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# An international, multi-centred, double masked randomised 4-week, placebo-controlled study to compare the efficacy of oral valganciclovir and topical 2% ganciclovir in the treatment of cytomegalovirus anterior uveitis

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The study sponsor was primarily involved in the study design; collection, management, analysis and interpretation of data; writing of the report and the decision to submit the report for publication.

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Committees/Collaborators: John Gonzales (Principal Investigator); Nisha Acharya and Thuy Doan (Co-investigators and Editorial Committee); Gerami Seitzman (Medical Monitor); Thomas Lietman, Jennifer Rose-Nussbaumer, Jeremy Keenan, and Charles Lin (Data and Safety Monitoring Committee), Travis Porco (Biostatistician), Catherine Oldenberg (Data Coordinating Centre Supervisor), Sarah Lopez (Study Site Coordinator) Ashlin Joye, Jaskirat Takhar, and Elisabeth Gebreegziabher (Data Managers and Study Coordinators), Thanapong Somkijrungroj (Site Director, King Chulalongkorn University); Wipada Laovirojjanakul (Site Director, Khon Kaen University); Chang-Ping Lin (Site Director, National Taiwan University), Ruedee Kumklee (Study Coordinator, Khon Kaen University)

Patient and Public Involvement: We did not involve patients or the public in our work.

Data statement: As this manuscript describes the protocol for a study that has yet to be implemented, no data are currently available to access. After completion of the trial, we will publish the data in a data repository.

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## Abstract

**Introduction** Cytomegalovirus (CMV) anterior uveitis is a recognised cause of anterior uveitis in immunocompetent patients and preventable cause of vision loss. Ocular sequela include corneal endothelial damage which can cause corneal oedema, and failure, as well as glaucoma. Recurrences of inflammation are common and therefore patients are often exposed to long-term therapy. Oral therapy is available in the form of valganciclovir albeit with the caveat of systemic side effects such as bone marrow suppression and renal failure necessitating regular interval laboratory monitoring. Recent reports have demonstrated that topical 2% ganciclovir solution may offer promising treatment outcomes in patients with CMV anterior uveitis with superior safety, cost effectiveness, and convenience profiles. An investigation into the relative equipoise of these therapies is warranted for these reasons.

**Methods and Analysis** The Systemic and Topical Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO) trial is designed as a multi-centre, block randomised by site, double-masked, placebo-controlled trial comparing the efficacy of oral valganciclovir, 2% topical ganciclovir, and placebo in treating PCR-proven CMV anterior uveitis. Participant clinical evaluation will occur at of 3 study timepoints by a masked study ophthalmologist over a 28-day period to assess resolution of ocular inflammation (secondary outcome). A control group will provide additional information about the possible impact that the infected host's immune response may play in controlling local viral replication. The primary analysis is an ANCOVA (3 arms) correcting for baseline to compare quantitative CMV viral load in the anterior chamber aqueous fluid pre and 7-days post treatment.

**Ethics and Dissemination** The University of California San Francisco Committee on Human Research, and the Khon Kaen University institutional review board (IRB) has given ethical approval. The results of this trial will be presented at local and international meetings and submitted for peer-reviewed journals for publication.

**Trial Registration Number** NCT03576898; Pre-enrollment

## Article Summary

### Strengths and limitations of this study

- This randomised clinical trial compares both clinical and quantitative microbiologic outcomes of CMV treatment as opposed to solely clinical response.
- Baseline and endline quantitative PCR viral loads from all sites that will be included in analysis are processed at the same laboratory ensuring a uniform assessment of reduction in viral load.
- This is a multi-centre clinical trial that includes 3 sites in Asia where the disease is most prevalent.
- Our limited sample size may not allow detection of the smallest meaningful difference between the treatments.
- Adherence is mainly assessed via self-report and may not accurately reflect treatment administration.

## INTRODUCTION

Cytomegalovirus (CMV) has been recognised as an important cause of posterior retinitis in HIV/AIDS patients, particularly prior to the introduction of highly active anti-retroviral therapy. However, CMV is now being implicated in immunocompetent individuals as a cause of anterior uveitis.[1-4] Definitive diagnosis requires laboratory diagnosis, such as directed polymerase chain reaction (PCR).[5]

CMV anterior uveitis is a preventable cause of vision loss. It can cause damage to the corneal endothelial cells,[6-9] which can lead to oedema, failure, and, ultimately, requirement for corneal transplant. CMV can recur in transplanted grafts, necessitating further surgeries and complications.[10-14] Additionally, recurrences of inflammation in CMV anterior uveitis can be associated with glaucoma, which can cause irreversible blindness if not appropriately managed.[15-17]

While CMV anterior uveitis is typically characterised by recurrent bouts of inflammation, it is unknown whether each recurrence is mediated by active viral replication or a sterile immune response. Consequently, patients may be prescribed frequent oral or topical antiviral therapy. An understanding of the most effective treatment strategy for CMV anterior uveitis will help inform future studies investigating ways of decreasing recurrences of inflammation in this condition.

One option for the management of CMV anterior uveitis is oral valganciclovir 900mg twice daily.[18,19] Prevention of recurrent inflammation in CMV anterior uveitis has been with valganciclovir 450mg twice daily.[18,19] Oral valganciclovir can be associated with serious systemic side effects including renal failure and bone marrow suppression, necessitating regular interval laboratory monitoring. Topical ganciclovir 2% has also been used to treat and prevent recurrences of CMV anterior uveitis.[20-22] Topical therapy is attractive as it does not require laboratory monitoring, though ocular side effects including corneal epitheliopathy and conjunctivitis could preclude long-term use.[23,24] Thus, comparing oral valganciclovir 900mg twice daily to topical ganciclovir 2% every 2 hours (up to six times daily) has equipoise in the uveitis/cornea specialist.

To gain insight into preferred practice patterns for the treatment of CMV anterior uveitis, we surveyed attendees of the Biennial Chulalongkorn-Khon Kaen-Proctor-UCSF (CKPU) meeting in Bangkok, Thailand on March 5, 2019. Survey questions were provided in a Likert scale, multiple-choice format with participants asked about their agreeability on 7 statements. Answer choices varied from 1 to 4; 1 representing “strongly disagree”, 2 representing “somewhat disagree”, 3 representing “somewhat agree”, and 4 representing “strongly agree”. Sixty-eight faculty level physicians responded to our survey, 5 practicing in North America, and 63 practicing in Asia. Results are summarised in Table 1. Physician preference for treatment medication of CMV anterior uveitis in presented in Table 2. The results demonstrate that a consensus regarding the best treatment for CMV anterior uveitis is not yet established.

Some cases of CMV anterior uveitis have been noted to resolve without antiviral treatment.[25] In this setting, having a trial in which oral valganciclovir is compared to

topical ganciclovir, and including a control group where no antiviral was used would provide additional information about the possible impact that the infected host's immune response may play in controlling local viral replication.

## METHODS AND ANALYSIS

### Study overview

The Systemic and Topical Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO) trial is a randomised, participant- and ophthalmologist-masked, placebo-controlled trial comparing the efficacy of oral valganciclovir, and 2% topical ganciclovir in treating PCR-proven CMV anterior uveitis. Study participants will be block-randomised with 1:1:1 allocation into the 3 study treatment arms. Participants will return to clinic for 3 study evaluations by a masked study ophthalmologist over a 28-day period at which point they will be assessed for resolution of ocular inflammation. An objective measure of treatment efficacy will be assessed via biological samples of anterior chamber aqueous fluid pre and 7 days post-treatment. An overview of study procedures and study timeline is provided in **table 3**. We used the SPIRIT checklist when writing our protocol.[26]

### Specific aims and outcomes

The specific aims of this trial are (1) to assess reduction in CMV viral load by treatment (as measured by $\log_{10}$-transformed quantitative viral load pre-treatment compared to 7-days post treatment and (2) to assess the proportion of participants achieving clinical quiescence after 7 and 28 days of treatment. As a nested observational aim within our primary aim, we will evaluate the effect of topical corticosteroid started prior to enrollment on viral load yield by directed quantitative PCR. The research hypothesis for this study is that oral valganciclovir therapy will demonstrate the greatest efficacy in treating CMV anterior uveitis compared to 2% topical ganciclovir and placebo therapy.

### Setting and Eligibility

The seroprevalence of CMV varies geographically. In East Asia it estimated to be as high as 87-100%, whereas in Western countries it is between 51.5 – 54.4%.[27-29] For comparative purposes, four tertiary academic centres located in three countries will be included, one in the United States of America, two in Thailand, and one in Taiwan.

Participants must provide written, informed consent before any study procedures can occur (see Appendix 1 for a sample informed consent). Study inclusion and exclusion criteria are outlined in Table 4.

### Recruitment

Each study centre will screen subjects independently adhering to the inclusion and exclusion criteria outlined. Patients who are found to be CMV positive by in-house PCR and who meet eligibility criteria will be approached about participating in the study. Participant enrollment and project activities are planned to begin in July 2019 and end August 2021.

## Assignment of Intervention

Competitive block (of 3 and 6) randomization of the participants, stratified by site will ensure that an equal number of participants at each site are randomised to study arms in a 1:1:1 ratio, not necessarily enrolling an equal number of participants at each site. Randomization lists will be created by the coordinating centre clinical coordinator and accessed online via REDCap database. After consenting participants for enrollment and confirming all eligibility requirements have been met, the study coordinators who are masked to the allocation sequence assign the participants an ID from a predetermined list and will log into REDCap and perform the randomisation. The now unmasked study coordinators will then give participants their assigned treatment.

## Masking

All study ophthalmologists and participants will remain masked to treatment assignment after enrollment. All participants will receive both pills and eye drops, and will not know which medications are active. Study doctors have no part in handling the medication bottles, all participants will be given dark bags to place and keep their bottles in throughout the trial. Additionally, participants will meet with the study coordinator first, before seeing their study doctor at follow-up visits to review the participants' medication calendars, and study coordinators will hold participant calendars and study bottles in their office for the entire visit. Before bringing participants to the study doctor, the study coordinator will remind participants not to disclose any information concerning their treatment to their study ophthalmologist. If the participant is experiencing intolerable side effects, the study coordinator and participant will speak with the study doctor concerning discontinuation of treatment.

The UCSF pharmacy will be responsible for purchasing and distributing bottles of the medications labeled with NDC/lot number and expiration dates to the Proctor Foundation, and then medications will be distributed to all study sites. Study ID labels, and dosing instructions will be provided to each site on separate label sheets. After enrollment, and randomization each site's study coordinator will place the study ID label on the bottle, before dispensing the drug to the participant. If personnel are unmasked at any time, a protocol deviation form must be completed.

## Intervention

Participants will initially present with anterior uveitis suspected to be due to a viral aetiology. Participants will receive a routine standard of care work-up for such a suspected aetiology, which will include an anterior chamber (AC) paracentesis.[30] Half of this fluid (~50μL) will be used for qualitative PCR to detect CMV, HSV, and VZV at each respective site. The other half (~50μL) will be stored at -80 degrees Celsius. Patients will have serologic testing for complete blood count, renal function, pregnancy status (in women of child-bearing age), and HIV status.

In the event a participant is ineligible for study inclusion, the stored 50 μL aliquot will be discarded. In eligible participants, this aliquot will undergo quantitative CMV PCR at a

US laboratory, where lab personnel will be masked to participant identification, treatment assignment, and site. This aliquot will serve as the baseline (prior to treatment randomization) CMV viral load.

Eligible participants will remain masked and randomised by the unmasked study site coordinator to one of three study treatment arms. After randomization, all participants will receive both tablets and topical eye drops. One-third of participants will receive oral valganciclovir tablets and placebo eye drops (balanced salt solution, BSS), one-third will receive topical ganciclovir 2% eye drops and placebo tablets, and one-third will receive placebo eye drops (BSS) and placebo tablets. Dosing will be 4 tablets daily (900mg PO BID) for oral medication and one drop every 2 waking hours (6 drops per day) for topical.

After 7 days of treatment therapy participants will return for clinical examination and repeat anterior chamber paracentesis. Approximately 80μL will be stored for eventual quantitative CMV PCR by the aforementioned US laboratory, providing a post-treatment viral load value. Approximately 20 μL will be stored for future studies, provided a participant provides consent.

Since it is standard of care to prescribe topical corticosteroids to reduce the severity of intraocular inflammation in participants with anterior uveitis, all participants (regardless of randomization) will be prescribed topical prednisolone acetate 1% and instructed to apply one drop to the affected eye three times daily.

**Harms and Modifications**

Non-serious adverse events may include any unfavorable medical occurrences in participants who have ever received study medication, regardless of any causal relationship with treatment. This may include: increased intraocular pressure (>24 mm Hg), abnormal lab findings (rise in creatinine to ≥1.5 to <2 mg/dL, reduction of white blood cell count to below >1,000 to <2,500/μL, platelet count from 20,000 to 75,000, or hemoglobin level >6.5 to <9 g/dL), concurrent accident or illness, increase in the frequency and severity of a pre-existing condition, side-effects intolerable to participants (gastrointestinal upset, nausea, vomiting, fatigue), or signs of corneal or conjunctival toxicity (keratitis or conjunctivitis).[23,24,31]

If a non-serious adverse event in the form of a laboratory abnormality occurs, dose reductions of valganciclovir to 1 tablet (450mg) BID and topical drop frequency to 3-times daily will be made. Repeat laboratory testing should be ordered at 7 days of treatment with the reduced dose to monitor for resolution.

Serious adverse events (SAEs) include any medical occurrence that results in the following outcomes, or any other adverse event classified as severe: creatinine ≥2mg/dL, leukocytes ≤1,000/μL, platelets <20,000/μL, hemoglobin <6.5 g/dL, death, non-elective surgery or hospitalization for any reason, myocardial infarction, stroke, any life-threatening event, cancer, seizure, congenital anomaly/birth defect, disability or

permanent damage, required intervention to prevent permanent impairment/damage.

An unmasked medical monitor will evaluate the details of all SAEs via a protocol deviation form and can request additional clinical information and recommend additional tests. The medical monitor will determine whether the event is a true SAE and whether it is likely related to the study drug. The medical monitor will recommend continuing the study medication versus dose reduction versus discontinuation. Additionally, if the investigator thinks the SAE is related to the study drug, he/she may stop the study medication anytime (including prior to evaluation by the medical monitor). Further medical management will be at the discretion of the treating ophthalmologist and any consulting services. These participants should continue with scheduled study visits if possible.

All serious adverse events will require the completion of a protocol deviation form and will be reported to the local IRB, and UCSF IRB.

**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 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.

**Concomitant Care**

Study ophthalmologists may use other topical adjunctive medications such as IOP lowering medications and cycloplegic agents. These should be documented on the study forms at each visit. The protocol does not allow for intravenous, or injection antiviral therapy, additional antivirals, or surgery during the study period. If such therapies are deemed necessary, the Protocol Deviation form should be completed.

**Retention**

Participants will be considered to have dropped out from the study only if they declare they are no longer interested in further participation and not willing to return for any study visits, or are deceased. If participants are not willing to return for any study visits, no further information will be collected.

Participants who stop the study treatment for reasons other than efficacy, tolerability and/or safety or miss study visits and do not respond to contact, are considered non-compliant participants. Non-compliance will be noted and participants will continue their study treatment.

## Outcome Assessments

*Viral Load Reduction*

Primary measurement of efficacy of the 3 treatments arms will be a comparison of the $\log_{10}$-transformed quantitative viral load PCR at baseline versus after 7 days of therapy. This measure provides an objective measurement of treatment efficacy. We note a dearth of published data regarding CMV viral loads pre- and post-treatment. An endpoint of 7 days was chosen as a prior study found viral loads to be frequently undetectable after 14 days of therapy.[32] All quantitative PCR measurements will be conducted at a single laboratory (masked to treatment and site) within the United States to ensure the same assay will be used on the provided samples, permitting direct comparison of quantitative viral load values.

*Effect of Topical Steroids on Baseline Viral Load*

Participants will be queried about the frequency of their topical steroids use to measure the effect of topical corticosteroid use prior to eligibility screening on CMV viral load. We anticipate that the dosing of corticosteroids prior to presentation or referral will vary depending on the practices of the referring physician. More frequent dosing of topical corticosteroids may affect the amount of virus recovered from the anterior chamber perhaps due to local immunosuppression.

*Clinical Response to Therapy*

For the clinical secondary outcome, participants will be classified as quiescent if they demonstrate on clinical examination: Less than or equal to 0.5+ AC cell **AND** resolution of other signs associated with active inflammation, including increased IOP, corneal oedema, and/or active keratic precipitates (KP). IOP must be controlled without the addition of new IOP medication. In addition, a subjective measure of efficacy will be assessed by asking participants to estimate how many days after initiating therapy they felt improvement in their symptoms.

## Methods: Data Collection, Management, and Monitoring

Paper forms for each participant will be completed by study personnel in real time when the participant is being assessed. The forms will be reviewed and crosschecked for consistency and completeness by the study coordinator within 24 hours of completion. Study coordinators at all sites will scan data collection forms and send them electronically to the Data Coordinating Centre (DCC) within 10 days of the participant visit and retain hard copies in a secure location. The DCC, is responsible for supervising data collection, data management, data quality control, data analysis, event adjudication, and training and certification of study site staff in the data management systems. All data will be entered into the official electronic research database, REDCap within 10 days of receipt of completed forms.

Discrepancies and missing values will be assessed by the data entry manager and resolved by queries sent to the study coordinator and appropriate observers. A logfile will preserve the date and time of any changes, together with who entered the changes.

### Data and Safety Monitoring Committee

The Data Safety and Monitoring Committee (DSMC) has been established and is independent with experts from diverse fields including biostatistics, epidemiology, and ophthalmology. Only after the DSMC reviews and approves the protocol will participants be enrolled. The DSMC monitors severe or unexpected events that threaten the safety of participants and oversees the data collected throughout the duration of the study. The DSMC is responsible for reviewing the results of the interim analysis and determining whether the trial should continue with or without modification.

## STATISTICAL ANALYSIS PLAN

### Sample Size

Limited data from the available studies reporting detectable CMV viral loads pre- and post-treatment were available to guide our power calculations. We estimate a pre-treatment viral load of 5.4 $\log_{10}$IU/mL[6,20,32,33] (standard deviation +/- 1.00 log), and post-treatment viral load of 4.88 $\log_{10}$IU/mL[6,32-34] (standard deviation +/- 0.95 log) with correlation of 0.7 between baseline and follow-up viral load. With a sample size of 33 participants per arm with estimated 10% total loss to follow-up, we anticipate at least 80% power to detect a 0.61 log difference in viral load (based on pairwise analysis subject to an alpha of 0.05/3).

### Interim Analysis

An interim analysis of efficacy is performed one-third of the way through the trial, with alpha set at 0.001. The DSMC reviews the unmasked interim analysis and makes recommendations on the continuation of the trial. No interim analysis for futility is performed. We anticipate 80% power to detect a difference of 1.7 log units between any two arms.

### *Specific Aim 1*

The outcome variable is log viral load as measured by quantitative PCR at 7 days. The primary prespecified analysis is a permutation P-value based on the Fisher F statistic, derived from a linear model predicting the log viral load (primary outcome variable) as a function of the baseline log viral load and the treatment arm (dummy coded), i.e., ANCOVA. Estimation will be conducted by ordinary least squares. Type I error rate (alpha) of 0.05 will be used.

Quantities of interest for analysis will be quantitative PCR data from anterior chamber paracentesis aqueous fluid collected at the pre-study visit for each participant enrolled in

the study. The second quantity of interest for comparative purposes will be quantitative viral load derived from the analysis of aqueous fluid from the second anterior chamber paracentesis conducted on study day 7 (exam #2). The outcome will be modelled as a linear function of baseline value and the treatment arm. Specifically, we will test the null hypothesis of a common intercept for all 3 treatment arms. We will also present the change scores for additional information and insight.

