Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Fungal Ulcer Treatment Augmented with Natamycin and Cyclosporine (FANCY): study protocol for a randomized, double-masked, parallel-arm clinical trial comparing 0.1% cyclosporine A, 2% cyclosporine A, and placebo as adjunctive therapy to natamycin for mild to moderate fungal keratitis

Josephine Christy
Aravind Eye Hospital Pondicherry
Miel Sundararajan
University of Washington
Kunal Mandlik
Aravind Eye Hospital Pondicherry
R. Rashmita
Aravind Eye Hospital Pondicherry
Venkatesh Prajna
Aravind Eye Hospital, Madurai
Anitha Venugopal
Aravind Eye Hostpital, Tirunelveli
Jeremy Keenan
University of California, San Francisco
Neel Pasricha
University of California, San Francisco
Brianna Colado
University of California, San Francisco
Vishawanath Prathikanti
University of California, San Francisco
Sarah Abdelrahman
University of California, San Francisco
Thomas L. Lietman
University of California, San Francisco
Gerami D. Seitzman
gerami.seitzman@ucsf.edu

University of California, San Francisco

Study protocol

Keywords: Fungal keratitis, mycotic keratitis, cyclosporine A

Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9646742/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

STUDY PROTOCOL

TITLE PAGE

Fungal Ulcer Treatment Augmented with Natamycin and Cyclosporine (FANCY): study protocol for a randomized, double-masked, parallel-arm clinical trial comparing 0.1% cyclosporine A, 2% cyclosporine A, and placebo as adjunctive therapy to natamycin for mild to moderate fungal keratitis.

Josephine Christy*[1], Miel Sundararajan*[2], Kunal Mandlik[1], R. Rashmita[1], N. Venkatesh Prajna[3], Anitha Venugopal[4], Jeremy D. Keenan[5,6], Neel Pasricha[5,6], Brianna Colado[5], Vishwanath Prathikanti[5], Sarah Abdelrahmen[5], Thomas M. Lietman[5,6] 5, Gerami D. Seitzman[5,6]

Affiliations:

[1]Aravind Eye Hospital, Pondicherry, India
[2] Department of Ophthalmology, University of Washington, Seattle, WA
[3]Aravind Eye Hospital, Madurai, India
[4] Aravind Eye Hospital, Tirinavelli, India
[5] Francis I. Proctor Foundation, University of California, San Francisco, USA
[6] Department of Ophthalmology, University of California, San Francisco, USA
*These authors contributed equally to this work.

Corresponding author: Gerami Seitzman, MD
490 Illinois Street, Floor 2
San Francisco, CA 94143
Gerami.Seitzman@ucsf.edu

Acknowledgments: Jayanthi Ramamurthy, Roja Yuvaraj

ABSTRACT

Background

Fungal keratitis is a vision threatening disease which leads to loss of the affected eye in 8-11% of cases. Topical antifungal agents are typically first line therapy. Cyclosporine A (CsA) has been shown to inhibit the growth of certain pathogenic filamentous fungi *in vitro*. This randomized controlled trial aims to determine the effectiveness of topical CsA as an adjunct to the standard anti-fungal therapeutic regimen.

Methods

A three-armed, parallel, randomized, masked, placebo-controlled clinical trial is presented. Participants with smear- or culture- confirmed fungal keratitis and mild to moderate disease, defined as best spectacle corrected visual acuity between LogMAR 0.2 and 1.3. In addition to standard topical anti-fungal therapy, patients will be randomized to one of three arms to receive topical CsA 2%, topical CsA 0.1%, or placebo. Best spectacle corrected visual acuity at 12 weeks will serve as the primary outcome.

Discussion

There is limited literature on the role of CsA in fungal keratitis. This study will provide insight into the use of adjunctive nonsteroidal anti-inflammatory therapies for this potentially sight-threatening disease.

Trial Registration

This trial was registered on Clinical Trials.org on October 23, 2024. (NCT06658002, https://clinicaltrials.gov/study/NCT06658002)

KEYWORDS: Fungal keratitis; mycotic keratitis; cyclosporine A

Background

Mycotic keratitis is a vision-threatening corneal infection with a substantial global burden. Approximately one million cases of fungal keratitis occur worldwide each year, with disease severity such that 8–11% of affected eyes require evisceration or enucleation.(1) In tropical and subtropical regions, particularly in economically developing countries, fungal pathogens account for up to 80% of cases of microbial keratitis and represents a leading cause of monocular blindness in working-age adults.(2)

Corneal ulcers caused by fungal pathogens are associated with worse visual outcomes compared to bacterial keratitis, even when treated appropriately.(3, 4) Although antifungal therapy may achieve microbiological cure, visual recovery often remains limited. During active infection, fungal organisms, keratocytes, and infiltrating inflammatory cells release proteolytic enzymes and cytokines. This results in stromal degradation, keratolysis, and corneal thinning, ultimately leading to visually limiting irregular astigmatism and corneal stromal scarring.(5-7) Because of this, “successful” treatment, defined by resolution of infection, commonly does not necessarily result into good visual outcomes.

First-line therapy for filamentous fungal keratitis is natamycin 5%.(8) Natamycin is the only topical antifungal agent approved by the U.S. Food and Drug Administration for this indication. Even with initiation of appropriate topical therapy, fungal keratitis may progress to corneal perforation. Because of this, additional topical antifungal agents are often used in clinical practice, including voriconazole, amphotericin B, and rarely caspofungin and flucytosine.(9)

However, none of these anti-fungal agents directly target the host inflammatory response which causes corneal tissue damage and long-term vision loss.

Adjunctive immunomodulatory therapy has proven beneficial in bacterial keratitis, where topical corticosteroids may reduce corneal scarring when used appropriately. In contrast, corticosteroids are contraindicated in fungal keratitis, as they promote fungal growth in vitro and have been associated with worsening stromal melting and poor clinical outcomes when administered during active fungal infection.(10-12) This highlights the need for alternative, adjunctive, non-steroidal anti-inflammatory agents that can modulate corneal inflammation during fungal keratitis without compromising antifungal activity.

