Systemic and Topical Antiviral Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO) - NCT03586284

Protocol Number: 1.0

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Sponsor: University of California, San Francisco

Version Number: v1.3

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale

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1 STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

All site staff responsible for the conduct, management, or oversight of the study has completed Human Subjects Protection Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

2 PROTOCOL SUMMARY

1.1 Synopsis

Title:	Systemic and Topical Antiviral Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO)
Study Description:	Systemic and Topical Antiviral Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO) study is a sequential double-masked randomized clinical trial comparing the efficacy of oral valganciclovir, topical ganciclovir 2%, and placebo for the treatment of PCR-proven CMV anterior uveitis (also known as keratouveitis) in Trial I. After participants achieve control of their inflammation, they will enter Trial II, where they will be re-randomized to one of three arms (oral valganciclovir prophylaxis, topical ganciclovir prophylaxis, or placebo) to determine which therapeutic and route of administration reduces recurrences of CMV anterior uveitis. Recurrences of inflammation in Trial II will be assessed for viral load to determine if recurrences are mediated by active viral replication. Participants who initially present with anterior uveitis suspected to be due to a viral aetiology and meet all eligibility criteria will be recruited from the clinic. Chulalongkorn University, Khon Kaen University, and Chiang Mai University (all in Thailand) along with the Francis I. Proctor Foundation at the University of California, San Francisco (UCSF) will jointly execute this clinical trial. UCSF will serve as the clinical and data coordinating center.
Objectives:	Specific Aim 1 (Trial I): To compare CMV viral load and inflammation after randomization to oral valganciclovir, topical ganciclovir, or placebo.

1a: We hypothesize that participants randomized to oral valganciclovir will have a lower viral load after 7 days compared to those randomized to either topical ganciclovir 2% or placebo (Trial I primary outcome).

1b: We hypothesize that a greater proportion of participants randomized to oral valganciclovir will have controlled inflammation compared to those randomized to either topical ganciclovir 2% or placebo.

Specific Aim 2 (Trial II): To compare the effect of long-term antiviral suppression on recurrence rate of inflammation.

2a: We hypothesize that participants randomized to placebo will have fewer recurrences over 12 months compared to those randomized to either oral valganciclovir or topical ganciclovir 2% (Trial II primary outcome).

2b: We hypothesize that aqueous obtained at the time of a recurrence of inflammation will demonstrate a detectable viral load and will correlate with level of inflammation.

Specific Aim 3 (samples from Trials I and II): To characterize host transcriptional signatures and viral genomic features in CMV anterior uveitis using RNA sequencing of aqueous samples.

3a: We hypothesize participants completing both trials who exhibit recurrent inflammation after antiviral suppression (Trial II) will have a higher prevalence of CMV mutations associated with antiviral resistance compared to baseline samples (Trial I).

3b: We hypothesize that (i) host transcriptional profiles will distinguish CMV anterior uveitis from anterior uveitis cases negative for CMV and unable to enter Trial I, (ii) we will identify non-CMV pathogens in cases unable to enter Trial I, and (iii) we will identify unique signatures associated with recurrent inflammation in Trial II participants.

Study Population:	We plan to enroll 117 participants (39 participants per arm) across all sites.
Description of Sites/Facilities Enrolling Participants:	Participants will be enrolled at 6 sites centered in the United States, Thailand and Taiwan. In Thailand, participants will be enrolled at Chulalongkorn University in Bangkok, Thailand, Khon Kaen University in Khon Kaen, Thailand and Chiang Mai University in Chiang Mai, Thailand. In Taiwan, participants will be enrolled at

	Chang Gung Memorial Hospital. In the United States, participants will be enrolled at the Francis I. Proctor Foundation/UCSF and the University of California, Los Angeles. UCSF will serve as the clinical and data coordinating center.
Outcomes:	Trial I: Primary Endpoint: Viral load after 7 days of treatment Secondary Endpoints: • Clinical quiescence Trial II: Primary Endpoint: Recurrence of inflammation over 12 months Secondary Endpoints: • Prevalence of mutations conferring antiviral resistance

Schema

Trial I

Pre-study visit (Exam 0)

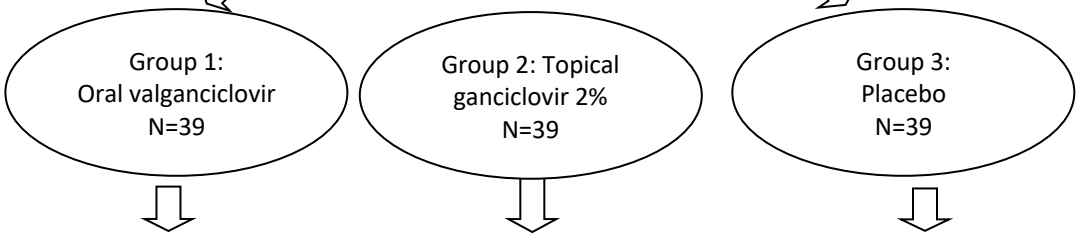
Clinical eye examination reveals active anterior uveitis (≥1+ AC cell) and suggests a viral aetiology

- **Anterior chamber paracentesis** is performed to establish a viral aetiology
  – Half of fluid will be used for in- house directed PCR for HSV, VZV and CMV
  – At least 50 µL of fluid will be preserved and used for quantitative CMV PCR to be conducted at a single US laboratory in eligible participants
- Laboratory screening orders (CBC, chemistry panel, pregnancy test, HIV status)
- Patients will be prescribed topical corticosteroid (prednisolone acetate 1%) to be used one drop in affected eye four times per day (this is typical standard of care)
- Patients requiring management of elevated IOP will be prescribed IOP-lowering medication according to treating ophthalmologist's discretion and best medical judgment

Exam 1 Randomization and Procedures

- Review of results of in- house CMV PCR and laboratory results
- Consent, enrollment, randomization to treatment arm if participants meet eligibility criteria
- Clinical eye examination (in the following order: visual acuity, slit lamp examination of anterior segment, intraocular pressure assessment, confocal microscopy)

