Systemic and topical antiviral control of cytomegalovirus anterior uveitis: treatment outcomes (NCT03586284)

Statistical Analysis Plan – January 14, 2025

1. Administrative Information

Trial registration:	TBD (hyperlink to clinicaltrials.gov)
Funder:	Application stage
SAP Version:	Version 3, Updated 2025-01-14 A revision history for this document is included at the end.
Protocol Version:	Refers to Protocol Version 1
Contributors and Signatures:	Person writing the SAP: Fanxiu Xiong, MAS[1], Benjamin Arnold, PhD MPH[1], John Gonzales, MD[1], Tom Lietman, MD[1] Statistician responsible: Benjamin Arnold, PhD MPH[1]_____ Principal Investigator: John A. Gonzales, MD[1] _____

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SAP Table of Contents

This SAP was organized following guidelines proposed in:

Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. *JAMA*. 2017;318: 2337–2343. PMID: 29260229

The companion computational notebook with underlying sample size calculations presented herein is entitled: `STACCATO_sample_size.Rmd / .html`; it is saved in the same directory as this document.

2. Introduction

2.1. Background and rationale

The systemic and topical antiviral control of cytomegalovirus anterior uveitis: treatment outcomes trial will enroll participants with PCR-proven CMV anterior uveitis into a sequential double-masked randomized clinical trial in the US and Asia. In Trial I, participants will be randomized to oral valganciclovir, topical ganciclovir 2%, or placebo and viral load after 7 days of treatment will be compared by arm. After participants achieve control of their inflammation, they will be offered enrollment to Trial II in which they will be re-randomized to one of the three arms in Trial I at prophylactic dosing and monitored for recurrence. Additionally, patients with known CMV anterior uveitis, but who are inactive, may enter Trial II directly. Recurrences of inflammation in Trial II will be assessed for presence of detectable viral load.

The purpose of this study is to identify the antiviral therapy best at reducing CMV load, assess use of long-term antiviral prophylaxis in decreasing recurrence, and examine the role of antiviral resistance mutations in CMV anterior uveitis.

2.2. Objectives

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

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

1b: We hypothesize that a greater proportion of participants randomized to oral valganciclovir will have clinical inactivity at day 7 compared to those randomized to either topical ganciclovir 2% or placebo.

1c: We hypothesize RNA sequencing of aqueous humor fluid obtained as part of eligibility determination for Trial I, but negative for CMV, will identify other pathogens and provide host transcriptional profiling.

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

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

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

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

3a: We hypothesize participants completing both trials who exhibit recurrent inflammation after antiviral suppression (Trial II) will have a higher prevalence of CMV mutations associated with antiviral resistance compared to baseline samples (Trial I).
3b: We hypothesize that host transcriptional profiles will (i) distinguish CMV anterior uveitis from anterior uveitis cases negative for CMV and unable to enter Trial I, (ii) we will identify non-CMV pathogens in cases unable to enter Trial I, and (iii) identify unique signatures associated with recurrent inflammation in Trial II participants.

3. Study Methods

3.1. Trial design

The trial is an individual sequential double-masked randomized placebo-controlled trial. In trial I, study subjects are randomized to three arms:

1) Oral valganciclovir
2) Topical ganciclovir 2%
3) Placebo

After achieving clinical quiescence, participants will be offered enrollment into Trial II where they will be randomized to one of the three arms:

1) Oral valganciclovir
2) Topical ganciclovir 2%
3) Placebo

3.2. Randomization

For both trials, individuals will be randomized to the three arms in a 1:1:1 ratio. Randomization will be stratified by recruitment site, using randomly permuted blocks in multiples of 3 stratified, permuted block randomization will ensure that an approximately equal number of patients are randomized to each treatment arm by site. The masked allocation sequences will be generated at the UCSF Data Coordinating Center and implemented through a REDCap randomization module to ensure allocation concealment.

