filled in by the investigator or study coordinator, and should be signed upon its completion.

Note: If patient refuses medication but stays in the study / is willing to return, they have not dropped out. If there is an adverse event, the patient has not dropped out. The failure to show up for follow-up visits does not count as a drop-out, either.

A.14. Cost-Effectiveness Forms

Cost-effectiveness data will be collected through the cost-effectiveness form and the quality of life and vision function questionnaires. Quality of life and vision function questionnaires are to be read to the patient by the study coordinator or ophthalmic assistant, and their answers are to be captured using the appropriate data form. Cost data is to be collected in the cost data form at 3 weeks and 3 months, and should be filled out by the study coordinator or ophthalmic assistant.

A.1. Study Forms Completion Schedule

	Patient History Form	Clinical Exam Form	Inclusion/ Exclusion Criteria Form	Patient Consent Forms	Micro-biology Report Form	Visual Acuity/ Refraction Forms	Follow-up Compliance Forms	Adverse Event Forms (if applicable)	Photo-graphy	Cost-effectiveness Forms	Dropout Form (if applicable)
Enrollment (Day 1)	✓	✓	✓	✓	✓	✓ (BSCVA not necessary)			✓		
Day 2						✓					
Every 3 Days (+/- 1 day) until Re-epithelialization		✓						(✓)			(✓)
7 days after Enrollment					✓						
At Re-epithelialization		✓						(✓)			(✓)
3 Weeks from Enrollment (whether re-epithelialized or not)		✓				✓	✓	(✓)	✓		(✓)
3 Months from Enrollment		✓				✓		(✓)	✓	✓	(✓)

A.2. Patient History Form

M.R. No.: _____ **Patient ID:** ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y

Site: ☐ AECS ☐ LEI ☐ BEH
Affected Eye: ☐ Right Eye ☐ Left Eye (For months, JAN = January, FEB =February, etc)

(To be completed after enrollment)

Age: _____ **Sex:** ☐ Male ☐ Female **Weight (kg): _____**

If female:
Lactating ☐ Yes ☐ No
Potential Pregnancy ☐ Yes ☐ No
Pregnancy test result ☐ Pos ☐ Neg ☐ Not done

Occupation:
☐ Manual Labour – Agriculture (Farmer, Agriculturalist, shepherd, landscaper, etc.)
☐ Manual Labour – Non-agriculture (Construction, Mason, Wood cutter, Electrician, Mill Worker, etc.)
☐ Driver
☐ Semi-skilled/Skilled Labour
☐ Business/Trade
☐ Student
☐ Unemployed/Not working
☐ Other_____
☐ Service/Clerical Work
☐ Domestic Work (house maker, etc.)
☐ Retired
☐ Professional Work

Ocular History:
Duration of symptoms (days) _____
Yes ☐ No ☐ Recent trauma:_____
Object of injury:
☐ None ☐ Metal ☐ Wood ☐ Vegetative Matter ☐ Unknown Object
☐ Other (specify) _____

Yes ☐ No ☐ Contact lens wear? If yes: Yes ☐ No ☐ Overnight wear?
Brand of lens_____ Brand of cleaning solution_____

Ocular surface disease:
☐ None
☐ meibomitis ☐ dry eye ☐ blepharitis ☐ neurotrophic cornea
☐ rosacea ☐ OCP ☐ toxicity ☐ atopic ocular disease
☐Other_____

Systemic immune or inflammatory disease:
☐ None
☐ diabetes mellitus ☐ asthma ☐ exzema ☐ Hansen's disease ☐ psoriasis
☐ rheumatoid arthritis ☐ hay fever ☐ icthyosis ☐ malnourished ☐ HIV
☐ Other_____

Clinical Exam:
Yes ☐ No ☐ Adjacent infection (e.g. dacryocystitis, canaliculitis, conjunctivitis, blepharitis)_____
Yes ☐ No ☐ Trichiasis
Yes ☐ No ☐ Abnormal lid anatomy (e.g. entropion/ectropion, exposure)_____
Yes ☐ No ☐ Corneal abnormality (e.g. keratoconus, bullous keratopathy)_____

Recent Medications: ☐ None

Topical ocular antifungals

Name	Dose	Duration
☐ None		
☐ Natamycin		
☐ Amphotericin B		
☐ Clotrimazole		
☐ Econazole		
☐ Voriconazole		
☐ Fluconazole		
☐ Itraconazole		
☐ Other _____		

Other topical ocular medications

Class of Drug	Name	Dose	Duration
☐ None			
☐ Fluoroquinolone			
☐ Chloramphenicol			
☐ Aminoglycoside			
☐ Sulfacetamide			
☐ Glycopeptide			
☐ Cephalosporin			
☐ Unknown			
☐ Other _____			

Systemic antifungals

Name	Dose	Duration
☐ None		
☐ Itraconazole		
☐ Ketaconazole		
☐ Voriconazole		
☐ Fluconazole		
☐ Other _____		

Other systemic medications

Name	Dose	Duration
☐ None		

KOH/Giemsa: ☐ Fungus ☐ No Fungus ☐ Not done
☐ Filamentous ☐ Yeast

Gram stain: ☐ Bacteria ☐ No Bacteria ☐ Not done
☐ Gram + ☐ Gram -
☐ Bacilli ☐ Cocci
☐ Fungus ☐ No Fungus ☐ Not done
☐ Filamentous ☐ Yeast

_____
Investigator's Signature
Initials: _____

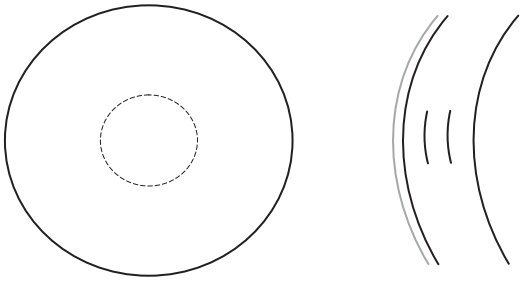
A.3. Clinical Examination Form

M.R. No.: _____ **Patient ID:** ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y

Affected Eye: ☐ Right Eye ☐ Left Eye (For months, JAN = January, FEB =February, etc)
Time-point: ☐ Enrollment ☐ Study Day _____ ☐ Re-epithelialization Day _____ ☐ 3 Weeks ☐ 3 Months

Cornea Examination of the Affected Eye: (*Drawing required for Enrollment, 3 weeks, and 3 months*)



(Required at every visit)

	Longest diameter in mm	Longest perpendicular width in mm
Size of epithelial defect		
Size of underlying stromal infiltrate/scar		

☐ glue

Infiltrate/Scar Depth: ☐ No infiltrate/scar ☐ >0-33% depth ☐ >33-67% depth ☐ >67-100% depth
Hypopyon: ☐ No hypopyon ☐ <0.5 mm ☐ _____ mm *(round to nearest 0.5 mm)*
IOP *(optional)*: ☐ **Not Done**
_____ **mmHg (by ☐ T_{App} or ☐ T_{Pneumo} or ☐ $T_{Tonopen}$) or ☐ $T_{Digital\ high}$ ☐ $T_{Digital\ normal}$**
Perforation: ☐ Yes ☐ No Recommend graft: ☐ Yes ☐ No Existing graft: ☐ Yes ☐ No

Check box if: ***(Required at Enrollment only)***
Check if any of the following are present:

☐ none
☐ feathery edges ☐ satellite lesions
☐ raised lesion ☐ endothelial plaque
☐ ring ulcer ☐ pigmented
☐ keratic precipitates ☐ the absence of corneal sensation

Yes ☐ No ☐ High suspicion of acanthamoeba by exam
Yes ☐ No ☐ High suspicion of herpetic keratitis by exam
Yes ☐ No ☐ Dense portion of infiltrate extends to limbus

Dilated Examination ***(Required at Enrollment and 3 months only):***

	Normal	Abnormal	No View	Not Examined	Comments:
Lens	☐	☐	☐	☐	_____
Retina	☐	☐	☐	☐	_____
Macula	☐	☐	☐	☐	_____
Optic Nerve	☐	☐	☐	☐	_____

_____
Investigator's Signature

A.4. Liver Function Test Form

M.R. No.: _____ **Patient ID:** ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y
(For months, JAN = January, FEB =February, etc)

Affected Eye: ☐ Right Eye ☐ Left Eye
Time-point: ☐ Enrollment ☐ 2 Weeks ☐Other Day _____

Liver function tests required at <u>Enrollment</u> and <u>2 weeks</u>, or weekly if previous elevated values

AST _____

ALT _____

Total Bilirubin _____

_____
Investigator's Signature

Initials: _____

A.5. Inclusion/Exclusion Criteria Form – MUTT I

M.R. No.: _____ Patient ID: 1 ☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y

(For months, JAN = January, FEB =February, etc)

FORM TO BE COMPLETED AT ENROLLMENT

Inclusion Criteria (All must be met)

At Enrollment:

☐ Presence of a corneal ulcer at presentation
☐ Evidence of filamentous fungus on smear (KOH wet mount, Giemsa, or Gram stain)
☐ The patient must be able to verbalize a basic understanding of the study after it is explained to the patient, as determined by physician examiner. This understanding must include a commitment to return for follow-up visits.
☐ Willingness to be treated as an in-patient or to be treated as an out-patient and return every 3 days +/- 1 day until re-epithelialization and every 1 week +/- 2 days to receive fresh medication for 3 weeks
☐ Appropriate consent
☐ Visual acuity between 6/12 (20/40, logMAR 0.3) and 6/120 (20/400, logMAR 1.3), inclusive

Presentation visual acuity: __ / ___ or ☐ CF ☐ HM ☐ LP ☐ NLP
Acuity by: ☐ Uncorrected ☐ Pinhole ☐ With correction ☐ BSCVA

Exclusion Criteria

At Enrollment:

☐ Impending perforation
☐ Evidence of bacteria on Gram stain at the time of enrollment
☐ Evidence of acanthamoeba by stain
☐ Evidence of herpetic keratitis by history or exam
☐ Corneal scar not easily distinguishable from current ulcer
☐ Age less than 16 years (before 16th birthday)
☐ Bilateral ulcers
☐ Previous keratoplasty in the affected eye
☐ Pregnancy (by history or urine test) or breast-feeding (by history)
☐ Acuity worse than 6/60 (20/200) in the fellow eye (note that any acuity, uncorrected, corrected, pinhole, or BSCVA 6/60 or better qualifies for enrollment)
☐ Acuity not between 6/12 (20/40) and 6/120 (20/400), inclusive in the affected eye
☐ Known allergy to study medications (antifungal or preservative)
☐ No light perception in the affected eye
☐ Not willing to participate, please indicate why_____

☐ **Patient meets ALL of the above inclusion criteria and does not meet any of the above exclusion criteria for MUTT I**
☐ **Eligible but not enrolled. Specify Reason: _____**

DO NOT ASSIGN STICKER UNTIL CONSENT FORM IS SIGNED

_____
Investigator's Signature

Initials: _____

A.6. Inclusion/Exclusion Criteria Form – MUTT II

M.R. No.: _____ **Patient ID:** | 2 | | | | | **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y
(For months, JAN = January, FEB =February, etc)

FORM TO BE COMPLETED AT ENROLLMENT

Inclusion Criteria (All must be met)

At Enrollment:

☐ Presence of a corneal ulcer at presentation
☐ Evidence of filamentous fungus on smear (KOH wet mount, Giemsa, or Gram stain)
☐ The patient must be able to verbalize a basic understanding of the study after it is explained to the patient, as determined by physician examiner. This understanding must include a commitment to return for follow-up visits.
☐ Willingness to be treated as an in-patient or to be treated as an out-patient and return every 3 days +/- 1 day until re-epithelialization and every 1 week +/- 2 days to receive fresh medication for 3 weeks
☐ Appropriate consent
☐ Visual acuity worse than 6/120 (20/400, logMAR 1.3)

Presentation visual acuity: __ / ___ or ☐ CF ☐ HM ☐ LP ☐ NLP
Acuity by: ☐ Uncorrected ☐ Pinhole ☐ With correction ☐ BSCVA

Exclusion Criteria

At Enrollment:

☐ Evidence of bacteria on Gram stain at the time of enrollment
☐ Evidence of acanthamoeba by stain
☐ Evidence of herpetic keratitis by history or exam
☐ Corneal scar not easily distinguishable from current ulcer
☐ Age less than 16 years (before 16th birthday)
☐ Bilateral ulcers
☐ Previous keratoplasty in the affected eye
☐ Ulcer <2 mm and depth less than anterior 1/3
☐ Pregnancy (by history or urine test) or breast-feeding (by history)
☐ Known liver disease, including hepatitis and cirrhosis (Child Pugh A-C)
☐ Liver function tests >2 times the upper limit of normal at baseline
☐ Acuity worse than 6/60 (20/200) in the fellow eye (note that any acuity, uncorrected, corrected, pinhole, or BSCVA 6/60 or better qualifies for enrollment)
☐ Acuity not worse than 6/120 (20/400) in the affected eye
☐ Known allergy to study medications (antifungal or preservative)
☐ Currently on contraindicated medication (see attached appendix)
☐ No light perception in the affected eye
☐ Not willing to participate, please indicate why_____

☐ **Patient meets ALL of the above inclusion criteria and does not meet any of the above exclusion criteria for MUTT II**
☐ **Eligible but not enrolled. Specify Reason: _____**

DO NOT ASSIGN STICKER UNTIL CONSENT FORM IS SIGNED

_____
Investigator's Signature
Initials: _____

Appendix: List of Contraindicated Medications for Voriconazole

- Rifampin
- Carbamazepine
- Long acting barbiturates
- Terfenadine
- Astemizole
- Cisapride
- Pimozide
- Quinidine
- Sirolimus
- Rifabutin
- Ergot alkaloids (egotamine, dihydroergotamine)
- Phenytoin
- HIV Protease Inhibitors
- NNRTIs
- Cyclosporine
- Tacrolimus
- Phenytoin
- Warfarin
- Omeprazole
- Benzodiazepines
- Statins
- Calcium Channel Blockers
- Vinca alkaloids

Please inform your doctor if you are on any of these medications before you begin the study medication, as they may be harmful when taken together.

A.7. Patient Consent Forms

Patient Consent Form for Aravind Eye Hospital Patients (MUTT I)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date:** D D / M M / Y Y
Enrollment Date: D D / M M / Y Y

Thank you for thinking about participating in this study at the Aravind Eye Hospital. In this study, Dr. Prajna, Dr. Srinivasan, Dr. Lietman and Dr. Acharya are trying to find out if giving a newer antifungal medicine called voriconazole to people with fungal corneal ulcers will help their eyes heal better than giving the conventional antifungal medicine called natamycin. This newer antifungal medicine is effective in treating fungal disease elsewhere in the body and has been used to treat eye infections. We are asking you to participate in the study because you have an infection affecting the front part of the eye (cornea) caused by fungus. By signing this form, you agree that you understand the following things:

1) By participating in this study at Aravind Eye Hospital, you agree to stay in the hospital and get a daily exam until your eye heals. We are asking you to stay at the hospital because we want to check on your eye often to make sure the medication is helping your eye heal.

2) If you do not want to stay in the hospital but still would like to participate in the study, you agree that we will take the medication as directed by the physician without skipping a dose. You will come back to the hospital every 3 days for an eye exam, and you will need a fresh bottle of medication every week for 3 weeks.. You also agree to bring back the old bottles you used so we can check if you took the medication as instructed. It is important that you know that you need to take the medication for three weeks but that the medication we are giving you is good only for 1 week. Therefore, it is crucial that the medication be replaced every week.

3) After the initial treatment, you agree to return to the hospital two more times to get an exam. We will ask you to come back at 3 weeks and 3 months from when you start receiving treatment. During these exams, we will measure your vision, look at your eye using an instrument called a slit lamp, and take photographs of your eye. Each exam will take about 1 hour.

4) You will receive an antifungal medication for your corneal ulcer. You will receive either

   a. An eye drop with medicine called natamycin

   OR

   b. An eye drop with medicine called voriconazole

   The medication you will receive will be determined by chance. A computer program will be used to randomly (like the flip of a coin) assign you to one medication or the other. This means that you have 50/50 chance of getting either drug. You or your doctor will not know which medication you are receiving.

5) As with all eye drops, there is the risk of irritation and/or allergy. Sometimes, the medicine may fail to heal your eye. There are not many risks reported with the use of voriconazole eye drops. However, it is reported that taking voriconazole in other ways may cause blurred vision, unusual liver function test, fever, rash, vomiting, nausea, diarrhea, headache, stomach pain, and difficulty breathing. If you experience any of these symptoms while taking the study eye drops, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine or oral contraceptives, please let your doctor know before you receive the eye drops because voriconazole may cause harm when taken with these drugs.

6) You may be asked to give a blood sample. We are asking ten percent of patients to give a blood samples to analyze the level of voriconazole present in their blood stream. These samples will be stored until the conclusion of the study to be analyzed and will only be connected to you through your patient ID number.

7) You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have,

how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

8) Pregnant and breast-feeding women cannot take part in the study because the medicine given in the study is not allowed during pregnancy and breast-feeding. If you are female, we will test if you are pregnant by urine test.

9) You will be assigned to a treatment program by chance, and the treatment you receive may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

10) The experimental treatments may have side effects that no one knows about yet, especially for new drugs like topical voriconazole. We will let you know if we learn anything that might make you change your mind about participating in the study.

11) The doctor may ask you to leave the study for any reason.

12) You do not have to pay for the medicine or for your hospital stay while part of the study. Also, if you are a part of the study, but later are taken out for any reason, the study will continue to pay for the medical treatment of your ulcer.

13) You will not receive any money from the study, since there will be no financial cost for you to participate.

14) We will hold medical records with personal medical information regarding the results of your eye exams. However, this information will be kept secret to others.

15) If you do not wish to participate in the study, you will be offered the standard treatment, natamycin.

16) If you are injured as a result of being in this study, treatment will be available. The costs of such treatment will be covered by Aravind Eye Hospital. We do not normally provide any other form of compensation for injury. For further information about this, you may contact the study coordinator.

I, the undersigned, understand the treatment offered to me in this study as well as its possible side effects. I volunteer freely and recognize that my identity and health records will remain confidential and will not be released to anyone else. I understand that if, for any reason, I decide to stop participating in the study, I will still be treated free of charge. If I have any questions regarding the study, I will contact the Aravind Eye Hospital Study Coordinator, Sister Sumithra, at:

Aravind Eye Hospital
1, Anna Nagar
Madurai - 625 020
Tamil Nadu
Phone: 91-452-4356100

_____	_____	_____
Printed Name	Signature	Date

Minor Assent Form for Aravind Eye Hospital Patients (MUTT I)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date:** D D / M M / Y Y
Enrollment Date: D D / M M / Y Y

Thank you for thinking about participating in this study at the Aravind Eye Hospital. In this study, Dr. Prajna, Dr. Srinivasan, Dr. Lietman and Dr Acharya are trying to find out if giving a newer antifungal medicine called voriconazole to people with fungal corneal ulcers will help their eyes heal better than giving the conventional antifungal medicine called natamycin. This newer antifungal medicine is effective in treating fungal disease elsewhere in the body and has been used to treat eye infections. We are asking you to participate in the study because you have an infection affecting the front part of the eye (cornea) caused by fungus. By signing this form, you agree that you understand the following things:

1) By participating in this study at Aravind Eye Hospital, you agree to stay in the hospital and get a daily exam until your eye heals. We are asking you to stay at the hospital because we want to check on your eye often to make sure the medication is helping your eye heal.

2) If you do not want to stay in the hospital but still would like to participate in the study, you agree that we will take the medication as directed by the physician without skipping a dose. You will come back to the hospital every 3 days for an eye exam, and you will need a fresh bottle of medication every week for 3 weeks.. You also agree to bring back the old bottles you used so we can check if you took the medication as instructed. It is important that you know that you need to take the medication for three weeks but that the medication we are giving you is good only for 1 week. Therefore, it is crucial that the medication be replaced every week.

3) After the initial treatment, you agree to return to the hospital two more times to get an exam. We will ask you to come back at 3 weeks and 3 months from when you start receiving treatment. During these exams, we will measure your vision, look at your eye using an instrument called a slit lamp, and take photographs of your eye. Each exam will take about 1 hour.

4) You will receive an antifungal medication for your corneal ulcer. You will receive either

   - An eye drop with medicine called natamycin

   OR

   - An eye drop with medicine called voriconazole

   The medication you will receive will be determined by chance. A computer program will be used to randomly (like the flip of a coin) assign you to one medication or the other. This means that you have 50/50 chance of getting either drug. You or your doctor will not know which medication you are receiving.

5) As with all eye drops, there is the risk of irritation and/or allergy. Sometimes, the medicine may fail to heal your eye. There are not many risks reported with the use of voriconazole eye drops. However, it is reported that taking voriconazole in other ways may cause blurred vision, unusual liver function test, fever, rash, vomiting, nausea, diarrhea, headache, stomach pain, and difficulty breathing. If you experience any of these symptoms while taking the study eye drops, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine or oral contraceptives, please let your doctor know before you receive the eye drops because voriconazole may cause harm when taken with these drugs.

6) You may be asked to give a blood sample. We are asking ten percent of patients to give a blood samples to analyze the level of voriconazole present in their blood stream. These samples will be stored until the conclusion of the study to be analyzed and will only be connected to you through your patient ID number.

7) You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how

many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

8) Pregnant and breast-feeding women cannot take part in the study because the medicine given in the study is not allowed during pregnancy and breast-feeding. If you are female, we will test if you are pregnant by urine test.

9) You will be assigned to a treatment program by chance, and the treatment you receive may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

10) The experimental treatments may have side effects that no one knows about yet, especially for new drugs like topical voriconazole. We will let you know if we learn anything that might make you change your mind about participating in the study.

11) The doctor may ask you to leave the study for any reason.

12) You do not have to pay for the medicine or for your hospital stay while part of the study. Also, if you are a part of the study, but later are taken out for any reason, the study will continue to pay for the medical treatment of your ulcer.

13) You will not receive any money from the study, since there will be no financial cost for you to participate.

14) We will hold medical records with personal medical information regarding the results of your eye exams. However, this information will be kept secret to others.

15) If you do not wish to participate in the study, you will be offered the standard treatment, natamycin.

16) If you are injured as a result of being in this study, treatment will be available. The costs of such treatment will be covered by Aravind Eye Hospital. We do not normally provide any other form of compensation for injury. For further information about this, you may contact the study coordinator.

I, the undersigned, understand the treatment offered to me in this study as well as its possible side effects. I volunteer freely and recognize that my identity and health records will remain confidential and will not be released to anyone else. I understand that if, for any reason, I decide to stop participating in the study, I will still be treated at no cost. If I have any questions regarding the study, I will contact the Aravind Eye Hospital Study Coordinator, Sister Sumithra, at:

Aravind Eye Hospital
1, Anna Nagar
Madurai - 625 020
Tamil Nadu
Phone: 91-452-4356100

_____	_____	_____
Printed Name	Signature of Minor	Date

The person being considered for this study is legally unable to consent for herself/himself because s/he is a minor. By signing this form, I am giving permission for my child or ward to participate in the study.

_____	_____	_____
Printed Name	Signature of a Parent	Date

Patient Consent Form for Aravind Eye Hospital Patients (MUTT II)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date:** D D / M M / Y Y
Enrollment Date: D D / M M / Y Y

Thank you for thinking about participating in this study at the Aravind Eye Hospital. In this study, Dr. Prajna, Dr. Srinivasan, Dr. Lietman and Dr. Acharya are trying to find out if giving a newer antifungal pill called voriconazole in addition to voriconazole eyedrops to people with fungal corneal ulcers will help their eyes heal better than giving them voriconazole eye drops alone. This newer antifungal medicine is effective in treating fungal disease elsewhere in the body and has been used to treat eye infections. We are asking you to participate in the study because you have an infection affecting the front part of the eye (cornea) caused by fungus. By signing this form, you agree that you understand the following things:

1) By participating in this study at Aravind Eye Hospital, you agree to stay in the hospital and get a daily exam until your eye heals. We are asking you to stay at the hospital because we want to check on your eye often to make sure the medication is helping your eye heal.

2) If you do not want to stay in the hospital but still would like to participate in the study, you agree that we will take the medication as directed by the physician without skipping a dose. You will come back to the hospital every 3 days for an eye exam, and you will need a fresh bottle of medication every week for 3 weeks.. You also agree to bring back the old bottles you used so we can check if you took the medication as instructed. It is important that you know that you need to take the medication for three weeks but that the medication we are giving you is good only for 1 week. Therefore, it is crucial that the medication be replaced every week.