Randomization to 1 of 3 arms



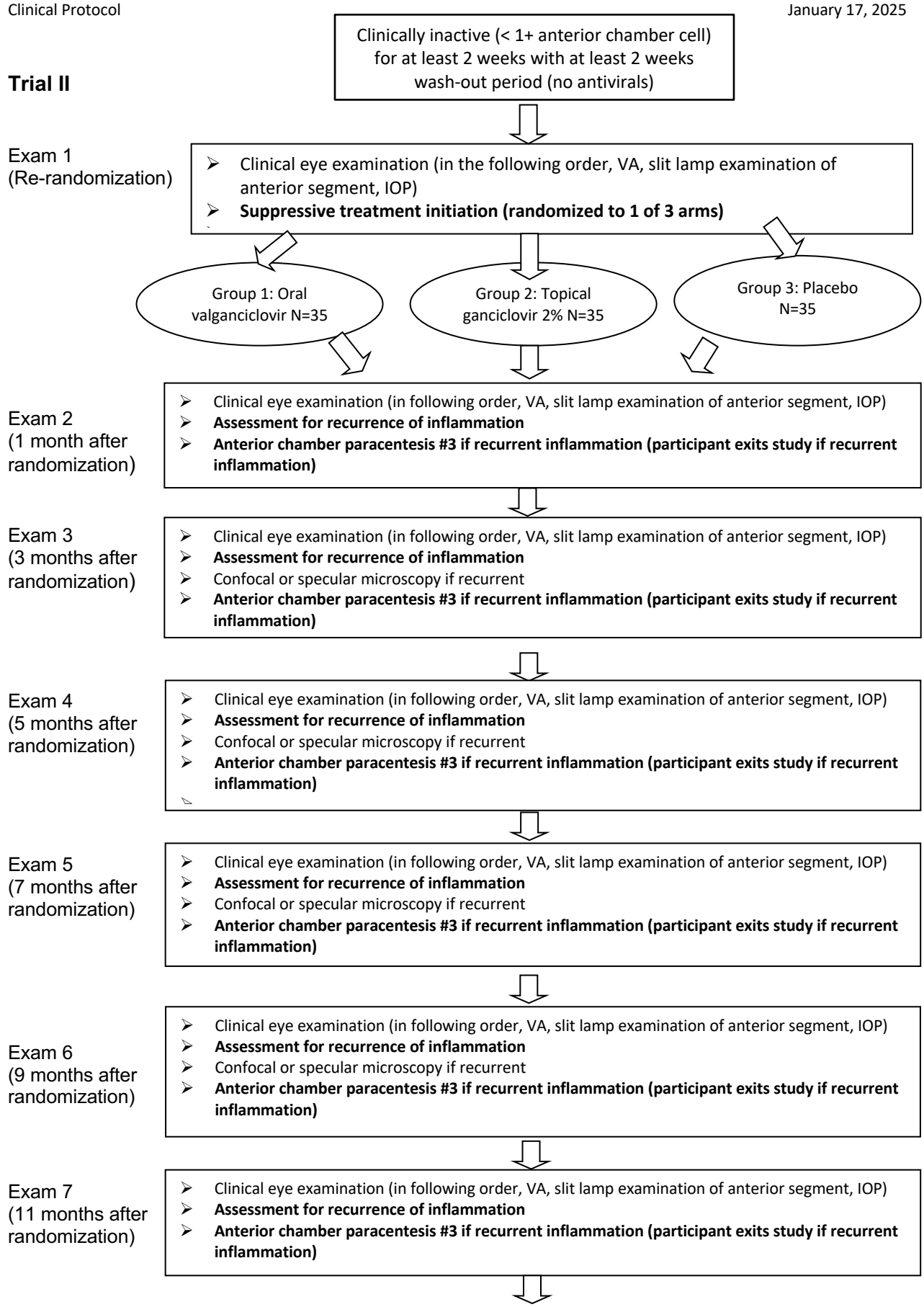
Exam 2 (Day 7) Follow-up

- Clinical eye examination (in the following order: visual acuity, slit lamp examination of anterior segment, intraocular pressure assessment)
- **Anterior chamber paracentesis #2**
- **Assessment of clinical quiescence (grading anterior chamber cell)**
- Laboratory monitoring orders (complete blood count, complete metabolic panel)

Exam 3 (Day 21) Final visit for trial I

- Clinical eye examination (in the following order: visual acuity, slit lamp examination of anterior segment, intraocular pressure assessment)
- **Assessment of clinical quiescence (grading anterior chamber cell)**
- Laboratory monitoring orders (complete blood count, complete metabolic panel)

Trial II


Clinically inactive (< 1+ anterior chamber cell) for at least 2 weeks with at least 2 weeks wash-out period (no antivirals)
Exam 1 (Re-randomization)
Clinical eye examination (in the following order, VA, slit lamp examination of anterior segment, IOP)
Suppressive treatment initiation (randomized to 1 of 3 arms)
Group 1: Oral valganciclovir N=35
Group 2: Topical ganciclovir 2% N=35
Group 3: Placebo N=35
Exam 2 (1 month after randomization)
Clinical eye examination (in following order, VA, slit lamp examination of anterior segment, IOP)
Assessment for recurrence of inflammation
Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)
Exam 3 (3 months after randomization)
Clinical eye examination (in following order, VA, slit lamp examination of anterior segment, IOP)
Assessment for recurrence of inflammation
Confocal or specular microscopy if recurrent
Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)
Exam 4 (5 months after randomization)
Clinical eye examination (in following order, VA, slit lamp examination of anterior segment, IOP)
Assessment for recurrence of inflammation
Confocal or specular microscopy if recurrent
Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)
Exam 5 (7 months after randomization)
Clinical eye examination (in following order, VA, slit lamp examination of anterior segment, IOP)
Assessment for recurrence of inflammation
Confocal or specular microscopy if recurrent
Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)
Exam 6 (9 months after randomization)
Clinical eye examination (in following order, VA, slit lamp examination of anterior segment, IOP)
Assessment for recurrence of inflammation
Confocal or specular microscopy if recurrent
Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)
Exam 7 (11 months after randomization)
Clinical eye examination (in following order, VA, slit lamp examination of anterior segment, IOP)
Assessment for recurrence of inflammation
Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)

Exam 8 (12 months after randomization, Final Visit)	➢ Clinical eye examination (in the following order, VA, slit lamp examination of anterior segment, IOP) ➢ **Assessment for recurrence of inflammation** ➢ **Anterior chamber paracentesis #3 if recurrent inflammation (participant exits study if recurrent inflammation)**

Note that participants will be advised to contact their local site clinical coordinator or study ophthalmologist if they are having symptoms of recurrent inflammation. Recurrent inflammation is typically symptomatic (characterized by ocular redness, decreased vision, light sensitivity, and pain). Thus, participants will be evaluated for symptoms of recurrent inflammation, which may occur between study visits, during which they would have clinical eye examination, anterior chamber paracentesis #3 (if there is recurrent (active) inflammation).