3.3. Sample size

Primary analysis for Trial I. The power calculation was based on the primary outcome, base-10 logarithm-transformed CMV viral load.[1] We informed the calculation with viral loads from our recently completed RCT, in which we measured viral load at baseline and 7 days post-treatment. The standard deviation (SD) of log-transformed viral load 7 days post-treatment in this RCT was 0.98. Since the primary analysis will adjust for baseline viral load, we used an estimate of the residual standard deviation, which is $SD_r = SD\sqrt{1-r^2}$, where r is the correlation between the baseline measure and primary endpoint. In our recently completed RCT, the correlation between baseline and post-treatment viral load was 0.74. Assuming a significance level of 0.05/2=0.025 to account for multiple comparisons and an SD_r of 0.66 in $\log_{10}$ viral load, we estimate that we will have 80% power to detect a difference of 0.50 in $\log_{10}$ viral load (from

3.11 to 2.61) between arms with 35 study participants per arm (117 total allowing for approximately 10% loss to follow up). A reduction of 0.50 in $\log_{10}$ viral load is equivalent to a $10^{0.50} = 3.2$-fold decrease in CMV viral load, which is small but clinically meaningful in the context of CMV antivirals that typically consider 10-fold reductions. This calculation is based on the standard power formula for the T-test using an estimated residual standard deviation.

Primary analysis for Trial II. The primary outcome for Trial II is the proportion of participants experiencing CMV recurrence over 12 months. We acknowledge that hypothesizing fewer recurrences in the placebo group compared to the oral antiviral group is contradictory to what one might think would typically occur with exposure to antivirals. However, data from a previous observational study showed a one-year recurrence risk of 25.0% (3/12) among participants who did not receive antiviral treatment and 75.0% (9/12) in participants receiving systemic antiviral treatment (oral ganciclovir or valganciclovir), which is similar to the experience of other research.[2, 3] Additionally, it is well documented that long-term suppressive-level dosing of antiviral in an immunodeficient host or in an immunologically privileged organ (such as the eye) can create an environment permissive to the development of antiviral resistance. Given 28 participants per arm (105 total from Trial I with 20% loss to follow-up) and a recurrence risk of 25% in the placebo group, we can detect a difference of 40 percentage points between the oral valganciclovir and the placebo groups (from 25% to 65%), using a two-sample z-test for proportions with 80% power and a two-sided alpha of 0.05/2.

For Specific Aim 3, we estimated the minimum detectable effect for the difference in prevalence of infections that have antiviral resistance mutations among recurrent infections in Trial II among patients who receive long-term antiviral prophylaxis compared with their infections at baseline. Aim 3 will focus on patients in the two active treatment arms in Trial II, because we would expect long-term use of topical and oral antivirals to lead to similar selection for antiviral resistance. From 117 patients enrolled, we conservatively assumed that 72% of patients would complete both Trial I and Trial II (n=84). We further assumed that 60% of patients will develop a recurrent infection over the monitoring period during the trials (Table 1)[5]. Participants who receive active treatment in Trial II (treatment regimens C+D) will contribute to the Aim 3 analysis (n=34). The analysis will be a paired analysis, comparing the proportion of recurrent infections with antiviral resistance mutations with that same proportion at baseline.

Table 1. Anticipated participant enrollment, and follow-up by treatment patterns

Treatment Pattern, Trial I → Trial II	**Number enrolled**	**Number completing Trial I** (assumed 90%)	**Number completing Trial I and Trial II** (assumed 80% from Trial I)	**Number with Recurrent Infections in Trial II, included in Aim 3** (assumed 60%)
A. Placebo → Placebo	13	12	10	
B. Treatment → Placebo Topical g. → Placebo Oral v. → Placebo	26	23	18	
C. Placebo → Treatment Placebo → Topical g. Placebo → Oral v.	26	23	18	11
D. Treatment → Treatment Topical g. → Topical g. Topical g. → Oral v. Oral v. → Topical g. Oral v. → Oral v.	52	47	38	23
Total	117	105	84	34