3) After the initial treatment, you agree to return to the hospital two more times to get an exam. We will ask you to come back at 3 weeks and 3 months from when you start receiving treatment. During these exams, we will measure your vision, look at your eye using an instrument called a slit lamp, and take photographs of your eye. Each exam will take about 1 hour.

4) You will receive an antifungal medication for your corneal ulcer. You will receive either

   c. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and a placebo taken by mouth

   OR

   d. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and voriconazole taken by mouth

   The medication you will receive will be determined by chance. A computer program will be used to randomly (like the flip of a coin) assign you to one medication or the other. This means that you have 50/50 chance of getting either drug. Neither you nor your doctor will know which medication you are receiving.

   5) As with all eye drops, there is the risk of irritation and/or allergy. Sometimes, the medicine may fail to heal your eye. There are not many risks reported with the use of voriconazole or natamycin eye drops.

   6) It is reported that taking voriconazole in other ways, including by mouth, may cause the following side effects. Please note that the rates reported below reflect previous studies of voriconazole done in patients who were seriously ill and were on long-term therapy for several months, sometimes including IV therapy. We therefore expect that these rates will be lower in this study.

      a. Visual changes: 30-40% of patients receiving systemic voriconazole (by mouth or injection) may experience visual changes including blurred vision and color vision changes. These changes often go away when you continue the treatment, and they always go away within 14 days of discontinuing treatment.

      b. Formed hallucinations: 10-15% of patients receiving systemic voriconazole (by mouth or injection) may experience formed or auditory hallucinations, including seeing and hearing things

that are not actually there. These hallucinations are not permanent and resolve upon termination of therapy.

c. Abnormal liver function tests: 10-20% of patients receiving systemic voriconazole (by mouth or injection) may have elevated liver function tests. These changes usually resolve upon completion of the therapy. There is a risk of liver failure, but it is very rare. You will be required to have two blood tests: one when you begin the study to rule out pre-existing liver disease, and one after 2 weeks, to monitor the function of your liver upon completion of your treatment. All liver function tests will be reviewed by a doctor who specializes in internal medicine. If an abnormal liver value is found, you will have weekly repeat blood tests until the values return to normal. If liver values are found to be significantly elevated or you have clinical symptoms of liver dysfunction (including jaundice), you will be evaluated by a doctor who specializes in internal medicine.

d. Skin reactions: Up to 6% of patients receiving systemic voriconazole (by mouth or by injection) may experience a skin reaction, including rash. These are usually mild. You will be advised to avoid exposure to strong, direct sunlight while taking the medication as there have been reports of sensitivity to sunlight while taking this medication. Skin reactions will be closely monitored.

e. Headache: 3-4% of patients receiving systemic voriconazole (by mouth or by injection) may experience headaches. These headaches are not serious or permanent and will resolve when therapy is stopped.

f. Dizziness: 1-3% of patients may experience dizziness while taking this medication. The dizziness is not serious.

g. Other potential side effects: fever, vomiting, nausea, diarrhea, stomach pain, and difficulty breathing.

If you experience any of these symptoms while taking the study medications, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine, sulfonylurea oral hypoglycemics, oral contraceptives, or any of the drugs on the attached appendix, please let your doctor know before you receive the medication because voriconazole may cause harm when taken with these drugs. Additional monitoring, including additional blood tests, may be required for some medications.

Blood collection at the two-week time point for liver function tests will also be used to analyze the level of voriconazole in your blood stream. A small amount of extra blood will be drawn to analyze these samples. These samples will be stored until the conclusion of the study to be analyzed and will only be connected to you through your patient ID number.

You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

7) Pregnant and breast-feeding women cannot take part in the study because the medicine given in the study is not allowed during pregnancy and breast-feeding. If you are female, we will test if you are pregnant by urine test.

8) You will be assigned to a treatment program by chance, and the treatment you receive may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

9) The experimental treatments may have side effects that no one knows about yet, especially for new drugs like topical voriconazole. We will let you know if we learn anything that might make you change your mind about participating in the study.

10) The doctor may ask you to leave the study for any reason.

11) You do not have to pay for the medicine or for your hospital stay while part of the study. Also, if you are a part of the study, but later are taken out for any reason, the study will continue to pay for the medical treatment of your ulcer.

12) You will not receive any money from the study, since there will be no financial cost for you to participate.

13) We will hold medical records with personal medical information regarding the results of your eye exams. However, this information will be kept secret to others.

14) If you do not wish to participate in the study, you will be offered the standard treatment, natamycin.

15) If you are injured as a result of being in this study, treatment will be available. The costs of such treatment will be covered by Aravind Eye Hospital. We do not normally provide any other form of compensation for injury. For further information about this, you may contact the study coordinator.

I, the undersigned, understand the treatment offered to me in this study as well as its possible side effects. I volunteer freely and recognize that my identity and health records will remain confidential and will not be released to anyone else. I understand that if, for any reason, I decide to stop participating in the study, I will still be treated free of charge. If I have any questions regarding the study, I will contact the Aravind Eye Hospital Study Coordinator, Sister Sumithra, at:

Aravind Eye Hospital
1, Anna Nagar
Madurai - 625 020
Tamil Nadu
Phone: 91-452-4356100

_____	_____	_____
Printed Name	Signature	Date

Minor Assent Form for Aravind Eye Hospital Patients (MUTT II)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date:** D D / M M / Y Y
Enrollment Date: D D / M M / Y Y

Thank you for thinking about participating in this study at the Aravind Eye Hospital. In this study, Dr. Prajna, Dr. Srinivasan, Dr. Lietman and Dr. Acharya are trying to find out if giving a newer antifungal medicine called voriconazole to people with fungal corneal ulcers will help their eyes heal better than giving them voriconazole eye drops alone. This newer antifungal medicine is effective in treating fungal disease elsewhere in the body and has been used to treat eye infections. We are asking you to participate in the study because you have an infection affecting the front part of the eye (cornea) caused by fungus. By signing this form, you agree that you understand the following things:

1) By participating in this study at Aravind Eye Hospital, you agree to stay in the hospital and get a daily exam until your eye heals. We are asking you to stay at the hospital because we want to check on your eye often to make sure the medication is helping your eye heal.

2) If you do not want to stay in the hospital but still would like to participate in the study, you agree that we will take the medication as directed by the physician without skipping a dose. You will come back to the hospital every 3 days for an eye exam, and you will need a fresh bottle of medication every week for 2 weeks. You also agree to bring back the old bottles you used so we can check if you took the medication as instructed. It is important that you know that you need to take the medication for three weeks but that the medication we are giving you is good only for 1 week. Therefore, it is crucial that the medication be replaced every week.

3) After the initial treatment, you agree to return to the hospital two more times to get an exam. We will ask you to come back at 3 weeks and 3 months from when you start receiving treatment. During these exams, we will measure your vision, look at your eye using an instrument called a slit lamp, and take photographs of your eye. Each exam will take about 1 hour.

4) You will receive an antifungal medication for your corneal ulcer. You will receive either

   a. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and a placebo taken by mouth

   OR

   b. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and voriconazole taken by mouth

The medication you will receive will be determined by chance. A computer program will be used to randomly (like the flip of a coin) assign you to one medication or the other. This means that you have 50/50 chance of getting either drug. Neither you nor your doctor will know which medication you are receiving.

5) As with all eye drops, there is the risk of irritation and/or allergy. Sometimes, the medicine may fail to heal your eye. There are not many risks reported with the use of voriconazole or natamycin eye drops.

6) It is reported that taking voriconazole in other ways, including by mouth, may cause the following side effects. Please note that the rates reported below reflect previous studies of voriconazole done in patients who were seriously ill and were on long-term therapy for several months, sometimes including IV therapy. We therefore expect that these rates will be lower in this study.

   a. Visual changes: 30-40% of patients receiving systemic voriconazole (by mouth or injection) may experience visual changes including blurred vision and color vision changes. These changes often go away when you continue the treatment, and they always go away within 14 days of discontinuing treatment.

   b. Formed hallucinations: 10-15% of patients receiving systemic voriconazole (by mouth or injection) may experience formed or auditory hallucinations, including seeing and hearing things

that are not actually there. These hallucinations are not permanent and resolve upon termination of therapy.

c. Abnormal liver function tests: 10-20% of patients receiving systemic voriconazole (by mouth or injection) may have elevated liver function tests. These changes usually resolve upon completion of the therapy. There is a risk of liver failure, but it is very rare. You will be required to have two blood tests: one when you begin the study to rule out pre-existing liver disease, and one after 3 weeks, to monitor the function of your liver upon completion of your treatment. All liver function tests will be reviewed by a doctor who specializes in internal medicine. If an abnormal liver value is found, you will have weekly repeat blood tests until the values return to normal. If liver values are found to be significantly elevated or you have clinical symptoms of liver dysfunction (including jaundice), you will be evaluated by a doctor who specializes in internal medicine.

d. Skin reactions: Up to 6% of patients receiving systemic voriconazole (by mouth or by injection) may experience a skin reaction, including rash. These are usually mild. You will be advised to avoid exposure to strong, direct sunlight while taking the medication as there have been reports of sensitivity to sunlight while taking this medication. Skin reactions will be closely monitored.

e. Headache: 3-4% of patients receiving systemic voriconazole (by mouth or by injection) may experience headaches. These headaches are not serious or permanent and will resolve when therapy is stopped.

f. Dizziness: 1-3% of patients may experience dizziness while taking this medication. The dizziness is not serious.

g. Other potential side effects: fever, vomiting, nausea, diarrhea, stomach pain, and difficulty breathing.

If you experience any of these symptoms while taking the study medications, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine, sulfonylurea oral hypoglycemics, oral contraceptives, or any of the drugs on the attached appendix, please let your doctor know before you receive the medication because voriconazole may cause harm when taken with these drugs. Additional monitoring, including additional blood tests, may be required for some medications.

Blood collection at the two-week time point for liver function tests will also be used to analyze the level of voriconazole in your blood stream. A small amount of extra blood will be drawn to analyze these samples. These samples will be stored until the conclusion of the study to be analyzed and will only be connected to you through your patient ID number.

You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

7) Pregnant and breast-feeding women cannot take part in the study because the medicine given in the study is not allowed during pregnancy and breast-feeding. If you are female, we will test if you are pregnant by urine test.

8) You will be assigned to a treatment program by chance, and the treatment you receive may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

9) The experimental treatments may have side effects that no one knows about yet, especially for new drugs like topical voriconazole. We will let you know if we learn anything that might make you change your mind about participating in the study.

10) The doctor may ask you to leave the study for any reason.

11) You do not have to pay for the medicine or for your hospital stay while part of the study. Also, if you are a part of the study, but later are taken out for any reason, the study will continue to pay for the medical treatment of your ulcer.

12) You will not receive any money from the study, since there will be no financial cost for you to participate.

13) We will hold medical records with personal medical information regarding the results of your eye exams. However, this information will be kept secret to others.

14) If you do not wish to participate in the study, you will be offered the standard treatment, natamycin.

15) If you are injured as a result of being in this study, treatment will be available. The costs of such treatment will be covered by Aravind Eye Hospital. We do not normally provide any other form of compensation for injury. For further information about this, you may contact the study coordinator.

I, the undersigned, understand the treatment offered to me in this study as well as its possible side effects. I volunteer freely and recognize that my identity and health records will remain confidential and will not be released to anyone else. I understand that if, for any reason, I decide to stop participating in the study, I will still be treated free of charge. If I have any questions regarding the study, I will contact the Aravind Eye Hospital Study Coordinator, Sister Sumithra, at:

Aravind Eye Hospital
1, Anna Nagar
Madurai - 625 020
Tamil Nadu
Phone: 91-452-4356100

_____	_____	_____
Printed Name	Signature	Date

The person being considered for this study is legally unable to consent for herself/himself because s/he is a minor. By signing this form, I am giving permission for my child or ward to participate in the study.

_____	_____	_____
Printed Name	Signature of a Parent	Date

Appendix: List of Contraindicated Medications for Voriconazole

- Rifampin
- Carbamazepine
- Long acting barbiturates
- Terfenadine
- Astemizole
- Cisapride
- Pimozide
- Quinidine
- Sirolimus
- Rifabutin
- Ergot alkaloids (egotamine, dihydroergotamine)
- Phenytoin
- HIV Protease Inhibitors
- NNRTIs
- Cyclosporine
- Tacrolimus
- Phenytoin
- Warfarin
- Omeprazole
- Benzodiazepines
- Statins
- Calcium Channel Blockers
- Vinca alkaloids

Please inform your doctor if you are on any of these medications before you begin the study medication, as they may be harmful when taken together.

Patient Consent Form for Lumbini Eye Institute and Bharatpur Eye Hospital Patients (MUTT II)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date: D D / M M / Y Y**
Enrollment Date: D D / M M / Y Y

Thank you for thinking about participating in this study at the Lumbini Eye Institute or Bharatpur Eye Hospital. In this study, Dr. Kavita and Dr. Khadka are trying to find out if giving a newer antifungal pill called voriconazole in addition to voriconazole eyedrops to people with fungal corneal ulcers will help their eyes heal better than giving them voriconazole eye drops alone. This newer antifungal medicine is effective in treating fungal disease elsewhere in the body and has been used to treat eye infections. We are asking you to participate in the study because you have an infection affecting the front part of the eye (cornea) caused by fungus. By signing this form, you agree that you understand the following things:

5) By participating in this study at Lumbini Eye Institute or Bharatpur Eye Hospital, you agree to stay in the hospital and get a daily exam until your eye heals. We are asking you to stay at the hospital because we want to check on your eye often to make sure the medication is helping your eye heal.

6) If you do not want to stay in the hospital but still would like to participate in the study, you agree that we will take the medication as directed by the physician without skipping a dose. You will come back to the hospital every 3 days for an eye exam, and you will need a fresh bottle of medication every week for 3 weeks.. You also agree to bring back the old bottles you used so we can check if you took the medication as instructed. It is important that you know that you need to take the medication for three weeks but that the medication we are giving you is good only for 1 week. Therefore, it is crucial that the medication be replaced every week.

7) After the initial treatment, you agree to return to the hospital two more times to get an exam. We will ask you to come back at 3 weeks and 3 months from when you start receiving treatment. During these exams, we will measure your vision, look at your eye using an instrument called a slit lamp, and take photographs of your eye. Each exam will take about 1 hour.

8) You will receive an antifungal medication for your corneal ulcer. You will receive either

   c. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and a placebo taken by mouth

   OR

   d. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and voriconazole taken by mouth

   The medication you will receive will be determined by chance. A computer program will be used to randomly (like the flip of a coin) assign you to one medication or the other. This means that you have 50/50 chance of getting either drug. Neither you nor your doctor will know which medication you are receiving.

   16) As with all eye drops, there is the risk of irritation and/or allergy. Sometimes, the medicine may fail to heal your eye. There are not many risks reported with the use of voriconazole or natamycin eye drops.

   17) It is reported that taking voriconazole in other ways, including by mouth, may cause the following side effects. Please note that the rates reported below reflect previous studies of voriconazole done in patients who were seriously ill and were on long-term therapy for several months, sometimes including IV therapy. We therefore expect that these rates will be lower in this study.

      a. Visual changes: 30-40% of patients receiving systemic voriconazole (by mouth or injection) may experience visual changes including blurred vision and color vision changes. These changes often go away when you continue the treatment, and they always go away within 14 days of discontinuing treatment.

      b. Formed hallucinations: 10-15% of patients receiving systemic voriconazole (by mouth or injection) may experience formed or auditory hallucinations, including seeing and hearing things

that are not actually there. These hallucinations are not permanent and resolve upon termination of therapy.

c. Abnormal liver function tests: 10-20% of patients receiving systemic voriconazole (by mouth or injection) may have elevated liver function tests. These changes usually resolve upon completion of the therapy. There is a risk of liver failure, but it is very rare. You will be required to have two blood tests: one when you begin the study to rule out pre-existing liver disease, and one after 2 weeks, to monitor the function of your liver upon completion of your treatment. All liver function tests will be reviewed by a doctor who specializes in internal medicine. If an abnormal liver value is found, you will have weekly repeat blood tests until the values return to normal. If liver values are found to be significantly elevated or you have clinical symptoms of liver dysfunction (including jaundice), you will be evaluated by a doctor who specializes in internal medicine.

d. Skin reactions: Up to 6% of patients receiving systemic voriconazole (by mouth or by injection) may experience a skin reaction, including rash. These are usually mild. You will be advised to avoid exposure to strong, direct sunlight while taking the medication as there have been reports of sensitivity to sunlight while taking this medication. Skin reactions will be closely monitored.

e. Headache: 3-4% of patients receiving systemic voriconazole (by mouth or by injection) may experience headaches. These headaches are not serious or permanent and will resolve when therapy is stopped.

f. Dizziness: 1-3% of patients may experience dizziness while taking this medication. The dizziness is not serious.

g. Other potential side effects: fever, vomiting, nausea, diarrhea, stomach pain, and difficulty breathing.

If you experience any of these symptoms while taking the study medications, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine, sulfonylurea oral hypoglycemics, oral contraceptives, or any of the drugs on the attached appendix, please let your doctor know before you receive the medication because voriconazole may cause harm when taken with these drugs. Additional monitoring, including additional blood tests, may be required for some medications.

Blood collection at the two-week time point for liver function tests will also be used to analyze the level of voriconazole in your blood stream. A small amount of extra blood will be drawn to analyze these samples. These samples will be stored until the conclusion of the study to be analyzed and will only be connected to you through your patient ID number.

You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

18) Pregnant and breast-feeding women cannot take part in the study because the medicine given in the study is not allowed during pregnancy and breast-feeding. If you are female, we will test if you are pregnant by urine test.

19) You will be assigned to a treatment program by chance, and the treatment you receive may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

20) The experimental treatments may have side effects that no one knows about yet, especially for new drugs like topical voriconazole. We will let you know if we learn anything that might make you change your mind about participating in the study.

21) The doctor may ask you to leave the study for any reason.

22) You do not have to pay for the medicine or for your hospital stay while part of the study. Also, if you are a part of the study, but later are taken out for any reason, the study will continue to pay for the medical treatment of your ulcer.

23) You will not receive any money from the study, since there will be no financial cost for you to participate.

24) We will hold medical records with personal medical information regarding the results of your eye exams. However, this information will be kept secret to others.

25) If you do not wish to participate in the study, you will be offered the standard treatment, natamycin.

26) If you are injured as a result of being in this study, treatment will be available. The costs of such treatment will be covered by Aravind Eye Hospital. We do not normally provide any other form of compensation for injury. For further information about this, you may contact the study coordinator.

I, the undersigned, understand the treatment offered to me in this study as well as its possible side effects. I volunteer freely and recognize that my identity and health records will remain confidential and will not be released to anyone else. I understand that if, for any reason, I decide to stop participating in the study, I will still be treated free of charge. If I have any questions regarding the study, I will contact the Study Coordinator at:

Lumbini Eye Institute
Shree Rana Ambika Shah Eye Hospital
P.O.Box no. 30,Siddharthanagar 3
Rupandehi 32901, Lumbini, Nepal
Phone: 00977-71-520265, 523827
Email: srij.gautam@yahoo.com

Bharatpur Eye Hospital
Chitwan, Nepal
Email: beh@nec.com.np
Telephone: 0097-56-520333 (523333, 522750)

_____ _____ _____

Printed Name Signature Date

Minor Assent Form for Lumbini Eye Institute and Bharatpur Eye Hospital Patients (MUTT II)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date:** D D / M M / Y Y
Enrollment Date: D D / M M / Y Y

Thank you for thinking about participating in this study at the Lumbini Eye Institute or Bharatpur Eye Hospital. In this study, Dr. Kavita and Dr. Khadka are trying to find out if giving a newer antifungal medicine called voriconazole to people with fungal corneal ulcers will help their eyes heal better than giving them voriconazole eye drops alone. This newer antifungal medicine is effective in treating fungal disease elsewhere in the body and has been used to treat eye infections. We are asking you to participate in the study because you have an infection affecting the front part of the eye (cornea) caused by fungus. By signing this form, you agree that you understand the following things:

16) By participating in this study at the Lumbini Eye Institute or Bharatpur Eye Hospital, you agree to stay in the hospital and get a daily exam until your eye heals. We are asking you to stay at the hospital because we want to check on your eye often to make sure the medication is helping your eye heal.

17) If you do not want to stay in the hospital but still would like to participate in the study, you agree that we will take the medication as directed by the physician without skipping a dose. You will come back to the hospital every 3 days for an eye exam, and you will need a fresh bottle of medication every week for 2 weeks. You also agree to bring back the old bottles you used so we can check if you took the medication as instructed. It is important that you know that you need to take the medication for three weeks but that the medication we are giving you is good only for 1 week. Therefore, it is crucial that the medication be replaced every week.

18) After the initial treatment, you agree to return to the hospital two more times to get an exam. We will ask you to come back at 3 weeks and 3 months from when you start receiving treatment. During these exams, we will measure your vision, look at your eye using an instrument called a slit lamp, and take photographs of your eye. Each exam will take about 1 hour.

19) You will receive an antifungal medication for your corneal ulcer. You will receive either

a. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and a placebo taken by mouth

OR

b. An eye drop with medicine called voriconazole and an eye drop with a medicine called natamycin and voriconazole taken by mouth

The medication you will receive will be determined by chance. A computer program will be used to randomly (like the flip of a coin) assign you to one medication or the other. This means that you have 50/50 chance of getting either drug. Neither you nor your doctor will know which medication you are receiving.

20) As with all eye drops, there is the risk of irritation and/or allergy. Sometimes, the medicine may fail to heal your eye. There are not many risks reported with the use of voriconazole or natamycin eye drops.

21) It is reported that taking voriconazole in other ways, including by mouth, may cause the following side effects. Please note that the rates reported below reflect previous studies of voriconazole done in patients who were seriously ill and were on long-term therapy for several months, sometimes including IV therapy. We therefore expect that these rates will be lower in this study.

h. Visual changes: 30-40% of patients receiving systemic voriconazole (by mouth or injection) may experience visual changes including blurred vision and color vision changes. These changes often go away when you continue the treatment, and they always go away within 14 days of discontinuing treatment.

i. Formed hallucinations: 10-15% of patients receiving systemic voriconazole (by mouth or injection) may experience formed or auditory hallucinations, including seeing and hearing things

that are not actually there. These hallucinations are not permanent and resolve upon termination of therapy.

j. Abnormal liver function tests: 10-20% of patients receiving systemic voriconazole (by mouth or injection) may have elevated liver function tests. These changes usually resolve upon completion of the therapy. There is a risk of liver failure, but it is very rare. You will be required to have two blood tests: one when you begin the study to rule out pre-existing liver disease, and one after 3 weeks, to monitor the function of your liver upon completion of your treatment. All liver function tests will be reviewed by a doctor who specializes in internal medicine. If an abnormal liver value is found, you will have weekly repeat blood tests until the values return to normal. If liver values are found to be significantly elevated or you have clinical symptoms of liver dysfunction (including jaundice), you will be evaluated by a doctor who specializes in internal medicine.

k. Skin reactions: Up to 6% of patients receiving systemic voriconazole (by mouth or by injection) may experience a skin reaction, including rash. These are usually mild. You will be advised to avoid exposure to strong, direct sunlight while taking the medication as there have been reports of sensitivity to sunlight while taking this medication. Skin reactions will be closely monitored.

l. Headache: 3-4% of patients receiving systemic voriconazole (by mouth or by injection) may experience headaches. These headaches are not serious or permanent and will resolve when therapy is stopped.

m. Dizziness: 1-3% of patients may experience dizziness while taking this medication. The dizziness is not serious.

n. Other potential side effects: fever, vomiting, nausea, diarrhea, stomach pain, and difficulty breathing.

If you experience any of these symptoms while taking the study medications, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine, sulfonylurea oral hypoglycemics, oral contraceptives, or any of the drugs on the attached appendix, please let your doctor know before you receive the medication because voriconazole may cause harm when taken with these drugs. Additional monitoring, including additional blood tests, may be required for some medications.

Blood collection at the two-week time point for liver function tests will also be used to analyze the level of voriconazole in your blood stream. A small amount of extra blood will be drawn to analyze these samples. These samples will be stored until the conclusion of the study to be analyzed and will only be connected to you through your patient ID number.

You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

22) Pregnant and breast-feeding women cannot take part in the study because the medicine given in the study is not allowed during pregnancy and breast-feeding. If you are female, we will test if you are pregnant by urine test.