Calcineurin inhibitors offer a potential strategy to address this unmet need. Cyclosporine A (CsA) and tacrolimus are members of this class and modulate the immune response by inhibiting T-cell activation and cytokine production via calcineurin pathway suppression, a mechanism distinct from corticosteroids.(13-15) In vitro, CsA inhibits the growth of common fungal keratitis pathogens, including *Fusarium* species and *Aspergillus fumigatus*.(16) This suggests that CsA may possess both anti-inflammatory and adjunctive antifungal properties.

Topical calcineurin inhibitors are widely used in ophthalmology for chronic and severe ocular surface inflammatory conditions, including vernal keratoconjunctivitis, rosacea keratoconjunctivitis, and ocular graft-versus-host disease.(17-20) In the context of fungal keratitis, small clinical studies have reported the use of topical cyclosporine and tacrolimus as immune modulators following therapeutic keratoplasty and as non-steroidal alternatives for

corneal transplant rejection prophylaxis.(21-23). One retrospective study reported that combination therapy with topical cyclosporine and natamycin was associated with a significantly higher likelihood of complete resolution of fungal keratitis at 2 and 4 weeks compared with natamycin monotherapy alone.(24) However, high-quality randomized controlled trials evaluating cyclosporine as an adjunctive treatment during active fungal keratitis are lacking.

Optimal dosing of adjunctive topical cyclosporine in the setting of fungal keratitis is unknown. Commercially available ophthalmic cyclosporine formulations typically range from 0.05% to 0.1%, while higher concentrations (1–2%) are available through compounding pharmacies and are used off-label in severe ocular surface disease. In vitro studies suggest that CsA exhibits dose-dependent antifungal activity.(16) However, the safety and efficacy of topical CsA, at any dose, during active corneal fungal infection has not been prospectively evaluated.

The goal of this study is to determine whether adjunctive topical CsA improves clinical outcomes and to evaluate its safety in patients with mild to moderate fungal keratitis receiving standard antifungal therapy**.** A secondary objective is to compare the efficacy of 2% CsA versus 0.1% CsA in achieving these outcomes. By evaluating a non-steroidal immunomodulatory agent with potential antifungal activity, this randomized controlled trial aims to assess whether CsA can meaningfully modify the host immune response during fungal keratitis. We hypothesize that adjunctive topical 2% CsA will result in improved 12-week best spectacle-corrected visual acuity compared with placebo.

Methods

The Fungal ulcer treatment Augmented with Natamycin and Cyclosporine (FANCY) trial is a three-armed, parallel, randomized, masked, placebo-controlled, superiority trial. Participants with a diagnosis of smear or culture positive fungal keratitis with mild to moderate disease, as defined by presenting best corrected visual acuity (BCVA) between and including 20/30 to 20/400 (logMAR 0.2 to 1.3), will be enrolled. All enrolled patients will receive standard therapy with topical natamycin for the first 48 hours. Following this, participants will be randomized in a 1:1:1 ratio to one of three study arms for adjunctive therapy: (A) topical CsA 2%, (B) topical CsA 0.1%, or (C) placebo. The assigned study medication will be administered four times daily for 4 weeks, followed by twice daily for an additional 4 weeks. The trial was registered at ClinicalTrials.gov (NCT06658002), where all required registration items are available, and began enrollment on August 28, 2025. This protocol adheres to the Recommendations for Interventional Trials (SPIRIT) guidelines (Supplementary Table S1). An abbreviated protocol is available on Clinical.trials.gov. This study was reviewed and approved by the Institutional Review Board at the University of California, San Francisco and the Institutional Ethics Committee of the Aravind Eye Hospital, Pondicherry and will be performed in accordance with the Declaration of Helsinki.

This is an investigator-initiated trial. The Proctor Foundation at the University of California, San Francisco serves as the institutional sponsor. Sponsor contact information: 490 Illinois St Second Floor, San Francisco, CA, USA.

Setting, participants, and eligibility

This trial will be conducted in the main cornea clinic at the Aravind Eye Hospital in Pondicherry, India. A study team member will identify potentially eligible patients upon presentation, explain the nature of the study, and obtain written informed consent. An informed consent form is provided in the appendix.

Patients may enter the study via two main pathways. First, patients presenting with a new diagnosis of fungal keratitis who have not yet received antifungal therapy will be identified as potential candidates and reassessed for eligibility after 48 hours of appropriate topical antifungal treatment. Second, patients who present having already received at least 48 hours of appropriate topical antifungal therapy may be randomized on the day of presentation.

Eligibility is based on best corrected visual acuity (BCVA) on day of randomization, with inclusion for eyes demonstrating BCVA between 20/30 and 20/400 (logMAR 0.2–1.3). Additional inclusion criteria are smear- or culture-positive fungal keratitis, age 18 years or older, and willingness to participate and provide written informed consent.

Exclusion criteria include: (1) co-infection with bacterial or viral pathogens, (2) corneal perforation, (3) imminent need for therapeutic keratoplasty, (4) inability to adhere to study follow-up, (5) use of topical or systemic cyclosporine within 7 days of presentation, (6) BCVA worse than 20/200 (logMAR 1.0) in the fellow eye, (7) pregnancy, (8) history of penetrating or endothelial keratoplasty, and (9) use of topical steroids within 7 days preceding presentation. In patients with bilateral disease meeting inclusion criteria in both eyes, only the eye with worse visual acuity will be randomized.

Strategies for participant enrollment and retention

To ensure achievement of the target sample size, participants will be recruited from patients presenting to the referral-based cornea clinic at Aravind Eye Hospital in Pondicherry, India. Screening logs will be maintained to document all eligible and enrolled patients. Based on institutional case volume, of approximately 4–6 participants per month is anticipated, allowing achievement of the target sample size within the projected timeline of 2.5-3 years.

