STATISTICAL ANALYSIS PLAN

Ganciclovir to Prevent Reactivation of Cytomegalovirus in Patients with Acute Respiratory Failure and Sepsis

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1. LIST OF ABBREVIATIONS AND ACRONYMS

Term/Abbreviation	Definition
ANC	Absolute Neutrophil Count
ARDS	Acute Respiratory Distress Syndrome
CMV	Cytomegalovirus
COMS	Core Outcome Measurement Set
ELISA	Enzyme-Linked Immunosorbent Assay
ETA	Endotracheal Aspirate
GCSF	Granulocyte-Colony Stimulating Factor
GRAIL	Ganciclovir/Valganciclovir for Prevention of CMV Reactivation in Acute Injury of the Lung and Respiratory Failure
HCT	Hematopoietic Cell Transplant
ICU	Intensive Care Unit
IG	Immunoglobulin
IL	Interleukin
ITT	Intent-To-Treat
LFA	Lateral Flow Assay
NPV	Negative Predictive Value
PEEP	Positive End Expiratory Pressure
PPV	Positive Predictive Value
RCT	Randomized Controlled Trial
RSFD	Respiratory-Support-Free Days
SBT	Spontaneous Breathing Trial
SOFA	Sequential Organ Failure Assessment
SOT	Solid Organ Transplant
VFD	Ventilator-Free Days
WBC	White Blood Cell
MNAR	Missing Not at Random
GCV	Ganciclovir

2. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide a description of the statistical analyses that will be conducted for the GRAIL3 final study reports.

2.1 General Design Considerations

The following is a summary of the protocol.

Short Title: A Phase 3 Study of Ganciclovir Use in Patients with Acute Respiratory Failure and Sepsis

Funder: NHLBI

Protocol Team: Renee Stapleton, MD, PhD
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Sample Size: Approximately 500 participants

Study Population: Immunocompetent, CMV seropositive adults hospitalized with sepsis and acute respiratory failure requiring respiratory support

Study Sites: Sites selected by the GRAIL Executive Committee

Study Hypotheses: We hypothesize that IV ganciclovir administered early in critical illness will effectively suppress CMV reactivation in CMV seropositive adults with sepsis-associated acute respiratory failure, thereby reducing lung damage and accelerating recovery from respiratory failure by direct and indirect mechanisms, and leading to improved clinical outcomes.

Study Design: Multicenter randomized placebo-controlled double-blind trail, [randomized in blocks for balance across study sites, with interim analyses of safety].

Study Duration: 180 days per patient

Study Product: Ganciclovir sodium: 2-amino-9-76,9-dihydro-3H-purin-6-one.
Placebo for ganciclovir: [normal saline].

Study Regimen: 500 adults will be randomized in a 1:1 ratio to receive either the study drug or placebo. Participants randomized to intervention will use IV ganciclovir only. Participants will receive ganciclovir or normal saline twice daily from day 1 through day 5 and once a day from day 6 through day 28, hospital discharge, death, whichever occurs earlier.

		Schedule of administration*	
		Day 1 through Day 5	Day 6 through Day 28 or hospital discharge, whichever occurs earlier
Arm	N	Twice daily	Once daily
1	250	Ganciclovir 5 mg/kg intravenously	Ganciclovir 5 mg/kg intravenously
2	250	Normal saline intravenously	Normal saline intravenously
Total	500		

* “Day” on this table refers to study day. Day 1 is the first day of study drug administration.

2.2 Study Objectives and Endpoints

Primary Objective: To evaluate whether administration of ganciclovir increases respiratory-support-free days (RSFDs) in immunocompetent patients with sepsis-associated acute respiratory failure.

Primary Endpoint: RSFDs will use a "last off" approach (detailed in Protocol V5 Section 6.1.1), meaning that RSFDs will be counted when a participant gets off and stays off of respiratory support (as defined in Protocol V5 Section 4.1.1) to day 28. Participants who do not survive through day 28 are assigned zero RSFDs.

Secondary Objectives:

1. To evaluate whether administration of ganciclovir increases VFDs in immunocompetent patients with sepsis-associated acute respiratory failure.
2. To evaluate whether administration of ganciclovir increases total RSFDs (all RSFDs, instead of last-off approach) in immunocompetent patients with sepsis-associated acute respiratory failure.
3. To evaluate whether mortality and time to death in the 28 and 180 days is different among ganciclovir recipients relative to placebo recipients, respectively.
4. To evaluate whether duration of mechanical ventilation among survivors in the first 28 days is different among ganciclovir recipients relative to placebo recipients.
5. To evaluate whether duration of respiratory support among survivors in the first 28 days is different among ganciclovir recipients relative to placebo recipients.
6. To evaluate whether oxygenation is different among ganciclovir recipients relative to placebo recipients.
7. To evaluate whether ICU-free days in the first 28 days are different among ganciclovir recipients relative to placebo recipients.
8. To evaluate whether CMV DNA detection in plasma and endotracheal aspirate (ETA) by day 28 is different among ganciclovir recipients relative to placebo recipients.
9. To assess the number and severity of reportable adverse events and reportable serious adverse events in the first 28 days in both groups.