All quantitative PCR viral loads will be $\log_{10}$-transformed, with units converting from IU/mL to $\log_{10}$ IU/mL. The baseline quantitative PCR for each participant, and treatment will be used as a predictor for quantitative PCR after 7 days of treatment.

The purpose of supplementary analyses reported in this section is to assess the role of statistical choices and data quality choices in shaping the result. A Kruskal-Wallis non-parametric one-way ANOVA (using change scores) will be an alternative consideration as a statistical analysis choice for the primary outcome. As a methodological analysis/sensitivity analysis, we will also conduct a Box-Cox transformation of our quantitative PCR log transformation.

If the results of the primary analysis are significant, then pairwise comparisons based on the Fisher Least Significant Difference will be conducted (P-values derived by permutation). We will also test for the hypothesis of interaction between study country in the 2 Asian sites (Taiwan and Thailand). This will be an assessment of the heterogeneity of effect between study sites. In addition, we will repeat the primary analysis including an additional random effect for study site and report the estimated treatment effects under this supplemental model.

***Specific Aim 2***

To compare the proportion of clinical quiescence at day 7 in the three treatment arms, a logistic regression model will be conducted. The outcome variable will be the binary classification of quiescence of inflammatory status determined by the masked study ophthalmologist. The analysis will be two-sided, with a type I error rate (alpha) of 0.05/2; the predictor is the study arm (as a factor). In addition, we will repeat this analysis for data collected on day 28. We propose a Bonferroni correction, for an alpha of 0.05/2. We will also tabulate the number of individuals who change status, reporting the 2x2 table of day 7 vs day 28 status, by arm. Missing data will be reported, and a sensitivity analysis will be conducted to assess the degree to which missing data could have affected the findings. Data collected at +/- two days will be used (day 7 data can be collected between days 5-9, and day 28 data can be collected at 26-30 days).

Participants will also be administered a questionnaire querying when felt symptomatic improvement other their condition. This analysis will be viewed as hypothesis generating and will be treated as supplementary and in addition to the main finding.

***Nested Observational Aim***

To assess the observational effect of topical corticosteroid administered prior to study enrollment on diagnostic yield of quantitative PCR an **ANCOVA** of log transformed mean viral load at initial anterior chamber paracentesis vs. frequency of pre-study topical steroid use will be conducted. The analysis will be **two-sided** with a **type 1 error rate** (alpha) of 0.05. We will report the findings unadjusted as hypothesis generating for a future larger trial. This will be sharply demarcated from the primary analysis. Bonferroni-Holm correction will be considered for this sub-aim.

***Secondary analyses***

In addition, an efficacy analysis will be conducted in which we include phenotype as a binary predictor (and the interaction with treatment). Phenotype of CMV anterior uveitis will be determined by the masked study ophthalmologist (acute/recurrent vs. chronic). Analysis will consist of an **ANCOVA** as before, with the additional covariates. We will perform a hypothesis test of the coefficient for the phenotype-treatment interactions, **two-sided**, with a **type I error rate** (alpha) of 0.05.

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.

## ETHICS AND DISSEMINATION

This protocol and informed consent forms have been approved by the IRB at the University of California, San Francisco, and King Chulalongkorn University. The trial is registered at clinicaltrials.gov (NCT03576898). Protocol modifications are submitted to the relevant parties for review and/or approval. Subsequent to initial review and approval, the responsible local IRB/ethics committees (ECs) will review the protocol annually. The investigators will make safety and progress reports to the IRBs/ECs at least annually and within 3 months of study termination or completion at their site. These reports will include the total number of participants enrolled, adverse events, and summaries of each DSMC review of safety and/or efficacy meeting. Upon completion of the 28 days study treatment protocol, the participant's treatment plan can be modified in any way the participant's ophthalmologist feels fit.

Written consent will be obtained for all study procedures after explaining the patient's disease, prognosis, and treatment options, discussing the risks and benefits of participation, and addressing the patient's questions and concerns. The protocol site-specific informed consent forms in local language (Thai, Chinese, or English), participant education and recruitment materials, other requested documented, and any subsequent modifications shall also be reviewed and approved by the ethical review bodies at each respective site. Table 5 summarises the study protocol and trial registration information.

Only key study personnel will have access to identifying information of participants, while administrative forms and specimens will be de-identified.

A portion of biological samples from the second anterior chamber paracentesis (approximately 20μL of aqueous fluid) will be obtained for use in a future study of virus genome assay. A consent will be obtained and included in the main study consent form to specifically address the collection and future studies of such aqueous fluid.

The results of this trial will be presented at local and international meetings and submitted to peer-reviewed journals for publication.

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## Tables

| | Strongly disagree | Somewhat disagree | Somewhat agree | Strongly agree |
|---|---|---|---|---|
| 1. I think that oral valganciclovir is an effective medication to treat CMV anterior uveitis | 0 (0%) | 8 (11.8%) | 36 (52.9%) | 24 (35.3%) |
| 2. I think that topical ganciclovir 2% eyedrops is an effective medication to treat CMV anterior uveitis | 1 (1.5%) | 11 (16.2%) | 33 (48.5%) | 23 (33.82%) |
| 3. I think that oral valganciclovir is a safe medication to treat short term ocular inflammation related to CMV anterior uveitis | 0 (0%) | 11 (16.2%) | 36 (52.9%) | 21 (30.9%) |
| 4. I think that oral valganciclovir is a safe medication to treat long term ocular inflammation and prevent recurrences of CMV anterior uveitis | 2 (2.9%) | 20 (29.4%) | 33 (48.5%) | 13 (19.1%) |
| 5. I think that topical ganciclovir eyedrops are a safe medication to treat short term ocular inflammation related to CMV anterior uveitis | 1 (1.5%) | 8 (11.8%) | 28 (41.2%) | 31 (45.6%) |
| 6. I think that topical ganciclovir eyedrops are a safe medication to treat long term ocular inflammation and prevent recurrences of CMV anterior uveitis | 1 (1.5%) | 17 (25.0%) | 31 (45.6%) | 19 (27.9%) |
| 7. I think that there is a benefit to treating CMV with long term therapy (beyond 6 months) | 1 (1.5%) | 8 (11.8%) | 26 (38.2%) | 33 (48.5%) |

**Table 1.** Survey results regarding CMV anterior uveitis practice patterns at the Biennial CKPU meeting in Bangkok, Thailand (n=68)

| | Oral valganciclovir | Topical ganciclovir eyedrops | No treatment |
|---|---|---|---|
| 1. The medication I am most likely to use to treat CMV anterior uveitis is… | 25 (36.8%) | 39 (57.4%) | 4 (5.88%) |
| 2. If cost were not an issue my preferred medication to treat CMV anterior uveitis would be… | 39 (56.5%) | 27 (39.1%) | 1 (1.5%) |

**Table 2.** Survey results regarding preferred treatment therapy of CMV anterior uveitis at the Biennial CKPU meeting in Bangkok, Thailand (n=68)

| Exam Visit | Procedures |
|---|---|
| Pre-study Visit (Exam #0) – routine care appointment | - Clinical eye exam suggests a viral aetiology<br>- Anterior chamber paracentesis is performed to establish a viral aetiology<br>  o half of fluid will be used for in-house directed PCR for HSV, VZV, and CMV<br>  o At least 50μL of fluid will be preserved and used for quantitative CMV PCR to be conducted at a single United States laboratory in eligible participants<br>- Laboratory screening orders (CBC, chemistry panel, pregnancy test, HIV status) |
| Exam #1 (Day 1 of study) | - Review of results of in-house CMV PCR, and laboratory results<br>- Consent, enrollment, randomization to treatment arm if participants meet eligibility criteria<br>- Treatment initiation<br>- Clinical eye exam (in the following order, VA, slit lamp exam of anterior segment, IOP)<br>- Endothelial cell morphology and density using specular microscopy or confocal microscopy |
| Exam #2 (Day 7 of study) | - Clinical eye exam (in the following order, VA, slit lamp exam of anterior segment, IOP)<br>- AC paracentesis #2<br>- Laboratory monitoring orders (CBC, Cr, BUN) |
| Exam #3 (Day 28 of study) | - Clinical eye examination (in the following order, VA, slit lamp exam of anterior segment, IOP)<br>- Endothelial cell density using specular microscopy or confocal microscopy<br>- Laboratory monitoring orders (CBC, Cr, BUN) |

**Table 3.** Timeline of Major Study Procedures. AC: anterior chamber; CBC: complete blood count; CMV: cytomegalovirus; Cr: creatinine; BUN: blood urea nitrogen; HSV: herpes simplex virus; IOP: intraocular pressure; PCR: polymerase chain reaction; VA: visual acuity; VZV: varicella-zoster virus

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| • CMV positivity by directed PCR from an anterior chamber paracentesis specimen conducted at any time in the past<br>• Active anterior uveitis using Standardization of Uveitis Nomenclature (SUN) Working group with clinical impression of CMV as the etiologic agent<br>  ○ ≥1+ anterior chamber cell AND/OR<br>  ○ Other signs consistent with active inflammation, such as elevated intraocular pressure (IOP), corneal oedema, and/or active keratic precipitates (KPs)<br>• Participant willingness to use an acceptable method of contraception during the study period (i.e. pharmacologic, barrier methods, or abstinence). | • Participants <20 years of age<br>• Inactive anterior uveitis<br>• Active intermediate or posterior inflammation (involvement of vitreous, choroid, or retina)<br>• Participants who have received antiviral therapy <14 days prior to enrollment<br>• Participants who have received periocular or intraocular corticosteroid injection < 8 weeks prior to enrollment<br>• Current use of oral corticosteroids<br>• Immunocompromised participants (primary or secondary immunodeficiency disorders)<br>• Prior immunosuppressive therapy in the past 3 months<br>• Directed PCR testing positive for herpes simplex virus (HSV) or varicella zoster virus (VZV)<br>• Plans to conceive during the study period, pregnant or breast-feeding mothers (blood or urine pregnancy test for all females of child-bearing age is mandatory within 4 weeks prior to enrollment)<br>• Complete blood count with white blood cell, absolute neutrophil, or platelet count lower than the lower limit of reference laboratory normal<br>• Blood urea nitrogen or creatinine above the upper limit of reference laboratory normal<br>• Recent ocular surgery within the past 30 days or planned surgery within the next 45 days<br>• Systemic autoimmune disease or ocular condition (besides anterior uveitis) anticipated to dictate or alter treatment course |

**Table 4.** Study participant inclusion and exclusion criteria. CMV: cytomegalovirus; HSV: herpes simplex virus; PCR: polymerase chain reaction; VZV: varicella-zoster virus

| Data Category | Information |
|---|---|
| Primary registry and trial identifying number | ClinicalTrials.gov<br>NCT03576898 |
| Date of registration in primary registry | July 3, 2018 |
| Secondary identifying numbers | |
| Source of monetary or material support | Francis I. Proctor Foundation, University of California, San Francisco |
| Contact for queries | John A. Gonzales, MD (john.gonzales@ucsf.edu) |
| Title | Systemic and Topical Antiviral Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes |
| Countries of recruitment | Thailand, Taiwan, USA |
| Health condition or problem studied | Cytomegalovirus anterior uveitis treatment |
| Intervention(s) | Intervention: treating with 900mg BID of oral valganciclovir, or topical 2% ganciclovir solution q2h for a duration of 28 days<br>Control: placebo tablets and eyedrops |
| Key eligibility criteria | CMV positive anterior chamber paracentesis, active signs of anterior uveitis, no history of immunodeficiency, normal laboratory values for CBC, and renal function |
| Study type | Competitive block randomised, placebo controlled clinical trial |
| Date of first enrolment | N/A |
| Target sample size | 99, 33 per treatment arm |
| Primary outcome | Change in log transformed viral load |
| Key secondary outcomes | Objective and subjective time to achieve clinical quiescence, and the relationship of topical steroid strength and dosing on initial anterior chamber paracentesis quantitative viral load for CMV |
| Project timeline | July 2019 - August 2021 |

**Table 5.** Trial registration data and protocol summary

**Appendix 1 – Informed Consent Document**

**UNIVERSITY OF CALIFORNIA, SAN FRANCISCO**
**CONSENT TO PARTICIPATE IN A RESEARCH STUDY**

**Study Title: Systemic and Topical Antiviral Control of CMV Anterior uveitis: Treatment Outcomes (STACCATO)**

| | |
|---|---|
| Research Project Director: | John A. Gonzales, M.D., Uveitis Specialist and Associate Professor of Ophthalmology at UCSF, Room S334, 513 Parnassus Ave, San Francisco, CA.<br>Phone: 415-502-2664; e-mail: john.gonzales@ucsf.edu |

| | |
|---|---|
| Study Coordinator: | Jaskirat Takhar<br>Phone: 415-502-2690; e-mail: jaskirat.takhar@ucsf.edu |

This is a clinical research study. Your study doctor, John Gonzales, M.D., from the UCSF Proctor Foundation, will explain the study to you.

Research studies include only people who choose to take part. Please take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask your study doctor.

You are being asked to take part in this study because you are suspected to have anterior uveitis (inflammation of the front portion of the eye) caused by the virus, cytomegalovirus (CMV).

**Why is this study being done?**

CMV anterior uveitis can cause increased pressure inside the eye, pain, cataract formation, and loss of vision. There are many treatment options, however we currently don't know which one is superior and there is no defined standard of care. The purpose of this study is to compare the most commonly used treatment options used in the management of your condition. After confirming the diagnosis, we will assign you to one of three treatment groups and compare the difference in outcomes between each group.

In addition, prior to this visit, the physician who referred you to our clinic may or may not have started you on steroid eye drops to control your inflammation. For our study we intend on giving all participants the same amount of steroid drops for the 28 day duration. We intend on investigating whether the amount of steroid given prior to you starting in this study affects the amount of virus found in the fluid from the eye.

**How many people will take part in this study?**

Overall, about 99 people will take part in this study. Approximately 33 people will be in each of the 3 treatment groups. The treatment options will be 28 days of oral antiviral therapy, 28 days of 2% antiviral eye drops, or 28 days of inactive therapy.

We expect that up to 70 people will be enrolled into this study here at UCSF. You will be randomly placed into a treatment group, and neither you nor your study doctor will know which treatment you are receiving.

**What will happen if I take part in this research study?**

**Before you can start the study:**

Pre-study visit: You will need to have the following exams, tests, and procedures to find out if you can be enrolled in the study. These exams, tests, and procedures are part of regular care for patients with suspected CMV anterior uveitis, and are routinely done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

- Initial removal of fluid from the front part of your eye (anterior chamber paracentesis): In our clinic we will anesthetise (or numb) your cornea with eye drops and carefully remove a small amount of fluid from inside the front portion of your eye using a small needle. The fluid will then be sent for laboratory testing to determine whether CMV is present in the fluid.
- Initial laboratory testing: we will order blood tests to look at your kidney function, red blood cell count, white blood cell count, and platelet count. Abnormal results will exclude you from participating in this study.
- Women of child-bearing age will undergo a blood pregnancy test, as some study medications can result in birth defects

**During the main part of the study:**

Exam #1: Approximately 7 days after completing the exams, tests, and procedures above, you will return for follow-up examination, and review of testing to determine if you can participate in this study. If eligible you will complete this form to give your consent to participate in this study. After completion, you will then be enrolled, and then "randomised" into one of the study groups described below. Randomization means that you are put into a group by chance. A computer program will place you in one of the groups. Neither you nor your doctor can choose the group you will be in. You will have an equal chance of being placed in any group. All groups will receive their assigned treatment for 28 days. You will receive both oral tablets and topical eye drops. You will not know which ones are active or inactive. You will be instructed to take 4 pills per day, 2 every morning and 2 every evening. You will also be instructed to apply 1 drop of eye solution every 2 hours while awake. You will repeat this regimen every day for all 28 days of treatment. Participants in all groups will be receiving a steroid eye drop regimen of prednisolone acetate 1% three times per day in the affected eye. It is important to note that all three treatment groups are used by practicing ophthalmologists to manage

your condition. A current standard of care has not been defined, which is the question this study hopes to answer.

- **If you are in group 1** you will receive valganciclovir pills and inactive eye drops.
- **If you are in group 2** you will receive inactive pills and topical ganciclovir 2% eye drops.
- **If you are in group 3** you will receive inactive pills and inactive eye drops.

You will also need follow-up exams and tests that are part of regular CMV anterior uveitis care:

- Two follow-up clinic visits will be scheduled at day 7 (Exam #2) and day 28 (Exam #3) of treatment. At each of these visits you will be seen by an ophthalmologist. On study day 7 (Exam #2) we will again remove fluid from your eye (a second anterior chamber paracentesis), to see if the amount of virus in the eye has decreased.
- At day 7 and day 28 blood tests will be repeated. These will be compared with baseline kidney function and blood counts. Any abnormal findings will require removal from the study.

**When you are finished with 28 days of treatment:**

Once you have finished 28 days of treatment, including your final clinic visit and evaluation, you will have completed the study. Your treating ophthalmologist will determine whether any continued treatment is necessary.

**Study location?**

All study procedures will be done at the UCSF Proctor Clinic.

**Study Chart**

| **Visits** | **What happens** |
|---|---|
| **Pre-study visit** | • Baseline fluid removal from the front of the eye using a needle<br>• Baseline blood tests<br>• Pregnancy testing for women of child-bearing age<br>• Baseline clinical exam |
| **Enrollment/ Randomization** (Day 1) | • Follow-up clinical examination<br>• Consent, Enrollment and randomization to treatment<br>• Begin treatment: 2 pills twice daily, 6 drops per day (1 every 2 hr)<br>• Corneal endothelial confocal microscopy |

| | |
|---|---|
| **Exam #2** (Day 7) | • Follow-up clinical exam<br>• Repeat removal of fluid from the eye using a needle (anterior chamber paracentesis #2)<br>• Repeat blood tests |
| **Exam #3** (Day 28) | • Follow-up clinical exam<br>• Repeat blood tests #2<br>• Corneal endothelial confocal microscopy |

**Can I stop being in the study?**

Yes. You can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop your participation safely.

It is important to tell the study doctor if you are thinking about stopping so any risks of the infection or stopping treatment can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest, if you do not follow the study rules, or if the study is stopped.

**What side effects or risks can I expect from being in the study?**

You may have side effects while in the study. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away soon after you stop taking these antiviral medications. In some cases, side effects can be serious, long lasting, or may never go away. In very rare cases, side effects may include death.

You should talk to your study doctor about any side effects you experience while taking part in the study.

**Procedural risks:**

The procedure to remove fluid from the eye (anterior chamber paracentesis) carries some risk whether they are performed in the study or as part of your routine care outside the study. The second anterior chamber paracentesis is being conducted as part of routine clinical care. It is a typically safe procedure, although some may experience pain or discomfort. To reduce discomfort, eye numbing drops will be given before the procedure is performed. A severe but extremely rare complication of this procedure includes blood accumulation in the eye (hyphema), persistent leakage of fluid from the eye (leakage of aqueous humour), infection inside the eye (endophthalmitis),

and traumatic cataract, which can affect vision. In extremely rare cases, endophthalmitis may require removal of the eye.