23) You will be assigned to a treatment program by chance, and the treatment you receive may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

24) The experimental treatments may have side effects that no one knows about yet, especially for new drugs like topical voriconazole. We will let you know if we learn anything that might make you change your mind about participating in the study.

25) The doctor may ask you to leave the study for any reason.

26) You do not have to pay for the medicine or for your hospital stay while part of the study. Also, if you are a part of the study, but later are taken out for any reason, the study will continue to pay for the medical treatment of your ulcer.

27) You will not receive any money from the study, since there will be no financial cost for you to participate.

28) We will hold medical records with personal medical information regarding the results of your eye exams. However, this information will be kept secret to others.

29) If you do not wish to participate in the study, you will be offered the standard treatment, natamycin.

30) If you are injured as a result of being in this study, treatment will be available. The costs of such treatment will be covered by Aravind Eye Hospital. We do not normally provide any other form of compensation for injury. For further information about this, you may contact the study coordinator.

I, the undersigned, understand the treatment offered to me in this study as well as its possible side effects. I volunteer freely and recognize that my identity and health records will remain confidential and will not be released to anyone else. I understand that if, for any reason, I decide to stop participating in the study, I will still be treated free of charge. If I have any questions regarding the study, I will contact the Study Coordinator at:

Lumbini Eye Institute
Shree Rana Ambika Shah Eye Hospital
P.O.Box no. 30,Siddharthanagar 3
Rupandehi 32901, Lumbini, Nepal
Phone: 00977-71-520265, 523827
Email: srij.gautam@yahoo.com

Bharatpur Eye Hospital
Chitwan, Nepal
Email: beh@nec.com.np
Telephone: 0097-56-520333 (523333, 522750)

_____ _____ _____
Printed Name Signature Date

The person being considered for this study is legally unable to consent for herself/himself because s/he is a minor. By signing this form, I am giving permission for my child or ward to participate in the study.

_____ _____ _____
Printed Name Signature of a Parent Date

Appendix: List of Contraindicated Medications for Voriconazole

- Rifampin
- Carbamazepine
- Long acting barbiturates
- Terfenadine
- Astemizole
- Cisapride
- Pimozide
- Quinidine
- Sirolimus
- Rifabutin
- Ergot alkaloids (egotamine, dihydroergotamine)
- Phenytoin
- HIV Protease Inhibitors
- NNRTIs
- Cyclosporine
- Tacrolimus
- Phenytoin
- Warfarin
- Omeprazole
- Benzodiazepines
- Statins
- Calcium Channel Blockers
- Vinca alkaloids

Please inform your doctor if you are on any of these medications before you begin the study medication, as they may be harmful when taken together.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT
MUTT I

M.R. No.: _____ **Patient ID:** | **1** | | | | | **Today's Date: __ __/__ __/__ __**

Enrollment Date: __ __/__ __/__ __

A. PURPOSE AND BACKGROUND

Drs. Nisha Acharya, Thomas M. Lietman, John P. Whitcher, and Stephen McLeod from the UCSF Proctor Foundation are conducting a study to determine if the use of a newer antifungal in the treatment of corneal ulcers improves visual acuity.

Patients with fungal infections in the cornea are often left with a corneal scar that affects vision.

You are being asked to participate in this study because you have a fungal corneal ulcer.

B. PROCEDURES

If you agree to be in this study, the following will happen:

1) A culture of your cornea will be performed. This is recommended whether or not you are involved in the study. This involves gently scraping the surface of the corneal ulcer and testing for the presence of fungus and bacteria in the microbiology laboratory.
2) If we find an evidence of fungus on your eye, you will be treated with one drop of an antifungal medication every 1 hour while awake for 1 week, and then every 2 hours while awake for 2 weeks. If the eye examination and laboratory test results show that you are eligible for the study and if you are interested in participating, you will be randomly assigned to one of two medications. This means that you have a 50/50 chance (like flipping a coin) of being in either group and that neither the researchers nor you will make the choice of which group you are in. One medication is called voriconazole and the other is called natamycin.
3) When we give you the medication, we will swab your eye to make sure the medicine is going into your eye. This procedure might feel uncomfortable and may make your ulcer worse on rare occasions. To lessen discomfort, you will be given numbing eye drops beforehand.
4) Regularly scheduled exams will be scheduled until healing after the corneal ulcer after treatment, and two exams will be done after healing occurs (at 3 weeks and 3 months). These are approximately the same number of exams that we would recommend if you were not in the study. Exams will take approximately the same amount of time whether or not you are in the study. An eye exam to find the best glasses and/or contact lenses will be done after the ulcer has healed and takes approximately 30 minutes.
5) You may be asked to give a blood sample. Blood samples will be collected from approximately 10% of patients to analyze the level of voriconazole in your blood stream. These samples will be stored until the conclusion of the study for analysis, and will only be linked to you through your patient ID number.
6) You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.
7) You may be withdrawn from the study without your consent if the researchers believe it is in your best interest or if you fail to follow study procedures.

A great deal of follow-up care is important with corneal ulcer treatment. We estimate that study participation will take an additional 1 hour past what is normally required. All study procedures will be done at the Proctor Foundation Cornea Clinic at the University of California, San Francisco.

C. RISKS/DISCOMFORTS

As with all eye drops, there is the risk of irritation and/or allergy.

1) Randomization: You will be assigned to a treatment program by chance. The treatment you receive (newer or conventional antifungal) may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

2) Because the risks to a fetus and a breast-fed from antifungal use are unknown, pregnant and lactating women must not participate in this study. Pregnancy testing will be offered if you would like to confirm your pregnancy status.

3) Voriconazole: It is a newer antifungal that is effectively being used for the fungal diseases affecting some parts of the body other than the eyes (such as lungs). We want to check if it is more effective than the conventional antifungal Natamycin for the fungal corneal ulcer. There are not many risks reported with the use of voriconazole eye drops. However, it is reported that taking voriconazole in other ways may cause blurred vision, abnormal liver function tests, fever, rash, vomiting, nausea, diarrhea, headache, stomach pain, and difficulty breathing. If you experience any of these symptoms while taking the study eye drops, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine or oral contraceptives, please let your doctor know before you receive the eye drops because voriconazole may cause harm when taken with these drugs.

4) Natamycin: Natamycin is the only FDA-approved antifungal medication that is currently being used for the treatment of fungal corneal ulcer. There are no known serious side effects with this medication.

5) The procedures (cultures, exams, checking for the best glasses or contact lenses) carry the same very small risk whether or not they are performed in the study or as part of your care outside the study. To minimize discomfort, topical anesthetic will be given before exams, scrapings, and cultures.

5) Unknown Risks: The experimental treatments may have side effects that no one knows about yet. The researchers will let you know if they learn anything that might make you change your mind about participating in the study.

Confidentiality:
Since the records will contain your information and since there will be a follow-up at 3 weeks and 3 months after the procedure, participation in this research study may involve a loss of privacy. However, information about you will be handled as confidentially as possible. This will be achieved by limiting access to the study records; storing study records in files in a locked office; and keeping computer databases secure with a password. In addition, the main study database will contain only an ID number and not your name.

Representatives from the sponsoring organizations may review information about the study.

Your name or individual identifiers will not be used in any published reports about this study.

Treatment and Compensation for Injury:
If you are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, depending on a number of factors.
The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at (415) 476-1814.

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CONSENT TO BE A RESEARCH SUBJECT

D. BENEFITS

If you are in the group that receives Voriconazole and it proves to treat your condition better and with fewer side effects than with Natamycin, then you may benefit from participating in the study; however, this cannot be guaranteed. Also, you will have participated in a study that may help future patients with a condition similar to your own.

E. ALTERNATIVES

If you choose not to participate in this study, you will be offered the standard therapy for your condition, at the discretion of your physician.

F. COSTS

The study will cover costs of tests and clinic visits that are a scheduled part of the study and which any insurance that you may have will not cover. The antifungal medication will be provided free of charge.

G. PAYMENT

You will not be paid or reimbursed for participating in this study.

H. QUESTIONS

This study has been explained to you by Dr. Thomas M. Lietman, Dr. Nisha Acharya, Dr. John P. Whitcher or one of their associates. If you have any other questions about the study, you may call Dr. Lietman at (415) 502-2662, Dr. Acharya (415) 476-8131, or the Study Coordinator at (415) 514-2163.

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CONSENT TO BE A RESEARCH SUBJECT

I. CONSENT

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled. If you do not wish to participate in the study, you will be offered the treatment as given in the study at your physician's discretion.

If you wish to participate in this study, you should sign below. Your signature will indicate your agreement to participate.

Once you have signed this consent form, you will be given copies of this consent form and the Experimental Subject's Bill of Rights to keep. You will also be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you. You will receive a copy of all three documents to keep.

Date	Subject's Signature for Consent
Date	Signature of Person Obtaining Consent

The person being considered for this study is unable to consent for himself/herself because he or she is a minor (age 16-17). You have been asked to give your permission to include your child in this study. You know of no reason why he/she would refuse were it possible to do so now.

Date	Signature of Parent or Legal Guardian of Study Subject
Date	Signature of Minor (16-17 years of age) indicating Assent
Date	Signature of Person Obtaining Consent

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT
MUTT II
Page 1 of 4

M.R. No.: _____ Patient ID 2 | | | | | **Today's Date: __ __/__ __/__ __**

Enrollment Date: __ __/__ __/__ __

A. PURPOSE AND BACKGROUND

Drs. Nisha Acharya, Thomas M. Lietman, John P. Whitcher, and Stephen McLeod from the UCSF Proctor Foundation are conducting a study to determine if the use of a newer antifungal in the treatment of corneal ulcers improves visual acuity.

Patients with fungal infections in the cornea are often left with a corneal scar that affects vision.

You are being asked to participate in this study because you have a fungal corneal ulcer.

B. PROCEDURES

If you agree to be in this study, the following will happen:

1) A culture of your cornea will be performed. This is recommended whether or not you are involved in the study. This involves gently scraping the surface of the corneal ulcer and testing for the presence of fungus and bacteria in the microbiology laboratory.
2) If we find an evidence of fungus on your eye, you will be treated with one drop of an antifungal medication every 1 hour while awake for 1 week, and then every 2 hours while awake for 2 weeks. If the eye examination and laboratory test results show that you are eligible for the study and if you are interested in participating, you will be randomly assigned to one of two medications. This means that you have a 50/50 chance (like flipping a coin) of being in either group and that neither the researchers nor you will make the choice of which group you are in. You will either receive voriconazole and natamycin eye drops and a placebo by mouth, or voriconazole and natamycin eye drops and voriconazole pills by mouth.
3) When we give you the medication, we will swab your eye to make sure the medicine is going into your eye. This procedure might feel uncomfortable and may make your ulcer worse on rare occasions. To lessen discomfort, you will be given numbing eye drops beforehand.
4) Regularly scheduled exams will be scheduled until healing after the corneal ulcer after treatment, and two exams will be done after healing occurs (at 3 weeks and 3 months). These are approximately the same number of exams that we would recommend if you were not in the study. Exams will take approximately the same amount of time whether or not you are in the study. An eye exam to find the best glasses and/or contact lenses will be done after the ulcer has healed and takes approximately 30 minutes.
5) You will be required to have two blood tests to monitor your liver function. The first blood test will occur prior to enrollment, to ensure that you are eligible for the study and that you do not have a pre-existing liver condition. The second blood test will occur at two weeks to monitor your liver function during the course of therapy. If it is found that you have elevated liver function tests at 2 weeks, you will receive a weekly blood test until your liver values have returned to normal. Some of the blood collected at the two week visit will be stored for analysis of your blood voriconazole levels. This additional test requires a small amount of extra blood over that which is required for the liver function tests. These samples will be stored until the completion of the study for analysis.
6) You will be asked to complete three short questionnaires to assess your quality of life and vision function. These are standardized questions used in many studies, and your answers will be kept

confidential. We will also ask you questions over the course of the study pertaining to the number of doctor visits you have, how many days of work you have missed, and number of days spent in the hospital. This information will be kept confidential.

6) You may be withdrawn from the study without your consent if the researchers believe it is in your best interest or if you fail to follow study procedures.

A great deal of follow-up care is important with corneal ulcer treatment. We estimate that study participation will take an additional 1 hour past what is normally required. All study procedures will be done at the Proctor Foundation Cornea Clinic at the University of California, San Francisco.

C. RISKS/DISCOMFORTS

As with all eye drops, there is the risk of irritation and/or allergy.

1) Randomization: You will be assigned to a treatment program by chance. The treatment you receive (voriconazole eye drops with or without voriconazole by mouth) may prove to be less effective or to have more side effects than the other study treatment or other available treatments.

2) Because the risks to a fetus and a breast-fed from antifungal use are unknown, pregnant and lactating women must not participate in this study. Pregnancy testing will be offered if you would like to confirm your pregnancy status.

3) Topical Voriconazole: It is a newer antifungal that is effectively being used for the fungal diseases affecting some parts of the body other than the eyes (such as lungs). We want to check if it is more effective than the conventional antifungal Natamycin for the fungal corneal ulcer. There are not many risks reported with the use of voriconazole eye drops.

4) Natamycin: Natamycin is the only FDA-approved antifungal medication that is currently being used for the treatment of fungal corneal ulcer. There are no known serious side effects with this medication.

5) Oral Voriconazole: It is reported that taking voriconazole orally and in other ways may cause the following side effects. Please note that the rates reported below reflect previous studies of voriconazole done in patients who were seriously ill and were on long-term therapy for several months, sometimes including IV therapy. We therefore expect that these rates will be lower in this study.

    a. Visual changes: 30-40% of patients receiving systemic voriconazole (by mouth or injection) may experience visual changes including blurred vision and color vision changes. These changes often go away when you continue the treatment, and they always go away within 14 days of discontinuing treatment.

    b. Formed hallucinations: 10-15% of patients receiving systemic voriconazole (by mouth or injection) may experience formed or auditory hallucinations, including seeing and hearing things that are not actually there. These hallucinations are not permanent and resolve upon termination of therapy.

    c. Abnormal liver function tests: 10-20% of patients receiving systemic voriconazole (by mouth or injection) may have elevated liver function tests. These changes almost always resolve upon completion of the therapy. There is a risk of liver failure, but it is very rare. You will be required to have two blood tests: one when you begin the study to rule out pre-existing liver disease, and one after 3 weeks, to monitor the function of your liver upon completion of your treatment. All liver function tests will be reviewed by a doctor who specializes in internal medicine. If an abnormal liver value is found, you will have weekly repeat blood tests until the values return to normal. If liver values are found to be significantly elevated or you have clinical symptoms of liver dysfunction (including jaundice), you will be evaluated by a doctor who specializes in internal medicine.

    d. Skin reactions: Up to 6% of patients receiving systemic voriconazole (by mouth or by injection) may experience a skin reaction, including rash. These are usually mild. You will be advised to avoid exposure to strong, direct sunlight while taking the medication as there have been reports of sensitivity to sunlight while taking this medication. Skin reactions will be closely monitored.

a. Headache: 3-4% of patients receiving systemic voriconazole (by mouth or by injection) may experience headaches. These headaches are not serious or permanent and will resolve when therapy is stopped.

e. Dizziness: 1-3% of patients may experience dizziness while taking this medication. The dizziness is not serious.

f. Other potential side effects: fever, vomiting, nausea, diarrhea, stomach pain, and difficulty breathing.

If you experience any of these symptoms while taking the study medications, please let your doctor know. If you are taking any drugs named rifampin, rifabutin, ritonavir, long acting barbiturates, phenytoin, carbamezepine, sulfonylurea oral hypoglycemics, oral contraceptives, or any of the drugs on the attached appendix, please let your doctor know before you receive the medication because voriconazole may cause harm when taken with these drugs. Additional monitoring, including additional blood tests, may be required for some medications.

6) The procedures (cultures, exams, checking for the best glasses or contact lenses) carry the same very small risk whether or not they are performed in the study or as part of your care outside the study. To minimize discomfort, topical anesthetic will be given before exams, scrapings, and cultures.

7) Unknown Risks: The experimental treatments may have side effects that no one knows about yet. The researchers will let you know if they learn anything that might make you change your mind about participating in the study.

Confidentiality:
Since the records will contain your information and since there will be a follow-up at 3 weeks and 3 months after the procedure, participation in this research study may involve a loss of privacy. However, information about you will be handled as confidentially as possible. This will be achieved by limiting access to the study records; storing study records in files in a locked office; and keeping computer databases secure with a password. In addition, the main study database will contain only an ID number and not your name.

Representatives from the sponsoring organizations may review information about the study.

Your name or individual identifiers will not be used in any published reports about this study.

Treatment and Compensation for Injury:
If you are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, depending on a number of factors.
The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at (415) 476-1814.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT

D. BENEFITS

If you are in the group that receives Voriconazole by mouth as well as eye drops and it proves to treat your condition better and with fewer side effects than with Voriconazole eye drops alone, then you may benefit from participating in the study; however, this cannot be guaranteed. Also, you will have participated in a study that may help future patients with a condition similar to your own.

E. ALTERNATIVES

If you choose not to participate in this study, you will be offered the standard therapy for your condition, at the discretion of your physician.

F. COSTS

The study will cover costs of tests and clinic visits that are a scheduled part of the study and which any insurance that you may have will not cover. The antifungal medication will be provided free of charge.

G. PAYMENT

You will not be paid or reimbursed for participating in this study.

H. QUESTIONS

This study has been explained to you by Dr. Thomas M. Lietman, Dr. Nisha Acharya, Dr. John P. Whitcher or one of their associates. If you have any other questions about the study, you may call Dr. Lietman at (415) 502-2662, Dr. Acharya (415) 476-8131, or the Study Coordinator at (415) 514-2163.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT

I. CONSENT

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled. If you do not wish to participate in the study, you will be offered the treatment as given in the study at your physician's discretion.

If you wish to participate in this study, you should sign below. Your signature will indicate your agreement to participate.

Once you have signed this consent form, you will be given copies of this consent form and the Experimental Subject's Bill of Rights to keep. You will also be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you. You will receive a copy of all three documents to keep.

Date	Subject's Signature for Consent
Date	Signature of Person Obtaining Consent

The person being considered for this study is unable to consent for himself/herself because he or she is a minor (age 16-17). You have been asked to give your permission to include your child in this study. You know of no reason why he/she would refuse were it possible to do so now.

Date	Signature of Parent or Legal Guardian of Study Subject
Date	Signature of Minor (16-17 years of age) indicating Assent

Appendix: List of Contraindicated Medications for Voriconazole

- Rifampin
- Carbamazepine
- Long acting barbiturates
- Terfenadine
- Astemizole
- Cisapride
- Pimozide
- Quinidine
- Sirolimus
- Rifabutin
- Ergot alkaloids (egotamine, dihydroergotamine)
- Phenytoin
- HIV Protease Inhibitors
- NNRTIs
- Cyclosporine
- Tacrolimus
- Phenytoin
- Warfarin
- Omeprazole
- Benzodiazepines
- Statins
- Calcium Channel Blockers
- Vinca alkaloids

Please inform your doctor if you are on any of these medications before you begin the study medication, as they may be harmful when taken together.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
EXPERIMENTAL SUBJECT'S
BILL OF RIGHTS

The rights below are the rights of every person who is asked to be in a research study. As an experimental subject I have the following rights:

1) To be told what the study is trying to find out,

2) To be told what will happen to me and whether any of the procedures, drugs, or devices is different from what would be used in standard practice,

3) To be told about the frequent and/or important risks, side effects, or discomforts of the things that will happen to me for research purposes,

4) To be told if I can expect any benefit from participating, and, if so, what the benefit might be,

5) To be told of the other choices I have and how they may be better or worse than being in the study,

6) To be allowed to ask any questions concerning the study both before agreeing to be involved and during the course of the study,

7) To be told what sort of medical treatment is available if any complications arise,

8) To refuse to participate at all or to change my mind about participation after the study is started. This decision will not affect my right to receive the care I would receive if I were not in the study,

9) To receive a copy of the signed and dated consent form,

10) To be free of pressure when considering whether I wish to agree to be in the study.

If I have other questions I should ask the researcher or the research assistant. In addition, I may contact the Committee on Human Research, which is concerned with protection of volunteers in research projects. I may reach the committee office by calling: (415) 476-1814 from 8:00 AM to 5:00 PM, Monday to Friday, or by writing to the Committee on Human Research, Box 0962, University of California, San Francisco, CA 94143.

Call (415) 476-1814 for information on translations.

University of California
Permission to Use Personal Health Information for Research

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date: __ __/__ __/__ __**
Study: ☐ MUTT I ☐ MUTT II **Enrollment Date: __ __/__ __/__ __**

IRB Approval Number**:**

Study Title (or IRB Approval Number if study title may breach subject's privacy):
Mycotic Ulcer Treatment Trial (MUTT)

Principal Investigator:	**Sponsor/Funding Agency (if funded):**
Thomas Lietman, MD	NEI
Nisha Acharya, MD MS	

A. WHAT IS THE PURPOSE OF THIS FORM?
State and federal privacy laws protect the use and release of your health information. Under these laws, the University of California San Francisco (UCSF) or your health care provider cannot release your health information to the research team unless you give your permission. The research team includes the researchers and people hired by the University or the sponsor to do the research. If you decide to give your permission and to participate in the study, you must sign this form, as well as the Consent Form. This form describes the different ways that the researcher, research team and research sponsor may use your health information for the research study. The research team will use and protect your information as described in the attached Consent Form. Once your health information is released it may not be protected by these privacy laws and might be shared with others. However, other laws protecting your confidentiality may still apply. If you have questions, please ask a member of the research team.

B. WHAT PERSONAL HEALTH INFORMATION WILL BE RELEASED?
If you give your permission and sign this form, you are allowing F. I. Proctor Foundation to release the following medical records containing your Personal Health Information. Your Personal Health Information includes health information in your medical records and information that can identify you. For example, Personal Health Information may include your name, address, phone number or social security number.

☐ Entire Medical Record	☐ Radiology Reports	☒ Laboratory Reports
☒ Outpatient Clinic Records	☐ Radiology Images	☐ Psychological Tests
☒ Progress Notes	☐ Diagnostic Imaging Reports	☐ Dental Records
☐ Consultations	☐ Operative Reports	☐ Discharge Summaries
☒ History & Physical Exams	☐ Pathology Reports	☐ Health Care Billing
☐ EKG	☐ Emergency Medicine Center Reports	

☐ Other: _____

C. DO I HAVE TO GIVE MY PERMISSION FOR CERTAIN SPECIFIC USES?
Yes. The following information will only be released if you give your specific permission by putting your initials on the line(s).

____ I agree to the release of information pertaining to drug and alcohol abuse, diagnosis or treatment.
____ I agree to the release of HIV/AIDS testing information.
____ I agree to the release of genetic testing information.
___ I agree to the release of information pertaining to mental health diagnosis or treatment as follows:

University of California

Permission to Use Personal Health Information for Research

D. HOW WILL MY PERSONAL HEALTH INFORMATION BE USED?

Your Personal Health Information may be released to these people for the following purposes:

1. To the research team for the research described in the attached Consent Form;
2. To others at UC who are required by law to review the research;
3. To others who are required by law to review the quality and safety of the research, including: U.S. government agencies, such as the Food and Drug Administration, the research sponsor or the sponsor's representatives, or government agencies in other countries. These organizations and their representatives may see your Personal Health Information. They may not copy or take it from your medical records unless permitted or required by law.

E. HOW WILL MY PERSONAL HEALTH INFORMATION BE USED IN A RESEARCH REPORT?

If you agree to be in this study, the research team may fill out a research report. (This is sometimes called a "case report".) The research report will ***not*** include your name, address, or telephone or social security number. The research report may include your date of birth, initials, dates you received medical care, and a tracking code. The research report will also include information the research team collects in the study. The research team and the research sponsor may use the research report and share it with others in the following ways:

1. To perform more research;
2. Share it with researchers in the U.S. or other countries;
3. Place it into research databases;
4. Use it to improve the design of future studies;
5. Use it to publish articles or for presentations to other researchers;
6. Share it with business partners of the sponsor; or
7. File applications with U.S. or foreign government agencies to get approval for new drugs or health care products.

F. DOES MY PERMISSION EXPIRE?

This permission to release your Personal Health Information expires when the research ends and all required study monitoring is over. Research reports can be used forever.

G. CAN I CANCEL MY PERMISSION?

You can cancel your permission at any time. You can do this in two ways. You can write to the researcher or you can ask someone on the research team to give you a form to fill out to cancel your permission. If you cancel your permission, you may no longer be in the research study. You may want to ask someone on the research team if canceling will affect your medical treatment. If you cancel, information that was already collected and disclosed about you may continue to be used. Also, if the law requires it, the sponsor and government agencies may look at your medical records to review the quality or safety of the study.