Secondary Outcomes:

1. VFDs (defined to be 28 days minus the duration of mechanical ventilation through day 28 since randomization [a key secondary endpoint]). Participants who do not survive through day 28 are assigned zero VFDs.
2. Total RSFDs (calculated as 28 days minus each day of respiratory support through day 28 since randomization). Participants who do not survive through day 28 are assigned zero respiratory-support-free ventilator-free days.
3. Mortality by day 180 (day 28, day 180, time-to-event).
4. Duration of mechanical ventilation (among survivors) by day 28.
5. Duration of respiratory support (among surviviors) by day 28.
6. Oxygenation (PaO_2/FiO_2 ratio) daily on study days 1–7.
7. ICU-free days by day 28.
8. CMV DNA detection in plasma and endotracheal aspirate (ETA) by day 28 (>0 IU/mL, >1000 IU/mL).
9. Number of patients with reportable adverse events of Grade 3 or higher by day 28.

Exploratory Objectives:

1. To evaluate whether static respiratory system compliance at randomization, day 4, and day 7 differs between ganciclovir recipients and placebo recipients.
2. To assess the occurrence of invasive bacterial and fungal infections in ganciclovir recipients compared to placebo recipients.
3. To evaluate whether organ dysfunction scores (regular SOFA variables, including respiratory, coagulation, liver, cardiovascular, CNS, and renal function, with the Sat/FiO_2 ratio replacing the

PaO_2/FiO_2 ratio used in the standard SOFA score) differ between ganciclovir recipients and placebo recipients.

4. To assess long-term life quality as measured by the Acute Respiratory Failure Core Outcome Measurement Set (COMS), which will include the Katz Index of Independence in Activities of Daily Living (ADL) and Lawton – Brody Instrumental Activities of Daily Living Scale (IADL) completed by legally authorized representatives (LARs) or participants at baseline; and the ADL, IADL, Hospital Anxiety and Depression Scale (HADS), EQ-5D-5L, and Impact of Events Scale – Revised (IES-R), completed by patients at day 180.
5. To assess risk factors associated with CMV reactivation kinetics, including demographics, comorbidities, severity of illness, organ dysfunction, lymphocyte count, time from hospital admission to enrollment, ventilation type, viral load prior to randomization, and duration of illness before hospital admission.
6. To characterize the relationship between CMV viral load kinetics in blood and lung compartments and respiratory-support-free days (RSFDs), ventilator-free days (VFDs), and secondary clinical outcomes, including oxygenation, static respiratory system compliance, mortality, and duration of mechanical ventilation in survivors.
7. To assess the performance of a rapid lateral flow CMV serostatus assay compared to clinically performed assays for CMV IgG antibodies, as conducted in CLIA-approved laboratories, throughout the study and at trial completion.
8. To evaluate assays characterizing immunity to CMV, including cellular immunity, neutralizing antibodies, antibody epitope expansion, and transcriptional signatures.
9. To determine whether the use and duration of ECMO differ between ganciclovir recipients and placebo recipients.
10. To assess the occurrence of neuromuscular blockade in ganciclovir recipients compared to placebo recipients.
11. To assess the use of prone positioning in ganciclovir recipients compared to placebo recipients.

Exploratory Endpoints:

1. Static respiratory system compliance at randomization, day 4 and day 7.
2. Invasive bacterial and fungal infections.
3. Organ dysfunction scores (regular SOFA variables including respiratory, coagulation, liver, cardiovascular, CNS and renal; as well as SpO2/FiO2 ratio).
4. The NHLBI-endorsed Acute Respiratory Failure Core Outcome Measurement Set (COMS) in survivors at day 180.
5. Risk factors for CMV reactivation (>0 IU/mL, >1000 IU/mL) in plasma and lung:
    1) Sex, age, race
    2) Co-morbidity
    3) APACHE III score
    4) SOFA score and individual components
    5) Lymphocyte count
    6) Time from hospital admission
    7) Viral load prior to randomization
6. Relationship of CMV viral load with RSFDs and VFDs and secondary clinical outcomes:
    1) Viral load: initial (baseline), peak, slope, area under the curve (AUC).
    2) Association with RSFD and VFD, day-28 mortality, duration of mechanical ventilation in survivors, static compliance, PaO2/FiO2.
    3) Baseline viral load in plasma and lung
7. Viral load kinetics among survivors in day 7 and 14.
8. Sensitivity, specificity, PPV and NPV of rapid lateral flow CMV serostatus assay.
9. CMV cellular immunity, neutralizing antibodies, antibody epitope expansion, and transcriptional signatures.
10. Use of ECMO at any time during the post-randomization period, and duration if used
11. Occurrence of neuromuscular blockade.