**Medication risks:**

| | Valganciclovir | Topical Ganciclovir |
|---|---|---|
| Rare but Serious Side Effects | - Low red blood cell count causing anemia and fatigue<br>- Low white blood cell count increasing risk of infection<br>- Low platelet count leading to risk of bleeding<br>- Kidney failure<br>- Infertility<br>- Birth defects (in pregnant women)<br>- Cancer | - Allergic reaction |
| More Common but Less Serious Side Effects | - Diarrhea<br>- Nausea<br>- Vomiting<br>- Stomach pain | - Burning sensation in eye<br>- Temporary blurred vision |
| Less common and Less Serious Side Effects | - Fever<br>- Headache<br>- Sleep disturbances | - Eye redness<br>- Eye irritation |

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

**Placebo (inactive) risks:**
If you are in the group that receives placebo, your condition will go without the active (antiviral) treatment for 28 days. Some uveitis specialists will observe their patients with CMV anterior uveitis to see if the inflammation will resolve on its own or with just steroid eye drops. In such cases, starting oral or topical antivirals is reserved for when the inflammation does not become subside after 4 weeks. The risks of delaying treatment include damage to the cornea with a decrease in vision or the development of glaucoma, which can result in vision loss. However, many uveitis specialists feel that a reasonable and safe option in CMV anterior uveitis is to monitor initially without starting oral or topical antiviral medication.

**Blood drawing (venipuncture) risks:**

Drawing blood may cause temporary discomfort from the needle stick, bruising, infection, and fainting.

**Reproductive risks:**
You should not become pregnant or father a baby while on this study because the drugs in this study can affect an unborn baby. Women should not breastfeed a baby while on this study. It is important to understand that you need to use birth control while in this study. Acceptable forms of birth control include intra-uterine (placed inside the uterus), oral (birth control pills), birth control patch placed on your skin, or barrier (i.e. condoms). Check with your study doctor about what kind of birth control methods to use and how long to use them. Some methods might not be approved for use in this study.

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

For more information about risks and side effects, ask your study doctor.

**Are there benefits to taking part in the study?**

It is possible that one of the treatment options proves to treat CMV anterior uveitis better than the others, in which case your infection will be treated more effectively. It is also possible that one of the treatment options will prove to have fewer side effects or complications, in which case you will be subjected to fewer side effects.

Additionally, we hope this study will help doctors learn more about CMV anterior uveitis, and that this information will help in the treatment of future patients with conditions like yours.

**What other choices do I have if I do not take part in this study?**

If you choose not to take part in this study, your quality of care will remain the same. The fluid in your eye will still be sampled at the first visit in order to diagnose your infection. Your treatment regimen will be chosen and initiated at your treating ophthalmologist's discretion. At UCSF the standard of care includes oral valganciclovir 900 mg given twice daily or topical ganciclovir 2% provided by a compounding pharmacy for a period of 28 days, with routine follow-up and blood tests. Occasionally, some patients are monitored with just steroid eye drops and are not started on antivirals if the inflammation does not become quiet after approximately 28 days. Additionally, patients treated outside of the study may have a repeat (or second anterior chamber paracentesis) to demonstrate that the amount of virus in their ocular fluid is decreasing. Therefore, this trial is very similar to what happens in "real life".

**How will information about me be kept confidential?**

Participation in research involves some loss of privacy. We will do our best to make sure that information about you is kept confidential, but we cannot guarantee total privacy. Some information from your medical records will be collected and used for this study. If you do not have a UCSF medical record, one will be created for you. Your signed consent form and some of your research tests will be added to your UCSF medical record. Therefore, people involved with your future care and insurance may become aware of your participation and of any information added to your medical record as a result of your participation. Study tests that are performed by research labs, and information gathered directly from you by the researchers will be part of your research records but will not be added to your medical record. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Authorised representatives from the following organizations may review your research data for the purpose of monitoring or managing the conduct of this study:

- Representatives of UCSF Proctor Foundation
- Representatives of the University of California
- Representatives of the Food and Drug Administration (FDA)

**Are there any costs to me for taking part in this study?**

Two types of procedures will be done during this study. Some are part of your standard medical care, such as the first eye fluid analysis, and others are primarily for research. You or your insurer will be billed for the standard medical care. You will be responsible for your co-pays, deductibles, and any other charges that your insurer will not pay. There is a possibility that your insurer may not cover all standard medical care costs if you are receiving medical services out of network. Any procedures done only for research will not be charged to you or your insurer. Although the second eye fluid removal is considered part of routine clinical care, the procedure and testing of fluid will be paid for at no cost to the participant.

All antiviral and inactive (placebo) therapy will be provided for you at no cost.

**Will I be paid for taking part in this study?**

You will not be paid for taking part in this study.

**What happens if I am injured because I took part in this study?**

It is important that you tell your study doctor, Dr. John Gonzales, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him at 415-502-2664.

**Treatment and Compensation for Injury:** If you are injured as a result of being in this study, the University of California will provide necessary medical

treatment. The costs of the treatment may be billed to you or your insurer just like any other medical costs, or covered by the University of California, depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Institutional Review Board at 415- 476-1814.

**What are my rights if I take part in this study?**

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

**Who pays for this study?**

Proctor Foundation/UCSF.

**Who can answer my questions about the study?**

You can talk to your study doctor about any questions, concerns, or complaints you have about this study. Contact your study doctor John Gonzales, M.D., at 415-502-2664.

If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please call the office of the Institutional Review Board at 415-476-1814.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

**CONSENT**

Please read each sentence below and think about your choice. After reading each sentence, put your initials in the "Yes" or "No" box. If you have any questions about this study, please talk to the study doctor or nurse.

No matter what you decide to do, it will not affect your care.

1. My specimens and associated data may be kept for use in research to determine the amount of virus in my sample.

| *YES* | *NO* |
|---|---|

2. Someone may contact me in the future to ask me to take part in more research.

| *YES* | *NO* |
|---|---|

3. Any leftover fluid from your anterior chamber paracentesis samples will be collected at stored at UCSF/Proctor Foundation laboratory. In the future the following tests may be performed on any leftover fluid:

- Genetic sequencing of the DNA of CMV, which means identifying the strain of virus you may have based on its genetic signature.
- The duration of specimen retention will be until the specimen is used up.
- Your name and any identifying information will not be included in this stored sample (will become an anonymous sample) to protect your confidentiality/privacy
- Only UCSF researchers will have access to the specimens and the data.
- Your specimen will not be used for commercial value or gain, and subjects will not be paid for their sample
- Should you decide to request destruction of your sample, this will not be possible as your specimen will have been made anonymous and cannot be traced back to you.
- There will be no genetic testing on your own, human DNA, only on the virus (CMV).

| *YES* | *NO* |
|---|---|

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

You will be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, you should sign below.

| | |
|---|---|
| Date | Participant's Signature for Consent |
| Date | Person Obtaining Consent |

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

## Manual of Operations & Procedures

## (MOP)

February 27, 2019
Version 0.9

**Table of Contents**

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**Background**

Recurrent anterior uveitis in immunocompetent individuals can be caused by members of the herpes virus group, including cytomegalovirus (CMV). Repeated or chronic bouts of CMV intraocular inflammation can be associated with ocular hypertension, glaucoma, pain, vision reduction or blindness. CMV anterior uveitis is commonly misdiagnosed as a non-infectious anterior uveitis and treated as such, which can beget further complications.[1-5] Definitive diagnosis requires directed polymerase chain reaction (PCR) testing. While antiviral therapy exists for CMV, identifying the appropriate therapy has been challenging because no randomized trials comparing routes of therapy (particularly oral or topical) have been performed. Currently, CMV anterior uveitis is typically treated with oral valganciclovir in the United States but carries the risk of serious systemic side effects that necessitate laboratory monitoring. There is evidence that suggests topical ganciclovir can be used to treat and prevent recurrences of CMV anterior uveitis, though the appropriate concentration is not well defined.[4,6,7] Topical ganciclovir is attractive because it does not require laboratory monitoring, though a unique side effect profile that includes corneal epitheliopathy and conjunctivitis may preclude long-term use. While anterior chamber paracentesis with polymerase chain reaction (PCR) testing demonstrates CMV during an initial flare of inflammation, it is unknown whether repeated recurrences of inflammation are mediated by viral reinfection and replication in the anterior chamber or if a sterile immune response is at play. Consequently, patients may be submitted to many years of oral or topical antiviral therapy. This strategy poses challenges without proper evaluation of the multiple treatment and long-term management approaches. Further studies are needed to elucidate the most appropriate antiviral therapies that balance efficacy and toxicity while treating CMV anterior uveitis.

## 1. Introduction to trial

Here we propose a double-masked randomized controlled 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). This study will provide valuable information concerning the treatment of CMV anterior uveitis with oral and topical medications, and side-effect profile of treatment. The information obtained from this study will help inform future larger clinical trials in CMV anterior uveitis.

### 1.1. Research question

Is topical or oral antiviral therapy better at reducing viral load in CMV anterior uveitis?

### 1.2. Specific Aim

1. To assess reduction in log transformed CMV viral load at 7 days after randomization to treatment with oral or topical antiviral. *We hypothesize that the greatest reduction in viral load will be with oral valganciclovir 900 mg PO BID compared to topical ganciclovir 2% six times daily and placebo.*

2. To assess the proportion of participants achieving clinical quiescence after randomization to 28 days of treatment. *We hypothesize that the proportion of participants that have achieved clinical quiescence at 28 days will be higher with oral valganciclovir compared to topical ganciclovir 2% and placebo.*

3. To evaluate the effect of topical corticosteroid started prior to eligibility screening on diagnostic yield of PCR. *We hypothesize that the yield of virus will be positively correlated with increased frequency of use of topical corticosteroids.*

## 2. Trial design

### 2.1. Design summary

**Study type**

- Randomized, double-masked comparative effectiveness trial
- Competitive block randomization by site:
  Sites: Khon Kaen University, and Francis I. Proctor Foundation at the University of California, San Francisco
- Sample size: 99 participants, 33 participants per arm

**Treatment arms**

- Oral valganciclovir and placebo eyedrops (BSS)
- Topical ganciclovir 2% and placebo tablets
- Placebo eyedrops (BSS) and placebo tablets

**Treatment timeline**

- Study Treatment
  - Day 1 to day 28

**Outcomes**

- **Primary outcome:**
  - Reduction in log transformed viral load as measured by quantitative PCR at 7 days of treatment **(1)**
- **Secondary outcomes:**
  - Percent achieving clinical quiescence by 28 days of treatment **(2)**
  - Effect of topical corticosteroid used prior to AC paracentesis #1 on PCR-determined viral load **(3)**

### 2.2. Study timeline

**Table 1: Timeline for study completion**

| Date | Planned Activity |
|---|---|
| September 2016 through January 2019 | Pre-study activities: refine/finalize MOP, IRB, conduct certification of sites and study personnel, earliest project start date |
| March 2019 through March 2021 | Enrollment and follow-up |
| April 2021 through June 2021 | Lock database, analyze, publish, and disseminate results |

## 2.3. Eligibility

### 2.3.1. Anatomical location of inflammation

All participants must currently meet criteria for anterior uveitis, as defined by the Standardization of Uveitis Nomenclature (SUN) Working Group. Participants should not have signs of active inflammation at any other primary sites.

The Standardization of Uveitis Nomenclature

| Type | Primary Site of Inflammation | Includes |
|---|---|---|
| Anterior Uveitis | Anterior Chamber | Iritis<br>Iridocyclitis<br>Anterior cyclitis |
| Intermediate Uveitis | Vitreous | Pars planitis<br>Posterior cyclitis<br>Hyalitis |
| Posterior Uveitis | Retina or Choroid | Focal, multifocal, or Diffuse choroiditis<br>Chorioretinitis<br>Retinochoroiditis<br>Retinitis<br>Neuroretinitis |
| Panuveitis | Anterior Chamber<br>Vitreous<br>Retina or Choroid | |

*Adapted from the SUN Working Group Anatomic Classification of Uveitis[8]

### 2.3.2. Criteria for active inflammation

Declaring active anterior uveitis is a subjective decision, especially in the case of CMV anterior uveitis where frank AC cell is not always present during flares. Therefore, eligibility uses the wording "**clinical impression consistent with CMV anterior uveitis**", at the discretion of the study ophthalmologist. Ideally, all study ophthalmologists will use similar criteria for declaring active inflammation.

For this reason, recommended criteria are as follows:

- ≥ 1+ anterior chamber cell **AND/OR**
- Other signs consistent with active inflammation, including increased intraocular pressure (IOP), corneal edema, active keratic precipitates (KP)

### 2.3.3. Criteria for phenotype classification

Uveitis experts have reported observation of a phenotypic spectrum in CMV anterior uveitis. This spectrum ranges from a more acute/recurrent phenotype, characterized by acute and recurrent bouts of inflammation, to a phenotype that features a more stable, chronic, smoldering inflammation. This classification is subjective and made at the discretion of the ophthalmologist.

However, it is felt that active viral infection is represented in both phenotypes so should not affect our statistical analysis. All study ophthalmologists will use similar criteria for declaring active inflammation.

For this reason, recommended criteria for identifying phenotype are as follows:

- **Acute/Recurrent phenotype:** First episode of inflammation or clear ocular history of acute inflammatory episodes followed by quiescence.
- **Chronic phenotype:** Ocular history features consistent anterior segment inflammation in the form of AC cell, continuously active keratic precipitates, or elevated IOP.

### 2.3.4. Inclusion Criteria

All the following criteria must be met at enrollment:

- Clinical impression consistent with CMV anterior uveitis (see 2.3.2 for details)
- Directed PCR positive for CMV **OR** previous PCR-proven CMV anterior uveitis
- Active anterior uveitis
- Willingness to use an acceptable method of contraception during the study period (i.e. pharmacologics, devices, barrier methods) or abstinence.

### 2.3.5. Exclusion criteria

Any one excludes patient:

- Participants <20 years of age
- Inactive anterior uveitis
- Intermediate or posterior inflammation (involvement of vitreous, choroid, or retina)
- Received antiviral therapy <14 days prior to enrollment
- Received periocular or intraocular corticosteroid injection < 8 weeks prior to enrollment
- Currently taking oral corticosteroids
- Immunocompromised (primary or secondary immunosuppressive disorders)
- Prior immunosuppressive therapy in the past 3 months
- Directed PCR positive for herpes simplex virus (HSV) or varicella zoster virus (VZV)
- Planning to conceive during the study period, pregnant or breast-feeding (blood or urine pregnancy test for all females of child-bearing age is mandatory within 4 weeks prior to enrollment)
- Complete blood count with white blood cell, absolute neutrophil, or platelet count lower than the lower limit of reference laboratory normal
- BUN or Cr 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

Beyond the tests listed above, the remainder of the work-up is at the discretion of the investigator and should be tailored to the clinical situation. Distinguishing between infectious and non-infectious uveitis is part of standard of care and should be dictated by the patient's clinical exam. If there is any clinical suspicion of non-infectious etiology, other tests should be considered at the

investigator's discretion.

## 2.4. Randomization/Masking

### 2.4.1. Randomization

Competitive block randomization of the participants will ensure that an equal number of participants are randomized to each study arm at each site. It does not mean that all sites will enroll the same number of participants. In fact, it is possible for a site to enroll many more participants than other sites. Patient phenotype or site location will not affect the treatment protocol. Randomization lists will be created by the coordinating center clinical coordinator (Jaskirat Takhar) and accessed online via REDCap database.

The study coordinators (listed below) will be aware of patient treatment assignment and will have a copy of the patient ID list for their site; however, they will not know future treatment assignments prior to randomizing participants. After consenting participants for enrollment and confirming all eligibility requirements have been met, study coordinators will log into REDCap and perform the randomization. Study coordinators will then give participants their assigned treatment.

The following study coordinators will have access to REDCap to perform randomizations but will not know the treatment assignments prior to randomizing participants:

- Dr. Sarah Lopez, Study Coordinator, University of California, San Francisco
- Ms. Ruedee Kumklee, Study Coordinator, Khon Kaen University Hospital, Khon Kaen, Thailand

Emergency Contact Personnel*

Emergency contacts designated above will log into REDCap to consult the list of randomized participants in case of an emergency in which unmasking is necessary for patient safety and Dr. Gerami Seitzman, medical monitor, cannot be reached.

### 2.4.2. Masking

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. This means that participants will receive either active pills with placebo drops, placebo pills with active drops, or placebo pills and placebo drops. Participants will not know which medications are active. Participants will be briefed before follow-up visits and instructed not to disclose information to the ophthalmologist concerning their treatment (i.e. stinging of the eyes with drop application, etc). This will help to prevent bias in study outcomes.

The UCSF Pharmacy's investigational drugs department will purchase and supply valganciclovir tablets, placebo tablets, basic saline solution, ganciclovir sodium and sterile water for compounding.

The UCSF pharmacy will be responsible for purchasing and distributing bottles of the medications labeled with NDC/lot number and expiration dates to Proctor Foundation, who will add dosing instructions and distribute medication to the Thailand sites where respective pharmacies will distribute the medication to study participants.

Study ID labels will be provided to each site on separate label sheets. Each site's study coordinator will place the study ID label on the bottle after randomization, before dispensing the

drug to the patient. The format of the study IDs is as follows: the first two characters will be standardized as "ST". The first digit following "ST" will represent the study site: "1" represents patients at UCSF, "3" represents patients at Khon Kaen University, "5" represents patients at King Chulalongkorn University, and "7" represents patients at National Taiwan University. The subsequent 2 digits represent the patient number at that site. There will be one final alphabetic character which is randomly generated as another level of data entry adjudication to limit erroneous data entry by patient ID. These digits will be specific to subjects enrolled at each site, and are independent from other sites (each site will have a patient designated "01").

**ST300C**

ST = standard code for STACCATO study patient
3 = site (in this instance Khon Kaen Univeristy)
00 = patient # (patients numbers start from 00 and go up by integers)
C = random alphabet character

After enrollment and randomization, the patient ID labels will be placed on the medication bottles by the study coordinator or pharmacist, depending on site-specific requirements. At no time will the study doctor handle labeling or distribution of the study medication. Study coordinators will record the study ID, drug, date dispensed, bottle number, lot number, and expiration date of each bottle dispensed during the trial in the drug accountability log.

Because there may be differences in appearance between active pills and placebo pills and between active drops and placebo drops, it is important that steps are taken to avoid study doctors discovering patient assignment. Not only will the study doctors have no part in handling the medication bottles, all participants will be given dark bags to place and keep their bottles in throughout the trial. They will be instructed to bring their medication to their visits enclosed in the bags, and an extra bag will be kept at the front desk of the clinics in case they forget. This will minimize the chances of the study doctor seeing the medications. Additionally, participants will meet with the study coordinator first, before seeing their study doctor at follow-up visits. The study coordinator will review the participants' medication calendars with them and keep the calendars and study bottles in his/her office for the entire patient visit. Before bringing participants to the study doctor, the study coordinator will remind them not to reveal their medications to their study doctor.

Clinical examiners will not be given any information on medication assignment. If personnel are unmasked at any time, a protocol deviation form must be completed, scanned and emailed to both the Medical Monitor (gerami.seitzman@ucsf.edu), Principal Investigator (john.gonzales@ucsf.edu) and Coordinating Center Manager (jaskirat.takhar@ucsf.edu). Note that these participants should still continue in the trial.

## 2.5. Outcomes

### 2.5.1. Reduction in viral load

Reduction in log transformed viral load will be measured by CMV-directed quantitative PCR of pre-enrollment aqueous, followed by measurements at day 7 of treatment. This procedure will be performed by Viracor.

#### 2.5.2. Proportion of participants achieving of clinical quiescence

Percent of participants that have achieved clinical quiescence by 28 days of treatment. Please refer to section 5.1.3 for criteria and guidelines.

#### 2.5.3 Topical corticosteroid effect on viral yield

At time of screening, the use of topical corticosteroid before presentation will be recorded for each participant and analyzed to determine whether corticosteroid use prior to Exam #0 had an effect on quantitative PCR viral load from aqueous fluid removed at Exam #0.