H. SIGNATURE

If you agree to the release and use of your Personal Health Information, please sign below. You will be given a signed copy of this form**.**

_____

Name of Subject (print)

_____ _____

Signature of Subject Date

Note: if the subject is a minor, an individual signing with an "X", an adult incapable of giving consent, or is unable to read the authorization, fill out and attach the "special signatures" page (sections "I" and "J").

University of California
Permission to Use Personal Health Information for Research

SPECIAL SIGNATURES PAGE

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date: __ __/__ __/__ __**

Enrollment Date: __ __/__ __/__ __

I. If the subject is a minor, or an individual signing with an "X", or an adult incapable of giving consent (where IRB approved), the legally authorized representative or witness signs here:

_____ _____
Name of Legally Authorized Representative or Witness to the "X" (print) Relationship to the Subject

_____ _____
Signature of Representative or Witness Date

J. If the subject is unable to read the authorization, the translator or reader and a witness sign here:

I have accurately and completely read this Authorization to _____ (subject's name) in _____ (language), the subject's primary language. **The subject has verbally affirmed his/her Authorization to me and to the witness.**

_____
Name of Translator or Reader (print)

_____ _____
Signature of Translator or Reader Date

_____
Name of Witness (print)

_____ _____
Signature of Witness Date

A.8. Microbiology Report Form

M.R. No.: _____ **Patient ID:** ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Affected Eye: ☐ Right Eye ☐ Left Eye **Enrollment Date:** _ _ ☐☐☐ _ _
D D M M M Y Y
(For months, JAN = January, FEB =February, etc)

Time point: ☐ Enrollment

☐ Repeat culture date : _ _ ☐☐☐ _ _
D D M M M Y Y

KOH :
☐ Not done
☐ Fungus present
☐ Fungus absent

Giemsa:
☐ Not done
☐ Fungus present
☐ Fungus absent

Gram stain:
☐ Not done
☐ Fungus present ☐ Bacteria present
☐ Fungus absent ☐ Bacteria absent

Fungal culture:
☐ Not done, explain _____
☐ Negative at one week in spite of positive smear
☐ Positive Date: D D / M M / Y Y
Media with growth (check all that applies)
☐Potato Dextrose Agar ☐Sabouraud Dextrose Agar ☐Other, specify_____

☐ *Fusarium* spp
☐ *Aspergillus flavus*
☐ *Aspergillus fumigatus*
☐ *Aspergillus niger*
☐ *Aspergillus terreus*
☐ Unidentified hyaline
☐ Other *Aspergillus* species, specify:
_____

☐ *Alternaria* spp
☐ *Bipolaris* spp
☐ *Curvularia* spp
☐ *Exserohilum* spp
☐ *Lasiodiplodia* spp
☐ Unidentified dematiaceous
☐ Other, specify:
_____

Bacterial culture:
☐ Negative at one week
☐ Positive Date: D D / M M / Y Y
Media with growth (check all that applies)
☐Blood Agar ☐Chocolate Agar ☐Other, specify_____

Please specify _____

_____
Signature of Microbiologist or Study Coordinator or Investigator

Initials: _____

A.9. Visual Acuity Forms

Visual Acuity Form S

M.R. No: _____ Patient ID ☐☐☐☐☐ Today's Date: __ __ ☐☐☐ __ __
D D M M M Y Y

To be completed after enrollment

TIME POINT: ☐ Enrollment ☐ 3 Weeks ☐ 3 Months
ACUITY WITH: ☐ Refraction

AT ENROLLMENT TEST BOTH EYES USING FORM A
4 METER VISUAL ACUITY CHART S: Precision Vision Chart 2305

☐ Study Eye ☐ Right Eye ☐ Left Eye

CIRCLE each letter the subject identifies correctly, and write the total correct for each row in the column at the right. If 3 letters or less are read correctly on any row from Row 3 or below, STOP TESTING on that row. If the total number of letters read correctly at 4 meters is less than 10, add a +0.75 sphere to the distance correction, then have the subject read the first 6 rows of the letters again at 1 meter.

SECTION I = 4 Meter Test
(MUST TRY FIRST TWO LINES)

Row	Snellen Equivalent (meters)	Precision Vision Chart 2305 Tumbling E's	Number correct at 4 meters	log MAR Equiv
1	6/60	E 3 W E M	_____	1.0
2	6/48	3 M E W 3	_____	0.9
3	6/38	W E M 3 W	_____	0.8
4	6/30	M W 3 M E	_____	0.7
5	6/24	3 E W E M	_____	0.6
6	6/19	E W M W 3	_____	0.5
7	6/15	3 M E 3 W	_____	0.4
8	6/12	M E W M 3	_____	0.3
9	6/9.5	W 3 M W E	_____	0.2
10	6/7.5	E M E 3 W	_____	0.1
11	6/6	M 3 W E M	_____	0.0
12	6/4.8	3 W M 3 E	_____	-0.1
13	6/3.8	E M W E 3	_____	-0.2
14	6/3	W 3 E M W	_____	-0.3

Total number correct at 4 meters _____
If <10, proceed to 1 meter testing

Please circle + or – cylinder:
Refraction: _____ ± _____ x _____

or ☐ Plano

☐ Done ☐ Not done

SECTION II = 1 Meter Test
ADD +0.75 SPHERE
(MUST TRY FIRST TWO LINES)

Row	Snellen Equivalent (meters)	Precision Vision Chart 2305 Tumbling E's	Number correct at 1 meter	log MAR Equiv
1	6/242	E 3 W E M	_____	1.6
2	6/194	3 M E W 3	_____	1.5
3	6/152	W E M 3 W	_____	1.4
4	6/121	M W 3 M E	_____	1.3
5	6/97	3 E W E M	_____	1.2
6	6/76	E W M W 3	_____	1.1

Total number correct at 1meter _____
if <10, proceed to Low Vision Testing

☐ Done ☐ Not done

SECTION III = Low Vision Testing
If actual visual acuity is not measurable, please test:

Count fingers (CF) ☐ Yes ☐ No *If no, test for HM*
Hand motion (HM) ☐ Yes ☐ No *If no, test for LP*
Light Perception (LP) ☐ Yes ☐ No

SNELLEN EQUIVALENT:
(Most difficult line with 4 or 5 correct) __ /_ _

☐ CF ☐ HM ☐ LP ☐ NLP

Signature: _____
Initials: _____

Visual Acuity Form A

M.R. No: _____ Patient ID ☐☐☐☐☐ Today's Date: _ _ ☐☐☐ _ _
D D M M M Y Y

To be completed after enrollment

TIME POINT: ☐ Enrollment ☐ 3 Months
ACUITY WITH: ☐ Refraction ☐ CL over refraction ☐ CL not done because: _____
TEST BOTH EYES AT ENROLLMENT ONLY
4 METER VISUAL ACUITY CHART A: Precision Vision Chart 2305A

☐ Study Eye ☐ Fellow Eye (at Enrollment only) ☐ Right Eye ☐ Left Eye

CIRCLE each letter the subject identifies correctly, and write the total correct for each row in the column at the right. If 3 letters or less are read correctly on any row from Row 3 or below, STOP TESTING on that row. If the total number of letters read correctly at 4 meters is less than 10, add a + 0.75 sphere to the distance correction, then have the subject read the first 6 rows of the letters again at 1 meter.

SECTION I = 4 Meter Test
(MUST TRY FIRST TWO LINES)

Row	Snellen Equivalent (meters)	Precision Vision Chart 2305A Tumbling E's	Number correct at 4 meters	log MAR Equiv
1	6/60		_____	1.0
2	6/48		_____	0.9
3	6/38		_____	0.8
4	6/30		_____	0.7
5	6/24		_____	0.6
6	6/19		_____	0.5
7	6/15		_____	0.4
8	6/12		_____	0.3
9	6/9.5		_____	0.2
10	6/7.5		_____	0.1
11	6/6		_____	0.0
12	6/4.8		_____	-0.1
13	6/3.8		_____	-0.2
14	6/3		_____	-0.3

Total number correct at 4 meters _____
If <10, proceed to 1 meter testing

Please circle + or – cylinder:
Refraction or
CL over refraction _____ ± _____ x _____
or ☐ Plano
Base curve _____ **power** _____

☐ Done ☐ Not done

SECTION II = 1 Meter Test
ADD +0.75 SPHERE
(MUST TRY FIRST TWO LINES)

Row	Snellen Equivalent (meters)	Precision Vision Chart 2305A Tumbling E's	Number correct at 1 meter	log MAR Equiv
1	6/242		_____	1.6
2	6/194		_____	1.5
3	6/152		_____	1.4
4	6/121		_____	1.3
5	6/97		_____	1.2
6	6/76		_____	1.1

Total number correct at 1 meter _____

if <10, proceed to Low Vision Testing

☐ Done ☐ Not done

SECTION III = Low Vision Testing

If actual visual acuity is not measurable, please test:

Count fingers (CF) ☐ Yes ☐ No *If no, test for HM*
Hand motion (HM) ☐ Yes ☐ No *If no, test for LP*
Light Perception (LP) ☐ Yes ☐ No

SNELLEN EQUIVALENT:
(Most difficult line with 4 or 5 correct) _ /_ _ _

☐ CF ☐ HM ☐ LP ☐ NLP

Signature: _____

Initials: _____

Visual Acuity Form R – REFRACTION

M.R. No: _____ Patient ID ☐☐☐☐☐ Today's Date: __ __ ☐☐☐ __ __
D D M M M Y Y

To be completed after enrollment

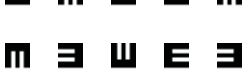
WORKSHEET ONLY – NOT FOR DATA ENTRY

TIME POINT: ☐ Enrollment ☐ 3 Weeks ☐ 3 Months

Precision Vision Chart 2305B

☐ Study Eye ☐ Fellow Eye (at Enrollment only) ☐ Right Eye ☐ Left Eye

SECTION I = 4 Meter Test

Row	Snellen Equivalent (meters)	Precision Vision Chart 2305 B Tumbling E's	Number correct at 4 meters	log MAR Equiv
1	6/60			1.0
2	6/48			0.9
3	6/38			0.8
4	6/30			0.7
5	6/24			0.6
6	6/19			0.5
7	6/15			0.4
8	6/12			0.3
9	6/9.5			0.2
10	6/7.5			0.1
11	6/6			0.0
12	6/4.8			-0.1
13	6/3.8			-0.2
14	6/3			-0.3

SECTION II = 1 Meter Test **ADD +0.75 SPHERE**

Row	Snellen Equivalent (meters)	Precision Vision Chart 2305 B Tumbling E's	Number correct at 1 meter	log MAR Equiv
1	6/242			1.6
2	6/194			1.5
3	6/152			1.4
4	6/121			1.3
5	6/97			1.2
6	6/76			1.1

Please circle + or – cylinder:

Refraction or

CL over refraction _____ ± _____ x _____

or ☐ Plano

Base curve _____ **power** _____

WORKSHEET ONLY
←NOT FOR DATA ENTRY

A.10. Follow-up Compliance Forms

In-Patient Compliance Form: Topical Medication

M.R. No.: _____ **Patient ID:** ☐☐☐☐☐ **Enrollment Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

- One drop of medication should be given every one hour while awake for one week. For another 2 weeks, one drop of medication should be given every 2 hours while awake.
- Mark when medication is given to a patient in this form.
- While being used, all medication should be kept in a refrigerator.
- Medication should be changed every 1 week +/- 1 day. If it's time to change medication for this patient, please contact the study coordinator.

	Day	Date	AM												PM											
			12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Every **one** hour while awake	1																									
	2																									
	3																									
	4																									
	5																									
	6																									
	7																									
Every **two** hours while awake	8																									
	9																									
	10																									
	11																									
	12																									
	13																									
	14																									
	15																									
	16																									
	17																									
	18																									
	19																									
	20																									
	21																									

Out-Patient Compliance Form: Topical Medication

M.R. No.: ______________ **Patient ID:** ☐☐☐☐☐ **Enrollment Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Any unusual circumstance with patient compliance should be recorded in the column titled "Note". If a medication bottle is not collected, the reason should be recorded as well.

Date Dispensed	Date Returned	Self-reported Compliance			Note
		Number Missing Dose (circle)	Why Missed	Given to others (circle)	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	0 1 2 3 4 5 Other:____		Y N	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	0 1 2 3 4 5 Other:____		Y N	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	0 1 2 3 4 5 Other:____		Y N	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	0 1 2 3 4 5 Other:____		Y N	

____
Study Coordinator's Signature

Initials: ___________

In-Patient Compliance Form: Oral Medication

M.R. No.: _____ Patient ID: | | | | | | **Enrollment Date:** _ _ □ □ □ _ _
D D M M M Y Y

✋ Mark when medication is given to a patient in this form.

✋ Give 400 mg of oral medication the first day twice a day followed by 200 mg twice a day for 3 weeks

	Day	Date	Time Given	
			AM	PM
400 mg BID	1			
200 mg BID	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
	11			
	12			
	13			
	14			
	15			
	16			
	17			
	18			
	19			
	20			
	21			

_____
Study Coordinator's Signature

Initials: _____

Out-Patient Compliance Form: Oral Medication

M.R. No.: _____ Patient ID: ☐☐☐☐☐ **Enrollment Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Any unusual circumstance with patient compliance should be recorded in the section titled "Note". If a medication bottle is not collected, the reason should be recorded as well.

Date Dispensed	Date Returned	Self-reported Compliance					Note
		Number of Pills Dispensed (Circle)	Number of Pills Returned (Circle)	Number Doses Missed (Circle)	Why Missed	Given to others (Circle)	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	16 18 20 22 Other: ____	0 1 2 3 4 5 Other: _____	0 1 2 3 4 5 Other: _____		Y N	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	16 18 20 22 Other: ____	0 1 2 3 4 5 Other: _____			Y N	
_ _ ☐☐☐ _ _ DD MMM YY	_ _ ☐☐☐ _ _ DD MMM YY	16 18 20 22 Other: ____	0 1 2 3 4 5 Other: _____	0 1 2 3 4 5 Other: _____		Y N	

_____
Study Coordinator's Signature

Initials: _____

A.11. Follow-up Reminder Letters

Aravind Eye Hospitals
Follow-up Reminder Letter

Date: _____

To:

Dear Mr/Ms/Mrs _____:

Vanakkam. Please come for the (3-week / 3-month) follow- up examination, for treatment of your eye / corneal ulcer, to Aravind Eye Hospital without fail on _____ as specified by your doctor. This is a very important examination to assess the progress. If you are unable to visit on that date due to unavoidable circumstances, please come to the hospital within 1 week / 2 weeks / 1 month (except on Sundays) from the date specified above for follow-up examination without fail. All of your travel expenses will be reimbursed by Aravind Eye Hospital.

Note: when you come to the hospital, bring the follow–up card already given to you along with this letter.

Thanks for your co-operation.

_____

Signature of Coordinator

F.I. Proctor Foundation - UCSF
Follow-up Reminder Letter

F.I. Proctor Foundation for Research in Ophthalmology
95 Kirkham Street
University of California
San Francisco, CA 94122

Date:
Patient name:
Study Identification number:

Name of Patient:
Address of Patient:
City, State, Zip Code:

Dear (Patient Name),

Thank you for participating in the Mycotic Ulcer Treatment Trial (MUTT).

As we discussed during your last visit, your next appointment for a follow-up is scheduled for:

"**Day of the week, Month Day, Year**" at the Proctor Foundation Ophthalmology Clinic, UCSF.

Time	**Appointment**	**Reception Desk**	**Suggested Parking Location**
TIME	Dr. Tom Lietman Dr. Nisha Acharya	95 KIRKHAM ST	REQUEST PARKING PERMIT AT RECEPTION DESK FOR 95 KIRKHAM LOT

Please, allow time before your appointment so that we can review your registration information and check your insurance card.

If you are unable to keep this appointment or if you have any questions, please call the following number:

MUTT Study Coordinator, Catie Oldenburg
415-514-2163

Sincerely,

_____
Signature of Study Coordinator

A.12. Medication Change and Procedure Log

M.R. No.: _____ Patient ID: ☐☐☐☐☐ **Today's Date:** _ _ □□□ _ _
D D M M M Y Y

Enrollment Date: _ _ □□□ _ _
D D M M M Y Y

☐ **Stopping Topical Study Medication (even if stopping at 3 weeks)**

Date stopped _ _ □□□ _ _
DD MMM YY

Reason _____

Was it re-started? Yes ☐ No ☐ If yes, what date re-started? _ _ □□□ _ _
DD MMM YY

☐ **Stopping Oral Study Medication (MUTT 2 only, even if stopping at 3 weeks)**

Date stopped _ _ □□□ _ _
DD MMM YY

Reason _____

Was it re-started? Yes ☐ No ☐ If yes, what date re-started? _ _ □□□ _ _
DD MMM YY

☐ **Change of Topical Antifungal Medication (after stopping study medication)**

Date changed _ _ □□□ _ _
DD MMM YY

New Regimen _____

Reason _____

☐ **Addition of Topical Antifungal Medication (to study medication)**

Date added _ _ □□□ _ _
DD MMM YY

Medication added _____

Reason _____

☐ **Addition of Systemic Antifungal Medication**

Date added _ _ □□□ _ _
DD MMM YY

Medication added _____

Reason _____

☐ **Addition of Antibacterial Medication**

Date added _ _ □□□ _ _
DD MMM YY

Medication added _____

Reason _____

☐ **Addition of Other Topical/Systemic Medication**

Date added _ _ □□□ _ _
DD MMM YY

Medication added _____

Reason _____

☐ **Procedure or Surgery**

Date _ _ □□□ _ _
DD MMM YY

Procedure performed:

☐ Cataract ☐ Tear duct/sac surgery ☐ Other: _____

☐ Cornea transplant ☐ Cornea glue

Reason _____

Investigator's Signature: _____

Initials: _____

A.13. Adverse Event Form – MUTT I

M.R. No.: _____ Patient ID: | 1 | | | | | **Today's Date:** _ _ □ □ □ _ _
D D M M M Y Y

Enrollment Date: _ _ □ □ □ _ _
D D M M M Y Y

Date of E-Mail to Medical Monitor: _ _ □ □ □ _ _
D D M M M Y Y

Email Address: mcleods@vision.ucsf.edu

NOTE: Any adverse event during the course of the study (5 months from enrollment) should be reported even when a study participant is no longer on study medication.
Adverse Events:

Date of event: D D / M M / Y Y

Non-serious Ocular: narrative form not required

☐ Local allergic reaction (conjunctiva, lid)

☐ Increase in hypopyon (increase >2mm)

☐ Increase in the longest diameter of infiltrate (>50% increase from baseline and >1mm)

☐ Intraocular pressure ≥35 mmHg for 1 week despite therapy

☐ Progressive corneal thinning, ≤ 50% of enrollment thickness

☐ Other (specify) _____

Non-serious Non-ocular: narrative form not required

☐ AST or ALT elevated to twice upper limit of normal

☐ Visual disturbances

☐ Dermatologic reaction

☐ Headache

Serious Ocular: (1) report within 24hrs (2) Pfizer Serious Adverse Event Form required

☐ Corneal perforation

☐ Endophthalmitis

☐ Other serious ocular event thought to be related to the study drug, requiring non-elective hospitalization, loss of function, or systemic treatment
(specify)_____

Serious Non-ocular: (1) report within 24hrs (2) Pfizer Serious Adverse Event Form required

☐ Death

☐ Non-elective surgery, hospitalization, or loss of function

☐ Myocardial infarction or stroke

☐ AST or ALT elevated to five times upper limit of normal

_____
Investigator's Signature

Initials: _____

MUTT 2 Adverse Event Checklist

M.R. No.: _____ Patient ID: ___ ___ ___ ___ ___ Today's Date: D D / M M M / Y Y

Visit: ☐ Enrollment ☐ Study day _____ ☐ 3 weeks ☐ 3 months

Weight (kg): _____

(To be completed at each study visit, each adverse event checked off MUST be recorded on the Adverse Event Log and each serious adverse event MUST be reported on the Pfizer Serious Adverse Event Form to Pfizer and the Medical Monitor within 24 hours)

Ocular Adverse Event	Code	Current Status (check one for each condition)		
		None	Non-Serious	Serious
Local allergic reaction (conjunctiva, lid)	**ALL**	☐	☐ Any occurrence	
Increase in hypopyon (increase >2 mm)	**HYP**	☐	☐ Any occurrence	
Intraocular pressure ≥ 35 mmHg for 1 week despite therapy	**IOP**	☐	☐ Any occurrence	
Progressive corneal thinning, ≤ 50% of enrollment thickness	**PCT**	☐	☐ Any occurrence	
Corneal perforation	**PER**	☐	☐ Any occurrence	
Endophthalmitis	**END**	☐		☐ Any occurrence is a serious adverse event
Other ocular event thought to be related to the study drug	**OTH**	☐	☐ Other ocular event thought to be related to the study drug, NOT requiring non-elective hospitalization nor systemic treatment, and not resulting in loss of function	☐ Serious ocular event thought to be related to the study drug, requiring non-elective hospitalization, loss of function, or systemic treatment
Ulcer not healing after 6 weeks of therapy	**NON**	☐	☐ Any occurrence	

Systemic Adverse Event	Code	Current Status (check one for each condition)		
		None	Non-Serious	Serious
SGOT (AST) or SGPT (ALT)	**LFT**	☐	☐ 2 to 5 times the upper limit of normal	☐ > 5 times the upper limit of normal
Clinical signs of liver failure	**CLF**	☐	☐ Jaundice	☐ Hospitalization due to liver failure
Visual disturbances	**VIS**	☐	☐ Color vision changes, halos, etc ☐ Formed hallucinations	☐ Formed hallucinations that do not resolve within 48 hours of discontinuing study treatment
Dermatologic reactions	**DER**	☐	☐ Any occurrence	☐ Stevens-Johnson Syndrome
Headache	**HEA**	☐	☐ Any occurrence	
Dizziness	**DIZ**	☐	☐ Any occurrence	
Nausea	**NAU**	☐	☐ Any nausea	☐ Severe discomfort, minimal food intake for 3 or more days **or** 2.5-5.0 kg weight loss resulting from nausea, or worse
Vomiting	**VOM**	☐	☐ Any vomiting	☐ severe: vomiting all food or fluids in 24 hours **or** orthostatic hypotension **or** 2.5-5.0 kg weight loss resulting from vomiting or worse
Diarrhea	**DIA**	☐	☐ Any diarrhea	☐ severe, bloody diarrhea **or** 8-9 loose stools in 24 hours **or** orthostatic hypotension **or** 2.5-5.0 kg weight loss resulting from diarrhea **or** worse
Stomach pain	**STO**	☐	☐ Any occurrence	
Fever	**FEV**	☐	☐ > 100.6 to 103°F/39.5°C	☐ ≥ 103°F/39.5°C
Difficulty breathing	**DIF**	☐	☐ Any occurrence	☐ Shortness of breath requiring medical

				intervention, such as severe asthmatic reaction
Lethargy reported by the patient	**LET**	☐	☐ Any occurrence	
Myocardial infarction or stroke	**MYO**	☐		☐ Any occurrence is a serious adverse event
Other systemic event thought to be related to the study drug	**OSE**	☐	☐ Other non-ocular event thought to be related to the study drug, NOT requiring non-elective hospitalization nor systemic treatment, and not resulting in loss of function	☐ Non-elective surgery, hospitalization or loss of function thought to be related to the study drug
Death	**DEA**	☐		☐ Any occurrence is a serious adverse event

MUTT 2 Adverse Event Log

M.R. No.: _____ Patient ID: ___ ___ ___ ___ ___ Today's Date: D D / M M M / Y Y

Instructions: Start the Adverse Event Log at the onset of the first adverse event for the patient. Maintain this log for the patient, updating with each new adverse event, over the course of the study. The Adverse Event Checklist must be filled out for each adverse event, and the Pfizer Serious Adverse Event form must be filled out for each serious adverse event and reported to the medical monitor within 24 hours. It is possible that the same adverse event may occur and resolve more than once – each new occurrence should be listed on a separate row.