12. Prone positioning status.

2.3 Randomization

Patients who met all inclusion and exclusion criteria were randomized to receive standard ICU care (including lung-protective ventilation and weaning protocols, if mechanically ventilated) plus either the intervention or placebo. Randomization was stratified by site and implemented using block randomization lists created by SCHARP. Assignment was conducted through the Medidata RTSM system.

2.4 Blinding

Participants and site staff (except site pharmacists) were blinded to treatment assignments. Study drug allocation was accessible only to site pharmacists, contract monitors (or the central site IDS pharmacist as backup), and unblinded statisticians responsible for trial oversight. Randomization assignment data were restricted to unblinded statisticians. Emergency unblinding was available 24/7 via Medidata RTSM. Communication regarding treatment allocation between clinical and pharmacy staff was strictly prohibited. DSMB members were unblinded to treatment assignment to permit ongoing safety monitoring, and closed DSMB reports included treatment group information.

2.5 Sample Size and Power

To estimate the required sample size for this study, we used ventilator-free days (VFD) data from the GRAIL Phase 2 trial and available literature on RSFDs, assuming similar variance. A clinically meaningful difference in RSFDs between treatment arms was assumed to be 2.74 days, with an empirically estimated standard deviation of 9.4 days. To detect this difference with 85% power using a two-sided test at a 5% significance level, a total sample size of 426 participants (213 per group) was required for the primary analysis.

To account for feasibility and potential dropout—including 1% lost to follow-up and 2% early discontinuation due to negative CMV ELISA results—the final sample size was increased to 500 participants. A pre-specified sample size re-estimation was conducted after 50% of participants had reached the primary endpoint; based on the observed data, the original sample size was retained.

Mortality was included as a secondary endpoint. Assuming a 20% mortality rate in the control arm, the final sample size of 500 provides 80% power to detect a hazard ratio of 0.52 between the Ganciclovir and placebo groups.

3. GENERAL DATA ANALYSIS CONSIDERATION

3.1 Analysis Set(s)

The **Intent-to-Treat (ITT) Analysis Set** will include all enrolled and randomized participants. However, one exception applies: participants who are withdrawn from the study before receiving any dose of the study drug will not undergo study procedures and will be excluded from the ITT analysis. Participants who are withdrawn after receiving at least one dose will continue to receive safety assessments (e.g., creatinine and complete blood count with differential) for up to 48 hours. Although no study bio-samples will be collected after withdrawal, clinical data available in the medical record (e.g., respiratory-support-free days) will continue to be collected per protocol, and such participants will be included in the ITT analysis. **The ITT set will serve as the primary analysis population.**

Two modified Intent-to-Treat (mITT) Analysis Sets will also be defined:

The first mITT set will exclude participants who are withdrawn from the study for any reason after receiving at least one dose of study drug. Reasons for withdrawal may include, but are not limited to: participant withdrawal of consent, inability or unwillingness to comply with study procedures, refusal to continue participation by the participant or their legally authorized representative (LAR), or an initial positive CMV LFA test followed by a confirmatory negative CMV ELISA result.

The second mITT set will include only those participants who survive and are followed through day 28.

In addition, a **classical ITT set**, which includes all randomized participants regardless of treatment receipt or study completion, will be retained for completeness.

3.2 Statistical Analysis Issues

Every effort will be made by the Data Coordinating Center (DCC) to ensure complete, accurate, and high-quality data collection and annotation. However, as with all clinical trials, challenges are anticipated. Given the high mortality rate in this population, missing data is a key issue that may affect both primary and secondary endpoints (e.g., CMV reactivation [missing viral load values], organ dysfunction, and outcomes from the NHLBI-endorsed acute respiratory failure core measurement set). This section outlines the anticipated sources of missing data and corresponding mitigation strategies, informed by experience from the GRAIL Phase 2 trial.

Missing Data in Primary Outcome
Missingness in the primary outcome, RSFDs, is expected to be minimal (~2%) and is likely to be missing not at random (MNAR), primarily due to participant withdrawal of consent. Such withdrawals typically occur in more severely ill participants under high stress. Given the low expected frequency, missing data in RSFDs will be excluded from the primary analysis without imputation.