### 2.6. Protocol Deviation

In rare cases, study ophthalmologists may determine that a deviation from protocol is necessary. This is at the discretion of the treating ophthalmologist, and could be undertaken for a variety of reasons, some of which are described subsequently. If a study ophthalmologist decides to deviate from the study protocol for any reason, they should complete a protocol deviation form and send it to Jaskirat Takhar (jaskirat.takhar@ucsf.edu) and Dr. Gonzales (john.gonzales@ucsf.edu). **Subjects that experience deviations in treatment protocol will continue in their initially assigned treatment group and their data will be analyzed accordingly.** Examples of instances where a protocol deviation may be necessary are as follows:

#### 2.6.1. Rescue Treatment

Study ophthalmologists may decide that continuing a participant's randomized treatment is futile. In cases of severe, persistent, uncontrolled inflammation that appears non-responsive to the current treatment, a switch to another medication may be deemed necessary by the treating ophthalmologist. If this occurs, a protocol deviation form must be completed. We believe this will be a rare occurrence, and should only occur if treatment with study medication seems completely unlikely to be able to control the inflammation. Since the medications may take multiple days to fully take effect, an increase in inflammation early on in the trial is not an indication for protocol deviation.

#### 2.6.2. Treatment discontinuation due to intolerability

Participants who experience severe symptoms thought to be related to the drug and are unable or unwilling to continue their medication due to symptoms may have their treatment discontinued. The protocol deviation form must be filled out.

#### 2.6.3. Treatment discontinuation due to safety

Participants who experience abnormal lab results meeting the designated threshold of a serious adverse event (SAE), will immediately stop taking their medication. Protocol deviation form must be filled out, and the severity of their adverse event will be documented for further analysis. Continued treatment will be at the study ophthalmologist's discretion.

Participants who experience abnormal lab results meeting the designated threshold of a non-serious adverse event will require a dose-reduction. The protocol deviation form must be filled out, and the severity of the participant's adverse event will be documented for further analysis.

The Medical Monitor will need to evaluate all situations of SAE. This will require the investigator notifying the Medical Monitor about the event, and the Medical Monitor

contacting the investigator regarding the situation. The Medical Monitor will determine whether the event is a true SAE and whether it 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 evalulation by the Medical Monitor).

Please refer to section 6 for specifics regarding adverse events, follow-up, and dose-reductions.

### 2.7. Dropouts, Non-Compliant Participants

#### 2.7.1. Patient dropouts

Participants will be considered to have dropped out from the study only if they **declare they are no longer interested in further participation and not willing to return for any study visits, or are deceased**. If participants are not willing to return for any study visits, no further information will be collected on those participants. We anticipate that a few participants may drop out due to unwillingness. In case of patient dropout, the patient dropout form should be filled out.

In the unlikely event of subject death, the subject will be removed from the remainder of the study. If the death is considered to be related to the study drug, the patient will be counted as a treatment failure. If death is not related to the study drug, the patient will be counted as a dropout. This would be at the discretion of the Medical Monitor.

#### 2.7.2. Non-compliant Participants

Participants who stop the study treatment for reasons other than efficacy, tolerability and/or safety (i.e. fear of potential side effects), or miss study visits and do not respond to contact, are considered non-compliant participants. These participants will continue their study treatment but their non-compliance will be noted in the data.

## 3. Treatment plan

### 3.1. Medications

All participants will receive both tablets and topical eye drops. Participants will be randomized to receive either oral valganciclovir tablets and placebo eye drops, ganciclovir 2% eye drops and placebo tablets, or placebo eye drops and placebo tablets. Dosing will be 4 tablets daily (900 mg PO BID) for oral medication and one drop every 2 waking hours (6 drops per day) for topical. **All study ophthalmologists and participants will be masked to treatment assignment.** For groups and dosing schedules, see Table 4.

To maintain masking, study coordinators will review patient side effects with the patient at each visit, prior to meeting with the physician. If the patient is experiencing intolerable side effects, the study coordinator and patient will speak with the study doctor concerning discontinuation of treatment.

**Table 4: Study groups, treatment, & dosing**

| | Days 0-28 | |
|---|---|---|
| | **Treatment** | **Dosing** |
| **Group 1** | **Valganciclovir tablets** | 900 mg PO BID (4 tablets daily) |
| | Placebo drops | 6 drops daily (~ q2h) |

| | | |
|---|---|---|
| **Group 2** | **Topical ganciclovir 2%**<br>Placebo tablets | 6 drops daily (~ q2h)<br>2 tablets PO BID (4 tablets daily) |
| **Group 3** | Placebo drops<br>Placebo tablets | 6 drops daily (~ q2h)<br>2 tablets PO BID (4 tablets daily) |

All individuals handling study drugs, including study participants and/or family members/others caring for and assisting study participants with their medications outside of the hospital/clinic, will be educated on the Safe Handling of Hazardous Medications guidelines outlined in the National Institute for Occupational Safety and Health manual, Preventing Occupational Exposures to Antineoplastic and Other Hazardous Drugs in Health Care Settings.

The National Institute for Occupational Safety and Health manual can be found at: http://www.cdc.gov/niosh/docs/2004-165/pdfs/2004-165.pdf

### 3.1.1. Tablets

**Packaging:** Oral valganciclovir tablets procured for this study will be packaged in identical blank bottles and labeled with study ID, bottle ID/drug code, and dosing instructions for the medication.

**Dosing:** 4 tablets daily split into two doses, one dose of 2 tablets in the morning and one dose of 2 tablets in the evening. This will total 1800 mg PO daily. Participants will be instructed to take their medication with food.

### 3.1.2. Eye drops

**Packaging:** All topical solutions will be packaged into identical bottles and labeled with study ID, drug code, packaging date, date dispensed, expiration date, and dosing instructions for eyedrop placement.

**Dosing:** One drop of topical solution every 2 hours while awake. This will total approximately 6 drops per day. Participants will be instructed to remove contact lenses before applying drops.

If there are tolerability issues such that the patient is unwilling to continue topical and/or oral therapy, treatment failure due to intolerability should be declared. If at any point treatment failure is declared due to tolerability, the patient should be treated according to best medical judgment of the treating ophthalmologist.

### 3.1.3. Topical corticosteroids

It is standard of care to prescribe topical corticosteroids to reduce the severity of intraocular inflammation in participants with anterior uveitis. Therefore, all participants will be prescribed topical prednisolone acetate 1% by the study ophthalmologist and instructed to apply one drop TID (at every other study drop application).

### 3.2. Packaging and distribution of study medications

Generic valganciclovir tablets will be purchased from UCSF's pharmacy and distributed to study sites. Jaskirat Takhar will be responsible for this distribution.

Lyophilized ganciclovir sodium powder will be purchased from UCSF's pharmacy. These bottles of powder will then be shipped by UCSF's Proctor Foundation to individual study sites. Respective study site pharmacies will then mix the powder with sterile water to make topical ganciclovir 2%.

Pre-packaged placebo sterile saline will be provided by the study manager.

All study medications, labeled with the appropriate dosing instructions, will be distributed by the study coordinator at each study visit. After randomization, before the drug is dispensed, the study coordinator will place the Study ID label on the bottles.

If at any time the patient fails to bring in their bottles, the study coordinator should refer to the randomization list to verify patient assignment. This will ensure that the patient is given the correct medications corresponding to their assignment.

### 3.3. Adherence to the treatment plan

Participants will be given a treatment diary in the form of a weekly calendar on which they will be asked to record the medications they are taking as well as reasons for missed doses. They will be asked to bring the calendars with them to each visit. Adherence will be monitored by the study coordinator through review of the calendars, and will be recorded on the Medication Log in each patient file. Pill counting will also be done for a more objective measure of adherence. If the patient fails to bring in their calendar, the study coordinator should ask the patient to estimate the number of doses they have missed.

#### 3.3.1. Missed Doses

The following instructions will be given to the participants if they miss scheduled doses of their medication:

**Tablets:** If a patient misses their scheduled dose they should skip that dose and continue with their next scheduled dose.

**Eye drops:** If the patient misses a dose, they should continue with the regular dosing schedule.

### 3.4. Other adjunctive treatments/procedures

Study ophthalmologists may use other topical adjunctive medications such as intraocular pressure (IOP) lowering medications and cycloplegic agents. These should be recorded on the Clinical Eye Exam form at each visit. The protocol does not allow for intravenous therapy, additional antivirals, or surgery during the study period. If such therapies are deemed necessary, the Protocol Deviation form should be completed and sent to the Medical Monitor, Principal Investigator, and Coordinating Center Manager.

## 4. Study Timeline and Procedures

### 4.1. Pre-study Visit (Examination #0 and AC paracentesis)

Per standard clinical care, participants suspected of having a herpetic etiology as a cause of their anterior uveitis will undergo routine clinical examination, and anterior chamber paracentesis. Baseline clinical eye exam and other baseline assessments should be collected (CBC, BUN, creatinine, urine or serum pregnancy test). Due to delay in laboratory measurements, specifically viral PCR panel, CBC, BUN, and Cr results, potential subjects are unable be consented, enrolled, or randomized at this visit. All patients at this point, however will be commenced on prednisolone acetate 1% TID in the affected eye, which is considered within the scope of standard of care.

#### 4.1.1. Procedures for Exam #0

- Medical history
- Clinical eye exam (including IOP, visual acuity, keratic precipitate location and morphology, and AC cell and flare grading)
- Anterior chamber paracentesis
- Laboratory screening orders (CBC, Cr, pregnancy testing, HIV status)

#### 4.1.2. Specimens for Exam #0

The following specimens will be collected for laboratory measurements *at Exam #0:*

- Anterior chamber aqueous fluid for CMV quantitative PCR
    - Aqueous humor will be divided into two parts, half 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 Viracor Laboratory.

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 (Creatinine, BUN)
- Blood or urine pregnancy test (all female participants of child-bearing age)
- Blood for HIV testing (unless HIV status is known)

#### 4.1.3. Forms for Exam #0

The following forms will be filled out as part of the Exam #0 visit:

- Patient Consent Forms for AC paracentesis

### 4.2. Consent, Enrollment, Randomization, Clinical Examination #1 (Day #1 of trial)

Consent, enrollment, and randomization to treatment assignment takes place 7 days after Exam #0. This process only occurs in cases where eligibility/screening criteria were met at Exam #0, and the patient meets all laboratory requirements (PCR criteria, no abnormal CBC, Cr, or positive pregnancy results).

#### 4.2.1. Consent

Procedures for obtaining consent include explaining the patient's disease, prognosis, and treatment options, discussing the risks and benefits of participation, and addressing the patient's questions and concerns. After explaining the nature of the study and the rights and responsibilities of the patient, the ophthalmologist and/or the study coordinator will obtain written consent from the subject. The subject is assured that participation in this study is voluntary and he/she can withdraw at any time if he/she feels uncomfortable.

#### 4.2.2. Enrollment, Randomization

Study coordinators should adhere to the following protocol for enrolling and randomizing subjects at this visit.

- After CMV status is confirmed positive and laboratory testing does not reveal any abnormal results, the study coordinator will review and sign the consent forms with the patient, enroll, and randomize the patient using RedCap, the study site coordinator will assign the subject a unique Patient ID (derived from the list provided by coordinating center manager, Jaskirat Takhar) as well as the randomization assignment. All of these steps should be done without observation by the study physician.
- The study coordinator will take note of the treatment assigned by REDCap, then label the appropriate study medications with the assigned Patient ID, dispense the assigned treatment to the patient, and review dosing instructions with the patient. Participants will continue prednisolone acetate 1% TID in the affected eye.
- The study coordinator will provide the subject with the medication and will be instructed to begin treatment. This handoff of medication should not involve the study ophthalmologist.

#### 4.2.3. Procedures for Exam #1

Required study forms include the following:

- Clinical eye exam in the following order
    - Visual Acuity Assessment
    - Slit lamp examination of the anterior segment and intraocular pressure
    - Anterior chamber paracentesis
    - Endothelial cell morphology and density using specular microscopy or confocal microscopy, if available (details in section 5)

#### 4.2.4. Specimens

No specimens will be collected at Exam #1.

#### 4.2.5. Forms for Exam #1

The following forms will be filled out as part of the Exam #1 visit:

- Eligibility/screening form
- Patient Consent Forms for clinical trial
- Baseline History Form
- Clinical Eye Exam Form

### 4.3. Examination #2 (Day 7 of clinical trial)

#### 4.3.1. Study Masking Procedure

- The patient comes for the study visit and meets with the study coordinator before seeing their study doctor.
- 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*)

*If the patient is experiencing intolerable side effects, the study coordinator and patient will speak to the doctor about the possibility of discontinuation of treatment.

#### 4.3.2. Procedures for Exam #2

Required study procedures include the following:

- Clinical eye exam in the following order
    - Visual Acuity Assessment
    - Slit lamp examination of the anterior segment and intraocular pressure
    - Anterior chamber paracentesis
- Laboratory monitoring orders (CBC, Cr, BUN)

After completion of the study visit, the patient will meet with the coordinator, review scheduling for the next study visit, and retrieve their medication.

**It is essential that visual acuity measurements, anterior segment eye exams and intra-ocular pressure are completed *before* dilation of the eyes. Dilation of the eyes, however, is not required for this study, but may be performed if deemed necessary by the study ophthalmologist.

#### 4.3.3. Specimens

The following specimens will be collected for laboratory measurements at Exam #2 (Day 7):

- Anterior chamber aqueous fluid for CMV quantitative PCR
- Blood for complete blood count (CBC with differential) and chemistry (Cr, BUN)

#### 4.3.4. Forms for data collection

The following forms will be filled out/reviewed as part of Exam #2:

- Clinical Eye Exam Form
- Medication Log (to be completed by patient)
- *Serious Adverse Event Narrative (if applicable)*
- *Patient Dropout Form (if applicable)*
- *Protocol Deviation Form (if applicable)*

For Exam #2, all the above procedures and forms should be completed for all participants, regardless of whether they are taking the medication or compliant with study visits. Participants will be instructed to continue to use prednisolone acetate 1% TID in the affected eye.

### 4.4. Examination #3 (Day 28 of clinical trial)

#### 4.4.1. Study Masking Procedure

- The patient comes for the study visit and meets with the study coordinator before seeing their study doctor.
- 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*

*If the patient is experiencing intolerable side effects, the study coordinator and patient will speak to the doctor about the possibility of discontinuation of treatment.

#### 4.4.2. Procedures for Exam #3

Required study procedures include the following:

- Clinical eye exam in the following order
    - Visual Acuity Assessment
    - Slit lamp examination of the front of the eye and intraocular pressure
    - Endothelial cell density using specular microscopy or confocal microscopy, if available (details in section 5)

**It is essential that visual acuity measurements, anterior segment eye exams and intraocular pressure are completed *before* dilation of the eyes. Dilation of the eyes, however, is not required for this study, but may be performed if deemed necessary by the study ophthalmologist.

#### 4.4.3. Specimens

Blood for complete blood count (CBC with differential) and chemistry (Cr, BUN).

#### 4.4.4. Forms for data collection

The following forms will be filled out/reviewed as part of Exam #3:

- Clinical Eye Exam Form
- Medication Log (to be completed by patient)
- *Serious Adverse Event Narrative (if applicable)*
- *Patient Dropout Form (if applicable)*
- *Protocol Deviation Form (if applicable)*

For Exam #3, all the above procedures and forms should be completed for all participants, regardless of whether they are taking the medication or compliant with study visits.

### 4.5. Non-study visits

Despite the regular follow-up outlined in the visit schedules above, it is anticipated that some participants may have complications or issues that require additional visits. These additional visits may not fall within the study visit window. It is possible that the circumstances leading to such

visits may result in changes to therapy, or a deviation in protocol and a need for alternative treatment. Required data collection during these non-study visits is limited to adverse event reporting and declaration of protocol deviation if it occurs.

#### 4.5.1. Study Procedures

Clinical eye exam

#### 4.5.2. Specimens

No specimens are required during non-study visits.

#### 4.5.3. Forms for data collection

The following forms will be filled out at non-study visits *only if treatment failure is declared.* Every attempt should be made to acquire information for all other forms. However, if this is not possible, at the minimum the following must be completed:

- *Protocol Deviation Form (if applicable)*
- *Clinical Eye Exam Form (if deviating from protocol)*
- *Serious Adverse Event Narrative Form (if applicable)*

### 4.6. Study Visit Summary

#### 4.6.1. Visit Schedule

**Table 9: Visit Schedule**

| Visit | Timing | Window | Study Procedures |
|---|---|---|---|
| Exam #0 - Pre-Study | N/A | N/A | AC paracentesis #1 |
| Enroll/Randomize/Exam #1 | Day 1 | ±2 days | Clinical features, Treatment initiation |
| Exam #2 | Day 7 | ±2 days | AC paracentesis #2, clinical exam |
| Exam #3 | Day 28 | ±2 days | Clinical exam, end of study |

#### 4.6.2. Task/Form Schedule

| Tasks/Forms | Visit | | | | |
|---|---|---|---|---|---|
| | Exam #0 | Enroll/ Randomize Exam #1 | Exam #2 | Exam #3 | Non-study visit |
| Eligibility/ Screening Form | X | Finish | | | |
| Consent/HIPAA | | X | | | |
| Baseline History Form | | X | | | |
| Clinical Eye Exam Form | | X | X | X | (x) |
| RedCap patient randomization | | X | | | |
| Treatment initiation | | X | | | |
| Laboratory orders (CBC, Cr, pregnancy test) | X | | X | X | |
| SAE/side effect evaluation | | | X | X | (x) |
| Calendar/ medication log*** | | | X | X | |
| Patient Dropout | (x) | (x) | (x) | (x) | (x) |
| Protocol Deviation Form | (x) | (x) | (x) | (x) | (x) |
| Adverse Event Narrative/Log | (x) | (x) | (x) | (x) | (x) |

**Note: protocol deviation can occur at a scheduled study visit or at a non-study visit; a protocol deviation form should be filled out in either scenario. All (x) are only to be filled out if applicable.**
*****Participants will complete this form and bring to the study coordinator for review**

### 4.7. – Data Handling and Data Entry

After completion of each study visit, study coordinators at all sites will scan data collection forms and send them electronically in PDF format to Jaskirat Takhar (jaskirat.takhar@ucsf.edu). Hard copies of the forms should be kept in a secure location at each site. See section 7 for further details on the data entry process.

## 5. Protocol for Study examinations and Procedures

### 5.1. Clinical assessment

#### 5.1.1. Anterior chamber inflammation

Slit lamp examination is an important part of our study: assessment of anterior chamber cell is an

important outcome. All of our study physicians are fellowship-trained uveitis specialists capable of measuring this variable.

The study ophthalmologist at each site will be required to perform an eye examination at each study visit. This examination should be similar to that performed in the routine care of uveitis participants. Several components will be assessed in detail per study protocol. The grading schemes to be used for these assessments are adapted from the Standardization of Uveitis Nomenclature (SUN) Working Group.[8]

**Anterior Chamber (AC) Cells**

**SUN Working Group Grading Scheme for Anterior Chamber Cells**

| Grade | Cells in Field* |
|---|---|
| 0 | <1 |
| 0.5+ | 1-5 |
| 1+ | 6-15 |
| 2+ | 16-25 |
| 3+ | 26-50 |
| 4+ | >50 |

*Field size is a 1mm by 1mm slit lamp beam. Grading should be done with the highest magnification and illumination and conducted in a completely dark room, prior to dilation.

**Anterior Chamber Flare**

Anterior chamber flare will be assessed by slit lamp exam but will not be part of our study criteria for controlled inflammation.

**SUN Working Group Grading Scheme for Anterior Chamber Flare**

| Grade | Description |
|---|---|
| 0 | None |
| 1+ | Faint |
| 2+ | Moderate (iris and lens details clear) |
| 3+ | Marked (iris and lens details hazy) |
| 4+ | Intense (fibrin or plastic aqueous) |

*Adapted from: Hogan MJ, Kimura SJ, Thygeson P. Signs and symptoms of uveitis: I. Anterior Uveitis. AM J Ophthal 1964;47:155-170; Grading should be conducted in a completely dark room

### 5.1.2. Criteria for active inflammation

Declaring active anterior uveitis requires the presence of active inflammatory cells or signs that are consistent with active inflammation when AC cells are not present.