Coding: Each adverse event in the log must be coded according to event and severity. See the Adverse Event Checklist for the event code. Severity is graded as: 1: Non-serious; 2: Serious

Event Code (refer to Adverse Event Checklist)	Adverse Event (Brief description or laboratory value and units)	Severity Grade (1: Non-serious; 2: Serious)	Date Started/Resolved (DD MMM YY)
___ ___ ___	1.	☐ 1 ☐ 2	Started: _ _ ☐☐☐_ _ Resolved: _ _ ☐☐☐_ _
___ ___ ___	2.	☐ 1 ☐ 2	Started: _ _ ☐☐☐_ _ Resolved: _ _ ☐☐☐_ _
___ ___ ___	3.	☐ 1 ☐ 2	Started: _ _ ☐☐☐_ _ Resolved: _ _ ☐☐☐_ _
___ ___ ___	4.	☐ 1 ☐ 2	Started: _ _ ☐☐☐_ _ Resolved: _ _ ☐☐☐_ _
___ ___ ___	5.	☐ 1 ☐ 2	Started: _ _ ☐☐☐_ _ Resolved: _ _ ☐☐☐_ _

Event Code (refer to Adverse Event Checklist)	**Adverse Event (Brief description or laboratory value and units)**	**Severity Code (1: Non-serious; 2: Serious)**	**Date Started/Resolved (DD MMM YY)**
__ __ __	6.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __
__ __ __	7.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __
__ __ __	8.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __
__ __ __	9.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __
__ __ __	10.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __
__ __ __	11.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __
__ __ __	12.	☐ 1 ☐ 2	Started: __ __ ☐☐☐ __ __ Resolved: __ __ ☐☐☐ __ __

*If all 12 rows have been filled, please continue on a new sheet.

A.14. Patient Dropout Form

M.R. No.: _____ Patient ID: ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y

If patient refuses medications but is willing to return for exams, he/she has ***NOT*** dropped out.

If patient has an adverse event but is willing to return for exams, he/she has ***NOT*** dropped out.

Reasons for dropout:

- ☐ Patient migration
- ☐ Patient not willing to return for exams

☐ Death of the patient (Notify Medical Monitor)

☐ Other reason (specify) _____

_____
Study Coordinator's Signature

Initials: _____

A.15. Cost-Effectiveness Forms

Cost-Effectiveness Data Form

M.R. No.: _____ Patient ID: ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y

Affected Eye: ☐ Right Eye ☐ Left Eye

Required at <u>3 months</u> only

Number of non-study visits since enrollment: _____

Number of internist visits related to study: _____

Total number of days in-hospital related to study: _____

How many hours of work did the patient miss because of the eye infection? (Record only <u>working</u> hours missed)

_____

How many rupees did you spend on a return trip to the hospital for visits related to your eye infection? _____

Did someone have to miss work in order to help take care of your eye? (ie, accompany you on hospital visits, in-home eye care, etc). ☐ Yes ☐ No

If yes:

What is their occupation? _____

How many hours of work did they have to miss? _____

_____

Investigator's Signature

Initials: _____

IND-VFQ33 Data Form (Aravind Only)

M.R. No.: _____ **Patient ID:** ☐☐☐☐☐ **Today's Date:** _ _ ☐☐☐ _ _
D D M M M Y Y

Enrollment Date: _ _ ☐☐☐ _ _
D D M M M Y Y

Affected Eye: ☐ Right Eye ☐ Left Eye

Required at <u>3 months</u> only

Question Number	**Response (Circle)**				
1.	1	2	3	4	5
2.	1	2	3	4	5
3.	1	2	3	4	5
4.	1	2	3	4	5
5.	1	2	3	4	5
6.	1	2	3	4	5
7.	1	2	3	4	5
8.	1	2	3	4	5
9.	1	2	3	4	5
10.	1	2	3	4	5
11.	1	2	3	4	5
12.	1	2	3	4	5
13.	1	2	3	4	5
14.	1	2	3	4	5
15.	1	2	3	4	5
16.	1	2	3	4	5
17.	1	2	3	4	5
18.	1	2	3	4	5
19.	1	2	3	4	5
20.	1	2	3	4	5
21.	1	2	3	4	5
22.	1	2	3	4	5
23.	1	2	3	4	5
24.	1	2	3	4	5
25.	1	2	3	4	5
26.	1	2	3	4	5
27.	1	2	3	4	
28.	1	2	3	4	
29.	1	2	3	4	
30.	1	2	3	4	
31.	1	2	3	4	
32.	1	2	3	4	
33.	1	2	3	4	

<u>Key:</u>
1: Not at all
2: A little
3: Quite a bit
4: A lot
5: Cannot do this because of my sight (Questions 1-26 only)

_____
Investigator's Signature

Initials: _____

VFQ25 Data Form (Proctor Only)

M.R. No.: _____ **Patient ID:** | | | | | | **Today's Date:** _ _ □ □ □ _ _
D D M M M Y Y

Enrollment Date: _ _ □ □ □ _ _
D D M M M Y Y

Affected Eye: ☐ Right Eye ☐ Left Eye

Required at 3 months only

Question	Response (Circle)					
1.	1	2	3	4	5	
2.	1	2	3	4	5	6
3.	1	2	3	4	5	
4.	1	2	3	4	5	
5.	1	2	3	4	5	6
6.	1	2	3	4	5	6
7.	1	2	3	4	5	6
8.	1	2	3	4	5	6
9.	1	2	3	4	5	6
10.	1	2	3	4	5	6
11.	1	2	3	4	5	6
12.	1	2	3	4	5	6
13.	1	2	3	4	5	6
14.	1	2	3	4	5	6
15.	1	2				
15a.	1	2				
15b.	1	2	3			
15c.	1	2	3	4		
16.	1	2	3	4	5	6
16a.	1	2	3	4	5	6
17.	1	2	3	4	5	
18.	1	2	3	4	5	
19.	1	2	3	4	5	
20.	1	2	3	4	5	
21.	1	2	3	4	5	
22.	1	2	3	4	5	
23.	1	2	3	4	5	
24.	1	2	3	4	5	
25.	1	2	3	4	5	

Key: Please see VFQ25 Questionnaire

_____
Investigator's Signature

Initials: _____

Appendix B: Certification Forms

B.1. Visual Acuity / Refraction Certification Form

B.2. Room Certification Form

B.3. Clinical Exam Certification Form

B.1. Visual Acuity / Refraction Certification Form

VISUAL ACUITY/REFRACTION CERTIFICATION FORM	**Site:**		
Date of Evaluation:			
Study Personnel Name (Print):			
Study Personnel Signature:		**Date:**	
Evaluator's Name (Print):		**Room Evaluated:**	
Indicate reason for evaluation:	☐ **New Certification** ☐ **Renewal Required**		

REFRACTION		
Yes	No	
□	□	Starting refraction determination
□	□	Proper testing distance (4m or 1m)
□	□	Refraction (lens selection, bracketing and end point)
□	□	Spherical refraction ("plus, minus, plus", check if more letters read with added power etc)
□	□	Cylinder axis determination
□	□	Cylinder Power determination (maintains spherical equivalent)
□	□	Proper patient instruction ("better/worse or the same" for sphere, "one or two" for cylinder, etc.
□	□	Final refinement

VISUAL ACUITY		
Yes	No	
□	□	Position patient properly, checking distance
□	□	Use trial frames properly (position on face, position of lens wells)
□	□	Properly occluding eye not being tested
□	□	Proper encouragement (encouraging patient to read smaller letter, place-keeping)
□	□	Determine need for 1-meter testing (3 letters or less correct)
□	□	Record/score properly, logMAR, Snellen
□	□	Test for count fingers (1/2 meter, 5 times, encouragement, 3/5)
□	□	Test for hand motion (Vertical &Horizontal, 5 times, 4/5, w/ or w/o direction)
□	□	Test for light perception (1/2 meter, 5 times, 3/5, with or without direction)

I have evaluated the requirements for visual acuity room set up according to the visual acuity specifications for the SCUT protocol.		
Certification Approved?	**Yes** ☐	**No** ☐ If No, please describe and comment on actions needed to meet requirements:
Evaluator's Signature:		
Certification Approval Date:		
Certification Expiration Date:		

B.2. Room Certification Form

ROOM CERTICATION FORM	Site:		
Date of Evaluation:			
Study Personnel Name (Print):			
Study Personnel Signature:		Date:	
Evaluator's Name (Print):		Room Evaluated:	
Indicate reason for evaluation:	☐ New Certification ☐ Renewal Required		

Retro-Illuminated Light Box		
Yes	No	
□	□	Wall Mounted: Height from floor_____(floor to top of third row of letters = 49 inches ±2 inches; 124.5± 5.
□	□	Stand Mounted: Height from floor_____(floor to top of third row of letters = 49 inches ±2 inches; 124.5± 5.
□	□	Modified charts R, 1 and 2
□	□	General Electric 20-watt fluorescent bulbs? Date bulbs last changed:_____
□	□	Were the bulbs "burned in" (left on for 96 hours)?
□	□	Are replacement bulbs available?
□	□	Have the replacement bulbs been "burned in"?
□	□	Fenestrated sleeves properly oriented? (Openings in the backs of the sleeves should be oriented to point directly toward the back of the box. Lower sleeve has a cut-out that should point down toward the ballast.)

Examination Room		
Yes	No	
□	□	4-Meter Lane (4 meters from front of box to patient's eye = approximately 157.5 inches)
□	□	1-Meter Lane (1 meter from front of box to patient's eyes = approximately 39.4 inches)
□	□	1-Meter measuring device (sturdy, non-flexible construction?)
□	□	Examination chair: sturdy chair with a back?
□	□	Light or Foot Candle Meter to measure? (No more than 15 f.c/ 161.4 Lux falling on center of light box when n Measure with room lights on and then off.)
□	□	Appropriate lighting scheme planned for use during visual acuity testing (e.g., shades drawn, doors open or closed, ambient illumination, etc.) Describe :_____

I have evaluated the requirements for visual acuity room set up according to the visual acuity specifications for the SCUT protocol.		
Certification Approved?	**Yes** ☐	**No** ☐ If No, please describe and comment on actions needed to meet requirements:
Evaluator's Signature:		
Certification Approval Date:		
Certification Expiration Date:		

B.3. Clinical Exam Certification Form

Clinical Exam Certification Form	**Site:**		
Date of Evaluation:			
Study Personnel Name (Print):			
Study Personnel Signature:		**Date:**	
Evaluator's Name (Print):		**Room Evaluated:**	
Indicate reason for evaluation:	☐ **New Certification** ☐ **Renewal Required**		

Slide presentation		
Yes	No	
□	□	Understands definition of infiltrate/scar axes
□	□	... multiple scars
□	□	... oddly shaped scars
□	□	... hypopyon
□	□	... depth of scar
□	□	... perforation

Photograph quiz		
Long	Short	Case Number
□	□	1
□	□	2
□	□	3
□	□	4
□	□	5
□	□	6
□	□	7
□	□	8
□	□	9
□	□	10
Yes □	No □	All judged to be within 1mm of examiner. (If not, each photo needs to be reviewed with trainee.)

Live patient exam		
Yes	No	
□	□	Acceptable measurement of infiltrate/scar

Certification Approved?	**Yes** ☐	**No** ☐ If No, please describe and comment on actions needed to meet requirements:
Evaluator's Signature:		
Certification Approval Date:		

Mycotic Ulcer Treatment Trial

Statistical Analysis Plan

Confidential

Version 2.2, 14 Feb 2012

Prepared by:
Travis C. Porco, PhD, MPH
Division of Preventive Medicine and Public Health
Department of Epidemiology and Biostatistics
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San Francisco, California 94143-0412
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1 Introduction

This document (Statistical Analysis Plan, SAP) describes the planned analysis and reporting for the **Mycotic Ulcer Treatment Trials**, University of California, San Francisco. It includes specifications for the statistical analyses and tables to be prepared for the final Clinical Study Reports.

The **Mycotic Ulcer Treatment Trials** consist of two separate trials for the treatment of fungal corneal ulcers. The proposed Mycotic Ulcer Treatment Trial I is a Phase III clinical trial to compare the efficacy of topical voriconazole (Pfizer, CAS 137234-62-9) to the standard treatment natamycin (CAS 7681-93-8) for the treatment of mycotic corneal ulcers. The proposed Mycotic Ulcer Treatment Trial II is a phase III clinical trial to compare the efficacy of oral voriconazole plus topical voriconazole to topical voriconazole alone for the treatment of mycotic corneal ulcers.

The content of this Statistical Analysis Plan meets the requirements stated by the US Food and Drug Administration (Department of Health and Human Services, Food and Drug Administration, 1998) and conforms to the American Statistical Association's Ethical Guidelines (American Statistical Association, 1999).

The following documents were reviewed in preparation of this Statistical Analysis Plan:

- Mycotic Ulcer Treatment Trial Manual of Operations
- ICH Guidance on Statistical Principles for Clinical Trials (Department of Health and Human Services, Food and Drug Administration, 1998)
- Statistical Analysis Plan (prepared by C. McCulloch), Steroids for Corneal Ulcers Trial (T. Lietman, principal investigator)

The planned analyses described in this Statistical Analysis Plan will be included in future manuscripts. Note, however, that exploratory analyses not necessarily identified in this Statistical Analysis Plan may be performed to support the analysis. All post-hoc or unplanned analyses which have not been delineated in this Statistical Analysis Plan will be clearly documented as such in the final Clinical Study Report, manuscripts, or any other document or submission.

Finalization of this document will take place prior to the enrollment of patients; the final version of this document will be numbered 2.0. All subsequent changes will be indicated by detailed change log in an Appendix.

N. Acharya, D. Glidden, J. Keenan, C. Kidd, T. Lietman, and K. Ray have contributed to this plan.

2 Investigational Plan

2.1 Study Design

The proposed **Mycotic Ulcer Treatment Trial** project consists of two separate international, multicenter, randomized, double-masked controlled clinical trials to determine whether (1) voriconazole treatment of mycotic corneal ulcers yields improvement in visual outcomes, when compared to the standard treatment using natamycin, and (2) the addition of oral voriconazole to topical voriconazole for the treatment of mycotic corneal ulcers yields reduction in perforations, when compared to topical voriconazole treatment. A placebo-controlled trial of voriconazole for this infectious process is not considered ethical, and the standard of care is to treat with the FDA-approved natamycin.

2.2 Study Population

2.2.1 MUTT I

Eligible volunteers will include men and women aged 16 or older. Enrollment will be restricted to individuals with baseline visual acuity between 20/40 (logMAR 0.3, inclusive) and 20/400 (logMAR 1.3, inclusive). Individuals with impending perforation are *excluded* from this trial. The full enrollment eligibility criteria are specified in the Manual of Operations.

2.2.2 MUTT II

Eligible volunteers will include men and women aged 16 or older. However, enrollment will be restricted to visual acuity worse than 20/400 (greater than logMAR 1.3, not inclusive). Individuals with impending perforation are *not excluded* from this trial. The full enrollment eligibility criteria are specified in the Manual of Operations.

2.3 Study Objectives and Endpoints

The study objectives are listed in the Manual of Operations, but are reviewed below.

2.3.1 Primary Objective

The primary objectives of the studies are

1. MUTT I: To assess whether topical treatment of mycotic corneal ulcers with 1% topical voriconazole yields noninferior visual acuity outcomes (**best spectacle-corrected visual acuity**) after **three months** than does standard treatment consisting of the topical administration of 5% natamycin (specifically, the noninferiority threshold is 0.15 logMAR, discussed below).
2. MUTT II: To assess whether topical treatment of mycotic corneal ulcers with 1% topical voriconazole together with oral administration of voriconazole yields lower perforation rates over three months of follow-up than treatment with topical voriconazole (1%) alone.

Subjects will undergo clinical examinations and acuity testing at enrollment, three weeks, and three months.

The primary endpoint for MUTT I is best spectacle-corrected visual acuity after three months, a time at which clinical experience suggests most ulcers will be healed. The primary endpoint for MUTT II is perforation status (yes or no) within three months. Here, perforation is taken to include therapeutic penetrating keratoplasty or other form of keratoplasty done within three months of enrollment.

All visual acuity data will be analyzed in logMAR units unless otherwise specified.

2.3.2 Secondary Objectives

- To determine whether patients treated with topical and oral voriconazole instead of topical voriconazole alone exhibit any improvement in best spectacle-corrected visual acuity within three months (MUTT II only).
- To determine whether patients treated with voriconazole instead of natamycin exhibit a difference in infiltrate/scar size at three weeks and at three months, as determined by slit lamp examination, and separately by photography (MUTT I). Similarly, we wish to compare infiltrate/scar size at three weeks and at three months between patients receiving both topical and oral voriconazole with those receiving only topical voriconazole (MUTT II).
- To determine whether patients treated with voriconazole have a different re-epithelialization time when compared to patients treated with natamycin (MUTT I). Similarly, we wish to compare re-epithelialization time between patients receiving both topical and oral voriconazole with those receiving only topical voriconazole (MUTT II).
- To determine whether voriconazole yields better best spectacle-corrected visual acuity outcomes than natamycin for particular fungal pathogens, in particular, for *Fusarium* spp (MUTT I). It is possible that any potential benefits of voriconazole vs. natamycin may be limited to particular fungal pathogens. Similarly, we wish to determine whether or not oral voriconazole together with topical voriconazole yields better perforation outcomes than only topical voriconazole for particular fungal pathogens (MUTT II). Visual acuity will also be examined by pathogen as well (MUTT II).
- To assess whether clinical outcomes, i.e., (a) best spectacle-corrected visual acuity, (b) infiltrate/scar size, and (c) reepithelialization time, are correlated with *in vitro* measures of fungal susceptibility to the medication (the minimum inhibitory concentration) (both MUTT I and MUTT II).
- To compare microbiological cure at 7 days between natamycin and voriconazole (MUTT I).

Secondary endpoints for both trials are (a) best spectacle-corrected visual acuity at 3 weeks, (b) infiltrate/scar size at 3 weeks and 3 months, (c) re-epithelialization time, (d) hard contact lens refraction at three months, and (e) microbiological cure at 7 days. Additionally, perforations within 3 months is a secondary endpoint for MUTT I (instead of being the primary outcome, as in MUTT II). As described elsewhere, reepithelialization time has been a standard measure of efficacy in other studies; examination at three weeks provides additional information regarding

response times, as well as some outcome information for patients who may be lost to follow-up by three months Analysis of outcomes will also be done by the following subgroups: causative organism, visual acuity group, duration of symptoms to enrollment, depth of ulcer, infiltrate scar size

3 Study Methods

3.1 Overall Design

3.1.1 MUTT I

The proposed study is a multicenter, randomized, double-masked trial designed to determine which topical anti-fungal preparation, voriconazole or natamycin, is most efficacious. Fungal corneal ulcer patients will be randomized to one of two study arms:

1. masked administration of 5% topical natamycin
2. masked administration of 1% topical voriconazole

Patients will be followed closely until re-epithelialization, and then rechecked 3 weeks, and 3 months after enrollment. The primary outcome will be best spectacle-corrected visual acuity at the 3-month visit.

The proposed schedule is listed in the Mycotic Ulcer Treatment Trial Manual of Operations.

3.1.2 MUTT II

This proposed study is a multicenter, randomized, double-masked trial designed to determine which treatment, topical voriconazole or topical voriconazole plus oral voriconazole, is most efficacious. Fungal corneal ulcer patients will be randomized to one of two study arms:

1. masked administration of 1% topical voriconazole plus a placebo
2. masked administration of 1% topical voriconazole plus oral voriconazole

Patients will be followed closely until re-epithelialization, and then rechecked 3 weeks, and 3 months after enrollment. The primary outcome will be whether or not a corneal perforation has occurred by the 3-month visit.

The proposed schedule is listed in the Mycotic Ulcer Treatment Trial Manual of Operations.

3.2 Study Population

After diagnosis with a corneal ulcer, patients will be approached and given the details, risks, and benefits of participating in one of the studies. Patients who wish to participate and sign the written consent form will be eligible to participate if they meet all inclusion and exclusion criteria. Assent for minors and consent from their legal guardians will be obtained. Full details are to be found in the Manual of Operations for the proposed trial.

The formal enrollment criteria are given in the Manual of Operations.

3.3 Randomization

3.3.1 Stratification between centers

Patients will be recruited from three centers: Aravind-Madurai, Aravind-Pondicherry, and Proctor. Following initial pinhole acuity evaluation, individuals are classified into (1) potentially eligible for **MUTT I**, or (2) potentially eligible for **MUTT II**. No individual can be eligible for both trials; the enrollment criteria are mutually exclusive (see Manual of Operations for details).

Individuals who are diagnosed with fungal corneal ulcers may be recruited into **MUTT I** or **MUTT II** depending on whether their initial classification revealed intermediate or poor vision (full eligibility criteria are listed in the Manual of Operations). The treatment protocols are specified in the Mycotic Ulcer Treatment Trial Manual of Operations.

Each center may contribute patients to each trial.

Each trial is operationally separate. The trials are expected to have differing start and ending dates as well as separate databases.

We will stratify the randomization between sites, using block randomization. This is to ensure that the data from Madurai alone and the data from Pondicherry alone are each approximately balanced with respect to the treatment assignments. While the Proctor site will also be approximately balanced, the expected recruitment from Proctor is small.

3.3.2 Randomization list

For each study, a list of sequential randomization assignments will be prepared for each site. The randomization lists consist of a unique identifier for each patient, together with the assignments to treatment arms. The assignment of patient ID numbers and randomization is thus performed on enrollment—after photography and slit-lamp examination.

The randomization lists for Madurai and Pondicherry will be prepared by the Proctor center (see Section 15.3) and communicated to the nurse at Aravind (Ms. Anushya), who will be responsible for reconstituting the drugs based on randomization assignment. Ms. Anushya will print a hard copy of the randomization list and keep it stored in a locked file cabinet after deleting the electronic copy from her computer and email account. She will not be involved in any other study-related activities. A randomization list in a sealed envelope will also be given to a senior faculty member at Aravind not involved in the trial, Dr. Usha, in case emergency masking is necessary and the coordinating center at Proctor can not be reached. The Proctor site's randomization list will be maintained by principal statistician T. Porco (hereinafter TP) and the Proctor compounding pharmacist, Dr. Charles Leiter.

Distribution of the randomization lists to Aravind will be accomplished using the University of California, San Francisco's encrypted email provision. Email is encrypted using the Advanced Encryption Standard (NIST FIPS 197) whenever the first four characters of the subject line are `PHI:`. The sender is notified when the recipient receives a secure email; the recipient receives a notification of a secure email and can view it using the UCSF Secure Messenger website. We have successfully used this method in the Pilot study. Ms. Anushya will certify that no other personnel will have access to her email account when the email is sent.

A backup copy of the full randomization lists for all three sites will be maintained by Proctor Director Todd Margolis, MD, PhD (hereinafter TM). These lists will be maintained as a hard copies stored in a locked file cabinet at the Proctor site.

The randomization lists for Madurai and the list for Pondicherry will each contain at least 300 consecutive randomization assignments. This number is expected to far exceed the requirements, based on the pilot data. The long list provides a measure of added safety in case one of these centers recruits far more patients than expected relative to the other center. The randomization list for Proctor will contain 40 consecutive randomization assignments, again, far more than we expect to need.

As discussed below, the randomization lists will be provided as Excel® worksheets. No technical knowledge will be required to use these lists.

3.3.3 Block randomization

We choose a permuted block randomization scheme with a randomly varying block size to protect the integrity of the assignment masking (Friedman *et al*., 1998). For definiteness, we choose blocks of size 4, 6, and 8 with probabilities 6/13, 4/13, and 3/13 respectively (so that approximately the same number of patients will be in blocks of a given size), though many other choices would serve equally well. Given the block size, a random permutation of assignment orders will be generated. This process will be repeated (with replacement) 75 times for each site, guaranteeing at least 300 assignments for each site. Selected specific computer commands that will be used to generate the sequence of assignments is provided in the Appendix (Section 15.3).

3.3.4 Unique patient identifiers

Unique patient identifiers will be generated as follows. For **MUTT I**, the first character will be`1`, which will serve to distinguish identifiers in the proposed Mycotic Ulcer Treatment Trial main trial from the pilot study and from subjects enrolled in arms of the Steroids for Corneal Ulcer Trial (a separate UCSF/Aravind clinical trial). For **MUTT II**, the first character will be `2`; it will never be possible for patients in any UCSF/Aravind trial to have the same identifier. The next four digits will be a serial number in the range 1100–1999. Each subject will receive a unique serial number, regardless of the study site. The final character will be a checksum, computed using the method used for ten-digit International Standard Book Numbers (2001). The use of the checksum identifies transposition errors and single-digit copying errors. Computational details are provided in the Appendix (Section 15.2).