Missing Data in Some Secondary/Exploratory Outcomes Due to Study Design
Some secondary endpoints (e.g., static respiratory compliance) may be unavailable due to early extubation, ICU discharge, or death prior to scheduled biospecimen collection. For instance, if ganciclovir reduces respiratory support duration, extubated participants may not be eligible for endotracheal aspirates, potentially biasing the analysis. To minimize missingness, biospecimen collection was scheduled at Day 7 (±1 day), when approximately 40% of participants are expected to remain alive and intubated. If missing data persist despite this timing, we will apply imputation techniques such as last observation carried forward (LOCF), multiple imputation (MI), or conduct sensitivity analyses. If missingness remains substantial, limitations will be acknowledged in interpreting results.

Missing Data in Covariates
Missing covariate data may result from death, withdrawal, or other causes. If covariate missingness exceeds 5%, it will be addressed analytically. Covariate missingness is expected to be MAR, and will be handled using either inverse probability weighting (IPW) or multiple imputation (MI) [1, 2].

- Weighting Adjustments:
  - Estimate the probability of missingness using logistic regression with baseline covariates as predictors.
  - Calculate IPW as the reciprocal of predicted probabilities.
  - Apply weighted generalized estimating equations (WGEE) or weighted regression models to reduce bias in treatment effect estimates.

- Multiple Imputation (MI):
  - Use Multivariate Imputation by Chained Equations (MICE) to impute missing covariates.
  - Perform 20–50 imputations to ensure stability.
  - Combine estimates across imputations using Rubin's rules to derive valid statistical inference.

4. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE

One interim analysis was planned and conducted after 50 deaths. The primary purpose of the interim analysis was to evaluate safety, efficacy, and sample size assumptions. This analysis included an assessment of the primary outcome (RSFDs) and mortality.

The interim analysis was conducted by the unblinded statistical team and reviewed by the independent Data and Safety Monitoring Board (DSMB). Based on pre-specified stopping boundaries and overall risk-benefit assessment, the DSMB recommended continuation of the trial without modification. No additional interim analyses were performed.

DSMB meetings were conducted on the following dates:

DSMB	1/10/2022
DSMB	7/8/2022
DSMB	8/30/2022
DSMB	1/26/2023
DSMB	8/11/2023
DSMB	2/8/2024
Interim Analysis	7/8/2024
DSMB	10/1/2024

The trial was paused for enrollment on July 22, 2022, to allow the DSMB time to review the updated dataset, and enrollment resumed on September 9, 2022. A closed enrollment decision was later made by NHLBI due to safety and futility concerns following the last DSMB meeting on October 1, 2024.

5. GENERAL ANALYSIS METHODS

Primary Endpoint

To evaluate the primary hypothesis—that the mean difference in respiratory support–free days (RSFDs) between the two treatment groups is significantly different from zero—we will use a two-sample parametric t-test as the primary statistical test. This test is known for its robustness to violations of normality when the sample size is sufficiently large ($n > 25$), as is the case in this trial.

To support and validate the primary analysis, we will also conduct a two-sample permutation t-test. This nonparametric test requires only the assumption of exchangeability, which is satisfied in the context of a randomized controlled trial. The permutation test provides a distribution-free confirmatory approach, reinforcing the robustness of our findings.

In addition, we will apply the semiparametric efficient and robust estimation procedure proposed by Davidian et al. [7,8] to estimate the mean treatment difference in RSFDs along with a 95% confidence interval. This method adjusts for baseline covariates predictive of the outcome, thereby improving both statistical efficiency and precision relative to unadjusted comparisons or traditional ANCOVA. This analysis also accommodates missingness in the primary endpoint (excluding death) by explicitly modeling the missing data mechanism, as described in Protocol Section 6.4.1.

In addition to the primary analysis using the main intent-to-treat (ITT) population, we will conduct sensitivity analyses using the classical ITT (cITT) and two modified ITT (mITT) analysis sets to assess the robustness of results.

Secondary endpoints
For quantitative secondary endpoints, including the key secondary endpoint of ventilator-free days (VFDs), we will apply the same analytic approach as described for the primary endpoint (RSFDs), including both unadjusted and adjusted methods using the semiparametric efficient and robust procedure proposed by Davidian et al. [7, 8]. In addition, analyses will be conducted on total RSFDs without the last-off approach, to assess consistency across definitions.

For time-to-event secondary endpoints, such as CMV reactivation at pre-specified thresholds, the Kaplan-Meier method will be used to estimate the probability of not experiencing the event by Day 28 for each treatment group. A 95% confidence interval for the between-group difference in event rates will be constructed using the Greenwood variance estimator. Between-group comparisons will be performed using a Z-test based on the Kaplan-Meier survival function estimates. Where applicable, Bonferroni correction will be used to adjust for multiple comparisons across secondary endpoints.