Participants will be encouraged to complete all scheduled follow-up visits through standardized visit scheduling and reinforcement of compliance at each study visit. Participants who miss scheduled visits will be actively contacted to minimize loss to follow-up. Study staff will attempt at least three telephone calls to reschedule missed visits and provide counseling on the importance of continued participation. If participants remain unreachable, a certified letter or locally appropriate equivalent method will be used to establish contact. Participants who cannot be contacted after these efforts will be classified as lost to follow-up. All contact attempts and outcomes will be documented in the REDCap database.

Participants who discontinue study intervention or deviate from the protocol will continue to be followed. Primary and secondary outcome data, including best spectacle-corrected visual acuity, corneal measurements (e.g., infiltrate/scar size), and adverse events, will continue to be collected at scheduled follow-up visits.

Patient and public involvement

There was no patient or public involvement in the design, conduct, or reporting of this trial.

Interventions and sample size

Topical CsA 2% is prepared by Aurolab (Madurai, India) under sterile conditions. CsA0.1% is supplied by Sun Pharmaceutical Industries (Mumbai, India). Patients assigned to the placebo group will receive an artificial tear formulation consisting of hydroxypropyl methylcellulose 0.3% supplied by Aurolab (Madurai, India).

Participants will instill one drop of study medication into the affected eye four times daily for 4 weeks, followed by twice daily for an additional 4 weeks.

All participants will receive standard topical natamycin 5% hourly while awake for the initial 48 hours prior to randomization, after which dosing frequency will be adjusted at the discretion of the treating physician in accordance with standard clinical care.

Study medications are dispensed by the study coordinator at the enrollment and 4-week visits and are handled exclusively by unmasked study personnel. Participants, treating physicians, and outcome assessors remain masked to treatment allocation.

Study medications should be stored at room temperature, and participants will be instructed on proper drop administration technique. Concomitant use of standard supportive therapies, including cycloplegic agents and intraocular pressure–lowering medications, is permitted. The use of topical or systemic corticosteroids is prohibited during the study period. Additional antifungal agents may be used at the discretion of the treating physician and will be recorded.

Target enrollment is 50 participants per arm (total N = 150). Participants will be instructed to bring medications to each visit. Compliance will be assessed by self-reported dosing frequency at each visit and by weighing medication bottles at the 12-week visit.

Power calculations were based on the primary comparison of 2% CsA versus placebo. We utilized results of the Mycotic Ulcer Treatment Trial to inform our calculations.(8) The MUTT trial demonstrated a standard deviation of 0.49 logMAR at the primary outcome (12-week BSCVA), and a correlation of 0.60 between baseline and 12-week BSCVA. The residual standard deviation was calculated as (SDr=$SD\sqrt{1-r^2}$, where r is the correlation between the baseline measure and primary endpoint. Given MUTT results, $SDr = 0.49\sqrt{1-0.60^2} = 0.392$. Assuming a significance level of 0.025 (Bonferroni-adjusted for two pairwise comparisons), 45 study participants per arm provide 80% power to detect a 0.257 logMAR (2.57-line difference) between arms. This amounts to 135 study participants. Accounting for an estimated 10% loss to follow up, the total recruitment target is 150 participants. Secondary analyses comparing 0.1% CsA versus placebo and 2% CsA versus 0.1% CsA will use the same power assumptions.

Randomization and masking

The study Analyst generated a simple randomization sequence and entered it into the REDCap (Research Electronic Data Capture) randomization module, aligning participant study identification numbers with treatment arms (2% CsA, 0.1% CsA, or placebo) in a 1:1:1 ratio. Upon enrollment, the study coordinator will randomize participants using REDCap, which assigns the participant a study identification number and a treatment arm. The randomization sequence is concealed within REDCap and inaccessible to enrolling investigators and clinicians.

The on-site study coordinator, the sole patient-facing team member with access to the randomization key, will relabel study medications to include the study title, expiration date, and participant study ID. A second, remote coordinator not directly involved in patient care will verify alignment of study labels with the REDCap randomization list. All medication bottles are identical in size and color, maintaining masking. Study medications are sourced from Aurolab (Madurai, Tamil Nadu, India).

Participants, study coordinators, physicians, technicians, and outcome data analysts will be masked to treatment allocation. Only three team members will remain unmasked: (1) the on-site study coordinator, (2) the remote study coordinator, and (2) the Data Analyst/biostatistician responsible for generating and entering the randomization sequence in REDCap.

Emergency unmasking will be permissible when required for participant safety. Requests for unmasking may be initiated by the treating physician and will be reviewed by the Medical Monitor, who may authorize unmasking if knowledge of the treatment assignment is necessary for clinical management. The Data and Safety Monitoring Committee (DSMC) may also request unmasking in the context of safety review, including when serious adverse events appear to be unbalanced across treatment arms. Unmasking will be performed by the unmasked data analyst using the REDCap randomization system. All instances of unmasking will be documented, and efforts will be made to ensure that investigators and outcome assessors remain masked whenever possible.