3.3.5 Random number generation

The choice of a random number seed determines the specific sequence of random numbers that will be produced by the random number generator. Once the seed is determined, the entire randomization assignments for all sites are determined. Details are given in the Appendix (Section 15.1).

3.3.6 Data Export

Study managers for each of the three sites will be provided with a Microsoft Excel® spreadsheet containing three columns: (1) the unique study identifier for the patient (see Section 3.3.4), (2) an empty field into which the date of enrollment may be entered, (3) the study drug assignment, written out in full as `Voriconazole` or `Natamycin`. As discussed in Section 3.3.2, these lists will be treated confidentially.

3.3.7 Quality assurance

For each study, three quality assurance steps are conducted. First, the output has been tested during the pilot phase, now nearing completion. Second, the software that generates the assignments verifies approximate balance of subjects in each group before writing the Microsoft Excel® files. Each file will contain the study site as the first line. Finally, the output files will be visually inspected. The software and procedures have already been developed and successfully used in the Pilot Study.

3.3.8 Summary of disposition of randomization list

The following individuals will receive a copy of the randomization lists:

- Ms. Anushya, Nurse, Aravind Eye Hospital EyeBank: Madurai lis
- Mr. Vaitilingam, Microbiologist, Aravind Eye Hospital, Pondicherry list
- Dr. Usha, Director of Oculoplastics, Aravind Eye Hospital, Madurai
- Dr. Charles Leiter, compounding pharmacist, Proctor study site
- Dr. Todd Margolis, Proctor Foundation Director
- Ms. Kathryn Ray, , statistician , Proctor Foundation
- Dr. Travis Porco, principal statistician, Mycotic Ulcer Treatment Trial

3.4 Masking

The patient, clinical examiner, refractionist, and microbiology laboratory will be masked to the treatment assignment. Note that only the individuals listed in Section 3.3.8 will have copies of the randomization list. Full details of procedures to maintain masking as well as for potential unmasking in the event it becomes necessary for safety reasons are provided in the Manual of Operations.

4 Planned Analyses

4.1 Interim Monitoring

Each study is to be monitored by a monitoring board appointed by the National Eye Institute. They regularly review enrollment, subject safety, data quality and study conduct; see Section 10.2. Frequency of monitoring will be decided by the DSMC.

4.1.1 Accrual Rate

A 120-patient two-by-two factorially-designed clinical trial of (1) voriconazole vs. natamycin, and (2) scraping vs. no scraping serves as the pilot study for the forthcoming MUTT trials. The outcome is best spectacle-corrected visual acuity at three months. This study took place at two sites, Madurai and Pondicherry. A total of 120 patients were enrolled in 167 days; 57 of these patients were in the MUTT I enrollment range (baseline visual acuity 0.3 to 1.6 inclusive), and 38 were in the MUTT II range (baseline visual acuity exceeding 1.6). Seventy-two out of 120 patients (60%) were recruited in Madurai; the remainder in Pondicherry.

Recruitment of 57 patients (who are in the visual acuity enrollment range 0.3 to 1.6) in 167 days with two centers is roughly 3 days per patient. The total of 346 patients (MUTT I) could be achieved in approximately 2.8 years. Recruitment of 38 patients (in the baseline visual acuity enrollment range 1.61- 1.9) is approximately 4.5 days per patient; the recruitment total of 240 would be achieved in approximately 2.9 years (MUTT II). In MUTT I, we would expect approximately 208 patients from Madurai, 138 from Pondicherry (Proctor is expected to contribute 2-5 cases). In MUTT II, we would expect approximately 144 cases from Madurai and 96 from Pondicherry.

The Steroids for Corneal Ulcers Trial (UCSF/Aravind) used two additional sites, Tirunelveli and Coimbatore. During 980 days, Madurai recruited 201 patients; Coimbatore and Tirunelveli recruited 203. Conservatively, if we assume that these two centers would boost enrollment by 50%, MUTT I and MUTT II recruitment would be completed in approximately two years.

We will establish weekly and monthly recruitment goals for each of the three sites, taking into careful consideration local holidays which may cause weekly recruitment rates to drop at certain times of the year. Careful monitoring of the recruitment process (including process control charts) will enable us to determine whether one of our centers may be falling behind in recruitment, precursory to further investigation and intervention. Monthly reports will be sent to each site. Standard graphs of realized cumulative recruitment together with cumulative recruitment goals for (a) the study as a whole, and (b) for each of the three sites will be prepared, and provided to the Data and Safety Monitoring Committee at each meeting (or more frequently, if requested). Additional control charts may be plotted if needed.

4.2 Interim Analysis

4.2.1 MUTT I

The planned interim analysis of efficacy will be conducted when one third of the data are available, i.e. when three months of follow-up are available for the first one third of the patients (115 patients), allowing time for analysis. We plan to examine the primary outcome variable (three month best spectacle-corrected visual acuity, using last-observation carried forward, but also imputing all perforations as 1.9 logMAR as a supplemental consideration) using the same statistical model we plan for the final analysis. Specifically, we will conduct linear regression of the primary outcome variable using the baseline best spectacle-corrected visual acuity and drug assignment as covariates, and test the null hypothesis that the regression coefficient for drug assignment is zero. Missing values will be handled as described in Section 8.2. See Section 5.3.2

for a discussion of the hypothesis test of the primary outcome, and Section 10.2.3 for a discussion of the critical value for the interim analysis.

Stopping rules. Stopping rules for benefit, harm, and futility are discussed in Section 10.2. These rules or guidelines would be determined at the first meeting of the DSMC (see Section 10.2).

Execution of interim analysis. The principal statistician (TP) will conduct the interim analysis in an unmasked manner, subject to independent statistical review by the DSMC. Quality assurance will be conducted by database manager KR.

4.2.2 MUTT II

Similar to MUTT I, the planned interim analysis of efficacy will be conducted when one third of the data are available, i.e. when three months of follow-up are available for the first one third of the patients (80 patients), allowing time for analysis. We plan to examine the primary outcome variable (perforation status by three months) using the same statistical model we plan for the final analysis. Specifically, we will conduct Cox proportional hazards regression of the primary outcome variable using the baseline infiltrate/scar size and drug assignment as covariates, and test the null hypothesis that the regression coefficient for drug assignment is zero. See Section 5.3.2 for a discussion of the hypothesis test of the primary outcome, and section 10.2.3 for a discussion of the critical value for the interim analysis.

Stopping rules. Stopping rules for benefit, harm, and futility are discussed in Section 10.2. These rules or guidelines would be determined at the first meeting of the DSMC (see Section 10.2).

Execution of interim analysis. The principal statistician (TP) will conduct the interim analysis in an unmasked manner, subject to independent statistical review by the DSMC. Quality assurance will be conducted by database manager KR.

4.3 Final Analyses

All final, planned analyses identified in this Statistical Analysis Plan will be performed only when the last patient has completed the final three-month visit. Further details of all statistical models and hypothesis testing are given in Section 9.

5 Sample Size Evaluation

5.1 MUTT I

The sample size was determined based on the primary objective (noninferiority comparison of voriconazole to natamycin) and primary endpoint (best spectacle-corrected visual acuity at three months).

5.1.1 Noninferiority margin

The primary comparison of voriconazole to natamycin will be conducted as a **noninferiority test**. The noninferiority margin will be specified as **one and one-half Snellen lines** (approximately 0.15 logMAR difference). In addition, we will also examine the possible inferiority of voriconazole to natamycin.

Little information is available to determine the effect of the active control in this trial (natamycin). It is likely that leaving an infectious fungal ulcer untreated would result in acuity decreasing after presentation, and often lead to perforation and blindness. MUTT suggests that treated ulcers improve an average of 4 lines from baseline. Thus we assume that if voriconazole-treated patients achieve a visual acuity within 1.5 lines of natamycin, then it would likely have at least 50% of the effect of natamycin.

5.1.2 Hypothesis tests

We will apportion our alpha level of 0.05 in equal measures to test two null hypotheses each at a level of 0.025—a test of non-inferiority and a test of inferiority.

The null hypothesis **for non-inferiority** states that the mean three-month visual acuity when topical voriconazole is used is 1.5 or more lines worse than when topical natamycin is used.

In principle, we could test the null hypothesis of non-inferiority in one of two ways:

- The change in the mean best spectacle-corrected visual acuity (BSCVA) from baseline to three months in the voriconazole group is at least 1.5 lines less than in the natamycin group. Comparison would be based on the confidence interval from Student's T test on the change scores.
- The mean best spectacle-corrected visual acuity at three months in the voriconazole group is at least 1.5 lines less than in the natamycin group, controlling for baseline best spectacle-corrected visual acuity. The statistical method is ANCOVA (in practice, multiple regression), and the test statistic is the T-statistic for the significance of the regression coefficient for the {0,1}-valued indicator variable for study arm. Noninferiority would be declared if the upper one-sided 97.5% confidence limit for this regression coefficient (indicating the effect of the drug controlling for baseline acuity) exceeds 1.5 (in Snellen units, or exceeds 0.15 in logMAR units). Note that larger values of β_2 indicate higher (worse) values for logMAR visual acuity and thus poorer drug performance.

For increased power, we chose the second of these alternatives, i.e. ANCOVA. Specifically, we model the best spectacle-corrected visual acuity at three months according to the linear model

$$Y_i=\beta_0+\beta_1 X_i+\beta_2 X'_i+\varepsilon_i \qquad (1)$$

where Y_i is the observed three-month best spectacle-corrected visual acuity for patient i, X_i is the baseline visual acuity for patient i, X'_i equals 0 if the i-th patient received natamycin, and 1 if the i-th patient received voriconazole, and the ε_i term is a normally-distributed random error component for the i-th patient.

Our approach is to create a two-sided 95% confidence interval for β_2. A margin of zero lines indicates equality; a margin of 1.5 lines indicates substantive inferiority. Hence, we can interpret the results of the study based on how the upper and lower limits of the confidence intervals fall on these margins.

5.2 MUTT II

The sample size was determined based on the primary objective (comparison of topical voriconazole only to topical plus oral voriconazole) and primary endpoint (perforation at three months).

5.2.1 Effect size

We assume a potential effect size of 0.15, i.e. a 15% (in absolute terms) difference between topical and oral plus topical voriconazole.

5.2.2 Hypothesis test

The comparison of oral plus topical voriconazole vs topical voriconazole alone will be a superiority comparison (overall two-sided α=0.05).

The null hypothesis states that the perforation risk within three months when oral and topical voriconazole is used is no different than when topical voriconazole alone is used.

We chose to use Cox proportional hazards regression correcting for infiltrate/scar size at baseline as well as treatment category. Specifically:

$$\log(\lambda(t)) = \log(\lambda_0(t)) + \gamma_1 Z + \gamma_2 Z',$$

where $\lambda(t)$ is the hazard for perforation, $\lambda_0(t)$ is the unspecified baseline hazard, γ_1 is the regression coefficient for the baseline infiltrate/scar size Z, and γ_2 the regression coefficient for the treatment assignment Z'.

5.3 Test Statistics

5.3.1 MUTT I

For the noninferiority limit, the test statistic is the upper (1-α/2) confidence interval for the value of the regression coefficient β_2, or

$$\hat{\beta}_2 + t_{1-\alpha/2,d} S$$

where α for the final comparison is slightly smaller than 0.05 because of the correction for the interim analysis, and d the number of degrees of freedom. However, we will declare superiority

in the event that the estimated T statistic falls in the rejection region for the hypothesis that β_2 does not equal zero.

For the inferiority limit, the test statistic is the lower $(1-\alpha/2)$ confidence interval for the value of the regression coefficient, where α is as above.

5.3.2 MUTT II

Proportional hazards regression will be conducted using standard methods (Kalbfleisch & Prentice, 1980). Ties will be broken using the Efron approximation. The test statistic is the Wald statistic for the coefficient for the treatment group:

$$Z = \frac{\hat{\gamma}_2}{SE(\hat{\gamma}_2)},$$

as given, for example, in (Kalbfleisch & Prentice, 1980).

5.4 Alpha level

5.4.1 MUTT I

A two-sided test would be conducted with an overall α of 0.05, corrected for the interim look. We propose to conduct the noninferiority comparison based on the lower one-sided 97.5% confidence limit of the regression coefficient in ANCOVA for study arm (again corrected for the interim look).

5.4.2 MUTT II

A two-sided test of the hypothesis that the log relative hazard $\gamma_2=0$ will be conducted at the 0.05 level, after adjusting for the interim look. See Section 10.2.3 for a discussion of the proposed flexible alpha spending function.

5.5 Power

For the first trial, we chose the noninferiority limit of 0.15 logMAR.

For the second trial, we choose to select sufficient samples for an 80% power to detect an effect size of 0.15.

5.6 Statistical properties of the primary outcome

To determine the expected standard deviation in the primary outcome variable, we used two sources of data: (1) three-month follow-up best spectacle-corrected visual acuity in the ongoing Steroids for Corneal Ulcers Trial being conducted at Aravind (T. Lietman, Principal

Investigator), and (2) the pilot study for this proposed Mycotic Ulcer Treatment Trial. This is consistent with the ICH recommendations for a phase III clinical trial (Department of Health and Human Services, Food and Drug Administration, 1998).

The sample standard deviation for the three-month best spectacle-corrected visual acuity in the overall Steroids for Corneal Ulcers Trial was 0.55 logMAR (source: Steroids for Corneal Ulcers Trial, Report to Data and Safety Monitoring Committee, 2008).

For the pilot study, 120 patients were randomized into four arms in a two-by-two design. The first factor was to scrape the cornea, or not; the second was the administration of voriconazole vs. natamycin. Note that in the main trial no subjects will undergo scraping. Based on the pilot study, we assume a standard deviation of 0.45 logMAR in the primary study endpoint (three month best spectacle-corrected visual acuity (using the patients with baseline visual acuity in the range 0.3 to 1.6 logMAR (inclusive), as specified above).

For the Steroids for Corneal Ulcers Trial, preliminary findings (after 192 enrollments) revealed a correlation of 0.645 between baseline best spectacle-corrected visual acuity and three month best spectacle-corrected visual acuity (source: Steroids for Corneal Ulcers Trial, Report to Data and Safety Monitoring Committee, 2008).

For the pilot data from the proposed Mycotic Ulcer Treatment Trial, a correlation of 0.09 between baseline best spectacle-corrected visual acuity and three month best spectacle-corrected visual acuity was found (when patients are restricted to the intermediate range of vision at baseline, i.e. 20/40 or worse but 20/400 or better, and using an imputed data set (see section on missing values below)).

5.7 Withdrawals and expected loss to follow-up

The expected number of withdrawals and loss to follow-up have been estimated from two sources: (1) the ongoing Steroids for Corneal Ulcers Trial being conducted at Aravind (T. Lietman, Principal Investigator), and (2) the pilot study for the proposed Mycotic Ulcer Treatment Trial. As of March 2008, 90% of enrolled cases presented for their 3-month follow-up examination. In the MUTT pilot study, we had primary outcome data on 106 of 120 cases (0.833%) (computed on all patients).

5.8 Sample size calculation methodology

5.8.1 MUTT I

As indicated above, based on the pilot study, we assume that the square root of the variance of the outcome (best spectacle-corrected visual acuity), after controlling for baseline best spectacle-corrected visual acuity, was 0.45 logMAR. This was derived by fitting a linear regression of the three-month best spectacle-corrected visual acuity to the baseline best spectacle-corrected visual acuity and using the estimated standard error from the regression (for conservatism, we assumed the variance was 10% larger). We used multiple imputation as described in Section 15.4.

To compute the required sample size, we used the formula in Chapter 3 of Chow et al. (Chow *et al.*, 2003) for a two-sample non-inferiority design. A total sample size of approximately 346

yields approximately 80% power to reject the hypothesis of noninferiority (with the limit at 1.5 lines), assuming a standard deviation of 0.45. The analysis includes loss to follow-up. Sample size curves are given in Figure 1 (see end of document); power curves are given in Figure 2.

Assuming a standard deviation of 0.464 yields a sample size of 368 (to achieve 80% power for the one-sided test of size 0.025). This value of the standard deviation was derived from the pilot study, by assigning a vision of 1.9 following transplant, but using last observation carried forward (or baseline, if there is no three week visual acuity) for missing three month visual acuities other than those following transplants (and using actual measurements following perforations). Following this procedure, we fit a linear regression of the three-month best spectacle-corrected visual acuity to the baseline best spectacle-corrected visual acuity using the estimated standard error from the regression.

The sample size for MUTT I is 368 patients.

5.8.2 MUTT II

For MUTT II, we ignore censoring and failure times, and determine the power to detect a change in risk of perforation. A sample size of 240 provides at least 80% power to detect a 15% change (in absolute terms) of the risk of perforation (using the baseline perforation risk in patients with severe vision deficit measured at baseline, as seen in the pilot study), and a two-sided α of 0.05. We obtained this result by regressing perforation on baseline scar size and drug (using logistic regression). We then simulated from the estimated regression function, but assuming a drug effect equal to a 15% reduction. On each simulated data set, we performed logistic regression, and recorded the fraction of times that the null hypothesis was correctly rejected. We anticipate slightly higher power when we use Cox regression (which will take into account perforation times as well).

5.9 Power for secondary analyses

In this section, we review the statistical power for key secondary analyses.

5.9.1 Infiltrate/scar size

Based on Mycotic Ulcer Treatment Trial pilot data, the unconditional variance of the three-month infiltrate/scar size was 1.91 mm, with a correlation of 0.871 with the baseline value. After regressing the baseline infiltrate/scar size, the variance (computed from the residual sum of squares) is 0.943 mm. For simplicity, we use the standard power formula (Chow *et al.*, 2003):

$$p=1-T_{n_1+n_2-2}(t_{\alpha/2},n_1+n_2-2,\lambda) \qquad (2)$$

where $T_m(x;\lambda)$ is the cumulative distribution function of a random variable with the noncentral T distribution with m degrees of freedom and noncentrality parameter λ. In this case,

$$\lambda = \frac{\delta}{\sigma\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}.$$

Based on this equation, we estimate that a difference of 0.26 could be detected with 80% power. This approximate power analysis was based on an assumed T-test of the residuals after modeling the three-month infiltrate/scar sizes by the baseline infiltrate/scar sizes.

5.9.2 Reepithelialization time

We computed an approximate sample size for the proposed survival analysis of reepithelialization time. Kaplan-Meier analysis of the subjects in the pilot study revealed a median reepithelialization time of approximately 12 days. Using the approximate formula given on page 115 of Friedman (Friedman *et al.*, 1998), we calculate that a 17% increase in the relative hazard for reepithelialization would require 330 subjects (in total) to be detected with 80% power.

5.9.3 Treatment effects within *Fusarium*

The statistical analysis of treatment effects within *Fusarium* cases is discussed in Section 9.1.3 (Specific Aim 2). We assumed the same analysis as for the primary comparison (and the same variance in the primary outcome), except that we assume we are restricted to cases known to be due to *Fusarium* (assuming 15% failure to grow fungus in culture, and assuming 36.7% of culture-positive cases were due to *Fusarium* as seen in the pilot study). Using Equation 2 shows that we can detect an effect size of 0.225 logMAR for three-month best spectacle-corrected visual acuity with 80%, and an effect size of 0.473 mm for three-month infiltrate/scar size with 80%.

5.9.4 Minimum inhibitory concentrations

Using Equations 3.120 and 3.122 of Searle (Searle, 1971), we estimated the power to detect an effect of a given size. We assumed a regression based on baseline best spectacle-corrected visual acuity, *Fusarium* or not, drug assignment, and minimum inhibitory concentration. Previous studies from our collaboration with Aravind provided an estimate of 1.14 dilutions (Lalitha *et al.*, 2007) for the standard deviation of the MIC (after modeling drug and organism). We otherwise conservatively assumed the same standard deviation for the three-month best spectacle-corrected visual acuity as for Specific Aim 1 (the primary outcome of the trial), we assumed 15% loss to follow-up, and that only 85% of subjects would yield fungal cultures. We computed that we have 80% power to detect an effect size (a difference in best spectacle-corrected visual acuity between the two drug assignments) of 0.062 logMAR per twofold dilution overall, and of 0.095 logMAR per twofold dilution restricted to *Fusarium* cases alone.

5.9.5 Other analyses

We estimate that the analysis of photographically determined infiltrate/scar sizes will have approximately the same power as for slit lamp estimates. Analysis of acuity measured with hard

contact lens is expected to have slightly *higher*, though comparable, power, based on findings from the Steroids for Corneal Ulcers Trial.

Analyses of perforations (MUTT I) or other adverse events (MUTT I and II) will be reported using confidence intervals and descriptive statistics, as failure to report a significant difference may be misinterpreted as evidence of no difference.

6 Analysis Populations

6.1 Summary

The following analysis populations are planned for this study:

- The **screening population**, which is to include all subjects who provide (a) baseline screening (including any demographic, visual acuity, or photographic data), and (b) informed consent.
- The **safety population**, which is to include all patients who receive any amount of planned study medication (voriconazole or natamycin).
- The **intent-to-treat efficacy population**, which is to include all patients who are randomized. This is the primary population for the efficacy analyses.
- The **per-protocol efficacy population**, which is to include all patients in the intent-to-treat efficacy population, excluding patients with any of the following: (a) non-fungal or mixed ulcer, (b) major protocol deviations, or (c) noncompliance with study medications (less than 50% of the study drug by self report).

Analyses as described elsewhere in this document will be applied to each of these populations, as appropriate. In particular, all analyses intended for the intent-to-treat population will be repeated for the per-protocol efficacy population, and will *always be clearly labeled as such*, and their secondary or supplemental nature unambiguously indicated in every report or presentation. Summary statistics for the screening and safety populations will be presented as needed, reported to the DSMC, and included in reports or publications.

6.2 Major protocol deviations

The incidence of deviations from the inclusion and exclusion criteria will be summarized using counts and percentages, and the treatment groups compared for the overall frequency of deviations using a $2\times N$ Fisher's exact test. Similar deviations will be grouped into general categories of deviations for a more condensed summary. A listing of deviations by participant will also be produced. Any major deviations from the protocol will be listed and/or summarized, including, but not limited to, participants who:

- never received study drug
- were subsequently found to be ineligible for the study
- never returned for a follow-up visit
- have follow-up visits outside the prescribed visit window

The number and percentage of randomized volunteers actually receiving study medication, switching antibiotics, or permanently discontinuing study drug (subdivided by reason) will be summarized. A summary of volunteers randomized by center will also be provided. Treatment groups will be compared for the proportion and reason for study drug discontinuation using the chi-square test. A summary of volunteer status at the end of the study period will also be generated with categories including or lost to follow-up (LFU).

Note that in particular, subjects who test positive for bacterial ulcers on culture will be excluded from the per-protocol population.

7 Data Collection and Quality Assurance

7.1 Quality assurance and security

Data collection forms, training, security, and quality assurance are discussed in the Manual of Operations for the proposed trials.

7.2 Analysis sets

For each separate trial, separate data sets for analysis will be produced at the Proctor central site by database manager KR. Each will be a Microsoft Excel® worksheet containing a single header line whose variable names match the Access database. Each analysis set will be in the form of a rectangular table in which each column corresponds to a single variable and each row to an observation. All missing values will be coded explicitly using the string `NA` (as used in the R software). Codes for categorical variables (such as 1 for male, and 2 for female) will be avoided in favor of self-documenting character strings (such as Male, Female) whenever possible. Automated checks will be made to ensure consistency and that each variable in the analysis set has in-range values (protecting against negative ages, spelling errors in categorical factors, and similar errors).

A detailed codebook will be prepared, containing for each variable, (a) the form from which the variable derived, (b) the text of the question, (c) all possible values for the variable, and (d) summary statistics for the variable. Note that all codes and character strings that represent categorical factors will be clearly defined in the codebook. Units for each continuous variable (e.g. mm vs. mm^2, logMAR, etc.) will be unambiguously indicated for each variable. Each release of the analysis set will be accompanied by the corresponding version of the codebook. Version numbering with dates will be strictly observed. Standard report-generation software included with the R statistical and data analysis package will be used to ensure consistency of the codebook and analysis set at all times.

7.3 Data monitoring reports

Data monitoring reports will be prepared based on analysis data sets. These will be prepared using report-generation software. Monitoring reports will include (a) recruitment reports for each center, (b) compliance reports, (c) retention reports, and (d) data quality reports. These will be

reviewed at the central site on a monthly basis, and communicated to the study sites on a monthly basis.

8 Statistical considerations

8.1 General

All alpha levels will be two-sided, set at an alpha of 0.05 (unless indicated otherwise or required because of interim analysis). All supplemental analyses will be conducted in both MUTT I and MUTT II unless indicated otherwise (of course, the primary outcomes differ for these two trials).