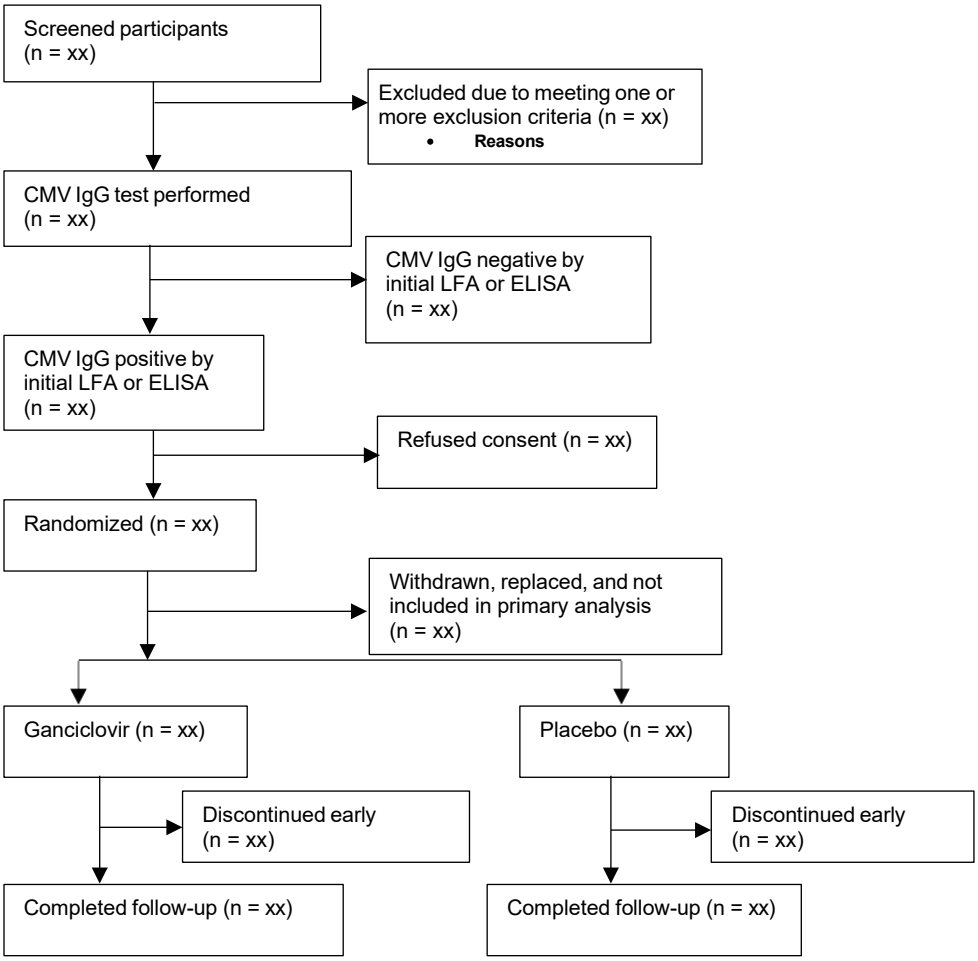
Additionally, multivariable models will be constructed for the primary endpoint (RSFDs) and other secondary outcomes to adjust for baseline characteristics and known risk factors for CMV reactivation. These models may include generalized linear models (GLMs) or the semiparametric method of Davidian et al., depending on the outcome type and distributional assumptions. The covariates considered will correspond to those outlined under exploratory objectives in Protocol Section 5.3.

As a secondary objective, we will assess whether the treatment effect varies by treatment period (i.e., during vs. post-drug exposure). Specifically, for mortality, we will test for treatment-by-period interaction to explore whether the timing of Ganciclovir exposure modifies the risk of death. If interaction is present, stratified or time-varying effect models will be considered in post hoc exploratory analyses.

6. TRIAL PARTICIPANT DISPOSITION

6.1 Disposition of Participants

Participant disposition will be summarized using a CONSORT flow diagram. This will display the number of participants screened for eligibility, the number excluded (with reasons), the number who underwent CMV IgG testing, the number randomized to each treatment arm, and the number who received or did not receive the assigned intervention (with reasons for non-receipt). The diagram will also present the number of participants who discontinued follow-up—such as due to adverse events, loss to follow-up, or protocol noncompliance—and the number included in the primary analysis. A summary table may also be provided to supplement the flow diagram with arm-specific details.