1.2 Schedule of Activities (SOA)

Tasks/Forms	**Trial I Visits**				**Trial II Visits**		
	Exam #0	Enroll/ Randomize Exam #1	Exam #2	Exam #3	Enroll/ Re-Randomize Exam #1	Exam #2-7	Exam #8
Eligibility/ Screening Form		X			X		
Consent/HIPAA		X			X		
Baseline History Form		X					
Clinical Eye Exam Form		X	X	X	X	X	X
REDCap patient		X			X		
Treatment initiation		X			X		
Laboratory orders (CBC, Cr, pregnancy	X		X	X		X	X
SAE/side effect evaluation			X	X		X	X
Calendar/ medication log***			X	X		X	X
Patient Dropout		(x)	(x)	(x)		(x)	(x)
Protocol Deviation Form	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Adverse Event Narrative/Log	(x)	(x)	(x)	(x)	(x)	(x)	(x)
AC paracentesis	X		X			(x)	(x)

Confocal microscopy				X			X

(x)-if applicable

2 INTRODUCTION

2.1 Background

Immunocompetence does not protect one from cytomegalovirus (CMV) ocular inflammation (uveitis). While CMV retinitis causes blindness in patients with HIV/AIDS, recurrent and chronic inflammation anterior uveitis in immunocompetent individuals can also lead to blindness by causing glaucoma and corneal decompensation.

There is debate whether each case of active CMV anterior uveitis requires treatment.[1,2] Nevertheless, treatment strategies include oral antiviral and topical antiviral therapy.[3-5] There is relative equipoise as to whether oral or topical antiviral treatment and management of CMV anterior uveitis is most effective.[6]

Once CMV anterior uveitis is controlled long-term antiviral prophylaxis has been suggested to decrease recurrences, but is not uniformly used.[7] There are no prospective randomized trials demonstrating whether long-term antiviral prophylaxis reduces recurrences and associated sequela of corneal decompensation or glaucoma.[3,4]

Clinical features of inflammation are the primary way that disease activity is assessed and monitored, but whether each recurrence is mediated by active viral replication and whether viral genome mutations and polymorphisms play a role in persistence and recurrence is largely unknown.

This proposal leverage's our team's expertise in metagenomic deep sequencing and the design and implementation of randomized controlled trials. We have led randomized placebo-controlled trials in infectious keratitis in the Steroids for Corneal Ulcers Trial (SCUT, U10 EY015114) and in non-infectious uveitis in the First-line Antimetabolites for Steroid-sparing Therapy (FAST, U10 EY021125) trial. Our group has significant prowess in identifying the entire genome of infectious pathogens as well as identifying mutations that confer resistance to medications.[8-10] Additionally, our group has characterized host transcriptome features in a variety of inflammatory conditions.[9,11] We also have substantial experience in conducting successful multicenter, international randomized trials. Including prior trials such as SCUT, FAST, the Mycotic Ulcer Treatment Trial (MUTT, U10 EY018573) and currently active trials like Azithromycin Reduction to Reaching Elimination of Trachoma (ARRET, UG1 EY030833), Adalimumab in JIA-associated Uveitis Stopping Trial (ADJUST, UG1 EY029658), and the Village-Integrated Eye Worker Trial (VIEW, UG1 EY028097).

There are no clear guidelines regarding the treatment for acute inflammation in CMV anterior uveitis and no guidelines regarding the most effective strategy for preventing recurrences of CMV anterior uveitis once clinical control is achieved. We seek to enroll participants with PCR-proven CMV anterior uveitis into a sequential double-masked placebo-controlled randomized clinical trial in the US and Asia.

2.2 Study Rationale and Significance

Cytomegalovirus made headlines in medicine and ophthalmology as a major cause of blindness during the HIV/AIDS epidemic. CMV retinitis causes a coagulative retinal necrosis of the peripheral and macular retina and can involve the optic nerve, all of which can lead to irreversible blindness. The risk factors for CMV retinitis are well known. Patients with CD4 counts lower than 50 cells/microliter have a 4-fold higher risk for developing CMV retinitis compared to those with CD4 counts greater than 100 cells/microliter. While the HIV/AIDS epidemic is over in developed countries, CMV retinitis is still encountered in patients with a variety of immunodeficient states or with advanced age.[12] The management for CMV retinitis is well developed. Systemic therapy consists of intravenous administration of antiviral medication that targets the viral kinases of CMV, which is necessary for its replication. Additionally, intraocular injection therapy using antivirals has been a standard approach, including, for a time, a ganciclovir-eluting implant. **There is, however, no defined therapeutic regimen for managing acutely active CMV anterior uveitis or whether long-term antiviral prophylaxis prevents recurrences.**

Antiviral resistance mutations play a role in CMV infection, particularly in HIV/AIDS and in organ transplant patients. The chronic exposure to prophylactic dosing of antiviral in an immunodeficient host plays a role in the development of CMV antiviral resistance.[13] This has implications for the eye as up to nearly 30% of patients on long-term antiviral prophylaxis for a history of CMV retinitis are found to have CMV resistant to standard antiviral therapy, which is associated with more areas of retinitis and worse vision as well as putting the other eye at risk for the development of CMV retinitis.[14,15] Additionally, plasma CMV viral loads > >400 copies/mL is associated with antiviral resistance due to mutations in the *UL97* and *UL54* genes.[16] **Does long-term antiviral prophylaxis for CMV anterior uveitis also promote the development of antiviral resistance?**

A detectable CMV viral load in the blood predicts the development of CMV disease (including retinitis) as well as death with or without antiretroviral therapy. Plasma CMV loads >400 copies/mL is associated with an increased risk of CMV retinitis progression.[16] Moreover, the viral load in the eye (as assessed from the aqueous or vitreous fluid in patients with CMV retinitis) is correlated with the area of retinitis involvement.[17] **CMV viral load may be correlated with disease severity in CMV anterior uveitis.**

CMV anterior uveitis may be characterized by a self-limiting course even without therapy, by frequently recurrent disease, or chronic inflammation. Such a wide array of phenotypes has led to a lack of precise treatment protocols. **Whether treatment of CMV anterior uveitis acutely with antiviral medication results in a more significant reduction in viral load, which may be associated with improved outcomes** with respect to corneal endothelial cell count, intraocular pressure, incidence or progressive glaucoma, and, visual acuity is unknown.