8.2 Missing data and loss to follow-up

The following considerations apply primarily to the intent-to-treat efficacy analysis, but may (*mutatis mutandis*) be used as needed for the other three analytic populations. Our treatment of missing values is intended to follow the principles outlined in Carpenter & Kenward (2007). These methods will be applied to both trials, MUTT I and MUTT II. Note in particular that the disadvantages of the last-observation carried forward method are appreciated, and that this method will never be used in a primary analysis, but occasionally reported because it is commonly used.

As emphasized in Carpenter & Kenward (2007), "there can be no universal analysis when data are missing". Our purpose is to vary the assumptions as well as the methods, to establish that the estimates of the treatment effect are robust as such assumptions are varied.

8.2.1 Missing values due to corneal transplant

Following Cook & DeMets (2007), we distinguish between missing values which are well-defined, but not observed due to failure to return or other reasons, and missing values that occur because of the state of the subject (cf. Sec. 11.3, Cook & DeMets). Subjects for whom the visual acuity, scar size, and epithelial defect cannot be measured because the subject has undergone a corneal transplant fall into this latter category. For visual acuity data missing at three months for this reason, we chose to use a logMAR vision of 1.7 or last observation carried forward (LOCF), whichever is larger for the three month vision (see Cook & DeMets, 2007, p. 364). For missing scar size and epithelial defect, we chose to use the most recent value for each patient prior to the surgery (because small scars may perforate, we avoid imputation of a very large scar size). Corneal transplants that occur beyond a three-month visual acuity measurement will not result in the assignment of the acuity value.

A sensitivity analysis will be performed to determine whether assignment of a different value (e.g., 1.9, the worst value on our scale) would affect any conclusions drawn.

An additional sensitivity analysis in which perforations are treated in the same way as transplants will be conducted (i.e. using a value of 1.7 or LOCF whichever is larger, instead of any measured visual acuity).

Inclusion of such patients is important if we are to avoid being misled in the event one drug were to be associated with more perforations, but yield better outcomes if perforation did not occur.

8.2.2 Missing values due to cataract surgery

When visual acuity data are missing because of cataract surgery, we have chosen to carry forward the last measured visual acuity. In principle the occurrence of cataract surgery is unrelated to the study outcome, and therefore sensitivity analyses in which the patients final acuities are omitted, in which last-observation carried forward is used, and in which any available acuity value (within the specified time period) is used will be conducted. We expect this to occur uncommonly.

8.2.3 Visual acuity following perforation

Following perforation, visual acuity values may be affected due to the presence of glue. We propose to use such acuity values in primary analyses. It is understood that the presence of glue reduces visual acuity. However, no additional penalization of acuity measures to reflect the fact that perforation is an undesirable outcome will be applied.

8.2.4 Missingness due to withdrawal (loss to follow-up)

We also will investigate whether or not any relationship between observed covariates (in particular age, gender, baseline visual acuity, baseline infiltrate/scar size, treatment assignment) and whether or not a three-week or three-month value is missing. Observation of such a relationship would provide evidence against the data being missing completely at random. Summary tables will be prepared for each outcome variable of interest (BSCVA, scar size, epithelial defect size, or perforation occurrence), and logistic regression will be used for statistical modeling of missingness. These analyses will be interpreted in an exploratory manner taking the problem of multiple comparisons into account. It is understood that the absence of any relationship between covariates and missingness can never demonstrate that data are MCAR.

We will report the number missing by treatment arm, as required by the CONSORT guidelines.

For each primary prespecified analyses for both MUTT I and MUTT II, we will propose to conduct the analysis using all available data, for visual acuity and infiltrate/scar size measurements. Specifically, the three-week data will be included as an additional outcome variable.

These analyses will be supplemented by a series of sensitivity analyses (indicated below).

8.2.5 Sensitivity analyses for data missing due to loss to follow-up

Last observation carried forward. As a sensitivity analysis, we will include patients with missing three-month values for visual acuity and for infiltrate/scar size by carrying forward the most recent observation of the variable. Because last-observation carried forward is a frequently-used method, we chose to include it (as well as baseline carried forward) despite well-founded criticism of carrying forward observations (Cook & DeMets, 2007; Carpenter & Kenward, 2007).

Baseline carried forward. As a sensitivity analysis, we will use baseline carried forward to three weeks and to three months. This analysis includes all patients.

Multiple imputation. For values missing due to loss to follow-up of the subject or other reasons unrelated to the state of the subject, or for death due to unrelated causes, missing values of the three-month best spectacle-corrected visual acuity, scar size, epithelial defect size, and perforation occurrence will be analyzed using the method of multiple imputation (with five replications), following the assignments made in the previous paragraph. Specifically, we propose to use a regression model-based imputation scheme (using a set of variables which will include age, sex, visual acuity at baseline, visual acuity at 3 weeks, assessed depth at enrollment, central/peripheral scar, study location, and follow-up time; see Section 15.4 for details). Several methods are available for model-based multiple imputation (Schafer, 1997); we will use normal-based regression models where appropriate. Sampling from a predictive distribution (possibly using Markov Chain Monte Carlo (MCMC) methods) of the response given the covariates can then be conducted to yield imputed values for the outcomes (a procedure which includes both the uncertainty in the regression coefficients themselves as well as the conditional variance of the outcome variable given the values of the regressors and the values of the regression coefficients). For each of the five replications, the effect size and standard deviation of the regression coefficient for the effect of drug (after controlling for baseline visual acuity) will be computed, and combined using the standard formulas in Little & Rubin (2002) (p. 86-87) to yield a T-test for the coefficient for the effect of drug. The same procedure will be used for scar size, epithelial defect size, re-epithelialization time (using Cox regression in this case), and perforation (using Cox regression rather than linear regression in this case). Note that we will not use scar size, epithelial defect size, visual acuity, re-epithelialization time, or perforation in imputing any of these (e.g. visual acuity will not be imputed based on epithelial defect, visual acuity, or perforation, etc.). The analysis will be repeated using the fourth-root transformation.

Note that imputation will never be used in a primary prespecified hypothesis test, but only as a supplemental sensitivity analysis. This will be clearly indicated in all reports or presentations based on these methods.

Model-based imputation using other outcome variables. Sensitivity analysis based on model-based imputation may be conducted by using baseline values, three-week values, and perforation time to model missing three month values using linear regression (Little & Rubin, 2002). Such analyses will be clearly labeled as exploratory sensitivity analyses.

Missingness not at random. We will follow the recommendations in Carpenter & Kenward (2007), Ch. 6, and explore models in which missingness is assumed to be nonrandom in the sense of depending on the unobserved value of the visual acuity or scar size (i.e., the outcome being regressed). In particular, a model in which the log odds of missingness is a linear function of the value will be explored by assuming particular values of this log odds and conducting the regression as this quantity varies as a sensitivity analysis. Such models will be presented as sensitivity analyses.

Missing covariates (regressors). Regression-based imputation will be used to predict missing covariates from other covariates. Multiple imputation will always be used (as described above). Such analyses will be supplemental (when needed) and will be labeled as such. Sensitivity

analyses will also be conducted, where appropriate. When using likelihood-based statistical models, inclusion of subjects with missing covariates is sometimes possible (in essence, integrating over the marginal distribution of the missing covariate).

8.3 Transformations and model adequacy

Because the legitimacy of the hypothesis test being conducted depends on the assumptions (i.e. normality and homoskedasticity for linear models, proportional hazards for Cox regression), the adequacy of the statistical model must be checked. Methods which will be employed will include (a) residual plots (vs. baseline value, vs. predicted values, and Q-Q plots), (b) jackknife influence estimates, (c) tests for normality (in particular, the Cramer-von Mises procedure as implemented in the `nortest` package of R), and (d) the Gill-Schumacher procedure for assessing the adequacy of the proportional hazards assumption for Cox regression (Gill & Schumacher, 1987) or the Therneau-Grambsch procedure, or examination of survival analysis residuals. Normality tests will be employed only as an interpretive guide; the low power of normality tests in a small sample setting where they may be most needed restricts their utility.

Analyses of the pilot study suggest that the square and cube root transformations will not achieve approximate normality of the residuals. The fourth root achieves acceptable results, although interpretation in the setting of a noninferiority trial may be considered problematic. We are proposing to analyze untransformed values for this reason. Sensitivity analysis will, however, include the following:

- Linear regression of the fourth root of the final (three month) BSCVA as the outcome, and treatment arm and the fourth root of the baseline BSCVA as regressors (predictors). As before, the treatment arm is an indicator of the drug used for MUTT I, and an indicator of whether oral voriconazole is used in addition to topical voriconazole (MUTT II).
- Robust regression (R command `rlm`, package MASS) of the untransformed visual acuity
- Quantile regression (R command `rq`, package quantreg; STATA command `qreg`)

8.4 Pooling across centers

The vast majority of patients are expected to come from the Madurai and Pondicherry sites, which are in the same hospital network in the geographic region serving the same patient population. UCSF/Proctor serves a very different population, although we expect few cases in the Proctor site.

8.5 Multiple comparisons

The noninferiority comparison in MUTT I will be conducted with a one-sided rejection probability for the null hypothesis of 0.025, and the inferiority comparison will also use a 0.025 level one-sided test—expending a total alpha in MUTT I of 0.05. An alpha of 0.05 will be used for the primary analyses in MUTT II. For pre-specified secondary analyses, we will report both the P-value and the number of pre-specified analyses performed. Note that the results of

secondary analyses are not independent events, making a Bonferroni correction very conservative.

8.6 Subgroup analyses

Sub-group analysis of the cases due to *Fusarium* spp will be performed as outlined elsewhere. Analysis of outcomes will also be done by the following subgroups: causative organism, visual acuity group, duration of symptoms to enrollment, depth of ulcer, infiltrate scar size, gender, and study location.

8.7 Software

The standard software package R 2.6 or higher (`http://www.r-project.org`) for the MacIntosh OS X will be used for all descriptive and inferential analyses. The package STATA 10 or higher for MacIntosh will also be used. Photographic analysis will be conducted using the program Optscore.

9 Hypothesis Tests

9.1 Analysis of primary efficacy variable

We describe an analysis template for continuous outcomes which will be repeated as indicated.

9.1.1 Primary analysis

The primary analysis (MUTT I) is for the best spectacle-corrected visual acuity at three months, measured in logMAR. We will perform a linear regression of the three-month best spectacle-corrected visual acuity with the enrollment best spectacle-corrected visual acuity and the treatment group as covariates. The treatment group is the primary predictor. Significance will be assessed using a one-tailed test at 0.025 level for assessing noninferiority and a one-tailed 0.025 level test of inferiority (see above). The analysis will include only those values collected within the 2.5 to 5 month window (using the value closest to 3-months if there is more than one record).

Residual plots (versus baseline value, versus predicted values and a Q-Q plot) will be investigated for outliers, nonlinearity, non-homogeneous variances and gross violations of normality.

If the hypothesis of noninferiority is rejected, we also prespecify that we will conduct a superiority comparison of voriconazole to natamycin using three month BSCVA as the primary outcome, baseline BSCVA and study arm as covariates (i.e., keeping everything the same as for the noninferiority comparison). No inflation of the alpha level occurs when this is done.

Many outcome measures have a baseline measure which should be included as a regressor in any analysis. Specifically, for three-month acuities, we would correct for baseline acuity (i.e. include baseline acuity in a regression model). For three-month scar size, we would correct for baseline infiltrate/scar size. For perforation, we would correct for depth.

For MUTT II, the primary analysis is Cox regression of the probability of perforation using baseline infiltrate/scar size and treatment group as covariates. Other variables (visual acuity, scar size) will be analyzed according to the template in this section for secondary analyses. Analysis is two-tailed with an alpha of 0.05.

9.1.2 Sensitivity analyses

Observations with large standardized residuals will be removed to assess sensitivity of results to their presence; bootstrap (BCA—bias corrected accelerated; see p. 178, Efron and Tibshirani, 1993) standard errors will be calculated if the residuals are highly non-normal or lack variance homogeneity; a nonlinear baseline value relationship with the outcome will be accommodated via higher-order polynomials. Where appropriate, we will follow the same template for every analysis of any variable using any predictors.

A series of sensitivity analysis are planned in the case of missing data or data not timed exactly at three months:

1. *Adjustment for measurement time*. A regression adjustment is added for the time at which the measurement is taken but the time window for a measurement to be considered a three-month measurement remains from 2.5 to 5 months.
2. *Expanded time window*. The time window is enlarged to 2.5 weeks to 6 months, choosing the closest measurement to three months.
3. *Mixed model analysis*. For the primary outcome we will also utilize a mixed model segmented regression of all measured values (including baseline). Predictors will be: treatment group, segments with piecewise linear components up to 3 weeks and after three weeks, and the interaction of treatment group and the segmented regression. Time is to be centered at 3 months to get "intercept" comparisons between the groups at 3 months as the primary test. Mixed model regression analysis with inclusion of random intercepts and, potentially, regression segments (unstructured covariance structure for the regression intercepts and segments). This analysis accommodates all available data, unequal visit spacing and corrects for data that are "missing at random".

Each of these sensitivity analyses will be compared to the primary analysis. If substantive or qualitative differences exist, the assumptions of the more complicated models will be carefully checked and, if appropriate, reported in the final manuscript.

9.1.3 Treatment by organism

We will conduct a subgroup analysis to examine a differential effect of voriconazole vs. natamycin on three month best spectacle-corrected visual acuity for *Fusarium* infections. The analysis will include only those values collected within the 2.5 to 5 month window (using the value closest to 3-months if there is more than one record).

9.2 Secondary analyses

9.2.1 Three week best spectacle-corrected visual acuity

The three-week best spectacle-corrected visual acuity measured in logMAR is a secondary outcome variable. The primary and exploratory (sensitivity) analyses will follow the template in Section 9.1. The three-week BSCVA will include values taken between 18 days (2.5 weeks) and 5 weeks, using the value closest to 3 weeks if multiple values are available.

9.2.2 Indian sites, best spectacle-corrected visual acuity

We will examine (a) three-month best spectacle-corrected visual acuity in Indian sites only, and (b) three-week best spectacle-corrected visual acuity in Indian sites only. The primary and exploratory (sensitivity) analyses will be identical to those in Section 9.1 but restricted to data collected from Madurai and Pondicherry.

9.2.3 Hard contact lens

Hard contact lens corrected visual acuity at three weeks and at three months, measured in logMAR will be analyzed according to the template in Section 9.1.

9.2.4 Infiltrate/scar size, slit lamp

Scar size measured by slit lamp exam will be calculated as the geometric mean of two principal axes in mm. The slit-lamp scar sizes at 3 weeks and at 3 months will be compared between the treatments using the template in Section 9.1.

9.2.5 Infiltrate/scar size, photography

Scar size measured by photography will be calculated as the square root of the encircled area in mm. The photography scar sizes at 3 weeks and at 3 months will be compared between the treatments using the template in Section 9.1.

9.2.6 Time to epithelial recovery

Time of epithelialization will be defined as the midpoint between the last observed date with an epithelial defect and the date of the first visit with no epithelial defect.

Primary analysis. Cox proportional hazards regression will be used with predictors of baseline epithelial defect size (via direct measurement in millimeters—a geometric mean of the two principal axes) and treatment group, the primary predictor, which will be tested with a two-sided test at level 0.05. Observations will be censored at the first occurrence of the following: reaching the 3-month time point, loss to follow-up, corneal transplant, or death. Multistate transition models (e.g. Andersen & Keiding, 2002) will be used to simultaneously estimate reepithelialization-related relative hazards as well as relative hazards related to the risk of perforation (in an analysis that will be labeled and interpreted as strictly exploratory) using baseline measurements, depth, treatment status at baseline, age, or other covariates as predictors.

Sensitivity analysis. The primary analysis will be compared with an analysis stratified on quartiles of the baseline epithelial defect size as a check of the proportional hazards assumption. If this sensitivity analysis is substantively or qualitatively different from the primary analysis, the stratified analysis will be adopted as the final one.

Treatment failure. The proportion of treatment failures will be compared between the arms using Fisher's exact test.

9.2.7 Microbiological cure

Microbiological cure at 7 days will be compared between the two treatment arms (both in MUTT I and MUTT II, separately) using logistic regression, with both treatment group and organism (*Aspergillus*, *Fusarium*, or other) as covariates.

9.2.8 Other subgroups

In both MUTT I and MUTT II, we propose (where applicable) to repeat all analyses in the following subgroups of patients: (a) culture-positive patients only, (b) cases which had not received an antifungal medication before presentation. Note that repeating all analyses restricted to Indian sites has been proposed above.

9.2.9 Study site as a predictor

Models will always be fit with and without study site as a fixed effect, and the results compared. Interaction terms may be included when justified.

9.2.10 Visual acuity at baseline

While our pilot data suggest that the method of correcting for baseline acuity is adequate given the restricted range of enrollment acuities, it is possible that a linear adjustment will be inadequate. Additional models including quadratic or higher-order terms will be systematically explored if they produce statistically significant improvement to the fit. A semiparametric adjustment will be explored and labeled as exploratory, however. Use of the data in both trials to improve our understanding of the effect of baseline acuity as a covariate will be conducted to help plan future trials. Such analyses will be clearly separated from the main prespecified results of the trial and will be labeled as such. Similar studies designed to understand better how to adjust for covariates in future trials may be conducted using infiltrate scar size, transplantation or perforation, or reepithelialization time, will be conducted as well, and such analyses may be conducted with multivariate methods.

9.2.11 Transplantation

A purely exploratory analysis to seek predictors of transplantation may be considered (in MUTT I and/or MUTT II). As before, such an analysis would be labeled as exploratory and kept separate from the main trial results. Presentation of such findings would center on age, depth of infiltrate/scar, size of scar, how long the patient had waited before seeking treatment, and/or acuity. Our studies are not powered to detect a difference in this outcome by drug.

9.2.12 Cost-effectiveness

Analyses of the cost-effectiveness of fungal ulcer treatment with voriconazole, oral voriconazole, or natamycin may be conducted using outcome data from this trial. The outcome variable will be the incremental cost per quality-adjusted life year (QALY). QALYs will be estimated from visual acuity measurements at baseline and three months, as well as published estimates of the health state utility for perforations and the transplant procedures themselves (if applicable). Medication side-effects will be included using published estimates. The cost-effectiveness analyses will be clearly separated from any primary prespecified hypothesis testing. We do, however, wish to explore the use of quality-adjusted life years as a combined outcome variable for the trial in a strictly exploratory manner (because both visual acuity and the occurrence of adverse outcomes such as perforation and/or transplantation are clinically important outcomes, we may wish to conduct methodological investigations of the use of QALYs as outcome variables). It is understood that this is strictly secondary and exploratory; such analyses, conducted to help prepare for future trials, do not yield independent information from the primary prespecified outcomes and will never be presented as though they were prespecified primary outcomes.

9.2.13 Photography

Photographs are graded by independent raters using the same system we use in previous UCSF-Aravind trials. Raters must achieve a reliability of 0.8 (Cohen's Kappa). Full details are presented in the Manual of Operations. Supplementary analyses of photographs may be conducted to determine whether certain features at baseline are associated with particular organisms.

9.2.14 Minimum inhibitory concentration

Minimum inhibitory concentration for the drug used will be used as a regression predictor for primary outcomes (three month BSCVA for MUTT I, perforation for MUTT II). In addition, it will be used as a predictor for secondary outcomes. Additional exploratory analyses will use BSCVA standardized within fungal organism, may use prior antifungal medication (of each type), or include organism-MIC-drug interactions. We will also use MIC as an outcome (to be predicted by organism or prior antifungal use).

9.2.15 Exploratory analyses using all variables

Exploratory analyses of visual acuity at three months, perforation, reepithelialization time, and transplant will be performed using all predictors. Such analyses will always be clearly labeled as such and will be kept separate from the primary prespecified outcome. Such analyses may be helpful in planning future trials.

9.3 Additional supplementary analyses

9.3.1 Visual acuity

We propose, in addition to the primary hypothesis tests, to conduct additional analyses to determine predictors of poor visual outcome (MUTT I) or perforation (MUTT I and II), other

than study medication. Of particular interest is what predictors are available at baseline. We will also determine statistical predictors for all other secondary outcome variables for both trials. These other variables will include, but are not necessarily limited to, date of presentation (either within the year or within the study), depth, use of prior antifungals, location of infiltrate/scar, or any other variable collected. Analyses of depth, prior antifungals, infiltrate/scar size, hard contact refraction, location, and the other secondary variables, will also be conducted on the same subsets, and will follow the same protocols listed above.

Analyses involving other predictors will be conducted using AIC-based stepwise regressions, multivariate adaptive regression splines (R package `polspline`), or other machine learning algorithms. Cross validation will be used to help avoid model selection bias. These analyses will be clearly labeled as exploratory and hypothesis-generating and will never be included in papers and reports containing primary study outcomes. We are sensitive to the issue of clearly separating exploratory, hypothesis-generating analyses from formal hypothesis testing, and understand the issues multiple comparisons may raise in this context.

Further analyses of MIC and outcome are also warranted. These may include causal mediation analysis or a conditional logistic regression.

The causes of visual loss with corneal scars will be explored also. We wish to determine what proportion of the final variance is due to the scar covering the pupil, irregular astigmatism, and/or glare.

9.3.2 Other analyses

Other exploratory or descriptive analyses will be of interest. Some (though not all) may be primarily of interest in planning future trials. We will conduct exploratory analyses of the differences between pinhole acuity measurements, BSCVA, and hard contact lens refractions. We will also examine the relationship between MIC and fungal species among our particular study population. Other analyses which are not currently planned but which may be considered in the future include analyses of features of photographs (do certain features correspond to particular species?), or of seasonal effects in incidence.

10 Human Subjects

10.1 Summary of final dispositions

All subjects who provide informed consent will be accounted for in this study. The frequency of subjects in each population will be presented. We will also present the frequency of subjects in each subgroup, the frequency of withdrawal and loss to follow-up, and any major protocol violations.

10.2 Data and Safety Monitoring Committee

10.2.1 Scope

A Data and Safety Monitoring Committee (DSMC) will be empaneled by the NEI. We propose that this committee consist of 5-7 individuals, and should include (a) cornea specialists, (b) an independent biostatistician, (c) a bioethicist, and (d) representation from both India and the United States. The committee will meet in person at least once per year, and will convene biannual teleconferences for progress reports. *Ad hoc* meetings as needed may also be convened. All study protocols will be subject to review and approval by Institutional Review Boards at the UCSF and Aravind sites, and by the DSMC.

The Data and Safety Monitoring Committee will meet to review the interim efficacy data for MUTT I when primary outcome data is available on one third of the study subjects—approximately 3 months after the 123th subject has been enrolled in the trial. For MUTT II, interim efficacy data will be available after the 80th subject has been enrolled in the trial; we expect these milestones to be predictable (and to occur at approximately the same time) and we will schedule this review for a face-to-face meeting. At this meeting, the DSMC will make one of the following recommendations:

- Continue the trial without modifications
- Continue the trial with study modifications
- Terminate enrollment or treatment in the trial because of safety concerns
- Terminate enrollment or treatment in the trial because of efficacy
- Terminate enrollment or treatment in the trial because of futility

10.2.2 Meetings

All in-person and teleconference meetings of the DSMC and study personnel will consist of (a) "open" sessions, which may be attended as needed by masked study personnel, and (b) "closed" sessions, which may only be attended by unmasked study personnel (TP, KR). Care will be taken so that *no* treatment assignments, data which would allow treatment assignments to be determined, or outcome data based on treatment assignments will be revealed during the open sessions.

Interim reports for the DSMC will be prepared by the central Proctor site (TP). These reports will include (a) recruitment overall, and by study site, (b) compliance, and (c) retention. The reports will also list study outcomes, and all adverse outcomes, including mortality and perforations. The DSMC will determine the database closure dates for each report in advance; archival copies of the (a) main SQL database, and (b) study analysis file as they exist at the time of each report will be maintained. All reports will be sent using secure email to the members of the DSMC two weeks prior to each meeting. Each interim report will be labeled clearly as confidential, printed in binding so that the contents are not visible from the outside, and labeled with the name of each person authorized to receive it. Reports will be kept in possession of KR and TP and only delivered in person; reports not delivered due to absences are to be shredded. In addition, redacted versions of the interim reports will be prepared which contain no masked study

information, and which are suitable for restricted distribution to other personnel on an as-needed basis. All hard copies will be destroyed at the end of each meeting, except for a copy to be kept in a locked file cabinet accessible only to TP and KR.