6.2 Treatment Exposure

Treatment exposure will be summarized in a table using data from the Daily Study Product Administration Form. The number of study product doses administered will be summarized using descriptive statistics, including the mean, standard deviation, median, and interquartile range (IQR), stratified by study site. The number of renal dose adjustments will also be summarized and categorized by site.

6.3 Protocol Deviations

All protocol deviations will be reviewed by the study team to ensure appropriate categorization. Assessment, remediation, and prevention measures will be documented and updated as needed. A summary table will present the number and types of protocol deviations by site, accompanied by a detailed listing of each deviation.

Listing: Protocol Deviations and Enrollment Violations by Site

Subject ID	Site	Type of Deviation	Deviation Date	Assessment	Remediation/prevention
Subject 1	Site 1				
Subject 2	Site 2				
…	…	…	…	…	…

7. BASELINE DATA

Unless otherwise specified, baseline characteristics will be summarized descriptively using appropriate summary statistics. Continuous variables will be summarized using the mean and standard deviation, median, interquartile range, and range. Categorical variables will be summarized using frequencies and percentages. All summaries will be presented overall and stratified by treatment group.

Screening
The number of participants screened and enrolled, along with the reasons for non-enrollment (e.g., ineligibility), will be summarized by site for all participants screened for the GRAIL 3 study.

Demographic
Baseline demographic characteristics, obtained from the Demographics Case Report Form (CRF), will be summarized by treatment group.

Respiratory Support
Baseline respiratory support data, as recorded in the Demographics CRF, will be summarized by treatment group.

Laboratory Results
Baseline laboratory values will be summarized by treatment group using data from the Participant Daily Status CRF, APACHE III – Acute Physiology CRF, and Safety Labs CRF.

CMV testing
CMV testing results from ELISA and LFA assays for all **screened** participants will be summarized using contingency tables.

8. PRIMARY ENDPOINT ANALYSES

The primary endpoint, respiratory-support-free days (RSFDs), is defined as 28 days minus the number of days a participant received respiratory support (including invasive mechanical ventilation, non-invasive ventilation with CPAP or bi-level CPAP and PEEP ≥ 5 cmH_2O, or high-flow nasal cannula oxygen with a flow rate ≥ 30 L/min) through Day 28 following randomization.

- Participants who do not survive through Day 28 will be assigned zero RSFDs.
- Participants who remain on respiratory support on Day 27 will be assigned zero RSFDs.
- Participants transferred to long-term acute care hospitals (LTACHs) will be assigned zero RSFDs.
- Participants who withdrew consent, refused further participation by themselves or a legally authorized representative (LAR), and for whom no further data were collected, or who withdrew due to study closure within the first 28 days will be considered missing for the RSFD endpoint.

RSFDs will be summarized descriptively using the mean, standard deviation, median, and range by treatment group. To formally test the primary hypothesis that the mean difference in RSFDs between treatment arms differs from zero, a two-sample parametric t-test will be used. This test is appropriate even if the normality assumption is mildly violated, given its robustness for moderate sample sizes ($n > 25$). As a confirmatory approach, a two-sample permutation t-test will also be performed. This nonparametric test does not require distributional assumptions and is valid under the weaker condition of exchangeability, which is satisfied in the randomized trial setting.

To further improve statistical efficiency and precision, the semiparametric efficient and robust method of Davidian et al. [7, 8] will be employed to estimate the mean difference between arms, along with its 95% confidence interval. This method adjusts for baseline characteristics predictive of the primary outcome and is more powerful than a conventional ANCOVA or t-test when baseline adjustment is warranted. If data on

the primary endpoint are missing for reasons other than death, the analysis will appropriately account for the missing data mechanism, as described in Protocol Section 6.4.1.

In addition to the ITT analysis, the primary endpoint will also be analyzed in a classical intent-to-treat (cITT) and two modified intent-to-treat (mITT) populations.

9. SAFETY ANALYSES

A summary table of MedDRA-coded adverse events (AEs) will be generated, displaying the frequency and percentage of participants experiencing each AE by treatment group, system organ class (SOC), and preferred term. In addition, a comprehensive listing of all reported AEs will be provided. This listing will include onset date, verbatim term, MedDRA code, SAE/EAE classification, study day, severity grade, relationship to study drug, outcome, and duration (in days).

Deaths will be summarized separately by treatment group, as described in Section 2.2. A Kaplan–Meier survival analysis will be conducted to estimate the time-to-death distribution for each arm. Survival curves will be compared using the log-rank test. A Cox proportional hazards model will also be used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for mortality between groups, adjusting for baseline creatinine clearance. In addition, subgroup analyses will be conducted stratified by baseline creatinine clearance ($\geqslant$ 60 vs. < 60 mL/min).