Criteria required for active inflammation:

- ≥ 1+ anterior chamber cell **OR**
- Other signs consistent with active inflammation, including increased IOP, corneal edema, and/or active KP

### 5.1.3. Criteria for clinical quiescence

All study ophthalmologists will use similar criteria for declaring clinical quiescence of inflammation.

Criteria required for clinical quiescence of inflammation:

- ≤ 1/2+ anterior chamber cell **AND**

- Resolution of other signs associated with active inflammation, including increased IOP, corneal edema, and/or active KP. IOP must be controlled without the addition of new IOP medications. For example: If a patient has increased IOP at Exam #2 and needs the addition of IOP medications, that patient's IOP is not controlled. If their IOP is within normal range by Exam #3 then that would indicate controlled IOP.

#### 5.1.4. Intraocular pressure (IOP)

Elevated intraocular pressure is associated with many of the complications that follow anterior uveitis. Therefore, IOP will be an important measure of treatment response throughout this study. Pressures will be measured at each visit by a study ophthalmologist at the slit lamp using Goldmann applanation tonometry or pneumotonometry.

#### 5.1.5. Endothelial cell density

Endothelial cell density will be an important clinical measure throughout this study. Participants should have density assessed via confocal microscopy or specular microscopy. Endothelial cell density should be recorded on the Clinical Eye Exam form at the day 1 and day 28 visits in number of cells/$mm^2$.

#### 5.1.6. Training and certification of ophthalmologists

All exams must be performed by uveitis specialists. The study protocol and classification system for grading inflammation will be reviewed by Dr. Gonzales (or another study ophthalmologist from the Proctor Foundation) with the investigators at all sites.

### 5.2. Anterior chamber paracentesis

#### 5.2.1. Procedural guidelines

All participants will have an anterior chamber paracentesis performed prior to enrollment in the study. Before paracentesis is performed, participants should have met all non-laboratory criteria (on the eligibility and screening form) and been consented for the procedure. All anterior chamber paracenteses will be performed by study ophthalmologists at the slit lamp or in the supine position using standard techniques that are described and validated by Trivedi et.al. in 2011.[9] Topical anesthetic drops will be instilled into the eye. Eyelids and ocular surface will then be prepared by using betadine 2% solution. A lid speculum will be placed into the procedure eye. A 27-guage or 30-guage needle will be inserted into the anterior chamber through the cornea via a temporal approach. Aqueous will then be removed from the anterior chamber ensuring the anterior chamber remains formed. For persistent wound leaks (as assessed by Seidel technique), a bandage contact lens will be placed. Topical antibiotics should be used 4 times daily for 1 week following each paracentesis.

#### 5.2.2. Aliquots for AC paracentesis #1 (at Prestudy visit, Exam #0)

Aqueous humor samples will be immediately split into two volumes. One volume of approximately 50 microliters will be sent for immediate diagnostic viral PCR testing on site in order to determine enrollment eligibility. At least 50 microliters will be immediately labeled and frozen on site. Samples from participants that do not eventually meet criteria for enrollment (for example, participants with viral PCR that is negative for CMV) will be destroyed. Samples from participants that meet criteria and are enrolled in the study will be saved. 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.

#### 5.2.3. Aliquots for AC paracentesis #2 (at Exam #2, day 7)

Aqueous humor samples will be immediately split into two volumes. One volume of approximately 50 microliters that is designated for CMV quantitative PCR will be immediately labeled and frozen on site. A second volume of approximately 20 microliters that is designated for RNA sequencing will be added to a RNA specific tube, 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.

### 5.3. Laboratory measurements

Hematology and serum chemistry will be measured in all participants prior to enrollment and monitored at Day 7 (Exam #2, day 7 of treatment), and Day 28 (Exam #3, day 28 of treatment). Each of the participating sites has an accredited onsite laboratory where all blood draws and laboratory measurements will be performed. The following will be measured and recorded:

#### 5.3.1. Hematology

- Hemoglobin and/or hematocrit
- Platelet count
- White blood cell count (WBC)
- % neutrophils
- % lymphocytes

#### 5.3.2. Serum Chemistry

- Creatinine (Cr)
- Blood Urea Nitrogen (BUN)

#### 5.3.3. Pregnancy

- Urine or serum pregnancy test (females of child-bearing age only)

#### 5.3.4. HIV status

- Blood for HIV serology (unless HIV status is known or HIV testing done within the past 6 months)

#### 5.3.4. Timing

Laboratory measurements used before enrollment will be collected between Exam #0 and Day 1. Lab orders from Exam #2 and Exam #3 should be conducted on the same day as their study visit.

## 6. Adverse events

**Table 3: Thresholds for classifying non-serious and serious lab abnormalities**

| | Non-Serious Adverse Event* | Serious Adverse Event |
|---|---|---|
| **Leukocytes** | >1,000 to [illegible] | [illegible]1,000[illegible] |
| **Platelets** | 20,000 to 75,000[illegible] | < 20,000[illegible] |
| **Hemoglobin** | [illegible]6.5 to < 9 g/dL | < 6.5 g/dL |
| **Creatinine** | [illegible]1.5 to < 2 mg/dL | [illegible]2 mg/dL |

### 6.1. Non-serious adverse event (AE)

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

- Increased intraocular pressure (>24 mm Hg)
- Abnormal lab findings (rise in creatinine to ≥ 1.5 to < 2 mg/dL, reduction of white blood cell count to below > 1,000 to <3000/mm3 platelet count from 20,000 to 75,000, or hemoglobin level >6.5 to <9 g/dL)
- Concurrent accident or illness
- Increase in the frequency and severity of symptoms of a pre-existing condition
- Side effects intolerable to patient (gastrointestinal upset, nausea, vomiting, fatigue)
- Signs of corneal or conjunctival toxicity (epitheliopathy, conjunctivitis)

#### 6.1.1. 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 from 2 tablets BID (900 mg) to 1 tablet BID (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.

### 6.2. 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*:

- Cr ≥ 2 mg/dL, leukocytes ≤1,000/mm3 platelets < 20,000/mm3 or hemoglobin < 6.5 g/dL
- Death
- Non-elective surgery or hospitalization for any reason
- Myocardial infarction or stroke
- Life-threatening adverse drug experience or any life threatening event
- Persistent or significant disability or incapacity
- Cancer
- Seizure
- Congenital anomaly/birth defect
- Disability or permanent damage
- Required intervention to prevent permanent impairment/damage

#### 6.2.1. 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 participants should continue with scheduled study visits if possible.

### 6.3. 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), Principal Investigator (Dr. John Gonzales john.gonzales@ucsf.edu) and the main study coordinator (Jaskirat Takhar jaskirat.takhar@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.

### 6.4. Patient death

Any patient death that occurs during the study will be reported by the study center's Study Coordinator to the Medical Monitor within 24 hours. The study arm of any deceased patient will be made available to the DSMC by the DCC if appropriate.

## 7. Data collection and management

This section discusses how the data will be collected from the different Study Centers, entered into the computer database and stored, and transferred to the Data Analysis Committee under the supervision of the Data Coordinating Center (DCC). The data management plan described below is similar to what we are already using for our NEI-funded Steroids for Corneal Ulcers Trial (SCUT), Mycotic Ulcer Treatment Trial (MUTT), and First-line Antimetabolite As Steroid-sparing Treatment (FAST) clinical trials, which enroll participants at Proctor/UCSF and international centers. Proctor/UCSF is the clinical and data coordinating center for these trials.

### 7.1. Data collection forms

The data collection forms are derived and modified from the SCUT, the MUTT pilot and main trial, and the FAST pilot and main trial. These forms have been extensively field-tested by UCSF investigators.

Study personnel at each site will be given specific training for each form for which they will be responsible. Paper forms for each patient will be completed by study personnel (study physicians, study coordinators, etc.) on paper in real time when the patient is being assessed.

### 7.2. Data review

Data will be reviewed in real time, as the patient is being assessed. The study coordinator will verify with the personnel filling out the forms that they are complete. Before scanning to be sent to Proctor for data entry, the forms will be reviewed and cross-checked for consistency and completeness by the study coordinator within **24 hours** of completing the form. If the forms are not filled out completely, the study coordinator will be responsible for completing the form to provide the missing data, or clarify any inconsistent data. The study coordinator or investigators who filled out the form are the only people who are authorized to add missing data or make any changes to the study forms. All changes should be made with a red ink pen, and then signed and dated. Further investigation may be conducted if certain fields or personnel are associated with a higher rate of paper form errors.

### 7.3. Data entry

Once the completed study forms have been reviewed and approved by the study coordinator, the study coordinator will scan and email the forms to the main study coordinator's email address (jaskirat.takhar@ucsf.edu) **within 10 days** of the patient visit. All data will be entered into the official electronic research data capture service at UCSF: REDCap. The goal is for data entries to occur within 10 days of receiving the completed forms in order to prevent the accumulation of un-entered forms. The database program contains an entry module for each form, prompting the user to enter the data in the same order as the form and clearly indicating each question.

#### 7.3.1. Data entry errors

Discrepancies and missing values will be assessed by the data entry manager, and resolved by queries sent to the study coordinator and appropriate observers (clinician, study coordinator, etc.). A logfile will preserve the date and time of any changes, together with who entered the changes.

#### 7.3.2. Data consistency and validity

A database data quality assessment program will be created in R programming software to help prevent inconsistencies or invalid data. The database program will check for the following errors: (1) improper entry of the patient ID based on the checksum, (2) data fields that are out of range, (3) inconsistent or illogical entries, (4) incompleteness, and (5) numerical values that are far outside the range of those previously entered. The software will create an error file with relevant data such as the form identification, field names and the data. Automated checks will be made to ensure consistency and that each variable in the analysis set has in-range values (protecting against negative ages, spelling errors in categorical factors, and similar errors). The DCC will conduct regular checks of the data at each site and will contact the study coordinator about any errors in order to resolve the inconsistencies and have the data entry operators enter the correct data.

#### 7.3.3. Data preparation and cleaning

Data sets for analysis will be produced in Microsoft Excel® worksheets (downloaded from the REDCap system) containing a single header line whose variable names match the REDCap database. Each analysis set will be in the form of a rectangular table in which each column corresponds to a single variable and each row to an observation. All missing values will be coded explicitly using the string "NA" (as used in the R software). Codes for categorical variables (such as

1 for male, and 2 for female) will be avoided in favor of self-documenting character strings (such as Male, Female) whenever possible.

A detailed codebook will be prepared, containing for each variable, (a) the form from which the variable derived, (b) the text of the question, (c) all possible values for the variable, and (d) summary statistics for the variable. Note that all codes and character strings that represent categorical factors will be clearly defined in the codebook. Units for each continuous variable (e.g. logMAR) will be unambiguously indicated for each variable. Each release of the analysis set will be accompanied by the corresponding version of the codebook. Version numbering with dates will be strictly observed.

#### 7.3.4. Monitoring

We will maintain a record of (1) changes to the initial entries on database forms, and (2) changes made to all entered data. Database errors include (a) missing information, (b) erroneous information that was initially entered, and (c) errors arising from difficulties with the forms themselves. Quality assurance reports will summarize the number of each of these. Most importantly, we will closely monitor the waiting time between the collection of the initial form and the entry of the data. If entry times exceed 30 days, then this will trigger a response, which may include investigation, retraining, and even reassignment.

### 7.4. Data analysis

Following data checks by the data analyst, the Data Analysis Committee will be responsible for analyzing the data. At designated time points, the unmasked data will be merged with the randomization list to perform statistical analyses and prepare reports.

Standard report-generation software included with the R statistical and data analysis package will be used to ensure consistency of the codebook and analysis set at all times.

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

### 7.5. Data storage and security

Paper forms will be maintained in locked file cabinets in locked rooms only accessible to research staff at each study site. All PDFs of forms will be sent to the study coordinator at Proctor. They should be sent through a secure email and saved on a secure server.

All electronic storage will be subject to standard security procedures in compliance with established enterprise information security standards. Each computer will be hardware-firewalled and will not be accessible outside the Local Area Network. Hard-disk encryption will be used for each machine, and the machine will not be accessible without a network account and password. Only one individual at each site will have password access to the machine; new accounts may be provided by the local network administrator only with the approval of the DCC. Accounts will be immediately deactivated for data entry or other personnel who leave the study. The computer used for data entry and storage will be kept in a physically locked room, and only authorized study personnel will be able to access this machine. All individuals who access the server room will be logged as to time

and date. At each center, the complete database (including all data tables as well as change logs) will be backed up weekly on the server and weekly on a CD/DVD, which will be kept at a safe, locked cabinet. Temperature logs will be maintained for the server room and reported.

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

## 8. Quality control

With the help of the Data Coordinating Center and the Data Analysis Committee, the Clinical Coordinating Center will evaluate the quality of study activities (clinical examination, treatment compliance, etc.).

### 8.1. Aqueous sample storage and shipment

Aqueous humor removed from the eye during anterior chamber paracentesis will be immediately frozen after being split into the appropriate aliquots (see section 5.2. for details concerning anterior chamber paracentesis aliquots). Upon study completion of all participants enrolled at a given site, those samples will be packaged together with sufficient dry ice to be shipped safely to UCSF while remaining frozen. Upon arrival at UCSF, shipments will be stored in Proctor freezers until samples from all enrollment centers have arrived and are ready to be analyzed.

### 8.2. Medication storage and expiration

Study tablets will be stored at room temperature. Study drops will be stored at 2-6 degrees Celsius. The expiration dates of the treatment kits will be regularly monitored and all expired study medicine will be discarded appropriately.

### 8.3. Periodic reports

The Study Coordinators will send weekly reports to the clinical coordinating center, on the number of eligible participants screened, the number of participants enrolled, reasons for ineligibility, and the number of participants who have come back for follow-up visits. This will be used to monitor the enrollment/follow-up progress and any protocol violation in enrollment.

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

### 8.4. Data management, security and quality assurance

See Section 7.

### 8.5. Monitoring compliance

Participants will be asked to record missed doses in individual treatment calendars and to bring them with their medication to each follow-up visit. The study coordinator will collect details of the number of doses missed by the patient and record these in the medication log. In addition, study coordinators will conduct pill counts at each visit.

### 8.6. Certification

For training and certification process, see Sections 5.2.3.

### 8.7. Data audits

Samples of data forms will be audited at each site to ensure consistency between the source documents and data entry. Patient charts will also be reviewed two times a year to confirm adherence to the protocol.

## 9. Duties and responsibilities of staff

### 9.1. Ophthalmologist

- Note: whenever possible, the primary study ophthalmologist should be the same person across all a patient's study visits, for consistency of measurements
- Responsible for enrolling study subjects
- Provide information for completing the clinical examination forms
- Obtain written consent from the subjects with the help of study coordinator
- Initiate study medication as per the randomization
- Responsible for the care of the patient throughout the course of the study
- Manage adverse events

### 9.2. Clinical Trial Manager

- Ensure the execution of the study as per the protocol
- Coordinate with the collaborating centers
- Prepare monthly reports regarding recruitment and follow-up progress
- Handle correspondence between centers
- Maintain IRB approval and renewals
- Communicate with central pharmacy regarding drug orders and distribution to sites

### 9.3. Study Coordinator

- Make sure that appropriate participants are screened and enrolled in to the study (including obtaining appropriate assent/consent)
- Prepare weekly reports regarding recruitment and follow-up progress
- Send reminders to study participants for follow-up visits
- Meet with the patient at each study visit, prior to the study ophthalmologist to help maintain masking
- Assist ophthalmologist with conforming to study procedures

- Verify data forms for completion and collect missing information
- Send study forms for prompt data entry
- Maintain stock of and dispense study medications

### 9.4. Data Analyst

- Develop data entry programs specific to the study forms under the supervision of DCC
- Monitor the flow of forms from the coordinator
- Supervise data entry operators for any errors or omissions
- Develop consistency checks
- Communicate with study coordinators and data entry operators to correct any mistakes in study forms and data entry
- Transfer data as and when requested by the DCC
- Back up all data appropriately

### 9.5. Data Entry Operator

- Enter all data from study forms when submitted by the study coordinator
- Verify inconsistencies in the forms and send them for correction to the study coordinator

### 9.6. Biostatistician (Proctor)

- Review data for quality control purposes
- Prepare reports for DSMC
- Lock database at completion of study after database is cleaned
- Conduct analyses at the conclusion of the study
- Prepare randomization list and distribute the list to a pharmacist and non-masked, senior physicians at the study sites in case of emergency.

### 9.7. Ophthalmic Assistants

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

## 10. Study Organization

### 10.1. Executive Committee

The Executive Committee will be chaired by Dr. Gonzales at the Proctor Foundation and will include Dr. Lietman and Dr. Oldenburg. This committee will act as the administrative and executive arm of the clinical trial and will meet once a month to provide overall oversight for the study and make decisions on day-to-day operational issues. The role of the Executive Committee includes monitoring study progress and data collection process, supervising the Clinical Coordinating Center (CCC) and Data Coordinating Center (DCC) for overall quality control, evaluating and adopting changes in study procedures as necessary, communicating with and implementing recommendations from the Data and Safety Monitoring Committee, making executive decisions on the allocation of resources, establishing policies on publications and authorship, and approving and overseeing ancillary studies.

### 10.2. Clinical Coordinating Center (CCC)

Dr. Gonzales will also be responsible for directing the CCC, located at the Proctor Foundation/UCSF. The responsibility of the CCC includes oversight and coordination of the clinical implementation of the trial at all sites. Specifically, this includes roles such as maintaining an up-to-date manual of operations, obtaining human research approvals from Institutional Review Boards, conducting training and certification of all personnel including physicians and coordinators, supervising preparation and dispensing of study medication, ensuring proper masking, and monitoring protocol adherence and recruitment. Dr. Gerami Seitzman will serve as medical monitor of the trial. He will receive all reports of serious adverse events from all study sites, ascertain whether further information is needed, and convey that information to the DSMC. As with our current NEI-funded studies, the study coordinator at Proctor/UCSF will serve as the manager of the coordinating center and will assist with many of the clinical coordination activities listed above and also check in weekly by email with the study coordinators at each site to discuss any study-related issues.

**10.3. Data Coordinating Center (DCC)**

The DCC, including Dr. Lietman and Dr. Oldenburg, is responsible for supervising data collection, data management, data quality control, data analysis, event adjudication, and training and certification of study site staff in the data management systems. The DCC will also be responsible for coordinating and supporting the activities of the Data Safety Monitoring Committee, including preparing interim and final data reports and organizing meetings with the Data Analysis Committee. The DCC will also be responsible for the dissemination of datasets for use by the Data Analysis Committee and other investigators. The DCC will meet weekly to monitor the progress and quality of data entry/management and address any issues. The DCC will be in close contact with the data analysts and data entry operators to ensure quality assurance of data entry. See MOP for further information on data management (Section 7).

**10.4. Data Analysis Center (DAC)**

Dr. Oldenburg will direct the Data Analysis Committee with assistance from the data manager. The primary functions of the DAC include designing a statistical analysis plan, preparing and distributing randomization lists, performing data analysis, and coordinating publications and presentations. The Committee is responsible for obtaining data from the Data Coordinating Center, performing unmasked data analyses and for preparing reports for the Data and Safety Monitoring Committee, and at the conclusion of the study, for the Principal Investigator. The DAC will also be responsible for conducting the prespecified interim analysis (when 1/3$^{rd}$ and 2/3$^{rds}$ of the primary outcome data has been collected) and also for providing data to the DSMC to evaluate the stopping guidelines.