Outcomes assessment, schedule, and procedures

The primary outcome of this study is best spectacle-corrected visual acuity (BSCVA) at 12-weeks measured in logMAR units using standardized ETDRS charts. Secondary outcomes

include corneal scar size, corneal re-epithelialization, corneal perforation rate, microbiologic cure at 1-week, ocular surface pain, and best scleral contact lens–corrected visual acuity at 12 weeks. Exploratory outcomes include ocular surface transcriptomic analysis from conjunctival swabs, intended to assess and quantify potential anti-inflammatory effects of adjunctive topical cyclosporine at both doses. These analyses are not currently funded and are included to generate preliminary data for future studies

The schedule of enrollment, interventions, and assessments is summarized in Figure 1. The following procedures are performed according to standardized protocols:

(1) Corneal smear and culture

Corneal culture, also known as corneal scraping or swab, is standard of care in all suspected cases of infectious keratitis. A drop of topical anesthetic is administered to the affected eye. Using slit lamp magnification, a sterile instrument (Kimura spatula or blade) is used to obtain a superficial corneal sample from the leading edge and the base of the corneal ulcer. Scrapings are transferred directly onto two glass microbiology slides for bacterial (Gram stain) and fungal (KOH wet mount, Gram stain, or Giemsa stain) identification. Additional scrapings are acquired and inoculated onto culture plates for bacterial (sheep's blood agar and chocolate agar) and fungal (potato dextrose agar or Sabouraud's agar) culture.

(2) Conjunctival swab

A drop of topical anesthetic is instilled in the study eye. A Dacron swab is used to sample the inferior palpebral conjunctiva within the inferior fornix. The swab is placed into a sterile tube containing Zymo DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and stored at -80°C.

This exploratory outcome, at the 4-week timepoint, aims to evaluate whether ocular surface transcriptomics can detect and quantify an anti-inflammatory response in patients treated with adjunctive 2% and 0.1% cyclosporine compared to placebo, generating preliminary data for future studies.

(3) History and medication form

At enrollment, patients' medical history will be reviewed to document the presence of diabetes mellitus, immune status (immunocompetent vs. immunocompromised), and history of contact lens wear. At each study visit, patients will be asked to report all current topical ophthalmic medications.

(4) BSCVA

The best spectacle corrected visual acuity is measured using refractive correction obtained by manifest refraction. Manifest refraction is performed with either a traditional phoropter or loose trial lenses. Spherical and cylindrical lenses are used to correct for the patient's refractive error until the best possible visual acuity is achieved.

The study eye is refracted at four meters unless the visual acuity measured at this distance is worse than 20/200, defined as missing 2 or more letters on the 6/60 (20/200) line. The subject is then moved to one meter from the study visual acuity chart and refraction is performed and recorded at this distance.

With the measured manifest refraction in place, the patient is placed in front of the ETDRS light

box at the standard 4-meter test distance. The bulbs in the ETDRS chart light box are to be two 20-watt frosted cool white bulbs. Room illumination should be at a level of 50 to 100 foot-candles as measured with a photometer held 4 feet from the floor and directed toward the ceiling.

The best visual acuity on the EDTRS light box is documented. If a patient misses 3 letters on one line, they are deemed to have failed that line, and the best acuity is documented as the line above. In the case that the patient reads less than 10 letters at 4 meters, the patient is to be moved to 1 meter from the ETDRS light box. Spherical power of +0.75D should be added to the prescription. Visual acuity should then be retested at this distance. Only the first 6 rows of letters need to be read at 1 meter. If less than 10 letters are read at 1 meter, then the examiner will proceed to Low Vision Testing; the patient will be asked to count fingers at 0.5 meter. If unable, the examiner will proceed to Hand Motions testing, followed by Light Perception.
BSCVA as detailed above is performed at the final study visit and is the primary outcome of this trial.

(5) BCVA and pinhole visual acuity

The patient is seated at the standard testing distance of 20 feet or 6 meters and the Snellen chart is used. The vision is measured initially in patient's current spectacles if corrected for distance. An occluder held by the patient will ensure that only the eye of interest is able to see the vision chart. The Snellen acuity is documented as the lowest line which can be read with 2 or fewer errors. The pinhole occluder is lowered over the study eye. The Snellen acuity is again measured by the above standards. These measurements are taken at each study visit.

(6) Pain rating

At the enrollment visit, the patient is asked to rate overall ocular surface pain on a scale ranging from 0 to 10. At all subsequent visits, the patient is asked to rate (1) overall ocular surface pain, (2) pain after administration of natamycin drop, and (3) pain after administration of study drug on a scale of 0 to 10. Zero denotes no pain, while 10 is the worst pain imaginable.

(8) Smart phone photography

At each study visit, a standardized image of the study eye will be captured using an iPhone 13 Pro. Patients will wear a mask to protect identity. An ophthalmic photographer, with assistance to retract eyelids, will center the cornea and include the entire limbus with the eye in primary gaze. The smartphone camera flash will be on, and the macro mode activated automatically by bringing the camera close to the eye. Images are captured at consistent magnification across visits.

(9) Ulcer measurements

The following clinical findings will be documented at each visit

(a) Infiltrate size:

The longest linear dimension of the ulcer is identified and measured in millimeters with the slit beam focused on the cornea. The beam is then rotated 90 degrees and the measurement in this dimension is taken. The ulcer area is approximated by multiplying these two values. Dimensions are measured to the nearest tenth of a millimeter.

(b) Epithelial defect size:

Fluorescein is administered and the epithelial defect is highlighted. The above measurements are then repeated, in the longest axis followed by that 90 degrees perpendicular, to approximate the area of the epithelial defect. Dimensions are measured to the nearest tenth of a millimeter.

(c) Hypopyon height:

The greatest vertical height of the layered hypopyon is measured in millimeters and reported as a continuous variable from 1 to 10 mm. A hypopyon of less than 1 mm will be recorded as 0.5 mm.

(d) Corneal stromal thinning assessment:

The area of greatest stromal thinning is identified. The amount of corneal tissue lost is estimated as a percentage of the normal, intact cornea. This value is recorded as a continuous variable ranging from 0% (no thinning) to 100% (complete loss). For example, if the cornea has 80% of its stroma remaining, the thinning is recorded as 20%.

(e) Infiltrate depth assessment

The depth of the corneal ulcer is clinically assessed by the examining physician using a slit-lamp biomicroscope. The deepest portion of the infiltrate is recorded and categorized as: anterior (involving the anterior one-third of the cornea), middle (involving up to the middle two-thirds), or deep (extending through the posterior one-third).