Survival analyses will be performed across multiple time windows: through Day 28 and Day 180 (± 42 days). Participants lost to follow-up will be censored at their last known survival date.

To further explore potential differences in mortality risk across treatment phases, an interaction analysis will be conducted between treatment assignment and treatment period (during-treatment vs. post-treatment). This analysis will help assess whether the effect of treatment on mortality differs between the period of active drug administration and the post-treatment period.

10. SECONDARY ENDPOINTS ANALYSES

Each secondary endpoint will be analyzed and summarized using appropriate statistical methods, and results will be presented by treatment group unless otherwise specified. Tables or figures will be used where appropriate.

1. Ventilator-Free Days (VFDs) defined as 28 days minus the total duration (in days) of invasive mechanical ventilation during the first 28 days post-randomization.
    - Participants who do not survive through Day 28 will be assigned 0 VFDs.
    - Participants who remain mechanically ventilated on Day 27 will be assigned 0 VFDs.
    - Participants transferred to long-term acute care hospitals (LTACHs) will be assigned 0 VFDs.
    - Participants who withdraw from the study will be considered as having missing data.

    Differences in VFDs between treatment arms will be analyzed using a two-sample parametric t-test. Descriptive statistics (mean, standard deviation, median, quartiles, and range) will also be presented in summary tables.

2. Total RSFDs without last-off approach defined as 28 minus the cumulative number of days on any form of respiratory support within 28 days of randomization, without the last-off rule applied. Participants who die before Day 28 will be assigned 0 RSFDs.

Differences in RSFDs between treatment arms will be analyzed using a two-sample parametric t-test. Descriptive statistics (mean, standard deviation, median, quartiles, and range) will also be presented in summary tables.

3. Mortality will be evaluated as a time-to-event outcome through 28 days and 180 (±42 days). Analyses will mirror those described in Section 9, including Kaplan–Meier survival curves, log-rank tests, and Cox proportional hazards models (adjusted and stratified by baseline creatinine clearance). In addition, we will test for an interaction between treatment group and period (during- vs. post-treatment) to determine if treatment effects on mortality vary over time.

4. Duration of mechanical ventilation among participants who survive through Day 28, the total number of days on invasive mechanical ventilation will be calculated. Participants transferred to LTACHs will be assigned 28 days. Differences between treatment arms will be analyzed using a two-sample parametric t-test. Descriptive statistics (mean, standard deviation, median, quartiles, and range) will also be presented in summary tables.

5. Duration of respiratory support (with Last-Off Approach) among Day 28 survivors, the number of days on any qualifying respiratory support (applying the last-off approach) will be summarized. Participants transferred to LTACHs will be assigned 28 days. Differences between treatment arms will be analyzed using a two-sample parametric t-test. Descriptive statistics (mean, standard deviation, median, quartiles, and range) will also be presented in summary tables.

6. Oxygenation (PaO_2/FiO_2 ratio) measured daily on study days 1–7 will be visualized using boxplots stratified by treatment arm.

7. ICU-free days, defined as 28 minus the number of days in the intensive care unit (ICU) during the first 28 days post-randomization.
    - Participants who do not survive through day 28 will be assigned zero ICU-free days.
    - Participants who remain in the ICU on day 27 will be assigned zero ICU-free days.
    - Participants who are transferred to LTACH facilities will be assigned zero ICU-free days.
    - Participants who withdraw from the study will be considered as having missing data.

    Differences in ICU-free days between treatment arms will be analyzed using a two-sample parametric t-test. Descriptive statistics (mean, standard deviation, median, quartiles, and range) will also be presented in summary tables.

8. CMV DNA detection in plasma and endotracheal aspirate (ETA) by day 28 (>0 IU/mL, >1000 IU/mL), defined as positive CMV PCR from ETA, plasma, or serum specimens.
    The analysis will be conducted by the unblinded protocol statistician.
    Differences in CMV DNA detection in plasma and endotracheal aspirate between treatment arms will be evaluated under the competing risk framework of Fine and Gray (10). Cumulative incidence estimates will be evaluated, and Gray's test p-value will be used to test the difference between arms accounting for death as competing risk.
    - This analysis will include death as a competing risk if it occurs on the same day of the last negative CMV test date.
    - Any CMV reactivation will be considered, even those which were below the level of quantification but greater than 0.

9. A comprehensive list of all reportable AEs of Grade 3 or higher by day 28 will be provided, including onset date, AE verbatim, MedDRA code, SAE/EAE, study day, severity grade, relationship to study drug, outcome, and duration days.