In the absence of longer-term antiviral therapy, we anticipate 5% of patients would experience recurrent infections with antiviral resistance mutations.[6, 7] A recent analysis of 2750 transplant recipients identified 30.4% patient samples had resistance to anti-CMV drugs.[8] We assumed 80% power and a two-sided alpha of 0.05. Under these assumptions, we estimate that we will be able to detect a 25% increase in the percentage of infections with antiviral resistance genes (from 5% to 30%), using a two-sample z-test for proportions with equal sample sizes. We note this detectable effect is conservative because it ignores possible correlation within-individuals in the paired outcomes, which would increase our power.

3.4. Statistical framework

For each trial, we propose two primary analyses:

- Comparison of oral valganciclovir and placebo arms (superiority comparison)
- Comparison of oral valganciclovir and topical ganciclovir 2% arms (superiority comparison)

Analyses of the trials will be intention to treat, with participants analyzed as they are randomized. We plan to use a frequentist approach to the analysis, with appropriate control for multiple testing and any interim analyses (details below).

3.5. Statistical interim analyses and stopping guidance

The interim analysis for both efficacy and futility will be conducted after one-third (1/3) and before one-half (1/2) of the patients have reached their primary endpoint; ideally when there are 15 patients per arm. With three arms and two primary comparisons of interest there are several scenarios for each interim analysis (Tables 2 and 3). The pre-specified action taken under each scenario will be agreed upon at the trial's first DSMC meeting. Separate interim analyses will be conducted for Trial I and Trial II, but will proceed similarly.

The DSMC will make one of the following recommendations:
• Continue the study without modifications
• Continue the study with modifications
• Terminate enrollment or treatment in the study because of safety concerns • Terminate enrollment or treatment in the trial because of futility

The DSMC will determine the database closure dates for each report in advance; archival copies of the (a) main database, and (b) study analysis file as they exist at the time of each report will be maintained. All reports will be sent using secure email to the members of the DSMC two weeks prior to each meeting.

Table 2. Treatment Arms

Arm 1: Oral valganciclovir	Arm 2: Ganciclovir 2% eyedrop	Arm 3: Placebo
A	B	C

Table 3. Possible Scenarios in Interim Analysis for Efficacy

Scenario	**Trial I/II interim result**	**Trial I/II interim result**	**Action for Trial I**	**Action for Trial II**
	A vs B	**A vs C**		
1	A ~ B	A ~ C	Continue all arms	Continue all arms
2	A ~ B	A < C	DSMC discussion[1]	DSMC discussion[1]
3	A ~ B	A > C	DSMC discussion[1]	DSMC discussion[1]
4	A < B	A ~ C	DSMC discussion[1]	DSMC discussion[1]
5	A < B	A < C	DSMC discussion[1]	DSMC discussion[1]
6	A < B	A > C	DSMC discussion[1]	DSMC discussion[1]
7	A > B	A ~ C	DSMC discussion[1]	DSMC discussion[1]

[1]DSMC discussion will be triggered in such a scenario to discuss the continuation or cessation of arm(s). Pre-specified actions for each scenario will be agreed upon between the investigators and the members of the DSMC at the trial's first DSMC meeting.

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

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

All subjects who provide informed consent will be accounted for in this study. We will present the number lost to follow-up and the number of protocol deviations by arm. The proportion of subjects reporting adverse events and serious adverse events will be reported by arm to the DSMC and will be compared using a Fisher's Exact Test.

Efficacy. Unmasked interim analyses will be conducted to determine whether or not sufficient evidence has accumulated to justify stopping the trial because one treatment is clearly superior

(and therefore should be extended to all future cases). The guidelines for efficacy will use group sequential boundaries for judging the statistical significance of the primary outcome measure. The Lan and DeMets5 flexible alpha spending approach with a power function is suggested, with a*(t*)= a (t*)Ɵ, where q= 3.561 chosen so that the two-sided P-value to stop the trial for efficacy is 0.0005 for each comparison.