(f) Centrality

The ulcer is classified as “central” if it lies within the central 4 mm of the cornea and peripheral if it lies outside this zone.

Adherence to study intervention

Adherence will be assessed by participant self-report of dosing frequency at each visit and by weighing returned study medication bottles at the 12-week visit.

Criteria for discontinuation or modification of study intervention

Study medication may be discontinued for individual participants if: (1) the study medication is considered by the investigator to be the likely cause of a serious adverse event or clinically significant adverse reaction; (2) there is clinical worsening requiring escalation of therapy or surgical intervention; (3) participant request. Discontinuation of study intervention does not constitute withdrawal from the study, and outcome assessments will continue according to the protocol whenever possible. Any clinically relevant new finding will be recorded and reported as an adverse event. Reasons for discontinuation and subsequent treatments will be documented in the treatment discontinuation form.

Figure 1: SPIRIT Schedule of Enrolment, Interventions, and Assessments

	STUDY PERIOD					
	Enrolment	**Allocation**	**Post-Allocation**			**Closeout**
TIMEPOINT	***-48 hours***	***Day 0***	***Week 1***	***Week 4***	***Week 8***	***Week 12***
ENROLMENT:						
Eligibility screen	X					
Informed Consent		X				
Confirm Inclusion/Exclusion	X	X				
Allocation		X				
INTERVENTIONS:						
Natamycin 5%	X[1]	X	X	X	X	X[4]
Cyclosporine A 2%		←	—	—	→	
Cyclosporine A 0.1%		←	—	—	→	
Placebo		←	—	—	→	
Supportive therapy		←	—	—	—	→
ASSESSMENTS:						
Cornea smear/culture	X		X[2]			
Conjunctival swab				X[3]		
History/medications	X	X	X	X		X
ETDRS (Pinhole) Acuity	X	X	X	X		X
Snellen (Pinhole) Acuity	X	X	X	X		X
BSCVA (SCL and RGP)						X
Pain score	X	X	X	X		X
Photography	X	X	X	X		X
Ulcer measurements	X	X	X	X		X
Adverse events		X	X	X		X

[1] Natamycin given hourly for 48 hours before randomization

[2] Repeat culture if epithelial defect > 3x3mm

[3] Stored for transcriptomic analysis

[4] Natamycin at least through week 8 and continued after per physician discretion until complete healing

BCVA – Best corrected visual acuity; BSCVA – Best spectacle corrected visual acuity; SCL – Scleral contact lens; RGP – Rigid Gas Permeable contact lens

Adverse events and assessment of harms

All adverse events (AEs) will be assessed systematically at each study visit through structured participant questioning and clinical examination by the study ophthalmologists. AEs will be recorded in REDCap with a reporting form. AEs will be classified as ocular or non-ocular. Serious adverse events (SAEs) include, but are not limited to, corneal perforation, therapeutic keratoplasty, hospitalization, or death.

The on-site study team will notify the medical monitor within 24 hours of identifying any SAE. The medical monitor will assess the event, determine the likelihood of its relationship to the study intervention, and inform the Data and Safety Monitoring Committee (DSMC) as appropriate. Emergency unmasking of the study arm may be performed on a case-by-case basis if the SAE is suspected to be related to the study medication.

Any SAEs determined to be related to the study intervention will be reported to the Aravind Eye Hospital Ethics Committee and the UCSF Committee on Human Research within 5 working

days. All other adverse events will be reviewed regularly by the DSMC to monitor participant safety throughout the trial.

Data collection, management, and monitoring

All study data are collected and entered directly into the REDCap electronic data capture system hosted at UCSF. Access to REDCap is restricted via secure, password-protected accounts, and role-based permissions control data entry, editing, and access. Data entry incorporates built-in logic and range checks to minimize errors and missing or inconsistent entries are flagged for review.

Demographic and clinical information including age, ocular conditions, history of contact lens wear, comorbidities (e.g., diabetes, immunocompromised status), and medication use is recorded at enrollment and at scheduled study visits. Sample material collected for exploratory analyses (e.g., conjunctival swabs) is de-identified and analyzed in aggregate.

Participant characteristics and equity, diversity, and inclusion (PRO EDI)

In accordance with PRO EDI reporting, additional participant characteristics will be collected to assess the diversity and representativeness of the study population. In addition to age and sex, data will be collected on occupation, residence (rural versus urban), and distance to subspecialty care facility. Occupations will be specifically recorded given the relevance to fungal keratitis risk, particularly among agricultural workers.

Time from symptom onset to presentation and referral pathway (eg, self-presentation versus referral) will also be documented as measures of healthcare access. These variables will be

summarized descriptively to characterize the study population and will be considered in exploratory analyses to evaluate whether treatment effects differ across participant subgroups.

No participants will be excluded based on socioeconomic status, occupation, or residence. Study procedures, including informed consent, will be conducted in the participant's preferred language to promote equitable participation

Periodic monitoring of study data will be conducted by the study team to ensure completeness, accuracy, and adherence to the protocol. Any discrepancies or data queries will be resolved promptly. Missing or incomplete data will be documented in the REDCap database, and available data will be used for intention-to-treat analyses.

All data are stored securely according to UCSF guidelines and retained for a minimum of 7 years following study completion. Regular backups are performed to prevent data loss. Access to study data is restricted to authorized study team members, and all procedures comply with applicable privacy and confidentiality regulations.

Study oversight

Medical Monitor: The Medical Monitor is responsible for reviewing all adverse events, both ocular and non-ocular, reported during the study. Serious adverse events (SAEs) are reviewed within 24 hours of reporting. The Medical Monitor assesses the likelihood that an SAE is related to the study intervention and provides a summary of all adverse events to the Data and Safety Monitoring Committee (DSMC). Emergency unmasking of treatment allocation for the Medical

Monitor can be performed on a case-by-case basis if an SAE is suspected to be related to study medication.