11. ADDITIONAL ANALYSES

Additional analyses will be specifically described in the companion document, GRAIL 3 Statistical Analysis Plan for Exploratory and Post Hoc Analyses. These analyses are outside the scope of the FSR.

1. Subgroup analyses will be conducted to explore potential treatment effects on RSFDs and mortality across the following baseline factors:
   - Study site
   - Covid-19
   - Ventilation type
   - Sex, age, race
   - Severity scores
   - Underlying conditions
   - CMV reactivation at randomization

2. To assess potential hematopoietic toxicities and their clinical relevance, we will conduct the following analyses:
   - Comparison of lymphocyte and monocyte counts between treatment arms.
   - Stratified analyses of RSFDs and mortality by baseline lymphopenia and monocytopenia severity at predefined thresholds: <1000 cells/μL, <500 cells/μL,<100 cells/μL

3. Ganciclovir (GCV) may cause hepatic, pancreatic, or renal toxicity in critically ill patients. Potential toxicities will be evaluated by comparing key laboratory markers between treatment arms:
   - Hepatic: alanine transaminase (ALT)
   - Pancreatic: lipase, amylase
   - Renal: cystatin C

4. Potential neurologic toxicities of Ganciclovir will be explored, including possible associations with delirium, seizure, or cerebrovascular events. The following measures will be compared between treatment arms:
   - Delirium scores
   - Incidence of seizures
   - Incidence of stroke

5. Ganciclovir exposure may increase the risk of arrhythmias in critically ill patients. Arrhythmic events will be summarized by treatment arm, and comparisons will be made to evaluate potential cardiotoxic effects of the study drug.

6. To explore potential toxicities or complications associated with Ganciclovir, clinical conditions present at or immediately prior to death will be summarized by treatment arm. Causes of death will be tabulated and compared using appropriate statistical methods (e.g., chi-square or Fisher's exact test).

7. To evaluate the immunologic effects of Ganciclovir and CMV suppression, we will assess cytokine and proteomic responses by treatment arm. For individual cytokines, peak levels and area under the curve (AUC) over the first 7 days will be compared between randomization groups, with adjustment for baseline values.

8. Ganciclovir Exposure and Pharmacokinetics (PK) analysis: Unusually high drug levels in critically ill patients may increase toxicity.

12. STATISTICAL ANALYSIS CHANGES FROM PROTOCOL

Additional analyses outlined in Section 11 will be incorporated separately in addition to the Final Study Report (FSR) statistical analysis to further investigate potential adverse outcomes associated with Ganciclovir (GCV) exposure.

13. REFERENCES

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14. CHANGE HISTORY

Version		Affected Section(s)	Activity Description
Number	**Effective Date**		
1.0	Date of last signature	All	New document

From:	Leisenring ScD, Wendy M
To:	Kalu, IJ
Cc:	Zhou, Zhipeng
Subject:	Re: Approval Request – Grail-3 SAP v1.0
Date:	Wednesday, October 22, 2025 2:16:49 PM
Attachments:	image001.png

I, Wendy Leisenring, Professor, approve. This email will serve as a substitute for a handwritten signature on the document.

Thank you!

--- Wendy Leisenring, SC.D / Professor / Clinical Biostatistics / 206.667.4374 / wleisenr@fredhutch.org

Fred Hutch Cancer Center

From: Kalu, IJ <ikalu@fredhutch.org>
Date: Wednesday, October 22, 2025 at 12:17 PM
To: Leisenring ScD, Wendy M <wleisenr@fredhutch.org>
Cc: Zhou, Zhipeng <zzhou2@fredhutch.org>
Subject: Approval Request – Grail-3 SAP v1.0

Dear Wendy,

Greetings. Please review and approve the attached Grail-3 SAP v1.0 document at your earliest convenience.

If you approve, kindly reply this email with the following statement in “quotation marks:”

"I, [Legal or approved chosen first and last name, Job Title], approve. This email will serve as a substitute for a handwritten signature on the document.”

We would greatly appreciate receiving your signed approval by **October 24th.**

Thank you in advance.

Warm regards,

- -
Ijeoma (IJ) Kalu, MSc.
She/her/hers
Research Coordinator
Data Analytics
Statistical Center for HIV/AIDS Research and Prevention (SCHARP)
Fred Hutchinson Cancer Center
ikalu@fredhutch.org

Signature Page for SCHARP-TMF-154136 v1.0

Reason for signing: Approved	Name: Zhipeng Zhou Role: I am an author of the document. Date of signature: 23-Oct-2025 16:40:21 GMT+0000

Reason for signing: Approved	Name: Sayan Dasgupta Role: I am an author of the document. Date of signature: 23-Oct-2025 17:23:44 GMT+0000

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