Futility. Early discontinuation due to the unlikeliness of significant findings conditional on interim results may be considered, based on the original sample size considerations. For evaluating futility in each trial, we propose to use a simulation-based approach6 that estimates conditional power under the assumed design effect for the remainder of participants and under the current trend observed at the interim analysis. We propose that the DSMC consider discontinuation for futility if the conditional power to detect a difference in viral load in Trial I and recurrence in trial II drops below 20% at the interim analysis under the assumed design effect for the remainder of participants yet to be enrolled. As for the efficacy analysis, the futility analysis will be conducted separately for each Aim/hypothesis. Our proposed futility guidance is one-sided, and is subordinate to efficacy analyses. We will not propose to discontinue an arm for futility at the interim analysis if that arm may still participate in an efficacy comparison.

3.6. Timing of the final analysis

The final analysis for Trial I will be conducted only after 9 days have elapsed since the treatment of the final enrollee. The final analysis for Trial II will be conducted only after 7 months have elapsed since the treatment of the final enrollee. We anticipate at least two months for data processing and resolution of potential errors, anomalies, or missing values.

3.7. Timing of outcome assessments

In Trial I, outcome assessments will be conducted each week since beginning of treatment, with a window of 5 to 9 days for the final outcome. In Trial II, outcome assessments will be conducted every 3 months since beginning of treatment, with a window of 5 to 7 months for the final outcome.

4. Statistical Principles

4.1. Confidence intervals and *P*-values

The primary analysis for Trial I and Trial II will be conducted with a two-sided alpha of 0.05/2 = 0.025 to account for the two primary comparisons of interest (*oral valganciclovir* vs. *topical ganciclovir 2%*, *oral valganciclovir* vs. placebo). We will report 95% confidence intervals for the estimated differences between groups, on an absolute scale.

We will use a single-step, max-T approach to estimate p-values adjusted for multiple comparisons in each analysis.[12] Alongside unadjusted p-values and 95% confidence intervals, we will present max-T adjusted p-values and simultaneous 95% confidence intervals for differences that account for multiple comparisons.

We will employ a multiple testing hierarchical approach in the following manner:

1. Primary endpointss (alpha = 0.025 each trial)
    a. Trial I: viral load comparison
    b. Trial II: Number of recurrences comparison
2. Key secondary endpoints (alpha = 0.025 shared):
    a. Inflammation control
    b. Antiviral resistance mutations

Gate-keeping procedure:
Primary endpoints must be significant before testing secondaries
Holm-Bonferroni correction within each family
Truncated testing if primary endpoint non-significant
Family-wise error rate controlled at 0.05 using:
Separate error rates for each trial
Sequential gatekeeping
Multiplicity adjustment within families

4.2. Protocol deviations

The primary analyses will be conducted on an intent-to-treat basis. Individuals who receive the mandated dose of the medication to which they have been randomized are assumed adherent.

Adherence will be assessed via a study calendar patients use to report adherence and objectively by pill counting and weighing of medication bottles. The details of the assessments are included in the Protocol. Participants who miss a scheduled dose of medication will be instructed to continue with their next scheduled dose of tablets or eye drops.

In the event an individual receives a medication to which they were not randomized, a protocol deviation will be reported. A per protocol analysis will be conducted using only individuals who were adherent (an analysis to be sharply distinguished from the primary intent-to-treat analysis).

Any individual who receives even one dose of study medication or placebo is included in the safety analysis population. Any individual who receives study medication and who has a follow-up visit during the prescribed period is included in the complete case analysis.

5. Trial Population

5.1. Screening data

We will tabulate the total number screened, enrolled, and excluded (along with the reasons for exclusion).

5.2. Eligibility

The eligibility criteria for Trial I and Trial II are listed in section 5 (study population) of the clinical protocol.

5.3. Recruitment

We will report the number of participants screened, enrolled, randomized, treated, and measured at the primary endpoint with reasons for exclusion at each step for Trial I and Trial II.