Data and Safety Monitoring Committee (DSMC): The DSMC is an independent committee appointed prior to study initiation, consisting of four subject matter experts. This DSMC consists of two cornea specialists from the Aravind Eye Hospital and two from UCSF. The DSMC is responsible for ongoing oversight of participant safety, study conduct, and data integrity. It reviews SAEs, adverse event summaries from the Medical Monitor, and study data quality, and provides recommendations for protocol modifications or other corrective actions as needed. The DSMC meets at least biannually or more frequently if prompted by safety concerns or other urgent issues.

Statistical Considerations

Treatment Arms for Analysis:

Arm 1: Topical antifungal + 2% CsA

Arm 2: Topical antifungal + 0.1% CsA

Arm 3: Topical antifungal + placebo

Primary Outcome: The primary outcome of this study is best spectacle-corrected visual acuity (BSCVA) at 12 weeks, measured in logMAR units. The primary comparison is between Arm 1 (topical antifungal + 2% CsA) and Arm 3 (topical antifungal + placebo). We hypothesize that BSCVA at 12 weeks will be superior in Arm 1.

Secondary Outcomes: Secondary outcomes include corneal scar size at 12 weeks, the rate of re-epithelialization, microbiologic cure at 1 week, and corneal perforation. Additional analyses will examine BSCVA at 12 weeks for other pairwise comparisons, including Arm 2 (topical antifungal + 0.1% CsA) versus Arm 1 (2% CsA) and Arm 2 versus Arm 3 (placebo). Ocular surface pain and best scleral contact lens–corrected visual acuity at 12 weeks will also be assessed.

For the primary analyses, multiple linear regression models will be used to evaluate the primary outcome, BSCVA at 12 weeks, and to test for interactions with covariates including age, sex, occupation, distance from care facility, presenting BSCVA, and ulcer size at presentation. Treatment arm will be included as a categorical variable, and baseline BSCVA measured at enrollment, prior to randomization, will be treated as a covariate. The primary analysis will follow an intention-to-treat approach and include all randomized participants in the groups to which they were assigned, regardless of adherence to the intervention or protocol deviations.

For participants who experience corneal perforation requiring intervention such as corneal gluing, therapeutic corneal transplantation), enucleation, or evisceration, the BSCVA recorded immediately prior to the intervention will be carried forward as the 12-week outcome.

Secondary outcomes will be analyzed using pre-specified models. Corneal scar size at 12 weeks will be evaluated using multiple linear regression, adjusting for baseline infiltrate measurements, with pairwise comparisons between treatment arms conducted at a significance level of 0.025 (Bonferroni-adjusted). Corneal perforation will be analyzed using Cox proportional hazards models to assess time to perforation, adjusting for baseline infiltrate depth. In participants with

persistent epithelial defects greater than 3 × 3 mm who are re-cultured at 1 week, a microbiologic cure at this timepoint will be compared between arms using Fisher's exact test. Ulcer re-epithelialization at weeks 1, 4, and 12 will be analyzed using logistic regression with treatment arm and baseline culture result as covariates; re-epithelialization is defined as an epithelial defect ≤0.5 mm in both the longest and perpendicular dimensions on slit lamp examination.

All models will be assessed for validity of assumptions, including normality of residuals tested by the Shapiro–Wilk method. ANCOVA will be used to examine potential effects of age and sex groups.

An interim analysis for safety and efficacy will be conducted when one-third of participants have completed the 12-week visit. The DSMC will determine whether to continue, modify, or terminate the trial based on safety and efficacy benchmarks, including primary outcome (12-week BSCVA) and the incidence of adverse events and SAEs in each arm. If outcomes in the placebo arm appear superior with respect to these benchmarks, the trial may be stopped for safety. Early efficacy stopping will be considered if Arm 1 (topical antifungal + 2% CsA) demonstrates a significant improvement in 12-week BSCVA compared with Arm 3 (placebo) at a stringent alpha of 0.001. Interim analysis will be conducted using ANCOVA, adjusting for baseline BSCVA.

Pre-specified subgroup analyses will be conducted based on baseline ulcer size, organism type (e.g., Fusarium vs Aspergillus), and baseline visual acuity. Adjusted analyses will include covariates such as age, sex, and clinical severity at presentation.

Discussion

This randomized controlled trial is designed to address a therapeutic gap in fungal keratitis therapy by evaluating whether adjunctive topical CsA can improve clinical outcomes without compromising antifungal efficacy. Despite advances in antifungal therapy, visual outcomes following fungal keratitis remain limited by corneal scarring and structural damage Both the active infection and the secondary host inflammatory response contribute to this vision loss. Currently, there are no approved adjunctive therapies to safely target inflammation during active fungal infection.

CsA was introduced in the 1980s as a systemic immunosuppressive agent for the prevention of solid organ transplant rejection.(25) Later, it was adopted as a systemic immunomodulatory therapy for ophthalmic inflammatory conditions, including uveitis associated with systemic autoimmune disease.(26) Topical formulations of cyclosporine are now used to treat many ocular surface inflammatory disorders, including dry eye disease, ocular rosacea, vernal and atopic keratoconjunctivitis, graft-versus-host disease.(27, 28)

The use of immunomodulatory therapy during active fungal infection raises important concerns. As a calcineurin inhibitor, cyclosporine suppresses T-cell activation and cytokine production through inhibition of interleukin transcription and downstream lymphocyte signaling pathways.(29) In principle, this mechanism could impair host immune responses necessary for effective fungal clearance. Experimental and clinical studies have demonstrated the importance of T-cell–mediated immunity in ocular infections, including the recruitment and persistence of tissue-resident memory T cells within the cornea following infection.(30) Counterbalancing these

concerns, cyclosporine has also demonstrated antifungal activity in vitro. Calcineurin inhibition has been shown to disrupt fungal growth and prevent hyphal elongation in organisms commonly implicated in fungal keratitis.(31, 32) This raises the possibility that cyclosporine may exert a dual effect by modulating the host inflammatory response while simultaneously limiting fungal pathogenicity. Retrospective clinical studies have suggested improved outcomes when cyclosporine is used adjunctively with natamycin, However, these data are limited by non-randomized designs and heterogeneous dosing strategies.