Long-term antiviral prophylaxis can beget the development of antiviral resistant CMV – in immunodeficient patients. In patient with acquired immunodeficiencies, such as HIV/AIDS, or organ transplant patients iatrogenically immunosuppressed to prevent graft rejection, antiviral resistant CMV may develop. Antiviral resistant CMV can be found in 19.5% of patients with

HIV/AIDS and in 11.4% of organ transplant patients.[13] Others have found that by 9 months of antiviral therapy, 27% of HIV/AIDS patients developed antiviral resistant CMV.[18] Further studies have identified mutations or polymorphisms in the *UL97* gene from the blood and vitreous in HIV/AIDS patients.[19] Thus, mutant CMV in the peripheral blood is reflected in the replicating virus in patients with antiviral resistant CMV retinitis.

Antiviral resistance is associated with progressive and new CMV ocular disease. In immunocompromised patients, the development of antiviral resistant CMV in their blood or urine portends poor clinical outcomes, including ocular disease as such patients develop progressive retinitis in those with prior CMV retinitis or development of retinitis in their previously affected contralateral eye.[14,18,20] Antiviral resistance most commonly involves mutations in the genes encoding viral kinases (*UL97* and *UL54*). Indeed, mutations in these two viral kinase genes is associated with a greater increase in the area of retina involved with CMV retinitis as well as a 9-fold increased risk of having contralateral involvement with CMV retinitis.[15] Whether recurrent CMV anterior uveitis is mediated by antiviral resistance is unknown. The present proposal will be able to identify such resistance.

2.3 Risk/Benefit Assessment

2.3.1 KNOWN POTENTIAL RISKS

The Medication risks:

Patients randomized to receive oral valganciclovir will be at risk of developing complications due to systemic toxicity. Although this drug is widely prescribed to treat CMV anterior uveitis in the United States, there are known adverse effects. Common ones include fever, headache, insomnia, diarrhea, nausea, vomiting, and abdominal pain. Rare side effects include hematologic abnormalities, acute renal failure, infertility, teratogenesis, and carcinogenesis. Patients randomized to receive topical ganciclovir eye drops will be subjected to the risks of topical toxicity. These include corneal epitheliopathy and conjunctivitis, both of which are almost always reversible upon discontinuation of the topical medication.

Minimizing medication risks:

Due to the potential complications associated with oral valganciclovir, patients will obtain pre-study labs, as well as monitoring labs throughout the study. The pre-study labs are to ascertain whether an individual is at greater risk of developing complications such as bone marrow suppression or acute renal failure. Patients identified as being high risk will be excluded from the study, as specified in our protocol. Patients enrolled in the study will receive regular laboratory tests in order to monitor CBC and renal function. Those that develop blood or renal abnormalities as specified in the protocol will be removed from the study. For patients that are randomized to receive topical antiviral therapy, the regular schedule of follow-up with clinical exam by a study ophthalmologist will provide ample opportunity to detect topical toxicity and discontinue medications as described in our protocol.

Procedural risks:

Although extremely rare, risks associated with anterior chamber paracentesis include endophthalmitis and traumatic cataract, both of which can lead to vision loss. In rare cases, endophthalmitis warrants need for eye removal.

Minimizing procedural risk:

All study ophthalmologists are trained to safely utilize anterior chamber paracentesis techniques to access fluid in the anterior chamber. Sterilization drops will be used in order to minimize the risks of intra-ocular infection.

2.3.2 KNOWN POTENTIAL BENEFITS

The benefits to the participant include a potential for a positive health outcome due to closer follow-up than standard care. Knowledge will be gained about their personal health through objective microbiological outcomes, which may help direct their care after study completion. Additionally, these participants will be contributing to work that could potentially guide treatment of CMV anterior uveitis for patients around the world. These benefits, in addition to the others mentioned above are reasonably balanced with the risks of partaking in the study.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Knowledge gained from this study may help improve the treatment of CMV anterior uveitis around the world. Given the risks to participants in this study, we feel the benefits of the important knowledge we expect to gain from this study outweigh the risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Specific Aim 1: To compare CMV viral load and inflammation after randomization to oral valganciclovir, topical ganciclovir, or placebo.	Reduction in log transformed viral load as measured by quantitative PCR at 7 days of treatment.	Viral load post 7 days of treatment using fluid from the eye is an important micro biologic outcome to compare the effect of treatment. This is primary outcome for Trial I
Specific Aim 2: To compare the effect of long-term antiviral suppression on recurrence of inflammation.	Proportion of participants with recurrence at the end of 6months.	CMV recurrence is a clinical outcome indicating effect of long-term treatment in controlling inflammation and preventing recurrence. This is primary outcome for Trial II
Specific Aim 3: To determine if antiviral resistance mutations develop with exposure to long-term antiviral suppression.	Prevalence of mutations associated with antiviral resistance	Using aqueous fluid from the eye, comparing prevalence of mutations conferring antiviral resistance in at baseline (Trial I) and recurrent disease (Trial II) is important to determine the relationship between viral and host genome features that mediate inflammation in CMV anterior uveitis.

4 STUDY DESIGN

4.1 Overall Design

The Systemic and topical antiviral control of cytomegalovirus anterior uveitis: treatment outcomes (STACCATO) study will enroll participants with PCR-proven CMV anterior uveitis into a sequential double-masked randomized clinical trial in the US and Asia. In Trial I, participants will be randomized to one of the three treatment groups:

Group 1: Valganciclovir tablets 900 mg PO BID (4 tablets daily) and Placebo drops 1 drop 6 times daily
Group 2: Topical ganciclovir 2% 1 drops 6 times daily and Placebo tablets 2 tablets PO BID (4 tablets daily)
Group 3: Placebo tablets 2 tablets PO BID (4 tablets daily) and Placebo drops 1 drop 6 times daily

After participants achieve control of their inflammation, they will enter Trial II at which point they will be re-randomized to one of the three arms in Trial I at a prophylactic dosing. Additionally, patients with known CMV anterior uveitis, but who are inactive, may enter Trial II directly. Recurrences of inflammation in Trial II will be assessed for presence of detectable viral load. Those who agree to participate will be randomized at baseline and after achieving clinical quiescence, get re-randomized to one of the three treatment groups:

Group 1: Valganciclovir tablets 450 mg PO BID (2 tablets daily) and Placebo drops 1 drop BID
Group 2: Topical ganciclovir 2% 1 drop BID and Placebo tablets 1 tablet PO BID (2 tablets daily)
Group 3: Placebo tablets 1 tablet PO BID (2 tablets daily) and Placebo drops 1 drop BID

All study ophthalmologists and participants will remain masked to treatment assignment after enrollment. Due to the treatment design, all participants will receive both pills and eye drops. An interim analysis will be performed for both trials once primary outcome data is available for one third of the patients.