5.4. Withdrawal/follow-up

We will tabulate the number of participants who withdraw, the number who die, and the number who are lost to follow-up before the primary outcome assessments in each Trial.

5.5. Baseline patient characteristics

We will report baseline characteristics by group, including sex, age in years, and clinical characteristics at enrollment.

6. Analysis

6.1. Outcome definitions

Trial I: Primary Endpoint: Log-transformed viral load after 7 days of treatment

Secondary Endpoints:

- Anterior chamber cell (days 7, 21)
- Intraocular pressure (days 7, 21)
- Visual acuity (days 7, 21)
- Controlled inactivity defined as ≤0.5+ anterior chamber cell AND intraocular pressure < 24 mmHg AND no cornea edema AND no active KPs (day 7, 21).

Trial II: Primary Endpoint: Proportion recurrent inflammation (≥1+ anterior chamber cell at any study visit) over 12 months

Secondary Endpoints:

- Prevalence of CMV associated with recurrence of uveitis

1a. The Aim 1 primary outcome **viral load after 7 days of treatment** will use the results from quantitative PCR at 7 days of treatment or placebo therapy (day 7 of trial). All quantitative PCR viral loads will undergo base-10 logarithm-transformation, with units converting from IU/mL to log IU/mL. Log-transformed viral loads will be used because the range of viral loads can be wide and dealing with very large and very small numbers is handled more efficiently using a logarithmic scale. Such normalization of the distribution of viral loads is consistent with standard practice in viral load analyses.[1]

1b. **Clinical outcomes and inactivity**: At each study visit participants will be clinically examined for ocular outcomes including AC inflammatory cells, intraocular pressure, and visual acuity. A study ophthalmologist will make a clinical determination as to whether the patient's inflammation is considered inactive. Clinical outcomes and the proportion of participants that

achieve quiescence by day 7 and 21 will be compared by arm. Patients will also be administered a questionnaire querying when they felt symptomatic improvement in their condition. This analysis will be viewed as hypothesis generating and will be treated as supplementary and in addition to the main finding.

2a. **Proportion recurrent inflammation over 12 months:** After achieving clinical quiescence, participants will be offered enrollment in Trial II where they would be randomized to long-term suppressive therapy to study the effect on recurrence rate of anterior uveitis. Each participant will be assessed for recurrence at each monthly visit during the 12 months or, alternatively, if participant's experience symptoms of a recurrence, then they will be instructed to call the local site's clinical coordinator to be scheduled for an evaluation within 1 to 2 days. Recurrence of uveitis in CMV is mostly typically symptomatic, so having participants call us is expected to be an effective strategy for catching recurrence of uveitis.[2, 13-15] If recurrence occurs, aqueous chamber fluid will be collected from participant at the time of recurrence to quantify viral load.

2b. **Prevalence of CMV associated with recurrence of uveitis:** Aqueous fluid obtained in participants exhibiting recurrence of uveitis during Trial II will be submitted for directed polymerase chain reaction testing to determine the proportion of uveitis flares associated with CMV (as compared to "sterile" inflammation).

3a. **Prevalence of mutations conferring antiviral resistance:** The analysis population will include individuals enrolled in both Trials I and II who are randomized to receive oral or topical antivirals in Trial II and experience a recurrent infection. Among patients that experience a recurrent infection (anticipated n=34, see Sample Size section 3.3), we will compare the proportion of infections with antiviral mutations upon recurrence with the proportion with antiviral mutations at baseline.

3b. **Pathogens other than CMV and host transcriptional profile**: (i) In aqueous we will describe the host transcriptional profile in participants with CMV anterior uveitis entering Trial I and in those who fail to enter Trial I due to having negative CMV PCR testing. (ii) We will identify and describe non-CMV pathogens in cases unable to enter Trial I. (iii) We will describe host transcriptional profile in participants during Trial II who have recurrent inflammation.

6.2. Analysis methods

Patients will be analyzed according to their assigned treatment (intention-to-treat).