10.2.3 Decisions

The DSMC will make decisions with the benefit of prespecified decision guidelines. These guidelines will be agreed upon at the initial meeting, and are expected to include (a) safety, (b) efficacy, (c) clinical importance, (d) effect of baseline covariates, or (e) validity.

Benefits. Unmasked interim analyses (See Section 4.2) will be conducted to determine whether or not sufficient evidence has accumulated to justify stopping the trial because one treatment is clearly superior (and therefore should be extended to all future cases). The guidelines for efficacy will use group sequential boundaries for judging the statistical significance of the primary outcome measure (three month best spectacle-corrected visual acuity, imputing all visual acuities after transplants as 1.9 logMAR acuity, and treating values missing for other reasons using multiple imputation as indicated below). The Lan and DeMets (DeMets & Lan, 1994) flexible alpha spending approach with a power use function is suggested:

$$\alpha^*(t^*)=\alpha t^{*\theta}$$

where $\theta=\log 50/\log 3\approx 3.561$ (chosen so that the two-sided *P*-value to stop the trial for efficacy is 0.001). (Note that the use of a standard O'Brien-Fleming function of the form $\alpha^*(t^*)=2-2\Phi(Z_{\alpha/2}/\sqrt{t^*})$ would yield a very conservative *P*-value of approximately 0.0007 for $t^*=1/3$, stopping one-third of the way through the trial.) The use of a flexible alpha-spending function protects the 0.05 alpha level of the overall trial while allowing for additional interim analyses for efficacy (if needed), without specifying the number and timing of the analyses at the start of the study. We note that the alpha spending function cannot be changed once the trial has begun.

Harm. Stopping for harm will be done at the judgment of the DSMC. Several endpoints will be examined, including (a) the best spectacle-corrected visual acuity at three months, (b) reepithelialization time, (c) adverse events, and especially (d) serious adverse events, including perforation or mortality. While the analysis would consider maldistribution of predictive factors such as (a) age, (b) infiltrate/scar size at presentation, and (c) organism, it is recognized that ethical considerations require careful considerations of statistical tests as well as qualitative judgments in the light of experience. Any additional analyses required by the DSMC will be conducted by TP and KR, as needed.

Note that serious adverse events (SAE) are reported directly to the DSMC Chair with 24 hours of the time the study site learns of them. The DSMC Chair receives notification of the event, the timing of the event, a medical narrative from the medical monitor, the site, the patient identification number. The statistician reports the study treatment assignment to the DSMC Chair. If voriconazole use clearly results in an unacceptable increase in the risk of treatment failures, then the study will be stopped. It is difficult to fully prescribe boundaries for monitoring

safety because there need not be strong evidence to discontinue the study if it appears that the treatment is harmful.

Futility. Early discontinuation due to the unlikeliness of significant findings conditional on interim results may be considered, based on the original sample size considerations. For evaluating futility, we propose using the B-value approach of Lan and Wittes (Lan & Wittes, 1988) to calculate conditional power of the study given the observed data. Such power calculations will be performed for a range of possible alternatives including the observed treatment difference in the data as well as the original alternative hypothesis of a 2-line (0.20 logMAR) benefit of voriconazole treatment at 3 months. We propose to stop the study if conditional power for a 2-line benefit of voriconazole at 3 months is less than 0.2. Since stopping the trial for futility does not reject the null hypothesis, there is no inflation of size associated with early stopping. Instead, it modestly decreases study power.

11 Baseline characteristics

11.1 Demographics and Patient History

All demographic and history variables (in particular, age, gender, occupation, and national origin) determined at presentation or enrollment will be summarized by counts and percentages tabulated by treatment assignment.

11.2 Prior and concurrent medication

We will present the percentage taking antifungals (and antibiotics) by gender, age, infiltrate/scar size at presentation, fungal organism, study site, affected eye, symptom duration, source of trauma, contact lens use, medications, organism genus, and in the closed report, treatment assignment (randomization arm).

11.3 Baseline comorbidities and history

Clinical variables at baseline (in particular, baseline acuity, fungal organism, baseline infiltrate/scar size) will be presented by gender, age, and study site. We will also tabulate the presence of trichiasis, abnormal lid anatomy, corneal anatomy, infiltrate/scar size, best spectacle-corrected visual acuity at baseline, and ocular surface disease at baseline.

11.4 Compliance

Compliance is assessed through patient self-report.

12 Safety and tolerability

The analysis of safety in this study will include summaries of the following:

- Exposure
- Adverse events

- Adverse events and serious adverse events (including perforations)
- Adverse events leading to withdrawal
- Any deaths

12.1 Exposure

Individuals are assumed to have exposure to the drug assigned to the arm they were randomized to.

12.2 Adverse Events

12.2.1 Individual events

The proportion of subjects with at least one of the following safety-related events will be compared using Fisher's Exact Test. Non-serious adverse events (not requiring narrative form) are listed in the Manual of Operations. Serious ocular and non-ocular adverse events must be reported within 24 hours and require a narrative form are also listed in the Manual of Operations.

In addition, we will compare the rate of each of the adverse events during the follow-up period using Poisson regression, which can take into account multiple instances of adverse events within a single subject. Negative binomial regression will be conducted as a sensitivity analysis.

12.2.2 Pooled adverse events

Adverse events will be analyzed according to five main categories:

- Proportion of subjects with *perforations*
- Proportion of subjects with *any ocular adverse event*
- Proportion of subjects with *any serious ocular adverse event*
- Proportion of subjects with *any non-ocular serious adverse event*
- Proportion of subjects with *any non-ocular adverse event*

The proportion of subjects with these events will be compared between the arms using Fisher's Exact Test. Poisson regression will be applied to compare the rates of overall adverse events, including recurrent events.

13 Reporting conventions

- All tables and data listings will be presented in landscape orientation, unless presented as part of the text of the final report.
- Figures will be presented in landscape orientation, unless the information is substantially easier to interpret in portrait orientation.
- Direct annotation of figures will be preferred to legends. All figures with more than one variable or item will contain either direct annotation or legends. All annotation will be unambiguously identifiable as such.

- Color will be used in figures only when needed to enhance clarity of communication. All color schemes will be evaluated for visual clarity for individuals with diminished color vision. All color encodings will be identified. Redundant encodings (such as the use of different plot symbols or line dash patterns) will be used in addition to color, so that all figures are interpretable after monochrome reproduction at 100 dots per inch. All dash patterns and line widths will be adequate to be distinguishable after monochrome reproduction at 100 dots per inch. Any distinction between plot symbols (circles, filled circles, diamonds, etc.) will remain clear after monochrome reproduction at 100 dots per inch.
- Fixed width sans serif fonts will be used for all labeling (Helvetica, Arial, Futura, or Computer Modern Sans Serif (cmss)).
- Boldface and italics will not be used unless substantial value is added.
- Decorative fonts and enhancements, including borders and shading, will not be used. Decorative presentation methods, such as ribbon graphs, will never be used.
- All information given in figures will also be presented in summary tables (perhaps only included in an Appendix or in supplementary materials).
- Only standard characters will be used in tables and data listings.
- All titles will be centered. The first title line will be the number of the table, figure, or listing. The second and possibly third lines will be the description of the table, figure, or data listing. The ICH numbering convention will be used for all.
- All footnotes will be left justified and at the page bottom. Footnotes will be used sparingly. Reference footnotes will be complete enough to locate any reference based on the information provided (Author, Journal, Pages, Date, or PubMed accession number).
- Missing values for numeric or character variables will be unambiguously identified as such using the special string `NA` (not available) in all settings; `NA` is the standard missing value code for our software. Each figure or table caption in which `NA` is used will indicate the meaning of `NA` in that figure or table. The abbreviation `NA` will never be used for any other purpose.
- All date values will presented in the form DDmmmYYYY format (e.g. 01jan2008), using four digit years. June will be encoded as `jne` (otherwise `jan` and `jun` would differ by only a single character), and July as `jly` (so that the lowercase letter `l`, easily confused with the digit `1`, will not be adjacent to any numerals).
- All tables, figures, and data listings will have the name of the program and a date/time stamp on the bottom of the output.

14 Abbreviations and acronyms

AES Advanced Encryption Standard
ANCOVA Analysis of covariance
CAS Chemical Abstracts Service
DSMC Data and Safety Monitoring Committee
FIPS Federal Information Processing Standard
KR K. Ray
MCAR Missing completely at random
NIST National Institute of Standards and Technology
TM T. Margolis
TP T. Porco
SM S. McLeod
UCSF University of California, San Francisco

15 Computational Appendix

In this Appendix, we provide details regarding the computational methods and commands we will use for the randomization and the analysis. All computations will be performed using the standard software package R (`http://www.r-project.org`). Statistician TP has twenty years of experience using R or very similar statistical computing environments (S, S-Plus).

15.1 Random Number Seed

Specification of the random number seed and pseudorandom number algorithm determines the entire randomization assignment (as is the case with any pseudorandom number generation method). Accordingly, the random number seed will be kept confidential, and the seed will be chosen carefully. In particular, easy-to-remember numbers or otherwise meaningful numbers (such as telephone numbers, birthdays, and so forth) are to be scrupulously avoided. We will use one random number seed during the testing and pilot phase (now complete), and a separate random number seed will be determined by the statistical administrator at Proctor. This seed will be used to generate the final randomization lists. A printed copy of the randomization lists for all three centers, the computer code used to generate them, and the random number seed will be maintained in a locked vault off site. The random number seed chosen will consist of at least eight digits, and a standard linear feedback shift-register (Matsumoto & Nishimura, 1998) algorithm ("Mersenne Twister") will be used for pseudorandom number generation.

15.2 Checksum

We propose to use compute the checksum by the following mod-11 algorithm. If d_i denotes the i-th consecutive digit of the serial number (reading left to right beginning at i=1), the checksum digit is $(\sum_i id_i) \bmod 11$. This checksum is guaranteed to correctly identify the occurrence of any single digit copying error and any single transposition error.

15.3 Randomization

15.3.1 Randomly choosing block sizes

The random sequence of block sizes will be generated by randomly choosing blocks of size 4, 6, and 8 *subjects* with probabilities 6/13, 4/13, and 3/13 respectively. Choosing 75 consecutive block sizes, not all of which will be used, guarantees at least 300 treatment assignments, more than is needed. This computation will be conducted using the `sample` function of R, using code we have already developed for the pilot study. Specifically, the random sequence of 75 block sizes will be obtained using the following command:

```
block.sizes <- sample(c(4,6,8),75,replace=TRUE,prob=c(6/13,4/13,3/13))
```

15.3.2 Randomly assigning treatment allocations within blocks

Once the length of a block has been determined, we construct the treatment assignment by randomly permuting the assignments as follows. Given a block size of *N* patients, a random ordering of the drug assignments will be achieved by concatenating *N*/2 copies of the sequence `Voriconazole`, `Natamycin` together and randomly permuting it. Specifically, in R, this is achieved by the command

```
sample( rep(c("Voriconazole","Natamycin"),nn/2), replace=FALSE )
```

where `nn` is *N*, the number of subjects in the block. This process will be repeated for each of the randomly chosen block sizes (details not shown).

We will repeat this process for each of the three centers, yielding a separate randomization list for each site.

Each subject will have a unique patient ID (care will be taken to ensure that patient IDs are not duplicated between sites, and that it will not be possible to determine the study site from the patient ID alone).

The randomization list generated in the statistics package will be exported to a Microsoft Excel® spreadsheet using code we have already tested in the study pilot. **No** statistical expertise, programming expertise, or familiarity with the R software will be needed to open or to use the randomization list.

Automated quality assurance checks are conducted by the software (which we have already used in the pilot study). Specifically, the software ensures that (a) the drug assignments are approximately balanced at each study site, (b) long runs of the same assignment do not occur (no run longer than 6 should ever occur, given the maximum block size of 12 subjects), (c) no patient ID is ever duplicated, and (d) the checksum field in the patient ID is correct.

15.3.3 Procedure for generating the randomization list at Proctor

This procedure is designed to (1) protect the masking of all Proctor personnel except the principal statistician TP and the Proctor pharmacist, (2) preserve multiple backup copies of the randomization lists and database encodings of the assignments (in the event of unforeseen circumstances, personnel turnover, etc.), and (3) ensure the confidence of study personnel and the Data and Safety Monitoring Committee in the integrity of the process and the accuracy of the information provided. Multiple redundant checks and duplication of information have been introduced for this purpose.

When the randomization list is to be generated, the principal statistician TP will meet with Proctor director Todd Margolis, and perform the randomization together using the following checklist:

- The principal statistician TP visually inspects the software to verify that only one call to `set.seed` is used. This is to certify that the provided randomization seed is used.
- The program is initiated by TP using an R console (R v. 2.6.1 for MacIntosh).
- A random seed of eight to ten digits will be determined arbitrarily by TP and TM. The seed should *not* consist of memorable digits such as phone numbers, etc.
- The random seed will be entered when prompted. This seed will be recorded, signed by TP and TM, and three copies of this will be kept.
- The random seed will be reentered a second time as a check.
- Three records of the random seed will be disposed as follows. One will be kept by TP in a locked cabinet inside a locked office at the Proctor International site, one will be kept by TM (also inside a locked cabinet inside a locked office at the Proctor site, and a third will be placed inside a sealed

envelope and stored offsite in a secure location. Note that the entire randomization list can be regenerated from the data on this form, and this provides an additional backup of this crucial information. Note also that three copies are kept, insuring that the integrity of the study is maintained in case of personnel turnover or unforseen events (meeting standard recommendations for enterprise information management).

- When the prompts have been completed, the computer will have generated all three randomization lists and performed automated quality assurance checks. Failure of the automated checks will lead to an error message displayed in the R workspace. In the event of such an error, the cause of the error must be documented in the final report, and the code must be corrected, validated, and the entire process repeated in a subsequent meeting of TP and TM.
- When the automated checks are completed, TP and TM will visually inspect the three lists by opening each successively in Excel: (a) the name must reflect the study site, (b) the first row should be STUDY ID, DATE ENROLLED, DRUG ASSIGNMENT, (c) for Madurai and Pondicherry should be at least 301 lines long, (d) Proctor should be at least 41 lines long, and (e) the columns appear to have the treatment assignments in an apparently unpredictable order.
- The Madurai and Pondicherry site lists are sent using secure email to Mr. Kennan of Aurolab (Aravind Eye Hospital pharmacy).
- The Proctor site list is sent to the Proctor laboratory (all UCSF internal email is automatically encrypted).
- All three lists are to be sent to the chair of the DSMC.
- All three randomization lists are printed out in two copies each. One hard copy is to be kept by TM, one to be kept by TP, and as before another copy is to be placed in an envelope to be stored securely offsite. Finally, the statistical administrator is to store an encrypted version of all three lists on the Proctor secure server and to provide TM with the encryption key. The encryption key is also to be placed into the offsite storage envelope.
- The offsite storage envelope containing (a) the randomization list generation records with the seed, (b) the randomization list itself, and (c) the encryption key, is to be sealed and signed by TM. This is to be stored securely offsite, so that in the event of emergency, the study investigators have access and the information is not lost.

15.4 Multiple imputation

Because of the random nature of the multiple imputation procedure, we will re-seed the random number generator with the (arbitrary, but now pre-specified) seed of 674533487 (interim analyses) or 963248738 (final analyses), we will use the Mersenne Twister algorithm (default in the R package), and we will conduct the five calls to the imputation routine sequentially; the software version of R (and any needed packages) which is current on the day the database is locked will be used. While such cautions are expected to play a very small role (as the test statistic is well estimated using the procedure described in Little & Rubin (2002)), prespecification of the algorithm and seed provides additional assurances of integrity, valuable in the event of a p-value very close to the boundary.

For multiple imputation, we propose the set of variables age, sex, MIC, topical antifungal use, fungal culture positivity, *Aspergillus* species, visual acuity at three weeks and three months, reepithelialization status, reepithelialization time, perforation status, TPK status, centrality of ulcer, study site, estimated depth at enrollment (and at three weeks and three months), and the

total follow-up time. MIC was included despite its being a secondary outcome of interest because it appeared to improve the imputation in the pilot study.

Multiple imputation is not used in any primary analysis.

Revision history

1. 2 July 2009. Finalization to take place prior to collection of any outcome data (changed from Introduction page 2; previously read "at the first meeting of the Data and Safety Monitoring Committee".

2. 4 November 2009. Enrollment criteria for MUTT I and MUTT II changed to reflect cutoff of 20/400, not 20/800 (Section 2.2).

3. 4 November 2009. Clarification in Section 8.3 (Transformations and model adequacy) that we will **not use a fourth root** transformation for the primary analysis

4. 4 November 2009. Page 24. 115th patient corrected to read 123d patient.

5. 4 November 2009. Sample size section was ambiguous regarding the sample size. An additional sentence was added to clarify this.

6. 19 January 2010. Weights for block randomization scheme changed, Section 3.3.3.

7. 21 January 2010. Missing parenthesis added to first equation on page 12.

8. 15 February 2010. Additional section added to Section 8.2 (Missing values) regarding whether or not the data are missing completely at random (MCAR), together with reference to Carpenter & Kenward. Acronyms added to Section 14. **Multiple imputation not to be used for any primary analysis.** Clarification also added to Appendix.

9. 22 February 2010. Software versions updated to read "R 2.6 or later" and "STATA v. 10 or later".

10. 22 February 2010. Wording change in section 9.1.2 regarding mixed model/segmented regression

11. Section 9.2.8-11, 9.2.9, and 9.3 added (supplemental exploratory analyses)

12. Section 9.2 renamed.

13. 3 April 2010. Superiority comparison to be conducted following the noninferiority comparison

14. 3 April 2010. Updated disposition of randomization list

15. 7 April 2010. Additional subgroup analyses added. Corrections to Randomization section regarding patient identifiers and format of spreadsheet.

16. 13 April 2010. Cost-effectiveness section, photography section added.

17. 13 April 2010. Clarification added regarding missing values following cataract surgery or perforation.

18. 13 April 2010. Optscore added to software section.

19. 15 April 2010. Elimination of hot-deck multiple imputation in favor of model-based imputation using methods from Schafer (1997). Addition of section on missing values for predictors (regressors).

20. 15 April 2010. Addition of additional procedures for testing proportional hazards assumptions, and disclaimer regarding normality testing.

21. 15 April 2010. Additional clarification regarding analytic plan for per protocol, safety, and screening populations

22. 16 April 2010. Exploratory analysis section added for emphasis; importance of separating exploratory (hypothesis generating) analysis from formal clinical trial inference clearly indicated and recognized.

23. 16 April 2010. Clarification regarding methods to be used in exploratory event history analysis.

24. 5 May 2010. The following sentence was added: "All visual acuity data will be analyzed in logMAR units unless otherwise specified." Also, because Sec. 9.2.4-5 did not clearly state that baseline infiltrate/scar size would be used in the regression for three-month scar size, we added this clarification to Sec. 9.1: "Many outcome measures have a baseline measure which should be included as a regressor in any analysis. Specifically, for three-month acuities, we would correct for baseline acuity (i.e. include baseline acuity in a regression model). For three-month scar size, we would correct for baseline infiltrate/scar size. For perforation, we would correct for depth." Section 9.2.14 was added to clarify our intent with respect to MIC (MIC was included as an outcome in the Proposal and in sections 2.3.2 and 5.9.4. The added text reads: "Minimum inhibitory concentration for the drug used will be used as a regression predictor for three month BSCVA for MUTT I, perforation for MUTT II. In addition, it will be used as a predictor for secondary outcomes. Additional exploratory analyses will use BSCVA standardized within fungal organism, may use prior antifungal medication (of each type), or include organism-MIC-drug interactions. We will also use MIC as an outcome (to be predicted by organism or prior antifungal use)." The phrase "at 7 days" was added to the Microbiological Cure section (note: this was already present in the MOP). No other changes have been made since 16 April 2010.

25. 6 October 2011. The hypothesis test section was clarified. Amendment added by D. Glidden, masked to all results. The hypothesis tests have not been changed.

26. 6 October 2011. Section 8.2.1. The method of handling missing values due to corneal transplant was revised to be consistent with that used in the Steroids for Corneal Ulcers Trial. Amendment added by D. Glidden, masked to all results.

27. 6 October 2011. Section 8.2.2. Revision of handling missing values for cataract surgery. Amendment by D. Glidden, masked to all results.

27. 6 October 2011. Clarification that the bias corrected accelerated method will be used in bootstrap standard error calculations. Amendment by D. Glidden, masked to all results.

28. 14 Feb 2012. Section 2.3.1. Clarification regarding the fact that keratoplasties (therapeutic and other) occurring within three months following enrollment will be counted as perforations for the purposes of analysis. Amendment by N. Acharya, masked to all results.

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Figure 1: **Sample size as a function of the assumed standard deviation. The vertical line is the assumed standard deviation. The solid red line is for eighty percent power; the orange line for ninety percent power. The one-sided alpha is 0.025; the noninferiority margin is 0.15.**

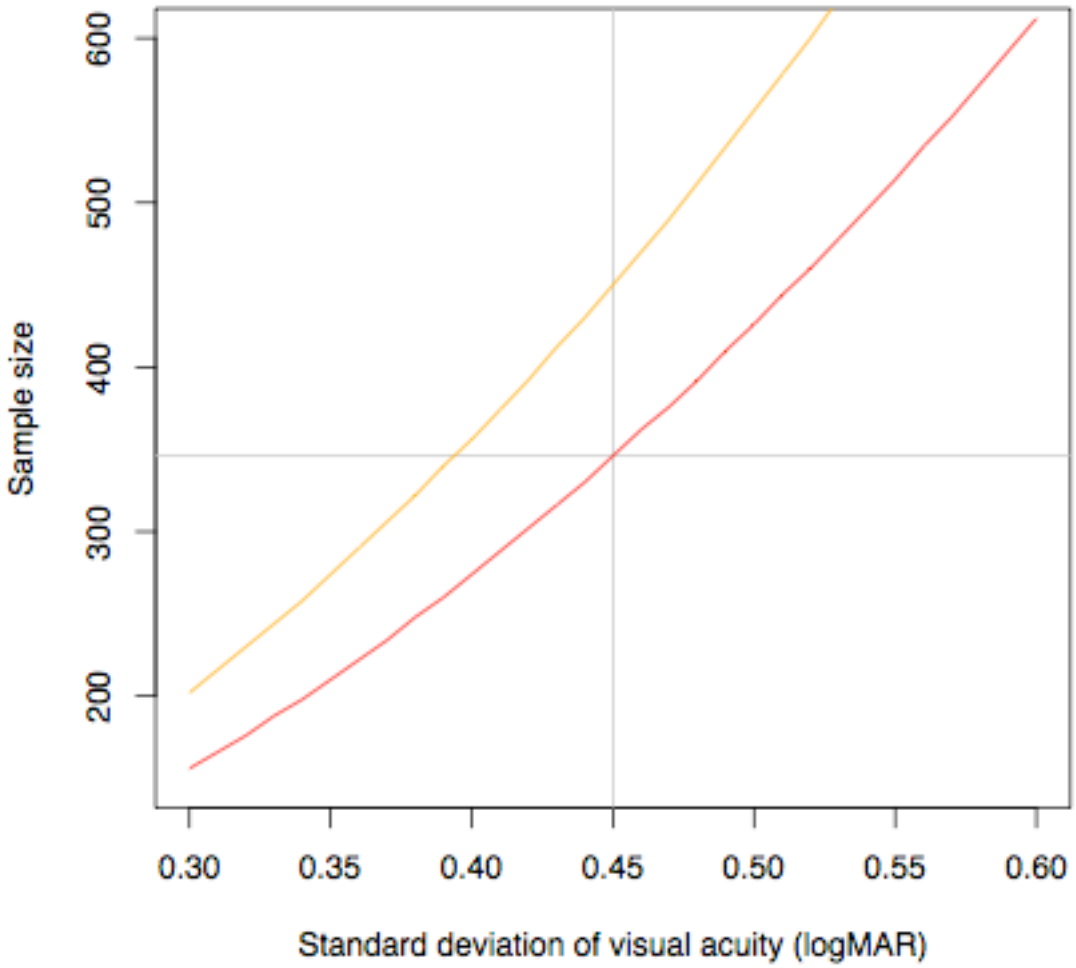

Figure 2: **Power as a function of the assumed standard deviation and effect size. The vertical line is the assumed standard deviation. The blue lines are for a sample size of 346 (173 in each group); the green lines for 306; the black for 370. Solid lines correspond to an effect size of 0.15, dotted to 0.1.**