**10.6. Data Safety and Monitoring Committee (DSMC)**

The Data Safety and Monitoring Committee (DSMC) is independent with experts from diverse fields including biostatistics, epidemiology, and ophthalmology. The committee will meet for the first time before the study begins. Only after the DSMC reviews and approves the protocol will participants be enrolled. We anticipate the group will meet regularly throughout the study and 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 1/3$^{rd}$ of the primary outcome has been collected). They will also receive reports of serious adverse events from the principal statistician, every 6 months.

### 10.7. Editorial Committee

The Editorial Committee is composed of the principal investigator Dr. Gonzales and co-investigators Dr. Acharya and Dr. Doan. This committee has the responsibility to assist in the preparation of the primary study results and to review secondary manuscripts produced by the study investigators. They have the responsibility to ensure the completion of the primary manuscript (Specific Aim 1) in a timely manner and direct its submission for publication.

### 10.8. Pharmacy

The UCSF Pharmacy can handle high volume orders and provide quick turn-around times to ensure a steady supply of medications over the course of the trial. They have participated in a number of clinical trials and will be able to provide the medications (valganciclovir and cytovene powder) over the course of the trial.

### 10.9. Study Communications

The study coordinator at the Proctor Foundation will be responsible for ensuring that communication between all facilities and committees is conducted seamlessly. Communication between study personnel at the Proctor Foundation and investigators at other sites will be performed by extensive use of secure email. Much of the communication will be through email, but we have found that regular, international, conference calls are valuable and that regular, face-to-face meetings are mandatory. Members of the CCC and the DCC at the Proctor Foundation will visit sites every 4-6 months through the duration of the study to discuss details of the study and monitor quality control.

## 11. References

1. Chee SP, Bacsal K, Jap A, Se-Thoe SY, Cheng CL, Tan BH. Clinical features of cytomegalovirus anterior uveitis in immunocompetent patients. *Am J Ophthalmol.* 2008;145(5):834-840.
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4. Su CC, Hu FR, Wang TH, et al. Clinical outcomes in cytomegalovirus-positive Posner-Schlossman syndrome patients treated with topical ganciclovir therapy. *Am J Ophthalmol.* 2014;158(5):1024-1031 e1022.
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6. Su CC, Wang IJ, Chen WL, Lin CP, His B, Hu FR. Topical ganciclovir treatment in patients with cytomegalovirus endotheliitis receiving penetrating keratoplasty. *Clin Exp Ophthalmol.* 2013;41(4):339-347.
7. Wong JX, Agrawal R, Wong EP, Teoh SC. Efficacy and safety of topical ganciclovir in the management of cytomegalovirus (CMV)-related anterior uveitis. *J Ophthalmic Inflamm Infect.* 2016;6(1):10.
8. Jabs DA, Nussenblatt RB, Rosenbaum JT, Standardization of Uveitis Nomenclature Working G. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol.* 2005;140(3):509-516.
9. Trivedi D, Denniston AK, Murray PI. Safety profile of anterior chamber paracentesis performed at the slit lamp. *Clin Exp Ophthalmol.* 2011;39(8):725-728.

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | #1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | #2a | Trial identifier and registry name. If not yet registered, name of intended registry | 1 |
| Trial registration: data set | #2b | All items from the World Health Organization Trial Registration Data Set | 22 |
| Protocol version | #3 | Date and version identifier | 1 |
| Funding | #4 | Sources and types of financial, material, and other support | 1 |
| Roles and responsibilities: contributorship | #5a | Names, affiliations, and roles of protocol contributors | 2 |

| | | | |
|---|---|---|---|
| Roles and responsibilities: sponsor contact information | #5b | Name and contact information for the trial sponsor | 1 |
| Roles and responsibilities: sponsor and funder | #5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 2 |
| Roles and responsibilities: committees | #5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 2 |
| **Introduction** | | | |
| Background and rationale | #6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5 |
| Background and rationale: choice of comparators | #6b | Explanation for choice of comparators | 5 |
| Objectives | #7 | Specific objectives or hypotheses | 6 |
| Trial design | #8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory) | 6 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | #9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be | 6 |

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|---|---|---|---|
| | | collected. Reference to where list of study sites can be obtained | |
| Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 |
| Interventions: description | #11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 7 |
| Interventions: modifications | #11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease) | 8 |
| Interventions: adherance | #11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) | 9 |
| Interventions: concomitant care | #11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 9 |
| Outcomes | #12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10 |
| Participant timeline | #13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 20 |
| Sample size | #14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 11 |
| Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 6 |

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| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: sequence generation | #16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 7 |
| Allocation concealment mechanism | #16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 7 |
| Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 7 |
| Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 7 |
| Blinding (masking): emergency unblinding | #17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 14 (on MOP-attachment) |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10 |

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| Data collection plan: retention | #18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 9 |
| Data management | #19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 10 |
| Statistics: outcomes | #20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 11 |
| Statistics: additional analyses | #20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 13 |
| Statistics: analysis population and missing data | #20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 13 |
| **Methods: Monitoring** | | | |
| Data monitoring: formal committee | #21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 11 |
| Data monitoring: interim analysis | #21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 11 |
| Harms | #22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 8 |

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| Auditing | #23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 34 (on MOP-attachment) |
| **Ethics and dissemination** | | | |
| Research ethics approval | #24 | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval | 13 |
| Protocol amendments | #25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators) | 13 |
| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 7 |
| Consent or assent: ancillary studies | #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | 14 |
| Confidentiality | #27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 28 |
| Declaration of interests | #28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 14 |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 13 |
| Ancillary and post trial care | #30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 25 & 29 |
| Dissemination policy: trial results | #31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 13 |

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|---|---|---|---|
| Dissemination policy: authorship | #31b | Authorship eligibility guidelines and any intended use of professional writers | 13 |
| Dissemination policy: reproducible research | #31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 14 |
| **Appendices** | | | |
| Informed consent materials | #32 | Model consent form and other related documentation given to participants and authorised surrogates | 23 |
| Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | 24 |

Notes:

- 17b: 14 (on MOP-attachment)
- 23: 34 (on MOP-attachment)  This checklist was completed on 22. July 2019 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

# A double masked randomised 4-week, placebo-controlled study in the US, Thailand and Taiwan to compare the efficacy of oral valganciclovir and topical 2% ganciclovir in the treatment of cytomegalovirus anterior uveitis: study protocol



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| Article Type: | Protocol |
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| Primary Subject Heading: | Ophthalmology |

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| Secondary Subject Heading: | Ophthalmology |
| Keywords: | Cytomegalovirus, Randomized clinical trial, Uveitis |
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# A double masked randomised 4-week, placebo-controlled study in the US, Thailand and Taiwan to compare the efficacy of oral valganciclovir and topical 2% ganciclovir in the treatment of cytomegalovirus anterior uveitis: study protocol

Clinicaltrials.gov – NCT03576898

Issue Date: March 26, 2019
Protocol Amendment Number: 01

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Funding: this work was supported by Huang Pacific Foundation and That Man May See Foundation.

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Sponsor and funder roles and responsibilities:

The study sponsor was primarily involved in the study design; collection, management, analysis and interpretation of data; writing of the report and the decision to submit the report for publication.

The study funders had no role in study design; collection, management, analysis or interpretation of data; writing of the report or the decision to submit the report for publication.

Committees/Collaborators: John Gonzales (Principal Investigator); Nisha Acharya and Thuy Doan (Co-investigators and Editorial Committee); Gerami Seitzman (Medical Monitor); Thomas Lietman, Jennifer Rose-Nussbaumer, Jeremy Keenan, and Charles Lin (Data and Safety Monitoring Committee); Travis Porco (Biostatistician); Catherine Oldenberg (Data Coordinating Centre Supervisor); Sarah Lopez (Study Site Coordinator); Ashlin Joye, Jaskirat Takhar, and Elisabeth Gebreegziabher (Data Managers and Study Coordinators); Thanapong Somkijrungroj (Site Director, King Chulalongkorn University); Wipada Laovirojjanakul (Site Director, Khon Kaen University); Chang-Ping Lin (Site Director, National Taiwan University); Ruedee Kumklee (Study Coordinator, Khon Kaen University)

Data statement: As this manuscript describes the protocol for a study that has yet to be implemented, no data are currently available to access. After completion of the trial, we will publish the data in a data repository.

Key words: Cytomegalovirus, Randomized clinical trial, Uveitis

Word Count: 4,307

Author Contributors: Gonzales JA, Somkijrungroj T, Laovirojjanakul W, Lietman TM, Joye AS, and Takhar JS conceived of the study. Gonzales JA, Somkijrungroj T, Laovirojjanakul W, Lietman TM, Lin C-P, Keenan JD, Seitzman GD, Rose-Nussbaumer JR, Doan TA, Acharya NR, Porco TC, Gebreegziabher EA, Joye AS, and Takhar JS initiated the study design and helped with implementation. Gonzales JA is the funding holder. Porco TC provided statistical expertise in clinical trial design. Porco TC and Gebreegziabher EA will conduct the primary statistical analysis. Takhar JS, Joye AS, Somkijrungroj T, Laovirojjanakul W, Lin C-P, Lietman TM, Porco TC, Keenan JD, Gebreegziabher EA, Seitzman GD, Rose-Nussbaumer JR, Doan TA, Acharya NR, Gonzales JA contributed to refinement of the study protocol and approved the final manuscript.

Competing Interests: None declared

## Abstract

**Introduction** Cytomegalovirus (CMV) anterior uveitis is a recognised cause of anterior uveitis in immunocompetent patients and preventable cause of vision loss. Ocular sequelae include corneal endothelial damage which can cause corneal oedema and failure, as well as glaucoma. Recurrences of inflammation are common and therefore patients are often exposed to long-term therapy. Oral therapy is available in the form of valganciclovir albeit with the caveat of systemic side effects such as bone marrow suppression and renal failure necessitating regular interval laboratory monitoring. Recent reports have demonstrated that topical 2% ganciclovir solution may offer promising treatment outcomes in patients with CMV anterior uveitis with superior safety, cost effectiveness, and convenience profiles. An investigation into the relative equipoise of these therapies is warranted for these reasons.

**Methods and Analysis** The Systemic and Topical Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO) trial is designed as a multi-centre, block randomised by site, double-masked, placebo-controlled trial comparing the efficacy of oral valganciclovir, 2% topical ganciclovir, and placebo in treating PCR-proven CMV anterior uveitis. Participant clinical evaluation will occur at 3 study timepoints by a masked study ophthalmologist over a 28-day period to assess resolution of ocular inflammation (secondary outcome). A control group will provide additional information about the possible impact that the infected host's immune response may play in controlling local viral replication. The primary analysis is an ANCOVA (3 arms) correcting for baseline to compare quantitative CMV viral load in the anterior chamber aqueous fluid pre and 7-days post treatment.

**Ethics and Dissemination** The University of California San Francisco Committee on Human Research, and the Khon Kaen University institutional review board (IRB) has given ethical approval. The results of this trial will be presented at local and international meetings and submitted for peer-reviewed journals for publication.

**Trial Registration Number** NCT03576898; Pre-enrollment

## Article Summary

### Strengths and limitations of this study

- This randomised clinical trial compares both clinical and quantitative microbiologic outcomes of CMV treatment as opposed to solely clinical response.
- Baseline and endline quantitative PCR viral loads from all sites that will be included in analysis are processed at the same laboratory ensuring a uniform assessment of reduction in viral load.
- This is a multi-centre clinical trial that includes 3 sites in Asia where the disease is most prevalent.
- Our limited sample size may not allow detection of the smallest meaningful difference between the treatments.
- Adherence is mainly assessed via self-report and may not accurately reflect treatment administration.

## INTRODUCTION

Cytomegalovirus (CMV) has been recognised as an important cause of posterior retinitis in HIV/AIDS patients, particularly prior to the introduction of highly active anti-retroviral therapy. However, CMV is now being implicated in immunocompetent individuals as a cause of anterior uveitis.[1-4] Definitive diagnosis requires laboratory diagnosis, such as directed polymerase chain reaction (PCR).[5]

CMV anterior uveitis is a preventable cause of vision loss. It can cause damage to the corneal endothelial cells, which can lead to oedema, failure, and, ultimately, requirement for corneal transplant.[6-9] CMV can recur in transplanted grafts, necessitating further surgeries and complications.[10-14] Additionally, recurrences of inflammation in CMV anterior uveitis can be associated with glaucoma, which can cause irreversible blindness if not appropriately managed.[15-17]

While CMV anterior uveitis is typically characterised by recurrent bouts of inflammation, it is unknown whether each recurrence is mediated by active viral replication or a sterile immune response. Consequently, patients may be prescribed frequent oral or topical antiviral therapy. An understanding of the most effective treatment strategy for CMV anterior uveitis will help inform future studies investigating ways of decreasing recurrences of inflammation in this condition.

One option for the management of CMV anterior uveitis is oral valganciclovir 900mg twice daily.[18,19] Prevention of recurrent inflammation in CMV anterior uveitis has been with valganciclovir 450mg twice daily.[18,19] Oral valganciclovir can be associated with serious systemic side effects including renal failure and bone marrow suppression, necessitating regular interval laboratory monitoring. Topical ganciclovir 2% has also been used to treat and prevent recurrences of CMV anterior uveitis.[20-22] Topical therapy is attractive as it does not require laboratory monitoring, though ocular side effects including corneal epitheliopathy and conjunctivitis could preclude long-term use.[23,24] Thus, comparing oral valganciclovir 900mg twice daily to topical ganciclovir 2% six times daily has equipoise in the uveitis/cornea specialist.

To gain insight into preferred practice patterns for the treatment of CMV anterior uveitis, we surveyed attendees of the Biennial Chulalongkorn-Khon Kaen-Proctor-UCSF (CKPU) meeting in Bangkok, Thailand on March 5, 2019. Survey questions were provided in a Likert scale, multiple-choice format with participants asked about their agreeability on 7 statements. Answer choices varied from 1 to 4; 1 representing “strongly disagree”, 2 representing “somewhat disagree”, 3 representing “somewhat agree”, and 4 representing “strongly agree”. Sixty-eight faculty level physicians responded to our survey, 5 practicing in North America, and 63 practicing in Asia. Results are summarised in **Table 1**. Physician preference for treatment medication of CMV anterior uveitis in presented in **Table 2**. The results demonstrate that a consensus regarding the best treatment for CMV anterior uveitis is not yet established.

Some cases of CMV anterior uveitis have been noted to resolve without antiviral treatment.[25] In this setting, having a trial in which oral valganciclovir is compared to

topical ganciclovir, and including a control group where no antiviral was used would provide additional information about the possible impact that the infected host's immune response may play in controlling local viral replication.

## METHODS AND ANALYSIS

### Study overview

The Systemic and Topical Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes (STACCATO) trial is a randomised, participant- and ophthalmologist-masked, placebo-controlled trial comparing the efficacy of oral valganciclovir, and 2% topical ganciclovir in treating PCR-proven CMV anterior uveitis. Study participants will be block-randomised with 1:1:1 allocation into the 3 study treatment arms. Participants will return to clinic for 3 study evaluations by a masked study ophthalmologist over a 28-day period at which point they will be assessed for resolution of ocular inflammation. All groups will receive their assigned treatment for 28 days. An objective measure of treatment efficacy will be assessed via biological samples of anterior chamber aqueous fluid pre and 7 days post-treatment. An overview of study procedures and study timeline is provided in **Table 3**. We used the SPIRIT checklist when writing our protocol.[26]

### Specific aims and outcomes

The specific aims of this trial are (1) to assess reduction in CMV viral load by treatment (as measured by $\log_{10}$-transformed quantitative viral load pre-treatment compared to 7-days post treatment and (2) to assess the proportion of participants achieving clinical quiescence after 7 and 28 days of treatment. As a nested observational aim within our primary aim, we will evaluate the effect of topical corticosteroid started prior to enrollment on viral load yield by directed quantitative PCR. The research hypothesis for this study is that oral valganciclovir therapy will demonstrate the greatest efficacy in treating CMV anterior uveitis compared to 2% topical ganciclovir and placebo therapy.

### Setting and Eligibility

The seroprevalence of CMV varies geographically. In East Asia it estimated to be as high as 87-100%, whereas in Western countries it is between 51.5 – 54.4%.[27-29] For comparative purposes, four tertiary academic centres located in three countries will be included, one in the United States of America, two in Thailand, and one in Taiwan.

Participants must provide written, informed consent before any study procedures can occur (see Appendix 1 for a sample informed consent). Study inclusion and exclusion criteria are outlined in **Table 4**.

### Recruitment

Each study centre will screen subjects independently adhering to the inclusion and exclusion criteria outlined. Patients who are found to be CMV positive by in-house PCR and who meet eligibility criteria will be approached about participating in the study. Participants who have a history of previously diagnosed CMV anterior uveitis are also eligible to participate in the study. However, like other participants, their participation in the trial would require that they agree to an AC paracentesis to confirm that their current

flare has demonstrable CMV present and to quantify the current flare's viral load to which it would be compared at 7 days post-randomization. Participant enrollment and project activities are planned to begin in October 2019 and end August 2021.

**Assignment of Intervention**

Competitive block (of 3 and 6) randomization of the participants, stratified by site will ensure that an equal number of participants at each site are randomised to study arms in a 1:1:1 ratio, not necessarily enrolling an equal number of participants at each site. Randomization lists will be created by the coordinating centre clinical coordinator and accessed online via REDCap database. After consenting participants for enrollment and confirming all eligibility requirements have been met, the study coordinators who are masked to the allocation sequence assign the participants an ID from a predetermined list and will log into REDCap and perform the randomisation. The now unmasked study coordinators will then give participants their assigned treatment.

**Masking**

All study ophthalmologists and participants will remain masked to treatment assignment after enrollment. All participants will receive both pills and eye drops, and will not know which medications are active. Study doctors have no part in handling the medication bottles, all participants will be given dark bags to place and keep their bottles in throughout the trial. Additionally, participants will meet with the study coordinator first, before seeing their study doctor at follow-up visits to review the participants' medication calendars, and study coordinators will hold participant calendars and study bottles in their office for the entire visit. Before bringing participants to the study doctor, the study coordinator will remind participants not to disclose any information concerning their treatment to their study ophthalmologist or any other participants they may come across. If the participant is experiencing intolerable side effects, the study coordinator and participant will speak with the study doctor concerning discontinuation of treatment.

The UCSF pharmacy will be responsible for purchasing and distributing bottles of the medications labeled with NDC/lot number and expiration dates to the Proctor Foundation, and then medications will be distributed to all study sites. Study ID labels, and dosing instructions will be provided to each site on separate label sheets. After enrollment, and randomization each site's study coordinator will place the study ID label on the bottle, before dispensing the drug to the participant. If personnel are unmasked at any time, a protocol deviation form must be completed.

**Intervention**

Participants will initially present with anterior uveitis suspected to be due to a viral aetiology. Participants will receive a routine standard of care work-up for such a suspected aetiology, which will include an anterior chamber (AC) paracentesis.[30] Half of this fluid (~50μL) will be used for qualitative PCR to detect CMV, HSV, and VZV at each respective site. The other half (~50μL) will be stored at -80 degrees Celsius. Patients will have serologic testing for complete blood count, renal function, pregnancy

status (in women of child-bearing age), and HIV status.

In the event a participant is ineligible for study inclusion, the stored 50 μL aliquot will be discarded. In eligible participants, this aliquot will undergo quantitative CMV PCR at a US laboratory, where lab personnel will be masked to participant identification, treatment assignment, and site. This aliquot will serve as the baseline (prior to treatment randomization) CMV viral load.