Primary Analysis Trial I

Aim 1a. The primary analysis will compare **viral load at 7 days** between each study arm. The Aim 1 treatment effect will be reported as a difference in log IU/mL between the two arms.

We will model mean log viral load using linear regression that includes indicators for treatment arm, indicators for each site used to stratify the randomization, and baseline viral load. We will additionally estimate max-T simultaneous confidence intervals and p-values adjusted for the two

pair-wise comparisons (*oral valganciclovir* vs. *topical ganciclovir 2%*, *oral valganciclovir* vs. placebo)[12].

We will examine residuals for approximate normality and to check for outliers and will report the variance within each arm.

Primary analyses will be repeated using multiple imputed datasets as described in Section 6.3 when missing data thresholds are met.

Secondary Analyses Trial I

Aim 1b. **Clinical outcomes and inactivity**: We will compare the clinical outcomes and the proportion of patients that reach clinical inactivity by day 7 and by day 21 in the three treatment arms. We will estimate the difference in proportion using a linear or log-binomial regression model, as appropriate, that includes indicators for treatment arms, indicators for each site used to stratify the randomization, and baseline viral load. We will use marginal predictions from the regression models to estimate the difference in proportion. We will report max-T simultaneous confidence intervals and p-values adjusted for the two pair-wise comparisons (*oral valganciclovir* vs. *topical ganciclovir 2%*, *oral valganciclovir* vs. placebo).[12, 16]

Primary Analysis Trial II

Aim 2a. **Recurrence rate of anterior uveitis over 12 months:** The proposed analysis for sub-Aim 2a (Trial II), which is a clinical outcome, is a log-binomial regression model to compare cumulative inflammatory recurrence (based on anterior chamber cell ≥ 1+ cell) between treatment groups. We will censor participants at the first recurrence of anterior uveitis (based on anterior chamber cell ≥ 1+ cell) or 12 months. The model includes indicators for the treatment arm and indicators for each site used to stratify the randomization. We will estimate the risk ratios from the model for the two primary comparisons, *oral valganciclovir* vs. *topical ganciclovir 2%*, and *oral valganciclovir* vs. *placebo*. We will use a simulation-based permutation test to estimate p-values, and will report max-T simultaneous confidence intervals and p-values adjusted for the two pair-wise comparisons (*oral valganciclovir* vs. *topical ganciclovir 2%, oral valganciclovir* vs. *placebo*). Pre-specified sub-group analyses by sex will be performed.

Secondary Analyses Trial II

Aim 2b. **Prevalence of detectable CMV with recurrence.** The proposed outcome for Aim 2b is prevalence of detectable CMV (CMV positive) based on nucleic acid amplification testing obtained from anterior chamber fluid (aqueous humor) obtained at recurrence during Trial II. Thus, this sub-aim is a microbiologic outcome. We will calculate the proportion of recurrence episodes with detectable CMV viral load by using log-binomial regression with treatment arm indicators and site stratification. We will report adjusted confidence intervals and p-values for treatment arm comparisons. For correlation between viral load and inflammation, we will assess the relationship between log-transformed viral load and anterior chamber cell grade at recurrence of inflammation using a log-binomial regression model adjusting for treatment arm and site. Pre-specified subgroup analyses by sex will be performed for both components. We will handle missing data through sensitivity analyses using multiple imputation.

Aim 2c. **Time to first CMV anterior uveitis recurrence within 12 months.** An exploratory outcome of Trial II will be the time to first CMV anterior uveitis recurrence within 12 months. We will use a Cox proportional hazards model to compare the time to recurrence by the arm within the 12 months of long-term suppressive therapy, including an indicator for each treatment arm and an indicator for site. We will assess the proportional hazards assumption by examining Kaplan-Meier curves by treatment group on the original and log-log scales and will examine Schoenfeld residuals to assess model fit. We will estimate the hazard ratio from the model for the two primary comparisons, *oral valganciclovir* vs. *topical ganciclovir 2%, oral valganciclovir* vs. *placebo*. Both comparisons will use max-T adjusted p-values and simultaneous 95% confidence intervals. Sensitivity analyses will examine 1) alternative censoring assumptions and 2) time-varying hazards if proportional hazards assumption is violated.