This study is a prospective, double-masked, randomized controlled design comparing two concentrations of topical CsA with placebo in patients receiving standard antifungal therapy. By focusing on patients with mild to moderate fungal keratitis, this trial is designed to evaluate whether early, non-steroidal immune modulation can improve visual and structural outcomes while maintaining safety. The results of this study will provide evidence to inform clinical decision-making and guide future investigations of targeted immunomodulatory strategies in infectious keratitis.

This study is conducted at a single tertiary referral center in South India, which may influence the demographic and socioeconomic composition of enrolled participants. Fungal keratitis disproportionately affects individuals in rural settings and those engaged in agricultural occupations. These factors may affect both disease severity and access to care. Collection and reporting of participant characteristics in accordance with PRO EDI recommendations will allow assessment of the representativeness of the study population and inform interpretation of the generalizability of trial findings.

Trial Status:

This manuscript describes Protocol version 1.0, dated August 21, 2025. Participant recruitment began on 28 of August 2025. As of 10 April 2026, 64 participants have been enrolled toward a target sample size of 150. Expected completion of recruitment: December 31, 2027.

DECLARATIONS

Ethics approval and consent to participate

This study was reviewed and approved by the Institutional Review Board at the University of California, San Francisco and the Institutional Ethics Committee of the Aravind Eye Hospital Pondicherry, India and will be performed in accordance with the Declaration of Helsinki. Written informed consent will be obtained from all patients or in the case of minors, their legal guardians. Collection and storage of conjunctival swab specimens for exploratory and future analyses are included in the main informed consent process.

Any protocol modifications will be communicated to the institutional review boards, trial registry, investigators, and relevant stakeholders. Amendments will be documented with version control and dates.

Following completion of the primary outcome assessment, participants will continue to receive standard clinical care for fungal keratitis. No additional post-trial interventions are planned and participants will be managed according to clinical need.

Compensation for research-related harm will be provided in accordance with institutional policies and applicable regulatory requirements

Consent for publication

No identifiable images are included in this submission, nor are any identifying features of any individual participant.

Availability of data and material

The data that will be collected in the proposed study will be maintained securely and in a deidentified fashion. It can be made available upon reasonable request to the corresponding author. De-identified individual participant data underlying the results reported in this study, including a data dictionary and statistical code, may be made available from the corresponding author upon reasonable request. Requests will be reviewed by the study investigators and are subject to institutional data-sharing policies at the University of California, San Francisco, including execution of appropriate data use agreements where applicable.

Ancillary and post-trial care

Participants will continue to receive standard clinical care following study completion. Any adverse events related to study participation will be managed according to institutional policies, and appropriate medical care will be provided.

Competing interests

There are no direct conflicts of interest to disclose.

Financial disclosures of the authors include: MS, Genentech; GDS: Dompè Pharmaceuticals, Kedrion Biopharma, Tarsus.

This study is primarily supported by internal funds from the University of California, San Francisco and the Proctor Foundation. Investigator effort is supported by a K12 award from the National Eye Institute of the National Institutes of Health (K12EY031372). This work was also supported in part by the National Eye Institute Core Grant for Vision Research (EY002162) and

an Unrestricted Grant from Research to Prevent Blindness (New York, NY). The funding sources had no role in the study design; collection, management, analysis, or interpretation of data; writing of the manuscript; or the decision to submit for publication

Dissemination policy

Study results will be disseminated through peer-reviewed publications, conference presentations, and reporting on ClinicalTrials.gov. Results may also be shared with participants upon request.

Authors' contributions

Conception and design of work (GDS, JC); acquisition of data (GDS, JC, BC); analysis and interpretation of data (GDS, JC, TA, SA, VP); draft of the work (MS, JC); substantial revision of the work (GDS, JC, JK, NP, NVP, KM, RR, AV). All authors have approved the submitted version of the manuscript and have agreed to be personally accountable for their own contributions. All authors agree that all questions regarding accuracy or integrity of the work, including those portions in which they were not directly involved, have been appropriately investigated and resolved, and the resolution documented in the literature.

List of Abbreviations:

BSCVA: Best Spectacle Corrected Visual Acuity, BCVA: Best Corrected Visual Acuity, CsA: Cyclosporine A, LogMAR: Logarithm of the Minimum Angle of Resolution, RGP: Rigid Gas Permeable contact lens

Appendix 1. Informed Consent Form

An informed consent form is provided as a supplementary appendix.