4.1.1 . STUDY MASKING PROCEDURES (EXAM #2 AND 3)

- The patient comes for the study visit and meets with the study coordinator before seeing their study doctor. This will facilitate masking of ophthalmologists and participants. All outcome assessors including lab technicians for trial I and ophthalmologists for trial II will remain masked. To facilitate interim and safety analysis, a data team member may also be unmasked.
- The patient's medication bottles are immediately placed in a secure, black bag and left at the study coordinators desk for the remainder of the visit. Patient medication calendars should also be placed in the black bag.
- The study coordinator will check-in with the patient and complete the following in any order:
    - Review of patient medication diaries
    - Review of adverse events since last visit*
    - Dispense new medication bottle (*if applicable*)

4.2 Scientific Rationale for Study Design

Randomized control trials are known to be the least biased form of evidence; we have chosen to do a randomized double- masked, placebo- controlled trial to reduce bias as much as possible.

4.3 End of Study Definition

A participant is considered to have completed the study if he or she has completed all visits and procedures and exams of the study described in Assessments and Procedures **Section 7.1**.

4.4 Adherence

Participants will be given a treatment diary in the form of a weekly calendar on which they will be asked to record adherence as well as reasons for missed doses. At each study visit, the study coordinator will review with the participant their treatment calendar, and document findings in the Medication Log in each participant file. Pill counting and medication bottle weighing will also be done for an objective measure of adherence. Participants who miss a scheduled dose of medication will be instructed to skip that dose and continue with their next scheduled dose of tablets or eye drops.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in Trial I of this study, an individual must meet all of the following criteria:

- CMV positivity by nucleic acid amplification test (e.g., polymerase chain reaction) from aqueous humor obtained via anterior chamber paracentesis obtained during an active flare of inflammation (uveitis) preceding enrollment into study
- Active anterior uveitis using Standardisation of Uveitis Nomenclature (SUN) Working group with clinical impression of CMV as the etiologic agent defined as ≥1+ anterior chamber cell[21]
- Participant willingness to use an acceptable method of contraception during the study period (i.e., pharmacologic, barrier methods or abstinence).
- Age over 18 years
- Basic understanding of the study as determined by the physician
- Commitment to return for follow up visits

To be eligible for Trial II, an individual must:

- Have a history of being CMV positive by nucleic acid amplification test (e.g., polymerase chain reaction) from aqueous humor obtained via anterior chamber paracentesis during an active flare of inflammation (uveitis)
- Have inactive inflammation (<1+ anterior chamber cell) for at least 2 weeks
- Have a period of 2 weeks of no antiviral prior to long-term treatment initiation

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

Trial I

- Negative for CMV by nucleic acid amplification test (e.g., polymerase chain reaction) of aqueous humor obtained via anterior chamber paracentesis during an active flare of inflammation (uveitis) preceding enrollment into study
- Participants <18 years of age
- Inactive anterior uveitis
- Active intermediate or posterior inflammation (involvement of vitreous, choroid or retina)
- Participants who have received antiviral therapy <14 days prior to enrolment
- Participants who have received periocular or intraocular corticosteroid injection <8 weeks prior to enrolment
- Current use of oral corticosteroids
- Immunocompromised participants (primary or secondary immunodeficiency disorders)
- Prior immunosuppressive therapy in the past 3 months
- Directed PCR testing positive for HSV or VZV
- Plans to conceive during the study period, pregnant or breastfeeding mothers (blood or urine pregnancy test for all females of childbearing age is mandatory within 4 weeks prior to enrolment)
- Complete blood count with white blood cell, absolute neutrophil or platelet count lower than the lower limit of reference laboratory normal
- Blood urea nitrogen or creatinine above the upper limit of reference laboratory normal
- Recent ocular surgery within the past 30 days or planned surgery within the next 45 days
- Systemic autoimmune disease or ocular condition (besides anterior uveitis) anticipated to dictate or alter treatment course

In Trial II, participants will be excluded if

- They have not had prior positive CMV by nucleic acid amplification test (e.g., polymerase chain reaction) of aqueous humor obtained via anterior chamber paracentesis during active flare of inflammation (uveitis)
- Have active inflammation
- Did not have a 2-week period of no antiviral (treatment) prior to randomization to long-term prophylaxis

5.3 Strategies for Recruitment and Retention

The investigating ophthalmologist is conducting the search for potential participants by reviewing results of the nucleic acid amplification test (e.g., polymerase chain reaction) performed on aqueous fluid obtained from anterior chamber paracentesis. He or she will then determine if the patient meets all non-laboratory eligibility criteria. If so, the patient will be approached and participation in the study will be discussed. If consent is obtained, the patient will be formally enrolled into the study and randomized to one of the 3 treatment groups by the study coordinator. These patients will be coming from the study sites' patient population, including patients referred for evaluation of a possible infectious cause of their uveitis.

Participants enrolled in Trial I, if willing, will get re-randomized to one of 3 arms in Trial II and

stay on that suppressive therapy for 12 months.

6 PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request. The reason for participant discontinuation or withdrawal from the study will be recorded on the final form. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may not be replaced and will be included in the intent to treat analysis. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced and will be included in the primary analysis.

7 STUDY ASSESSMENTS AND PROCEDURES

7.1 Assessments and procedures

7.1.1 PROCEDURES

- Visual acuity (best-spectacle correction using Snellen)
- Clinical eye exam (including slit lamp examination to examine cornea, evaluate for keratic precipitates and their morphology and location, to assess for anterior chamber cell and flare)
- Intraocular pressure assessment
- Anterior chamber paracentesis: Trial I-Exam 0 (pre-study visit) and Exam 2 (day 7), and Trial II- during recurrence
- Endothelial cell morphology and density using specular microscopy or confocal microscopy: Trial I- Exam 1 and Trial II- Exam 7
- Laboratory screening/monitoring orders (complete blood count, CBC, and complete metabolic panel, CMP): Trial I-Exam 0,2, and 3 and Trial II- Exam 2-7

7.1.2 SPECIMENS

The following specimens will be collected

- *Anterior chamber aqueous fluid for CMV quantitative PCR: Trial I- at Exam 0 and Exam 2, Trial II- during recurrence*
    - Aqueous humor from Exam 0 will be divided into two parts, some of fluid will be used for in-house directed PCR for HSV, VZV, and CMV. The results will determine study eligibility. 50 microliters of fluid will be preserved in the event the patient is CMV positive, and amenable to participating in the trial, ultimately to be used for quantitative CMV PCR to be conducted at Proctor Foundation/UCSF.
    - Aqueous humor from Exam 2 in trial I and during recurrence in trial II designated for CMV quantitative PCR and RNA sequencing will be immediately labeled and frozen on site. Once all enrolled participants at the site have completed the study, their aqueous samples will be packaged together with sufficient dry ice and shipped to UCSF for testing.

- The following specimens will be collected for laboratory measurements *after Exam #0 and prior to enrollment and randomization (Day #1 of clinical trial)*

  - Blood for complete blood count (CBC with differential)
  - Blood for serum chemistry panel (complete metabolic panel)
  - Blood or urine pregnancy test (all female participants of child-bearing age)
  - Blood for HIV testing (unless HIV status is known)

- Blood for complete blood count (CBC with differential) and chemistry (complete metabolic panel): (Trial I; Exam 2 and 3, Trial II Exam 2-7)

7.1.3 FORMS FOR DATA COLLECTION

- Patient Consent Forms for AC paracentesis- *Trial I- at Exam 0 and Exam 2, trial II- during recurrence*

- Eligibility/screening form: Trial I-Exam 1 and Trial II-Exam 1
- Patient Consent Forms for clinical trial: Trial I-Exam 1 and Trial II-Exam 1 (for new participants)
- Baseline History Form: Trial I-Exam 1
- Clinical Eye Exam Form: All Exams in Trial I and Trial II
- Medication Log (to be completed by patient): Trial I- Exam 2, and 3, Trial II- Exam 2-7
- Serious Adverse Event Narrative (if applicable): All Exams in Trial I and Trial II
- Patient Dropout Form (if applicable): Trial I- Exam 2, and 3, Trial II- Exam 2-7
- Protocol Deviation Form (if applicable): All Exams in Trial I and Trial II

7.2 Adverse Events and Serious Adverse Events

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.2.1 NON-SERIOUS ADVERSE EVENTS

Non-serious adverse events may include any unfavorable medical occurrences in patients who have ever received study medication, regardless of any causal relationship with treatment. Examples may include:

1. Increased intraocular pressure (>24 mm Hg)
2. Abnormal lab findings (rise in creatinine to ≥ 1.5 to < 2 mg/dL, reduction of white blood cell count to below 2.5)
3. Concurrent accident or illness
4. Increase in the frequency and severity of symptoms of a pre-existing condition
5. Side effects intolerable to patient (gastrointestinal upset, nausea, vomiting, fatigue)
6. Signs of corneal or conjunctival toxicity (epitheliopathy, conjunctivitis)

7.2.2 DOSE REDUCTION DUE TO SAFETY

In the event of a non-serious adverse event in the form of a laboratory abnormality, dose reductions in study treatments should be made. This will entail decreasing the dose of valganciclovir to 450 mg PO BID. Topical drop frequency will be decreased to 3 times daily. In the event of a non-serious lab abnormality, repeat testing should be ordered at 7 days of treatment with the reduced dose in order to monitor for resolution.

7.2.3 SERIOUS ADVERSE EVENT (SAE)

Serious adverse events include any medical occurrence that results in the following outcomes, or any other adverse event classified as severe*:

1. Cr ≥ 2 mg/dL, leukocytes ≤ 1,000/ L, platelets < 20,000/ L, or hemoglobin < 6.5 g/dL
2. Death
3. Non-elective surgery or hospitalization for any reason
4. Myocardial infarction or stroke
5. Corneal perforation
6. Life-threatening adverse drug experience or any life-threatening event
7. Persistent or significant disability or incapacity
8. Cancer
9. Seizure
10. Congenital anomaly/birth defect
11. Disability or permanent damage
12. Required intervention to prevent permanent impairment/damage

7.2.4 MEDICATION CESSATION

In the event of a serious adverse event, all study medications should be stopped and the medical monitor should be informed via a protocol deviation form. Further medical management will be at the discretion of the treating ophthalmologist and any consulting services. These patients should continue with scheduled study visits if possible.

7.2.5 ADVERSE EVENT REPORTING

Non-serious and serious adverse events will be noted at each study visit by the study coordinator while meeting with the patient at the beginning of each study visit. If there are any reported symptoms (not only severe symptoms), the study physician will review them with the patient while remaining masked to determine if any action is needed.

In case of a Serious Adverse Event (SAE), the Investigator needs to write a summary of the SAE and submit to the Medical Monitor (Dr. Gerami Seitzman; gerami.seitzman@ucsf.edu), and Principal Investigator (Dr. John Gonzales; john.gonzales@ucsf.edu) within 24 hours of the SAE. Information recorded in this message will include the nature of the event, date of onset, date of resolution, date of notification to Medical Monitor, and action taken. The Medical Monitor will review and decide if the serious adverse event was related to the study drug and collect additional information if needed.

The Medical Monitor will determine two things:

1) Whether this is a true adverse event and

2) Whether this is likely related to the study drug.

If the investigator thinks the SAE is related to the study drug, he/she can stop the study medication anytime (including prior to investigation by the medical monitor).

Any significant study drug-related adverse events will be reported by the coordinating center to the FDA and CHR office as appropriate. The principal statistician will inform the DSMC of serious adverse events by arm every month.

Any complications will be reported within 24 hours to the Principal Investigator (Dr. John Gonzales, *john.gonzales@ucsf.edu*).

7.3 Unanticipated Problems

7.3.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP.
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within 7 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 30 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee),

and the Office for Human Research Protections (OHRP) within 30 days of the IRB's receipt of the report of the problem from the investigator.]

7.3.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants will be notified of UPs in aggregate. When UPs affect an individual, they will be notified individually by the treating physician.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical Hypotheses

Specific Aim 1 (Trial I): To compare CMV viral load and inflammation after randomization to 7 days of oral valganciclovir, topical ganciclovir 2%, or placebo.

1a: We hypothesize that participants randomized to oral valganciclovir will have a lower viral load after 7 days compared to those randomized to either topical ganciclovir 2% or placebo (Trial I primary outcome).

1b: We hypothesize that a greater proportion of participants randomized to oral valganciclovir will have controlled inflammation compared to those randomized to either topical ganciclovir 2% or placebo.

Specific Aim 2 (Trial II): To compare the effect of long-term antiviral suppression on recurrence rate of inflammation.

2a: We hypothesize that participants randomized to placebo will have fewer recurrences over 12 months compared to those randomized to either oral valganciclovir or topical ganciclovir 2% (Trial II primary outcome).

2b: We hypothesize that aqueous obtained at the time of a recurrence of inflammation will demonstrate a detectable viral load and will correlate with level of inflammation.

Specific Aim 3 (samples from Trials I and II: **To characterize host transcriptional signatures and viral genomic features in CMV anterior uveitis using RNA sequencing of aqueous samples.**

3a: We hypothesize participants completing both trials who exhibit recurrent inflammation after antiviral suppression (Trial II) will have a higher prevalence of CMV mutations associated with antiviral resistance compared to baseline samples (Trial I).

3b: We hypothesize that host transcriptional profiles will (i) distinguish CMV anterior uveitis from anterior uveitis cases negative for CMV and unable to enter Trial I, (ii) we will identify non-CMV pathogens in cases unable to enter Trial I, and (iii) identify unique signatures associated with recurrent inflammation in Trial II participants.

Primary Outcome:

- Viral load after 7 days of treatment (Trial I)
- Recurrence of inflammation over 12 months (Trial II)

Secondary outcomes:

- Anterior chamber cell (Trials I and II)
- Intraocular pressure (Trials I and II)
- Visual acuity (Trials I and II)
- Corneal endothelial cell count and morphology (Trials I and II)
- Number of glaucoma medications (ocular hypertensive medication) (Trials I and II)
- Prevalence of mutations conferring antiviral resistance (Trials I and II)

8.2 Sample Size Determination

Primary analysis for Trial I. We power the study for each pairwise comparison. We will be comparing each arm against the others: oral valganciclovir vs placebo, topical ganciclovir 2% vs. placebo and oral valganciclovir vs. topical ganciclovir 2%. The power calculation was based on the primary outcome, log-transformed CMV viral load. We informed the calculation with viral loads from the pilot study, STACCATO. The STACCATO trial measured viral load at baseline and 7 days post-treatment. The standard deviation (SD) of log-transformed viral load 7 days post-treatment in STACCATO was 0.98. Since the primary analysis will adjust for baseline viral load, we used an estimate of the residual standard deviation, which is $SD_r = SD\sqrt{1-r^2}$, where r is the correlation between the baseline measure and primary endpoint. In STACCATO, the correlation between baseline and post-treatment viral load was 0.50. Assuming a significance level of 0.05/2=0.025 to account for multiple comparisons and an SD_r of 0.66 in $\log_{10}$ viral load, we estimate that we will have 80% power to detect a difference of 0.50 in $\log_{10}$ viral load (from 3.11 to 2.61) between arms with 35 study participants per arm (117 total allowing for approximately 10% loss to follow up). This calculation is based on the standard power formula for the T-test using an estimated residual standard deviation. While a global test of any difference between the three arms is possible, it is not clinically informative and the 2 pair-wise comparisons are of highest clinical interest. The proposed max-T procedure for P-value and simultaneous confidence interval estimation are a correct, valid approach for the two comparisons.[22]

Primary analysis for Trial II. The primary outcome for Trial II is recurrence of inflammation (≥1+anterior chamber cell) over 12 months. We calculated the effect size we would be able to observe with 80% power, a two-sided alpha of 0.05/2, and 28 participants per arm (105 total from Trial I with 20% loss to follow-up) based on a two-sample z-test for proportions. Assuming the risk of recurrence is 25% in the placebo group, we can detect a difference of 36 percentage points between the oral valganciclovir and the placebo groups. We acknowledge that having fewer recurrences in the placebo group compared to oral antiviral group may be contradictory to what one might think would typically occur with exposure to antivirals. However, it is well documented in the literature that long-term suppressive-level dosing of antiviral in an immunodeficient host or in an immunologically privileged organ (such as the eye) can create an environment permissive to the development of antiviral resistance.[13-15,18,20]

For Specific Aim 3, we estimated the minimum detectable effect for the difference in prevalence of infections that have antiviral resistance mutations among recurrent infections in Trial II among patients who receive long-term antiviral prophylaxis compared with their infections at baseline. Aim 3 will focus on patients in the two active treatment arms in Trial II, because we would expect long-term use of topical and oral antivirals to lead to similar selection for antiviral resistance. From 117 participants enrolled, we conservatively assumed that 72% of patients would complete both Trial I and Trial II (n=84). We further assumed that 60% of patients will develop a recurrent infection over the monitoring period during the trials (Table 1)[7]. Participants who receive active treatment in Trial II (treatment regimens C+D) will contribute to the Aim 3 analysis (n=34). The analysis will be a paired analysis, comparing the proportion of recurrent infections with antiviral resistance mutations with that same proportion at baseline. *Please refer to the Statistical Analysis Plan (SAP) for further details.*

Randomization

For both trials, individuals will be randomized to the three arms in a 1:1:1 ratio. Randomization will be stratified by site. Stratified, block randomization will ensure that an approximately equal number of patients are randomized to each treatment arm by site. UCSF biostatistician will prepare the randomization and it will be implemented in REDCap.

8.3 Statistical Analyses

1a. The Aim 1 primary outcome **viral load after 7 days of treatment** will use the results from quantitative PCR at 7 days of treatment or placebo therapy (day 7 of trial). All quantitative PCR viral loads will undergo log transformation, with units converting from IU/mL to log IU/mL.

1b. **Clinical inactivity of inflammation**: At each study visit patients will be clinically examined, and a study ophthalmologist will make a clinical determination as to whether the patient's inflammation is considered inactive (defined as ≤0.5+ anterior chamber cell). The proportion of participants that achieve clinically inactive inflammation by day 7 and 21 will be compared by arm. Patients will also be administered a questionnaire querying when they felt symptomatic improvement in their condition. This analysis will be viewed as hypothesis generating and will be treated as supplementary and in addition to the main finding.

2a. **Recurrence of inflammation over 12 months:** After achieving clinical quiescence, participants will be offered enrollment into Trial II where they would be re-randomized to long-term suppressive treatment to study the effect on recurrence of inflammation (defined as ≥1+ anterior chamber cell). Each participant will be assessed for recurrence at each visit. Alternatively, if participants experience symptoms of a flare up, they will be instructed to contact the local site clinical coordinator or study ophthalmologist for an examination within 3 days. If recurrence of inflammation occurs, aqueous chamber fluid will be collected (an anterior chamber paracentesis will be performed) from participant to quantify viral load.

3a. **Prevalence of mutations conferring antiviral resistance:** The analysis population will include individuals enrolled in both Trials I and II who are randomized to receive oral or topical antivirals in Trial II and experience a recurrent infection. Among patients that experience a

recurrent infection (anticipated n=34, see Sample Size section 3.3 in SAP), we will compare the proportion of infections with antiviral mutations upon recurrence with the proportion with antiviral mutations at baseline.

Interim Analysis

The interim analysis for each trial should be conducted after one-third and before one-half of the patients have been enrolled; ideally when there are 15 patients per arm.

8.3.1 SAFETY ANALYSES

Interim reports for the DSMC will be prepared by the central Proctor site.

The Data and Safety Monitoring Committee will meet to review the interim efficacy data when primary outcome data is available on one third of the study subjects. The DSMC will make one of the following recommendations:

- Continue the study without modifications
- Continue the study with modifications
- Terminate enrollment or treatment in the study because of safety concerns
- Terminate enrollment or treatment in the trial because of futility

The DSMC will determine the database closure dates for each report in advance; archival copies of the (a) main 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.

The DSMC will make decisions with the benefit of pre-specified decision guidelines. These guidelines will be agreed upon at the initial meeting, and are expected to include (a) safety, (b) efficacy, (c) futility, (d) clinical importance, or (e) validity.

Efficacy. Unmasked interim analyses 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. The Lan and DeMets[23] flexible alpha spending approach with a power function is suggested, with $\alpha^*(t^*)= \alpha\ (t^*)^{\theta}$, where $\theta= 3.561$ chosen so that the two-sided *P*-value to stop the trial for efficacy is 0.001.

Harm. Stopping for harm will be done at the judgment of the DSMC. Several endpoints will be examined, including adverse events, and especially abnormal lab results meeting the designated threshold of a serious adverse event.

All subjects who provide informed consent will be accounted for in this study. We will present the number lost to follow-up and the number of protocol deviations by arm. The proportion of

subjects reporting adverse events and serious adverse events will be reported by arm to the DSMC and will be compared using a Fisher's Exact Test.

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 to use a simulation-based approach[24] that estimates conditional power under the assumed design effect for the remainder of participants and under the current trend observed at the interim analysis. We propose that the DSMC consider discontinuation for futility if the conditional power to detect a difference in viral load in Trial I and recurrence in trial II drops below 20% at the interim analysis under the assumed design effect for the remainder of participants yet to be enrolled.

Population for Analyses

Individual level missing data (due to loss to follow-up or dropouts) is expected. Complete analysis will be reported. Missing values will be tabulated by treatment arm, and study site. Sensitivity analysis assigning outcomes to the missing values will be reported. Additionally, analyses will be reported in which we adjust for any baseline covariate known to be a predictor of missing outcome data. **Please refer to the SAP for further details regarding analyses.**

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 Regulatory, Ethical, and Study Oversight Considerations

9.1.1 INFORMED CONSENT PROCESS

9.1.1.1 Consent/assent and Other Informational Documents Provided to participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting study procedures. Consent forms will be given to the participant in their native language.

9.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

9.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

9.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the Data Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Data Coordinating Center at UCSF.

9.3.1 CERTIFICATE OF CONFIDENTIALITY

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

9.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the Data Coordinating Center at UCSF. After the study is completed, the de-identified, archived data will be transmitted to and stored on the Research Electronic Data Capture system (REDCap).

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the enrollment site.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data will be provided through REDCap.

10 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
John Gonzales, MD Associate Professor	Gerami Seitzman, MD Professor of Ophthalmology
Francis I. Proctor Foundation, University of California, San Francisco	Francis I. Proctor Foundation, University of California, San Francisco
490 Illinois street, second floor San Francisco, CA 94158	490 Illinois street, second floor San Francisco, CA 94158
415-476-1442	415-476-1442
john.gonzales@ucsf.edu	gerami.seitzman @ucsf.edu

10.1 Safety Oversight

A Data and Safety Monitoring Committee (DSMC) will be empaneled by the NEI. 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. The committee will review information on data quality, enrollment, patient retention, and study outcomes, etc. Committee members will be unmasked and receive reports of the data with ARM information at one interim time-point (when 1/3rd of the primary outcome has been collected). All study protocols will be subject to review and approval by Institutional Review Boards.

10.2 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of study participants are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Clinical monitoring will be conducted by the Clinical Coordinating Center (CCC) at UCSF. One person will make site visits to all enrollment sites once a year to monitor study activities. During each visit the CCC will conduct a complete chart review of all patient charts to ensure data is being recorded in a complete fashion. The CCC will conduct regular off-site reviews of data entered at UCSF to ensure 100% data verification.

10.3 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the study is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP)).

The investigational site will provide direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

11 DATA HANDLING AND RECORD KEEPING

11.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the study staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Enrollment sites will upload these forms to Box.com, where the Data Coordinating Center at UCSF will access these forms for data entry. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Research Electronic Data Capture (REDCap), a 21 CFR Part 11-compliant data capture system provided by the Data Coordinating Center at UCSF. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be double data-entered directly from the source documents.

11.2 Study Records Retention

Study documents should be retained for a minimum of 2 years. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

11.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to National Eye Institute Program Official and the Coordinating Center at UCSF. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

11.4 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy. In addition, every attempt will be made to publish results in peer-reviewed journals.

11.5 Conflict of Interest Policy

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this study. The study leadership in conjunction with the National Eye Institute has established policies and procedures

for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11.6 Additional Considerations

None

12 ABBREVIATIONS

AC	Anterior Chamber
AE	Adverse Event
ANCOVA	Analysis of Covariance
BID	Twice a day
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CCC	Clinical Coordinating Center
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
Cr	Creatinine
DCC	Data Coordinating Center
DSMC	Data Safety Monitoring Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GLP	Good Laboratory Practices
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
ICH	International Conference on Harmonisation
IOP	Intraocular Pressure
IRB	Institutional Review Board
MOP	Manual of Operations and Procedures
NEI	National Eye Institute
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PO	By mouth
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
UCSF	University of California, San Francisco
UP	Unanticipated Problem
US	United States
VA	Visual Acuity
VZV	Varicella-zoster Virus

VA	Visual Acuity

13 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale

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