Aim 3. **Prevalence of mutations conferring antiviral resistance:** The analysis population will include individuals enrolled in both Trials I and II who are randomized to receive oral or topical antivirals in Trial II and experience a recurrent infection. Among patients that experience a recurrent infection (anticipated n=34, see Sample Size section 3.3), we will compare the proportion of infections with antiviral mutations upon recurrence with the proportion with antiviral mutations at baseline. The analysis will thus be a pair-matched analysis (within-patient). We will use a linear binomial model to estimate the difference in prevalence of resistant infections, including fixed effects for each participant that will control for all between-participant characteristics in this observational analysis. Additional covariates in the model will include treatment arm and site. This pair-matched analysis is similar to McNemar's test, but allows us to control for type of retroviral and enrollment site using regression.

Sensitivity Analyses

In all analyses, model adequacy will be checked by examination of residuals or other goodness of fit tests as needed. Inadequate model fit will prompt us to report alternative models. If there are chance imbalances in prognostic baseline patient characteristics, including age, sex, and prednisolone use prior to enrollment, a secondary analysis will include them as covariates in the regression model.

6.3. Missing data handling

In Trial I and Trial II, we will report the fraction missing or lost to follow-up by study arm. If ≤10% of enrolled participants are missing outcome data, complete case analyses will be conducted. If >10% of enrolled participants are missing outcome data, inverse probability weighting will be used to weight complete cases by the inverse of an estimate of the probability of an outcome being observed as a sensitivity analysis. Weights will be constructed with baseline characteristics likely to predict missingness that are also associated with the outcome, including primary variables (viral load measurements, time to recurrence, treatment assignment, site) and auxiliary variables (baseline characteristics, partial outcome data, adherence measures, prior treatment history).

6.4. Additional analyses

As an alternative analysis for Aim 1a, we will assess the results of an analysis of change score controlling for baseline, which is statistically equivalent to our primary analysis but will report the results in terms of difference in the change in log viral load.[22]

6.5. Harms

Serious adverse events suspected of being drug-related will be reported to the DSMC, medical monitor and to the drug provider within 24 hours of our notification. In the event of an adverse event the medical monitor can make a request to be unmasked as to participant treatment arm. We will report safety events and gain additional insight into this aspect of therapy. Failure to find compelling statistical evidence of lack of safety will not prevent us from learning potentially valuable information that may help guide practice. Adverse events will be tabulated by comorbidities.

6.6. Statistical software

Analyses will be conducted using R, version 4.0 or higher (R Foundation for Statistical Computing, Vienna, Austria).

7. Appendix

ABBREVIATIONS AND ACRONYMS

DSMC	Data and Safety Monitoring Committee
SAP	Statistical Analysis Plan
PCR	Polymerase Chain Reaction
CMV	Cytomegalovirus
UCSF	University of California, San Francisco

8. Revision history

Version	Date	Summary of Changes, Justification, and Timing vis-à-vis key trial events (enrollment completion, interim analyses, unmasking, etc)
1	2021-05-13	First version

2	2022-05-24	• Added pre-specified subgroup analyses by sex and by study site (section 6.4) • Added per-protocol analysis (section 6.4) • Added analyses of specific gene mutations (section 6.4) • Added details for interim analysis scenarios (section 3.5)
3	2025-01-15	• Updated sample sizes for Trial I and II based on results from prior trial and studies (section 3.3) • Changed primary aims for Trial II and updated the statistical methods accordingly (section 6.1, 6.2) • Removed per-protocol analysis because all participants adhered to their assigned treatment; removed subgroup analysis by site because there was one site with only a few participants, which would not provide sufficient statistical power for a meaningful analysis (section 6.4)

9. References

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