Eligible participants will remain masked and randomised by the unmasked study site coordinator to one of three study treatment arms. After randomization, all participants will receive both tablets and topical eye drops. One-third of participants will receive oral valganciclovir tablets and placebo eye drops (balanced salt solution, BSS), one-third will receive topical ganciclovir 2% eye drops and placebo tablets, and one-third will receive placebo eye drops (BSS) and placebo tablets. Dosing will be 4 tablets daily (900mg PO BID) for oral medication and 6 drops per day for topical.

After 7 days of treatment therapy participants will return for clinical examination and repeat anterior chamber paracentesis. Approximately 80μL will be stored for eventual quantitative CMV PCR by the aforementioned US laboratory, providing a post-treatment viral load value. Approximately 20 μL will be stored for future studies, provided a participant provides consent. In addition, corneal endothelium will be assessed using confocal or specular microscopy (depending on availability of the particular imaging modality at each centre) and will be used to measure endothelial cell morphology and density on Day 1 and 28.

Since it is standard of care to prescribe topical corticosteroids to reduce the severity of intraocular inflammation in participants with anterior uveitis, all participants (regardless of randomization) will be prescribed topical prednisolone acetate 1% and instructed to apply one drop to the affected eye three times daily. Additionally, patients requiring management of elevated intraocular pressure (IOP) will be prescribed IOP-lowering medication according to treating ophthalmologist's discretion and best medical judgement.

Once participants have finished 28 days of treatment, including the final clinic visit and evaluation, they will have completed the study. The treating ophthalmologist will determine whether any continued treatment is necessary. Any continued treatment will be based upon the treating ophthalmologist's clinical best clinical judgement.

**Harms and Modifications**

Non-serious adverse events may include any unfavorable medical occurrences in participants who have ever received study medication, regardless of any causal relationship with treatment. This may include: increased intraocular pressure (>24 mm Hg), abnormal lab findings (rise in creatinine to ≥1.5 to <2 mg/dL, reduction of white blood cell count to below >1,000 to <2,500/μL, platelet count from 20,000 to 75,000, or hemoglobin level >6.5 to <9 g/dL), concurrent accident or illness, increase in the

frequency and severity of a pre-existing condition, side-effects intolerable to participants (gastrointestinal upset, nausea, vomiting, fatigue), or signs of corneal or conjunctival toxicity (keratitis or conjunctivitis).[23,24,31]

If a non-serious adverse event in the form of a laboratory abnormality occurs, dose reductions of valganciclovir to 1 tablet (450mg) BID and topical drop frequency to 3 times daily will be made. Repeat laboratory testing should be ordered at 7 days of treatment with the reduced dose to monitor for resolution.

Serious adverse events (SAEs) include any medical occurrence that results in the following outcomes, or any other adverse event classified as severe: creatinine ≥2mg/dL, leukocytes ≤1,000/μL, platelets <20,000/μL, hemoglobin <6.5 g/dL, death, non-elective surgery or hospitalization for any reason, myocardial infarction, stroke, any life-threatening event, cancer, seizure, congenital anomaly/birth defect, disability or permanent damage, required intervention to prevent permanent impairment/damage.

An unmasked medical monitor will evaluate the details of all SAEs via a protocol deviation form and can request additional clinical information and recommend additional tests. The medical monitor will determine whether the event is a true SAE and whether it is likely related to the study drug. The medical monitor will recommend continuing the study medication versus dose reduction versus discontinuation. Additionally, if the investigator thinks the SAE is related to the study drug, he/she may stop the study medication anytime (including prior to evaluation by the medical monitor). Further medical management will be at the discretion of the treating ophthalmologist and any consulting services. These participants should continue with scheduled study visits if possible.

All serious adverse events will require the completion of a protocol deviation form and will be reported to the local IRB, and UCSF IRB.

**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 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.

**Concomitant Care**

Study ophthalmologists may use other topical adjunctive medications such as IOP lowering medications and cycloplegic agents. These should be documented on the study forms at each visit. The protocol does not allow for intravenous, or injection antiviral therapy, additional antivirals, or surgery during the study period. If such therapies are deemed necessary, the Protocol Deviation form should be completed.

**Protocol Deviation**
In rare cases, study ophthalmologists may determine that a deviation from protocol is necessary. This is at the discretion of the treating ophthalmologist and could be undertaken for a variety of reasons including rescue treatment and treatment discontinuation due to intolerability or safety. Subjects that experience deviations in treatment protocol will continue in their initially assigned treatment group and their data will be analyzed accordingly.

**Retention**
Participants will be considered to have dropped out from the study only if they declare they are no longer interested in further participation and not willing to return for any study visits, or are deceased. If participants are not willing to return for any study visits, no further information will be collected.

Participants who stop the study treatment for reasons other than efficacy, tolerability and/or safety or miss study visits and do not respond to contact, are considered non-compliant participants. Non-compliance will be noted and participants will continue their study treatment.

**Outcome Assessments**

*Viral Load Reduction*
Primary measurement of efficacy of the 3 treatments arms will be a comparison of the $\log_{10}$-transformed quantitative viral load PCR at baseline versus after 7 days of therapy. This measure provides an objective measurement of treatment efficacy. We note a dearth of published data regarding CMV viral loads pre- and post-treatment. An endpoint of 7 days was chosen as a prior study found viral loads to be frequently undetectable after 14 days of therapy.[32] All quantitative PCR measurements will be conducted at a single laboratory (masked to treatment and site) within the United States to ensure the same assay will be used on the provided samples, permitting direct comparison of quantitative viral load values.

*Effect of Topical Steroids on Baseline Viral Load*
Participants will be queried about the frequency of their topical steroids use to measure the effect of topical corticosteroid use prior to eligibility screening on CMV viral load. We anticipate that the dosing of corticosteroids prior to presentation or referral will vary depending on the practices of the referring physician. More frequent dosing of topical corticosteroids may affect the amount of virus recovered from the anterior chamber perhaps due to local immunosuppression.

*Clinical Response to Therapy*
For the clinical secondary outcome, participants will be classified as "quiescent" if they demonstrate on clinical examination: Less than or equal to 0.5+ AC cell **AND** resolution of other signs associated with active inflammation, including increased IOP, corneal oedema, and/or active keratic precipitates (KP). Since active inflammation can feature elevated IOP, if IOP is not controlled at a study visit, the participant will be considered as "not quiescent". This designation, however, does not preclude the participant from receiving additional IOP-lowering medication and the treating ophthalmologist may institute additional IOP-lowering medications as deemed necessary. In addition, a subjective measure of efficacy will be assessed by asking participants to estimate how many days after initiating therapy they felt improvement in their symptoms.

**Methods: Data Collection, Management, and Monitoring**

Paper forms for each participant will be completed by study personnel in real time when the participant is being assessed. The forms will be reviewed and crosschecked for consistency and completeness by the study coordinator within 24 hours of completion. Study coordinators at all sites will scan data collection forms and send them electronically to the Data Coordinating Centre (DCC) within 10 days of the participant visit and retain hard copies in a secure location. The DCC, is responsible for supervising data collection, data management, data quality control, data analysis, event adjudication, and training and certification of study site staff in the data management systems. All data will be entered into the official electronic research database, REDCap within 10 days of receipt of completed forms.

Discrepancies and missing values will be assessed by the data entry manager and resolved by queries sent to the study coordinator and appropriate observers. A logfile will preserve the date and time of any changes, together with who entered the changes.

**Data and Safety Monitoring Committee**

The Data Safety and Monitoring Committee (DSMC) has been established and is independent with experts from diverse fields including biostatistics, epidemiology, and ophthalmology. Only after the DSMC reviews and approves the protocol will participants be enrolled. The DSMC monitors severe or unexpected events that threaten the safety of participants and oversees the data collected throughout the duration of the study. The DSMC is responsible for reviewing the results of the interim analysis and determining whether the trial should continue with or without modification.

**Patient and Public Involvement**
No patient involved.

# STATISTICAL ANALYSIS PLAN

## Sample Size

Limited data from the available studies reporting detectable CMV viral loads pre- and post-treatment were available to guide our power calculations. We estimate a pre-treatment viral load of 5.4 $\log_{10}$IU/mL[6,20,32,33] (standard deviation +/- 1.00 log), and post-treatment viral load of 4.88 $\log_{10}$IU/mL[6,32-34] (standard deviation +/- 0.95 log) with correlation of 0.7 between baseline and follow-up viral load. With a sample size of 33 participants per arm with estimated 10% total loss to follow-up, we anticipate at least 80% power to detect a 0.61 log difference in viral load (based on pairwise analysis subject to an alpha of 0.05/3).

## Interim Analysis

An interim analysis of efficacy is performed one-third of the way through the trial, with alpha set at 0.001. The DSMC reviews the unmasked interim analysis and makes recommendations on the continuation of the trial. No interim analysis for futility is performed. We anticipate 80% power to detect a difference of 1.7 log units between any two arms.

### *Specific Aim 1*

The outcome variable is $\log_{10}$ viral load as measured by quantitative PCR at 7 days. The primary prespecified analysis is a permutation P-value based on the Fisher F statistic, derived from a linear model predicting the log viral load (primary outcome variable) as a function of the baseline log viral load and the treatment arm (dummy coded), i.e., ANCOVA. Estimation will be conducted by ordinary least squares. Type I error rate (alpha) of 0.05 will be used.

Quantities of interest for analysis will be quantitative PCR data from anterior chamber paracentesis aqueous fluid collected at the pre-study visit for each participant enrolled in the study. The second quantity of interest for comparative purposes will be quantitative viral load derived from the analysis of aqueous fluid from the second anterior chamber paracentesis conducted on study day 7 (exam #2). The outcome will be modelled as a linear function of baseline value and the treatment arm. Specifically, we will test the null hypothesis of a common intercept for all 3 treatment arms. We will also present the change scores for additional information and insight.

All quantitative PCR viral loads will be $\log_{10}$-transformed, with units converting from IU/mL to $\log_{10}$ IU/mL. The baseline quantitative PCR for each participant, and treatment will be used as a predictor for quantitative PCR after 7 days of treatment.

The purpose of supplementary analyses reported in this section is to assess the role of statistical choices and data quality choices in shaping the result. A Kruskal-Wallis non-parametric one-way ANOVA (using change scores) will be an alternative consideration as a statistical analysis choice for the primary outcome. As a methodological

analysis/sensitivity analysis, we will also conduct a Box-Cox transformation of our quantitative PCR log transformation.

If the results of the primary analysis are significant, then pairwise comparisons based on the Fisher Least Significant Difference will be conducted (P-values derived by permutation). We will also test for the hypothesis of interaction between study country in the 2 Asian sites (Taiwan and Thailand). This will be an assessment of the heterogeneity of effect between study sites. In addition, we will repeat the primary analysis including an additional random effect for study site and report the estimated treatment effects under this supplemental model.

***Specific Aim 2***

To compare the proportion of clinical quiescence at day 7 in the three treatment arms, a logistic regression model will be conducted. The outcome variable will be the binary classification of quiescence of inflammatory status determined by the masked study ophthalmologist. The analysis will be two-sided, with a type I error rate (alpha) of 0.05/2; the predictor is the study arm (as a factor). In addition, we will repeat this analysis for data collected on day 28. We propose a Bonferroni correction, for an alpha of 0.05/2. We will also tabulate the number of individuals who change status, reporting the 2x2 table of day 7 vs day 28 status, by arm. Missing data will be reported, and a sensitivity analysis will be conducted to assess the degree to which missing data could have affected the findings. Data collected at +/- two days will be used (day 7 data can be collected between days 5-9, and day 28 data can be collected at 26-30 days).

Participants will also be administered a questionnaire querying when they felt symptomatic improvement other their condition. This analysis will be viewed as hypothesis generating and will be treated as supplementary and in addition to the main finding.

***Nested Observational Aim***

To assess the observational effect of topical corticosteroid administered prior to study enrollment on diagnostic yield of quantitative PCR an **ANCOVA** of log transformed mean viral load at initial anterior chamber paracentesis vs. frequency of pre-study topical steroid use will be conducted. The analysis will be **two-sided** with a **type 1 error rate** (alpha) of 0.05. We will report the findings unadjusted as hypothesis generating for a future larger trial. This will be sharply demarcated from the primary analysis. Bonferroni-Holm correction will be considered for this sub-aim.

***Secondary analyses***

In addition, an efficacy analysis will be conducted in which we include phenotype as a binary predictor (and the interaction with treatment). Phenotype of CMV anterior uveitis will be determined by the masked study ophthalmologist (acute/recurrent vs. chronic). Analysis will consist of an **ANCOVA** as before, with the additional covariates. We will

perform a hypothesis test of the coefficient for the phenotype-treatment interactions, **two-sided**, with a **type I error rate** (alpha) of 0.05.

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.

## ETHICS AND DISSEMINATION

This protocol and informed consent forms have been approved by the IRB at the University of California, San Francisco, and Khon Kaen University. The trial is registered at clinicaltrials.gov (NCT03576898). Protocol modifications are submitted to the relevant parties for review and/or approval. Subsequent to initial review and approval, the responsible local IRB/ethics committees (ECs) will review the protocol annually. The investigators will make safety and progress reports to the IRBs/ECs at least annually and within 3 months of study termination or completion at their site. These reports will include the total number of participants enrolled, adverse events, and summaries of each DSMC review of safety and/or efficacy meeting. Upon completion of the 28 days study treatment protocol, the participant's treatment plan can be modified in any way the participant's ophthalmologist feels fit.

Written consent will be obtained for all study procedures after explaining the patient's disease, prognosis, and treatment options, discussing the risks and benefits of participation, and addressing the patient's questions and concerns. The protocol site-specific informed consent forms in local language (Thai, Chinese, or English), participant education and recruitment materials, other requested documented, and any subsequent modifications shall also be reviewed and approved by the ethical review bodies at each respective site. Table 5 summarises the study protocol and trial registration information.

Only key study personnel will have access to identifying information of participants, while administrative forms and specimens will be de-identified.

A portion of biological samples from the second anterior chamber paracentesis (approximately 20μL of aqueous fluid) will be obtained for use in a future study of virus genome assay. A consent will be obtained and included in the main study consent form to specifically address the collection and future studies of such aqueous fluid.

The results of this trial will be presented at local and international meetings and submitted to peer-reviewed journals for publication.

## DATA SHARING STATEMENT

Data will not be made publicly available; investigators interested in data from this study should contact the principal investigator (john.gonzales@ucsf.edu) for inquiries.

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## Tables

| | Strongly disagree | Somewhat disagree | Somewhat agree | Strongly agree |
|---|---|---|---|---|
| 1. I think that oral valganciclovir is an effective medication to treat CMV anterior uveitis | 0 (0%) | 8 (11.8%) | 36 (52.9%) | 24 (35.3%) |
| 2. I think that topical ganciclovir 2% eyedrops is an effective medication to treat CMV anterior uveitis | 1 (1.5%) | 11 (16.2%) | 33 (48.5%) | 23 (33.82%) |
| 3. I think that oral valganciclovir is a safe medication to treat short term ocular inflammation related to CMV anterior uveitis | 0 (0%) | 11 (16.2%) | 36 (52.9%) | 21 (30.9%) |
| 4. I think that oral valganciclovir is a safe medication to treat long term ocular inflammation and prevent recurrences of CMV anterior uveitis | 2 (2.9%) | 20 (29.4%) | 33 (48.5%) | 13 (19.1%) |
| 5. I think that topical ganciclovir eyedrops are a safe medication to treat short term ocular inflammation related to CMV anterior uveitis | 1 (1.5%) | 8 (11.8%) | 28 (41.2%) | 31 (45.6%) |
| 6. I think that topical ganciclovir eyedrops are a safe medication to treat long term ocular inflammation and prevent recurrences of CMV anterior uveitis | 1 (1.5%) | 17 (25.0%) | 31 (45.6%) | 19 (27.9%) |
| 7. I think that there is a benefit to treating CMV with long term therapy (beyond 6 months) | 1 (1.5%) | 8 (11.8%) | 26 (38.2%) | 33 (48.5%) |

**Table 1.** Survey results regarding CMV anterior uveitis practice patterns at the Biennial CKPU meeting in Bangkok, Thailand (n=68)

| | Oral valganciclovir | Topical ganciclovir eyedrops | No treatment |
|---|---|---|---|
| 1. The medication I am most likely to use to treat CMV anterior uveitis is… | 25 (36.8%) | 39 (57.4%) | 4 (5.88%) |
| 2. If cost were not an issue my preferred medication to treat CMV anterior uveitis would be… | 39 (56.5%) | 27 (39.1%) | 1 (1.5%) |

**Table 2.** Survey results regarding preferred treatment therapy of CMV anterior uveitis at the Biennial CKPU meeting in Bangkok, Thailand (n=68)

| Exam Visit | Procedures |
|---|---|
| Pre-study Visit (Exam #0) – routine care appointment | - Clinical eye exam suggests a viral aetiology<br>- Anterior chamber paracentesis is performed to establish a viral aetiology<br>  o Half of fluid will be used for in-house directed PCR for HSV, VZV, and CMV<br>  o At least 50□L of fluid will be preserved and used for quantitative CMV PCR to be conducted at a single United States laboratory in eligible participants<br>- Laboratory screening orders (CBC, chemistry panel, pregnancy test, HIV status)<br>- Patients will be prescribed topical corticosteroid (prednisolone acetate 1%) to be used 1 drop in affected eye 4 times daily (this is typical standard of care)<br>- Patients requiring management of elevated intraocular pressure (IOP) will be prescribed IOP-lowering medication according to treating ophthalmologist's discretion and best medical judgement |
| Exam #1 (Day 1 of study (7 days after Exam #0)) | - Review of results of in-house CMV PCR, and laboratory results<br>- Consent, enrollment, randomization to treatment arm if participants meet eligibility criteria<br>- Treatment initiation<br>- Clinical eye exam (in the following order, VA, slit lamp exam of anterior segment, IOP)<br>- Endothelial cell morphology and density using specular microscopy or confocal microscopy |
| Exam #2 (Day 7 of study) | - Clinical eye exam (in the following order, VA, slit lamp exam of anterior segment, IOP)<br>- AC paracentesis #2<br>- Laboratory monitoring orders (CBC, Cr, BUN) |
| Exam #3 (Day 28 of study) | - Clinical eye examination (in the following order, VA, slit lamp exam of anterior segment, IOP)<br>- Endothelial cell density using specular microscopy or confocal microscopy<br>- Laboratory monitoring orders (CBC, Cr, BUN) |

**Table 3.** Timeline of Major Study Procedures. AC: anterior chamber; CBC: complete blood count; CMV: cytomegalovirus; Cr: creatinine; BUN: blood urea nitrogen; HSV: herpes simplex virus; IOP: intraocular pressure; PCR: polymerase chain reaction; VA: visual acuity; VZV: varicella-zoster virus

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| • CMV positivity by directed PCR from an anterior chamber paracentesis specimen conducted at any time in the past<br>• Active anterior uveitis using Standardization of Uveitis Nomenclature (SUN) Working group with clinical impression of CMV as the etiologic agent<br>  ○ ≥1+ anterior chamber cell AND/OR<br>  ○ Other signs consistent with active inflammation, such as elevated intraocular pressure (IOP), corneal oedema, and/or active keratic precipitates (KPs)<br>• Participant willingness to use an acceptable method of contraception during the study period (i.e. pharmacologic, barrier methods, or abstinence). | • Participants <20 years of age<br>• Inactive anterior uveitis<br>• Active intermediate or posterior inflammation (involvement of vitreous, choroid, or retina)<br>• Participants who have received antiviral therapy <14 days prior to enrollment<br>• Participants who have received periocular or intraocular corticosteroid injection < 8 weeks prior to enrollment<br>• Current use of oral corticosteroids<br>• Immunocompromised participants (primary or secondary immunodeficiency disorders)<br>• Prior immunosuppressive therapy in the past 3 months<br>• Directed PCR testing positive for herpes simplex virus (HSV) or varicella zoster virus (VZV)<br>• Plans to conceive during the study period, pregnant or breast-feeding mothers (blood or urine pregnancy test for all females of child-bearing age is mandatory within 4 weeks prior to enrollment)<br>• Complete blood count with white blood cell, absolute neutrophil, or platelet count lower than the lower limit of reference laboratory normal<br>• Blood urea nitrogen or creatinine above the upper limit of reference laboratory normal<br>• 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 |