References

1. Brown L, Leck AK, Gichangi M, Burton MJ, Denning DW. The global incidence and diagnosis of fungal keratitis. Lancet Infect Dis. 2021;21(3):e49-e57.
2. Hoffman JJ, Burton MJ, Leck A. Mycotic Keratitis-A Global Threat from the Filamentous Fungi. J Fungi (Basel). 2021;7(4).
3. Arboleda A, Ta CN. Overview of Mycotic Keratitis. Cornea. 2024;43(9):1065-71.
4. Sharma N, Bagga B, Singhal D, Nagpal R, Kate A, Saluja G, et al. Fungal keratitis: A review of clinical presentations, treatment strategies and outcomes. Ocul Surf. 2022;24:22-30.
5. Menda SA, Das M, Panigrahi A, Prajna NV, Acharya NR, Lietman TM, et al. Association of Postfungal Keratitis Corneal Scar Features With Visual Acuity. JAMA Ophthalmol. 2020;138(2):113-8.
6. Ichikawa K, Ono T, Chen L, Kitamoto K, Taketatni Y, Toyono T, et al. Quantitative evaluation of corneal irregularity and scarring after infectious keratitis using anterior segment optical coherence tomography. Graefes Arch Clin Exp Ophthalmol. 2024;262(1):133-41.
7. Cabrera-Aguas M, Khoo P, Watson SL. Infectious keratitis: A review. Clin Exp Ophthalmol. 2022;50(5):543-62.
8. Prajna NV, Krishnan T, Mascarenhas J, Rajaraman R, Prajna L, Srinivasan M, et al. The mycotic ulcer treatment trial: a randomized trial comparing natamycin vs voriconazole. JAMA Ophthalmol. 2013;131(4):422-9.
9. Thomas PA, Kaliamurthy J. Mycotic keratitis: epidemiology, diagnosis and management. Clin Microbiol Infect. 2013;19(3):210-20.
10. Keenan JD. Steroids in the Management of Infectious Keratitis. Cornea. 2023;42(11):1333-9.
11. Stern GA, Buttross M. Use of corticosteroids in combination with antimicrobial drugs in the treatment of infectious corneal disease. Ophthalmology. 1991;98(6):847-53.
12. Newmark E, Ellison AC, Kaufman HE. Combined pimaricin and dexamethasone therapy of keratomycosis. Am J Ophthalmol. 1971;71(3):718-22.
13. Carr WW. Topical calcineurin inhibitors for atopic dermatitis: review and treatment recommendations. Paediatr Drugs. 2013;15(4):303-10.
14. Stuetz A, Baumann K, Grassberger M, Wolff K, Meingassner JG. Discovery of topical calcineurin inhibitors and pharmacological profile of pimecrolimus. Int Arch Allergy Immunol. 2006;141(3):199-212.
15. Sousa IS, Tavares LFS, Silva BA, Moreno DSA, Alviano CS, Santos ALS, et al. Calcineurin activity in Fonsecaea pedrosoi: tacrolimus and cyclosporine A inhibited conidia growth, filamentation and showed synergism with itraconazole. Braz J Microbiol. 2024.
16. Bell NP, Karp CL, Alfonso EC, Schiffman J, Miller D. Effects of methylprednisolone and cyclosporine A on fungal growth in vitro. Cornea. 1999;18(3):306-13.
17. Kauss Hornecker M, Charles Weber S, Brandely Piat ML, Darrodes M, Jomaa K, Chast F. [Cyclosporine eye drops: A 4-year retrospective study (2009-2013)]. J Fr Ophtalmol. 2015;38(8):700-8.
18. Perez VL, Mah FS, Willcox M, Pflugfelder S. Anti-Inflammatories in the Treatment of Dry Eye Disease: A Review. J Ocul Pharmacol Ther. 2023;39(2):89-101.
19. Flts A, Medina R, Akpek EK. The evolution of cyclosporine treatments for treatment of ocular surface diseases. Curr Opin Allergy Clin Immunol. 2024;24(5):360-7.

20. Erdinest N, Noyman DBE, London N, Lavy I, Levinger N, Landau D, et al. Applications of topical immunomodulators enhance clinical signs of vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC): a meta-analysis. Int Ophthalmol. 2024;44(1):157.
21. Chatterjee S, Agrawal D. Use of Topical Cyclosporine 0.1% in Therapeutic Penetrating Keratoplasty for Fungal Keratitis. Cornea. 2022;41(9):1116-21.
22. Perry HD, Doshi SJ, Donnenfeld ED, Bai GS. Topical cyclosporin A in the management of therapeutic keratoplasty for mycotic keratitis. Cornea. 2002;21(2):161-3.
23. Ziaei M, Manzouri B. Topical cyclosporine in corneal transplantation. Cornea. 2015;34(1):110-5.
24. Yang C, Cai J, Wei Q, Lian H, An L. Clinical efficacy of cyclosporin and natamycin for fungal keratitis. Pak J Med Sci. 2024;40(5):974-8.
25. Miach PJ. Cyclosporin A in organ transplantation. Med J Aust. 1986;145(3-4):146-50.
26. Nussenblatt RB, Palestine AG, Chan CC, Stevens G, Jr., Mellow SD, Green SB. Randomized, double-masked study of cyclosporine compared to prednisolone in the treatment of endogenous uveitis. Am J Ophthalmol. 1991;112(2):138-46.
27. Donnenfeld E, Pflugfelder SC. Topical ophthalmic cyclosporine: pharmacology and clinical uses. Surv Ophthalmol. 2009;54(3):321-38.
28. Rao SN. Treatment of herpes simplex virus stromal keratitis unresponsive to topical prednisolone 1% with topical cyclosporine 0.05%. Am J Ophthalmol. 2006;141(4):771-2.
29. Fruman DA, Klee CB, Bierer BE, Burakoff SJ. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. Proc Natl Acad Sci U S A. 1992;89(9):3686-90.
30. Loi JK, Alexandre YO, Senthil K, Schienstock D, Sandford S, Devi S, et al. Corneal tissue-resident memory T cells form a unique immune compartment at the ocular surface. Cell Rep. 2022;39(8):110852.
31. Steinbach WJ, Cramer RA, Jr., Perfect BZ, Asfaw YG, Sauer TC, Najvar LK, et al. Calcineurin controls growth, morphology, and pathogenicity in Aspergillus fumigatus. Eukaryot Cell. 2006;5(7):1091-103.
32. Wong SSW, Rasid O, Laskaris P, Fekkar A, Cavaillon JM, Steinbach WJ, et al. Treatment of Cyclosporin A retains host defense against invasive pulmonary aspergillosis in a non-immunosuppressive murine model by preserving the myeloid cell population. Virulence. 2017;8(8):1744-52.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SPIRIT2025editablechecklistFANCY4.28.26.docx