**Table 4.** Study participant inclusion and exclusion criteria. CMV: cytomegalovirus; HSV: herpes simplex virus; PCR: polymerase chain reaction; VZV: varicella-zoster virus

| Data Category | Information |
|---|---|
| Primary registry and trial identifying number | ClinicalTrials.gov NCT03576898 |
| Date of registration in primary registry | July 3, 2018 |
| Secondary identifying numbers | |
| Source of monetary or material support | Francis I. Proctor Foundation, University of California, San Francisco |
| Contact for queries | John A. Gonzales, MD (john.gonzales@ucsf.edu) |
| Title | Systemic and Topical Antiviral Control of Cytomegalovirus Anterior uveitis: Treatment Outcomes |
| Countries of recruitment | Thailand, Taiwan, USA |
| Health condition or problem studied | Cytomegalovirus anterior uveitis treatment |
| Intervention(s) | Intervention: treating with 900mg BID of oral valganciclovir, or topical 2% ganciclovir solution q2h for a duration of 28 days Control: placebo tablets and eyedrops |
| Key eligibility criteria | CMV positive anterior chamber paracentesis, active signs of anterior uveitis, no history of immunodeficiency, normal laboratory values for CBC, and renal function |
| Study type | Competitive block randomised, placebo controlled clinical trial |
| Date of first enrolment | N/A |
| Target sample size | 99, 33 per treatment arm |
| Primary outcome | Change in log transformed viral load |
| Key secondary outcomes | Objective and subjective time to achieve clinical quiescence, and the relationship of topical steroid strength and dosing on initial anterior chamber paracentesis quantitative viral load for CMV |
| Project timeline | July 2019 - August 2021 |

**Table 5.** Trial registration data and protocol summary

**Appendix 1 – Informed Consent Document**

**UNIVERSITY OF CALIFORNIA, SAN FRANCISCO**
**CONSENT TO PARTICIPATE IN A RESEARCH STUDY**

**Study Title: Systemic and Topical Antiviral Control of CMV Anterior uveitis: Treatment Outcomes (STACCATO)**

| | |
|---|---|
| Research Project Director: | John A. Gonzales, M.D., Uveitis Specialist and Associate Professor of Ophthalmology at UCSF, Room S334, 513 Parnassus Ave, San Francisco, CA.<br>Phone: 415-502-2664; e-mail: john.gonzales@ucsf.edu |

| | |
|---|---|
| Study Coordinator: | Jaskirat Takhar<br>Phone: 415-502-2690; e-mail: jaskirat.takhar@ucsf.edu |

This is a clinical research study. Your study doctor, John Gonzales, M.D., from the UCSF Proctor Foundation, will explain the study to you.

Research studies include only people who choose to take part. Please take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask your study doctor.

You are being asked to take part in this study because you are suspected to have anterior uveitis (inflammation of the front portion of the eye) caused by the virus, cytomegalovirus (CMV).

**Why is this study being done?**

CMV anterior uveitis can cause increased pressure inside the eye, pain, cataract formation, and loss of vision. There are many treatment options, however we currently don't know which one is superior and there is no defined standard of care. The purpose of this study is to compare the most commonly used treatment options used in the management of your condition. After confirming the diagnosis, we will assign you to one of three treatment groups and compare the difference in outcomes between each group.

In addition, prior to this visit, the physician who referred you to our clinic may or may not have started you on steroid eye drops to control your inflammation. For our study we intend on giving all participants the same amount of steroid drops for the 28 day duration. We intend on investigating whether the amount of steroid given prior to you starting in this study affects the amount of virus found in the fluid from the eye.

**How many people will take part in this study?**

Overall, about 99 people will take part in this study. Approximately 33 people will be in each of the 3 treatment groups. The treatment options will be 28 days of oral antiviral therapy, 28 days of 2% antiviral eye drops, or 28 days of inactive therapy.

We expect that up to 70 people will be enrolled into this study here at UCSF. You will be randomly placed into a treatment group, and neither you nor your study doctor will know which treatment you are receiving.

**What will happen if I take part in this research study?**

**Before you can start the study:**

Pre-study visit: You will need to have the following exams, tests, and procedures to find out if you can be enrolled in the study. These exams, tests, and procedures are part of regular care for patients with suspected CMV anterior uveitis, and are routinely done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

- Initial removal of fluid from the front part of your eye (anterior chamber paracentesis): In our clinic we will anesthetise (or numb) your cornea with eye drops and carefully remove a small amount of fluid from inside the front portion of your eye using a small needle. The fluid will then be sent for laboratory testing to determine whether CMV is present in the fluid.
- Initial laboratory testing: we will order blood tests to look at your kidney function, red blood cell count, white blood cell count, and platelet count. Abnormal results will exclude you from participating in this study.
- Women of child-bearing age will undergo a blood pregnancy test, as some study medications can result in birth defects

**During the main part of the study:**

Exam #1: Approximately 7 days after completing the exams, tests, and procedures above, you will return for follow-up examination, and review of testing to determine if you can participate in this study. If eligible you will complete this form to give your consent to participate in this study. After completion, you will then be enrolled, and then "randomised" into one of the study groups described below. Randomization means that you are put into a group by chance. A computer program will place you in one of the groups. Neither you nor your doctor can choose the group you will be in. You will have an equal chance of being placed in any group. All groups will receive their assigned treatment for 28 days. You will receive both oral tablets and topical eye drops. You will not know which ones are active or inactive. You will be instructed to take 4 pills per day, 2 every morning and 2 every evening. You will also be instructed to apply 1 drop of eye solution every 2 hours while awake. You will repeat this regimen every day for all 28 days of treatment. Participants in all groups will be receiving a steroid eye drop regimen of prednisolone acetate 1% three times per day in the affected eye. It is important to note that all three treatment groups are used by practicing ophthalmologists to manage

your condition. A current standard of care has not been defined, which is the question this study hopes to answer.

- **If you are in group 1** you will receive valganciclovir pills and inactive eye drops.
- **If you are in group 2** you will receive inactive pills and topical ganciclovir 2% eye drops.
- **If you are in group 3** you will receive inactive pills and inactive eye drops.

You will also need follow-up exams and tests that are part of regular CMV anterior uveitis care:

- Two follow-up clinic visits will be scheduled at day 7 (Exam #2) and day 28 (Exam #3) of treatment. At each of these visits you will be seen by an ophthalmologist. On study day 7 (Exam #2) we will again remove fluid from your eye (a second anterior chamber paracentesis), to see if the amount of virus in the eye has decreased.
- At day 7 and day 28 blood tests will be repeated. These will be compared with baseline kidney function and blood counts. Any abnormal findings will require removal from the study.

**When you are finished with 28 days of treatment:**

Once you have finished 28 days of treatment, including your final clinic visit and evaluation, you will have completed the study. Your treating ophthalmologist will determine whether any continued treatment is necessary.

**Study location?**

All study procedures will be done at the UCSF Proctor Clinic.

**Study Chart**

| **Visits** | **What happens** |
|---|---|
| **Pre-study visit** | • Baseline fluid removal from the front of the eye using a needle<br>• Baseline blood tests<br>• Pregnancy testing for women of child-bearing age<br>• Baseline clinical exam |
| **Enrollment/ Randomization** (Day 1) | • Follow-up clinical examination<br>• Consent, Enrollment and randomization to treatment<br>• Begin treatment: 2 pills twice daily, 6 drops per day<br>• Corneal endothelial confocal microscopy |
| **Exam #2** | • Follow-up clinical exam |

| | |
|---|---|
| (Day 7) | • Repeat removal of fluid from the eye using a needle (anterior chamber paracentesis #2)<br>• Repeat blood tests |
| **Exam #3**<br>(Day 28) | • Follow-up clinical exam<br>• Repeat blood tests #2<br>• Corneal endothelial confocal microscopy |

**Can I stop being in the study?**

Yes. You can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop your participation safely.

It is important to tell the study doctor if you are thinking about stopping so any risks of the infection or stopping treatment can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest, if you do not follow the study rules, or if the study is stopped.

**What side effects or risks can I expect from being in the study?**

You may have side effects while in the study. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away soon after you stop taking these antiviral medications. In some cases, side effects can be serious, long lasting, or may never go away. In very rare cases, side effects may include death.

You should talk to your study doctor about any side effects you experience while taking part in the study.

**Procedural risks:**

The procedure to remove fluid from the eye (anterior chamber paracentesis) carries some risk whether they are performed in the study or as part of your routine care outside the study. The second anterior chamber paracentesis is being conducted as part of routine clinical care. It is a typically safe procedure, although some may experience pain or discomfort. To reduce discomfort, eye numbing drops will be given before the procedure is performed. A severe but extremely rare complication of this procedure includes blood accumulation in the eye (hyphema), persistent leakage of fluid from the eye (leakage of aqueous humour), infection inside the eye (endophthalmitis),

and traumatic cataract, which can affect vision. In extremely rare cases, endophthalmitis may require removal of the eye.

**Medication risks:**

| | Valganciclovir | Topical Ganciclovir |
|---|---|---|
| Rare but Serious Side Effects | - Low red blood cell count causing anemia and fatigue<br>- Low white blood cell count increasing risk of infection<br>- Low platelet count leading to risk of bleeding<br>- Kidney failure<br>- Infertility<br>- Birth defects (in pregnant women)<br>- Cancer | - Allergic reaction |
| More Common but Less Serious Side Effects | - Diarrhea<br>- Nausea<br>- Vomiting<br>- Stomach pain | - Burning sensation in eye<br>- Temporary blurred vision |
| Less common and Less Serious Side Effects | - Fever<br>- Headache<br>- Sleep disturbances | - Eye redness<br>- Eye irritation |

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

**Placebo (inactive) risks:**
If you are in the group that receives placebo, your condition will go without the active (antiviral) treatment for 28 days. Some uveitis specialists will observe their patients with CMV anterior uveitis to see if the inflammation will resolve on its own or with just steroid eye drops. In such cases, starting oral or topical antivirals is reserved for when the inflammation does not become subside after 4 weeks. The risks of delaying treatment include damage to the cornea with a decrease in vision or the development of glaucoma, which can result in vision loss. However, many uveitis specialists feel that a reasonable and safe option in CMV anterior uveitis is to monitor initially without starting oral or topical antiviral medication.

**Blood drawing (venipuncture) risks:**

Drawing blood may cause temporary discomfort from the needle stick, bruising, infection, and fainting.

**Reproductive risks:**
You should not become pregnant or father a baby while on this study because the drugs in this study can affect an unborn baby. Women should not breastfeed a baby while on this study. It is important to understand that you need to use birth control while in this study. Acceptable forms of birth control include intra-uterine (placed inside the uterus), oral (birth control pills), birth control patch placed on your skin, or barrier (i.e. condoms). Check with your study doctor about what kind of birth control methods to use and how long to use them. Some methods might not be approved for use in this study.

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

For more information about risks and side effects, ask your study doctor.

**Are there benefits to taking part in the study?**

It is possible that one of the treatment options proves to treat CMV anterior uveitis better than the others, in which case your infection will be treated more effectively. It is also possible that one of the treatment options will prove to have fewer side effects or complications, in which case you will be subjected to fewer side effects.

Additionally, we hope this study will help doctors learn more about CMV anterior uveitis, and that this information will help in the treatment of future patients with conditions like yours.

**What other choices do I have if I do not take part in this study?**

If you choose not to take part in this study, your quality of care will remain the same. The fluid in your eye will still be sampled at the first visit in order to diagnose your infection. Your treatment regimen will be chosen and initiated at your treating ophthalmologist's discretion. At UCSF the standard of care includes oral valganciclovir 900 mg given twice daily or topical ganciclovir 2% provided by a compounding pharmacy for a period of 28 days, with routine follow-up and blood tests. Occasionally, some patients are monitored with just steroid eye drops and are not started on antivirals if the inflammation does not become quiet after approximately 28 days. Additionally, patients treated outside of the study may have a repeat (or second anterior chamber paracentesis) to demonstrate that the amount of virus in their ocular fluid is decreasing. Therefore, this trial is very similar to what happens in "real life".

**How will information about me be kept confidential?**

Participation in research involves some loss of privacy. We will do our best to make sure that information about you is kept confidential, but we cannot guarantee total privacy. Some information from your medical records will be collected and used for this study. If you do not have a UCSF medical record, one will be created for you. Your signed consent form and some of your research tests will be added to your UCSF medical record. Therefore, people involved with your future care and insurance may become aware of your participation and of any information added to your medical record as a result of your participation. Study tests that are performed by research labs, and information gathered directly from you by the researchers will be part of your research records but will not be added to your medical record. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Authorised representatives from the following organizations may review your research data for the purpose of monitoring or managing the conduct of this study:

- Representatives of UCSF Proctor Foundation
- Representatives of the University of California
- Representatives of the Food and Drug Administration (FDA)

**Are there any costs to me for taking part in this study?**

Two types of procedures will be done during this study. Some are part of your standard medical care, such as the first eye fluid analysis, and others are primarily for research. You or your insurer will be billed for the standard medical care. You will be responsible for your co-pays, deductibles, and any other charges that your insurer will not pay. There is a possibility that your insurer may not cover all standard medical care costs if you are receiving medical services out of network. Any procedures done only for research will not be charged to you or your insurer. Although the second eye fluid removal is considered part of routine clinical care, the procedure and testing of fluid will be paid for at no cost to the participant.

All antiviral and inactive (placebo) therapy will be provided for you at no cost.

**Will I be paid for taking part in this study?**

You will not be paid for taking part in this study.

**What happens if I am injured because I took part in this study?**

It is important that you tell your study doctor, Dr. John Gonzales, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him at 415-502-2664.

**Treatment and Compensation for Injury:** If you are injured as a result of being in this study, the University of California will provide necessary medical

treatment. The costs of the treatment may be billed to you or your insurer just like any other medical costs, or covered by the University of California, depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Institutional Review Board at 415- 476-1814.

**What are my rights if I take part in this study?**

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

**Who pays for this study?**

Proctor Foundation/UCSF.

**Who can answer my questions about the study?**

You can talk to your study doctor about any questions, concerns, or complaints you have about this study. Contact your study doctor John Gonzales, M.D., at 415-502-2664.

If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please call the office of the Institutional Review Board at 415-476-1814.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

**CONSENT**

Please read each sentence below and think about your choice. After reading each sentence, put your initials in the "Yes" or "No" box. If you have any questions about this study, please talk to the study doctor or nurse.

No matter what you decide to do, it will not affect your care.

1. My specimens and associated data may be kept for use in research to determine the amount of virus in my sample.

| YES | NO |
|---|---|

2. Someone may contact me in the future to ask me to take part in more research.

| YES | NO |
|---|---|

3. Any leftover fluid from your anterior chamber paracentesis samples will be collected at stored at UCSF/Proctor Foundation laboratory. In the future the following tests may be performed on any leftover fluid:

- Genetic sequencing of the DNA of CMV, which means identifying the strain of virus you may have based on its genetic signature.
- The duration of specimen retention will be until the specimen is used up.
- Your name and any identifying information will not be included in this stored sample (will become an anonymous sample) to protect your confidentiality/privacy
- Only UCSF researchers will have access to the specimens and the data.
- Your specimen will not be used for commercial value or gain, and subjects will not be paid for their sample
- Should you decide to request destruction of your sample, this will not be possible as your specimen will have been made anonymous and cannot be traced back to you.
- There will be no genetic testing on your own, human DNA, only on the virus (CMV).

| YES | NO |
|---|---|

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

You will be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, you should sign below.

| | |
|---|---|
| Date | Participant's Signature for Consent |
| Date | Person Obtaining Consent |

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | #1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | #2a | Trial identifier and registry name. If not yet registered, name of intended registry | 1 |
| Trial registration: data set | #2b | All items from the World Health Organization Trial Registration Data Set | 22 |
| Protocol version | #3 | Date and version identifier | 1 |
| Funding | #4 | Sources and types of financial, material, and other support | 1 |

| | | | |
|---|---|---|---|
| Roles and responsibilities: contributorship | #5a | Names, affiliations, and roles of protocol contributors | 2 |
| Roles and responsibilities: sponsor contact information | #5b | Name and contact information for the trial sponsor | 1 |
| Roles and responsibilities: sponsor and funder | #5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 2 |
| Roles and responsibilities: committees | #5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 2 |
| **Introduction** | | | |
| Background and rationale | #6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5 |
| Background and rationale: choice of comparators | #6b | Explanation for choice of comparators | 5 |
| Objectives | #7 | Specific objectives or hypotheses | 6 |
| Trial design | #8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory) | 6 |
| **Methods:** | | | |

| | | | |
|---|---|---|---|
| **Participants, interventions, and outcomes** | | | |
| Study setting | #9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 6 |
| Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 |
| Interventions: description | #11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 7 |
| Interventions: modifications | #11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease) | 8 |
| Interventions: adherance | #11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) | 9 |
| Interventions: concomitant care | #11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 9 |
| Outcomes | #12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10 |
| Participant timeline | #13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly | 20 |

| | | | |
|---|---|---|---|
| | | recommended (see Figure) | |
| Sample size | #14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 12 |
| Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 6 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: sequence generation | #16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 7 |
| Allocation concealment mechanism | #16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 7 |
| Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 7 |
| Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 7 |
| Blinding (masking): emergency unblinding | #17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 14 (on MOP-attachment) |
| **Methods: Data collection,** | | | |

| | | | |
|---|---|---|---|
| **management, and analysis** | | | |
| Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10 |
| Data collection plan: retention | #18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 10 |
| Data management | #19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 11 |
| Statistics: outcomes | #20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 12 |
| Statistics: additional analyses | #20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 13 |
| Statistics: analysis population and missing data | #20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 13 |
| **Methods: Monitoring** | | | |
| Data monitoring: formal committee | #21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement | 11 |

| | | | |
|---|---|---|---|
| | | of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | |
| Data monitoring: interim analysis | #21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 12 |
| Harms | #22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 8 |
| Auditing | #23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 34 (on MOP-attachment) |
| **Ethics and dissemination** | | | |
| Research ethics approval | #24 | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval | 14 |
| Protocol amendments | #25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators) | 13 |
| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 7 |
| Consent or assent: ancillary studies | #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | 14 |
| Confidentiality | #27 | How personal information about potential and enrolled participants will be collected, shared, and | 6 (on Appendix- |

| | | | |
|---|---|---|---|
| | | maintained in order to protect confidentiality before, during, and after the trial | attachment) |
| Declaration of interests | #28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 2 |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 14 |
| Ancillary and post trial care | #30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 3 & 7(on Appendix-attachment) |
| Dissemination policy: trial results | #31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 14 |
| Dissemination policy: authorship | #31b | Authorship eligibility guidelines and any intended use of professional writers | 14 |
| Dissemination policy: reproducible research | #31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 14 |
| **Appendices** | | | |
| Informed consent materials | #32 | Model consent form and other related documentation given to participants and authorised surrogates | 1 (on Appendix-attachment) |
| Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | 9 (on Appendix-attachment) |

Notes:

- 17b: 14 (on MOP-attachment)
- 23: 34 (on MOP-attachment) The SPIRIT checklist is distributed under the terms of the Creative Commons Attribution License CC-BY-ND 3.0. This checklist was completed on 22. July 2019

using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai