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PROTOCOL

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

Clinical Trial Funded by the

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Bethesda, Maryland, USA

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Date 07/02/2025

Version 5.0

ABBREVIATIONS

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

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1 PROTOCOL SUMMARY

Title

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

Study drugs

Ganciclovir sodium: 2-amino-9-76,9-dihydro-3H-purin-6-one.

Placebo for ganciclovir: [normal saline]

Patients

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

Protocol Schema

		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.

Primary Objective

To evaluate whether administration of ganciclovir increases respiratory-support-free days in immunocompetent patients with sepsis-associated acute respiratory failure.

Primary 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 trial, [randomized in blocks for balance across study sites, with interim analyses of safety].
Study Duration	180 days per patient
Trial Safety Monitoring	Safety and Protocol Adherence Review Team (see Section 14.3) Data Safety Monitoring Board (see Section 14.5)
Study drug provider	Commercially acquired
Funding Agency	U.S. National Institutes of Health (NIH) National Heart, Lung, & Blood Institute (NHLBI)
Coordinating Center	Fred Hutchinson Cancer Center (Fred Hutch)/Vaccine & Infectious Disease Division (VIDD)
Statistical and Data Management	Fred Hutchinson Cancer Center/Vaccine & Infectious Disease Division (VIDD), Statistical Center for HIV/AIDS Research & Prevention (SCHARP)
Endpoint Laboratory(ies)	Boeckh Lab, Fred Hutch
Protocol Leadership Team	Renee Stapleton, MD, PhD, Division of Pulmonary and Critical Care Medicine, University of Vermont, Burlington, VT Michael Boeckh, MD, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, WA Ajit Limaye, MD, Division of Infectious Diseases , Univ. of California San Francisco, San Francisco, CA Gordon Rubenfeld, MD, MSc Sunnybrook Medical Centre, Univ. of Toronto, Toronto, Canada
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2 BACKGROUND

2.1 Critical illness due to sepsis

One million people per year in the US are hospitalized with sepsis and, as the population ages, its incidence is expected to increase **[1]**. Sepsis is considered the most expensive condition treated in US hospitals, with an overall cost to society of ~$38 billion in 2013, and the subset with acute respiratory failure accounts for 10% of all intensive care unit (ICU) admissions **[1, 2]**.

2.2 Acute respiratory failure

Acute respiratory failure is a common and serious complication in hospitalized patients. Sepsis, and particularly sepsis from pneumonia, accounts for 73% of patients with acute respiratory failure [3]. Among patients with sepsis, 40% develop acute respiratory failure, with mortality rates of >30% [4, 5]. Despite this burden of illness for sepsis-associated acute respiratory failure, no pharmacologic treatments have been proven effective, and care is generally supportive [6] . The proposed intervention (IV ganciclovir to prevent cytomegalovirus [CMV]-mediated exacerbation of respiratory failure), if proven effective, would be applicable to all CMV seropositive adults, who account for at least 60% of all patients with sepsis, representing a significant improvement in care for a common, deadly, and expensive health condition.

For this study, acute respiratory failure will be defined as in Section 4.1.1 of this protocol.

2.3 Cytomegalovirus (CMV) overview

CMV is a human herpesvirus known to infect more than 50-90% of US adults, and prevalence of CMV infection increases with age [7]. CMV has generally been considered a pathogen only in severely immunocompromised patients (transplant, HIV), and CMV reactivation is a well-established cause of morbidity and mortality in these populations. There are now multiple studies and meta-analyses demonstrating that CMV reactivation is also common in critically ill, otherwise immunocompetent patients with sepsis, pneumonia, and trauma and is associated with worse clinical outcomes [8-15] in this setting.

CMV infection can be acquired through multiple means: mother-to-child (in utero, breast milk), infected body fluids (saliva, genital secretions), blood transfusion, or organ transplant. In immunocompetent persons, following primary infection by any of these routes, CMV is controlled by the immune system and establishes latency in multiple organs/cell-types for the life of the host. Importantly, the lung represents one of the largest reservoirs of latent CMV in seropositive hosts, which may explain the propensity for CMV-associated pulmonary disease in predisposed hosts [16]. During periods of immunosuppression (or as a result of specific stimuli), CMV can reactivate from latency (preferentially in the lung) and replicate, producing active infection. In persons with impaired cellular immunity, reactivation can progress to high-grade CMV replication and commonly leads to clinically evident disease such as CMV pneumonia. Lower-grade CMV reactivation that is otherwise clinically silent (“subclinical”) can also be detected in apparently immunocompetent persons with critical illness using sensitive techniques such as PCR [8].

Even low-level, subclinical CMV reactivation can produce significant biologic effects such as inflammation, fibrosis and immunosuppression. Each of these effects of subclinical CMV infection has either previously been demonstrated or could theoretically be important in sepsis-associated acute respiratory failure and its complications. These biological effects of CMV have been shown to occur through various mediators and other indirect means (reviewed in [17, 18]).

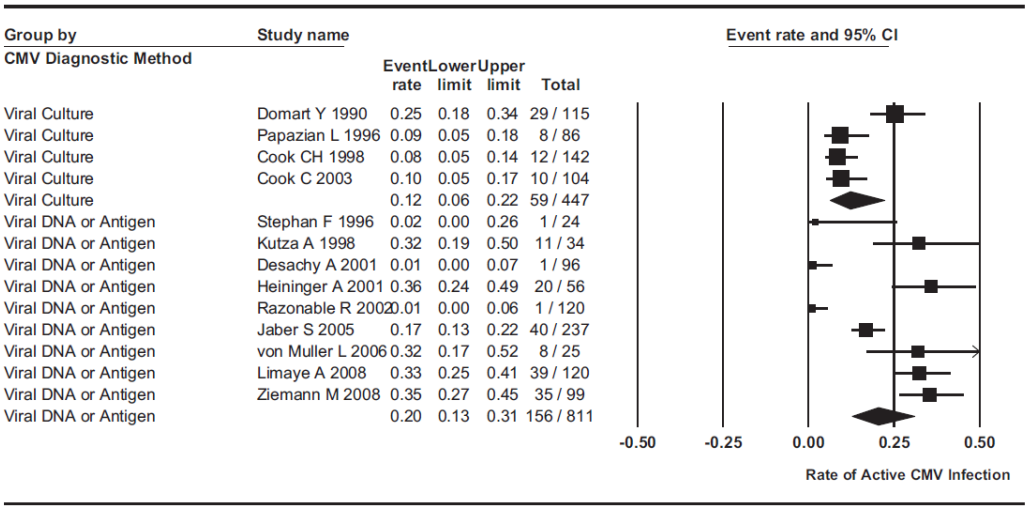
Importantly, several important CMV-associated adverse clinical outcomes in transplant populations [allograft rejection, secondary infections] are not necessarily accompanied by overt CMV disease and can only be detected by sensitive means of virus detection such as PCR [19-21]. In the context of critical illness caused by sepsis [8, 12, 14, 22, 23], it has been postulated that the underlying mechanism behind CMV reactivation is the immune perturbations that occur during sepsis: intense and dysregulated inflammation, and a compensatory anti-inflammatory response [24-26]. To date, specific mediators have not been defined.

2.4 CMV in immunocompetent ICU patients

2.4.1 CMV reactivation is common in immunocompetent ICU patients

CMV viremia occurs in ≥30% of CMV seropositive ICU patients (**Figure 2-1**) [12, 22, 27]. Importantly, CMV also preferentially reactivates in the lung compartment [28], which results in an overall reactivation rate of ~40% [23, 28]. However, predicting reactivation in individuals remains elusive, as specific risk factors for CMV reactivation are inconsistent across existing studies. In a prospective observational study performed by our group and published in JAMA [8], severity of illness at baseline was not independently associated with subsequent CMV reactivation, diminishing the possibility that reactivation is simply a marker for worse outcomes [8].

Figure 2-1: CMV reactivation in the ICU setting [22].



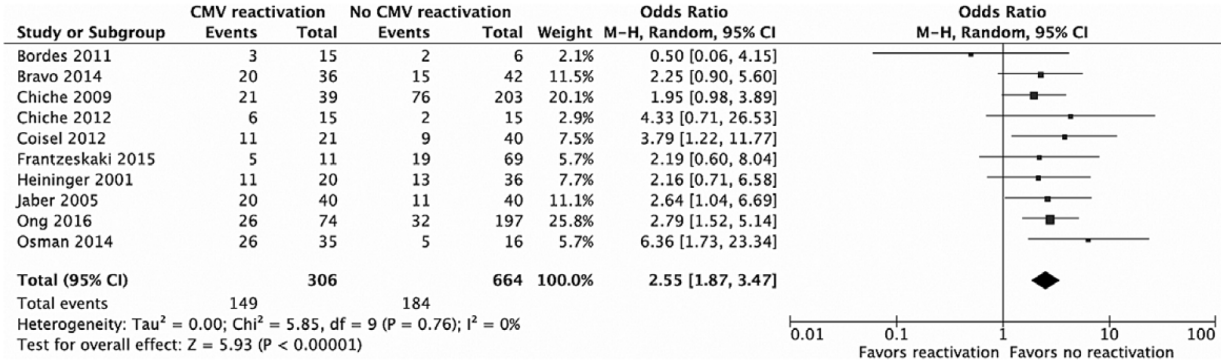
*Cytomegalovirus. Z=7.05; P<0.0001; Q=87.07; I2=86%

2.4.2 CMV reactivation is associated with adverse clinical outcomes in immunocompetent ICU patients with sepsis.

A compelling body of evidence implicates CMV reactivation as a *causal* contributor to morbidity and mortality in critically ill adults with sepsis-associated acute respiratory failure, based on observational studies, animal models, and data from our NHLBI-funded multicenter randomized controlled trial (RCT) of ganciclovir (Ganciclovir/Valganciclovir for Prevention of Cytomegalovirus Reactivation in Acute Injury of the Lung [GRAIL]) in 160 immunocompetent CMV seropositive adults, recently published in JAMA [8-10, 12, 15, 23]. In a recent meta-analysis, CMV reactivation (compared to no reactivation) was associated with a 2-fold increased

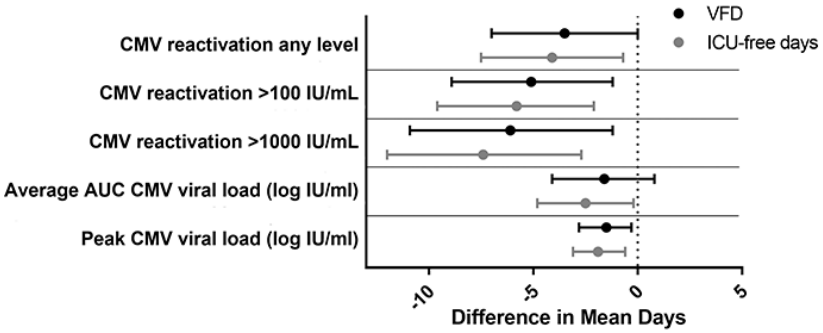
odds of mortality in ICU patients (**Figure 2-2**) [29]. In addition to mortality, recent studies have demonstrated a strong and independent association between CMV reactivation and increased hospital and ICU length of stay [8] and duration of mechanical ventilation [10].

Figure 2-2: Meta-analysis of mortality of in patients with CMV reactivation [12].



We performed a secondary pooled analysis of two prospective cohorts with sepsis: an observational cohort of ICU patients (n=40) and the placebo cohort from our phase 2 GRAIL RCT (n=66). In a multivariate model, CMV reactivation (assessed in multiple ways) was independently associated with worse clinical outcomes: fewer ventilator-free days (VFDs) and ICU-free days (**Figure 2-3**) [30].

Figure 2-3. Quantitative Association of CMV Reactivation with Clinical Outcomes in Critically Ill Patients with Sepsis, in the Absence of Ganciclovir Treatment [30]. [a] Estimates represent the difference in mean days and associated 95% CIs.



[a] After adjustment for age, race, gender, baseline transfusion status, study cohort, and a standardized score created from APACHE II and III scores used in the individual studies.

2.4.3 Putative mechanisms linking CMV reactivation with sepsis-associated acute respiratory failure.

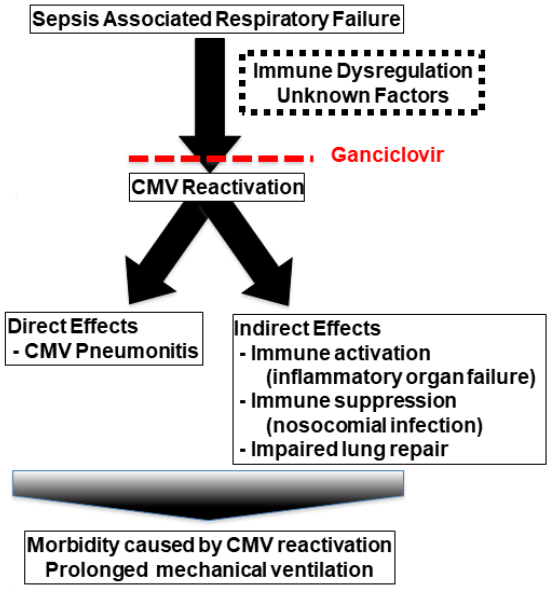
Based on a large body of evidence (natural history data, animal data, evidence that CMV can cause tissue invasive lung disease in critically ill immunocompetent patients, laboratory studies of CMV immunomodulatory capacity, and our phase 2 GRAIL RCT), it appears less likely that CMV initiates acute respiratory failure in patients with sepsis, as CMV reactivation tends to occur after the development of respiratory failure [8, 22]. Rather, despite adequate antimicrobial and supportive care for sepsis, CMV reactivation occurs frequently and secondarily contributes to the

severity of injury to the lung, worsening clinical outcomes. Results from our phase 2 GRAIL RCT support our hypothesis that preventing CMV reactivation interrupts this cascade, thereby improving clinical outcomes. The putative mechanisms by which CMV increases severity of respiratory failure are likely to involve several previously well-characterized biological effects of CMV (**Figure 2-4**).

2.4.3.1 CMV causes direct tissue injury and is a potent immune modulator.

Cell lysis caused by rapid viral replication during CMV reactivation is directly destructive to cells, and this process is thought to exacerbate damage to tissues in which CMV reactivates, such as the lungs [10, 14, 31]. Remarkably, although CMV is typically considered to cause tissue-invasive disease only in severely immunocompromised patients, histologic evidence of CMV pneumonitis was reported in 25-50% of selected previously immunocompetent critically ill patients with persistent unexplained respiratory failure and lung infiltrates who underwent lung biopsy or autopsy [31-33].

Figure 2-4. Proposed Mechanism. Effects of CMV and ganciclovir on sepsis-associated respiratory failure (solid lines) and clinical sequelae.



Numerous studies in both immunocompetent and immunocompromised patients show the profound effect of CMV on the human immune system [34-36]. While the single inflammatory pathway (interleukin [IL]-6) we studied in our phase 2 trial was not influenced by CMV prophylaxis, CMV interacts with the immune system and inflammatory pathways in many other ways, all of which could affect patients with sepsis. For example, CMV influences a broad range of immune cells, including effector CD8+ and gamma-delta T cells, and macrophage and cytokine response including IL-10 production [34, 37]. CMV also promotes immune activation [38] and upregulates adhesion molecules necessary for recruitment of inflammatory cells into the lung leading to tissue damage [37, 39, 40]. These immune activating and immunosuppressive properties of CMV may directly interact with or enhance key aspects of sepsis pathogenesis and profoundly interfere with compensatory mechanisms associated with recovery, especially because it is widely accepted that sepsis pathogenesis involves early aberrant activation of innate immune cells and is associated with a profound immunosuppression [41, 42]. Finally, active CMV

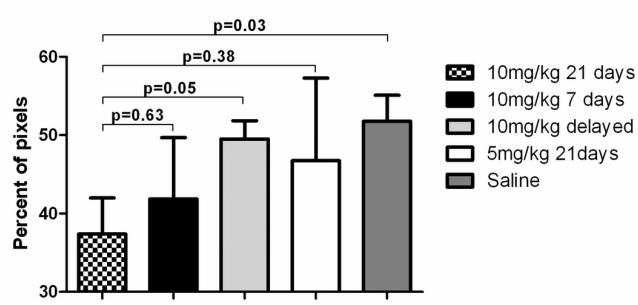
infection is known to increase risk of invasive bacterial and fungal infections [43, 44], which could also profoundly affect the course of critical illness in sepsis.

2.5 Use of ganciclovir in the context of respiratory failure

2.5.1 Ganciclovir antiviral therapy decreases CMV-associated lung injury in a murine model.

Studies in a murine CMV sepsis model (cecal ligation and perforation) demonstrate that in mice with latent CMV infection, CMV reactivation during sepsis causes lung injury (fibrosis) [45, 46]. Ganciclovir prophylaxis prevents the development of lung fibrosis after lung injury in this model by blocking murine CMV reactivation [45]. This effect is dependent on both the dose and timing of antiviral therapy, providing strong evidence of a mechanistic link between CMV reactivation and lung injury (**Figure 2-5)**, consistent with improvement of lung-related outcomes with ganciclovir prophylaxis in our phase 2 GRAIL RCT.

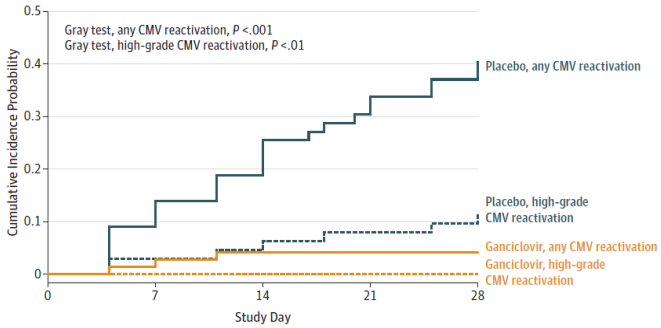
Figure 2-5. Ganciclovir Decreases Sepsis-associated Pulmonary Fibrosis (Represented by Percent of Pixels) in Mice: Effect of Dose and Duration [45]



2.5.2 Phase 2 RCT: Efficacy of ganciclovir for preventing CMV reactivation in patients with sepsis-associated acute respiratory failure.

Our phase 2 GRAIL RCT showed that ganciclovir was effective in suppressing CMV reactivation (**Figure 2-6)**. Specifically, CMV was suppressed in plasma and endotracheal aspirate (ETA) and bronchoalveolar lavage fluid (BAL). Safety data for this trial are detailed in Section 3.6.1.7. Similar success in preventing CMV reactivation was observed in a single-center, open-label RCT of valganciclovir for CMV seropositive, critically ill patients [47].

Figure 2-6. Cumulative Incidence of any CMV Reactivation and High-Grade CMV Reactivation in Plasma through Day 28 [23].



2.5.3 Phase 2 trial results demonstrate that ganciclovir prophylaxis improves several respiratory clinical outcomes in critically ill patients with sepsis-associated acute respiratory failure.

The phase 2 GRAIL RCT did not demonstrate a difference in the primary outcome of serum IL-6 levels between groups [23]. For that trial, IL-6 was selected as the primary endpoint because the trial was intentionally designed to measure a candidate biomarker with potential for predicting clinical outcomes of sepsis-associated acute respiratory failure. IL-6 was associated with mortality in prior ICU studies [48], and preliminary data linked increased IL-6 levels with CMV reactivation [49]. However, as described above (Section 2.4.3.1), CMV interacts with the immune system through multiple mechanisms, collectively resulting in profound impacts. It is likely that the failure to detect changes in IL-6 indicates that the observed clinical effects were mediated through pathways other than IL-6.

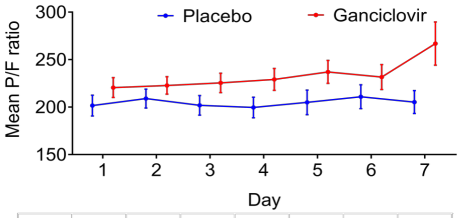
In contrast to the negative findings for the primary endpoint, several clinical respiratory outcomes (all secondary outcomes) were improved among patients who received CMV prophylaxis, as compared to placebo [23]. In the pre-specified sepsis subgroup, the ganciclovir group had a statistically significant increase of 3 VFDs compared to placebo, corresponding to a reduction in duration of mechanical ventilation of 2.5 days (p=0.006) among survivors at day 28 and of 1 day overall (p=0.06) (**Table 2-1**), and no statistically significant difference in mortality. Patients

Table 2-1. Key Secondary Outcomes Among Patients with Critical Illness Receiving Ganciclovir vs Placebo in the Sepsis Subgroup [23].

Outcome Day 28	Placebo Group, N=66	Ganciclovir Group, N=71	Absolute Difference (95% CI)	P-value
VFDs median (IQR), days	20 (9-24)	23 (16-25)	3 (0-4)	0.03
Duration of Mechanical ventilation, median (IQR), days	6 (3-11)	5 (3-8)	-1 (0-4)	0.06
Duration of mechanical ventilation in survivors, median (IQR), days	6.5 (3-12.25)	4 (2.25-7)	-3 (-4,0)	0.006

receiving ganciclovir also had a trend toward improved oxygenation as measured by PaO_2/FiO_2 ratio (**Figure 2-7**).

Figure 2-7. Median PaO2 to FiO_2 Ratio over First 7 Days of Mechanical Ventilation (Intent-to-Treat [ITT] population, Sepsis Subset). Error bars indicate first and third quartiles. Blue = placebo, red = ganciclovir [23].



N							
Placebo	66	64	59	50	44	40	34
Ganciclovir	71	68	62	56	45	39	33

A recent separate single-center RCT of antiviral prophylaxis did not identify differences in the few clinical outcomes that were measured [47]. However, a careful assessment of this negative trial provides useful insights that have guided our study. First, it was a small (N=124), complex, 3-arm trial primarily designed to investigate viral suppression efficacy for both herpes simplex virus and CMV using different drugs. Second, they used oral valganciclovir at half the standard dose used for prevention in other populations [23, 44, 50]. Third, only 20% of the subjects in this trial had sepsis/infection as their reason for ICU admission; the remainder were a heterogeneous population of trauma, cardiac arrest, and post-operative diagnoses. Finally, while the study did show an antiviral effect of valganciclovir, it did not examine clinical outcomes that are likely to be the main effect of CMV reactivation in sepsis (i.e. the exacerbation of existing respiratory failure).

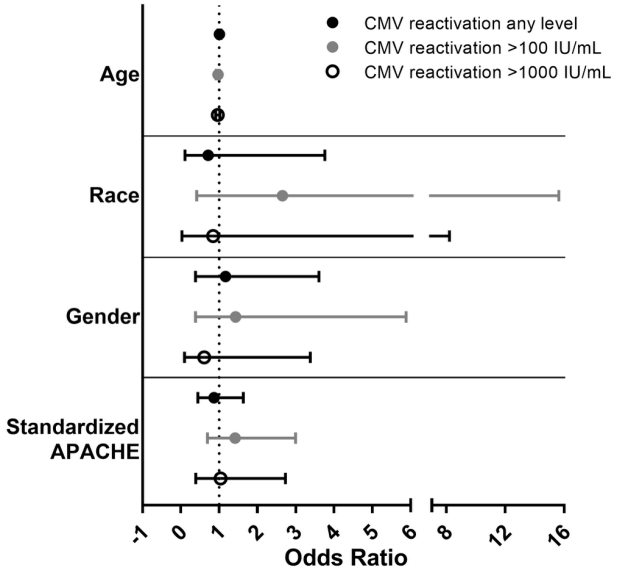
2.5.4 An assessment of risk factors for CMV reactivation to define a target population for the phase 3 RCT (Figure 2-8).

One key question is whether CMV prophylaxis can be targeted to a high-risk group, thereby enriching the study population for the phase 3 trial. The valuable data obtained from our phase 2 GRAIL RCT and our initial observational study allowed us to examine this question by performing analyses of risk factors for CMV reactivation. However, we were unable to identify any such baseline patient variables that were predictive for CMV reactivation, and that would therefore allow us to enrich the study population for the proposed phase 3 trial. Specifically, the association of the APACHE score with CMV reactivation measures was found to be inconsistent and with small effect size. We also did not identify other patient variables (e.g. age, race, gender and baseline transfusion status) associated with subsequent CMV reactivation. No protective threshold of lymphocyte counts was identified. These findings provide the rationale to conduct the phase 3 trial in all CMV seropositive patients.

2.6 A rapid, point-of-care, lateral flow assay (LFA) for CMV IgG serostatus detects CMV seropositivity with high sensitivity.

Prompt identification and initiation of prophylactic drug treatment in CMV seropositive patients is crucial both for this trial and for ultimate effective implementation of this strategy if the trial is

Figure 2-8. Baseline patient variables are not predictive of CMV reactivation in critically ill patients with sepsis, in the absence of ganciclovir treatment.[a] Data represent odds ratios for binary CMV responses (cross-sectional). Transfusion status was also not associated with CMV reactivation (data not shown).



[a] After adjustment for age, race, gender, baseline transfusion status, study cohort, and a standardized score created from APACHE II and III scores used in the individual studies.

positive. Standard enzyme-linked immunosorbent assays (ELISA) are routinely available, but the turnaround time varies by laboratory, and can require up to 48-72 hours. Delays in receiving results increase the likelihood that CMV reactivation has already occurred. However, empirically treating everyone would lead to unnecessary exposure to ganciclovir in the ~40% of patients who are CMV seronegative (and thus not at risk for CMV reactivation and associated adverse effects). To address this challenge, we will utilize a new, investigational, easy to perform, rapid, lateral flow assay (LFA) for CMV IgG antibodies (QooLabs, Inc). This technology is similar to that used for home pregnancy tests, and is easy to perform and interpret. The FDA has determined this to be a nonsignificant risk device study [Reference #Q202303]). All rapid testing results will be confirmed with a standard assay performed in a CLIA-certified lab. This approach will allow physicians to rapidly identify CMV seropositive patients in the ICU, so as to promptly initiate therapy.

We directly compared a novel, NIH SBIR-supported, point-of-care LFA platform (QooLabs, Inc., N43AI170072), versus the clinically validated ELISA assay performed in the CLIA-certified UW laboratory, in 200 samples. Our results indicate that the assay from QooLabs, Inc, had excellent performance characteristics for serum and plasma. For serum, the LFA had 97% sensitivity and 97% specificity when read by QNow UV Flashlight (the method that will be used for this trial) and 99% sensitivity and 93% specificity when read by QNow Automated reader (**Table 2-2**). For plasma, the LFA had 70% sensitivity and 98% specificity by UV Flashlight. Assuming a seroprevalence of ~60% as observed in our phase 2 RCT, the positive (PPV) and negative (NPV) predictive values for the test were 98% and 96% for serum by QNow UV Flashlight, 96% and 98% for serum by QNow Automated reader, and 97% and 77% for plasma by UV Flashlight, respectively. Serum will be the preferred sample for this study, but plasma will be acceptable if serum is not readily available. Thus, the assay will identify ~98% of CMV seropositive patients within an hour, facilitating rapid trial initiation in the majority of patients. The false negative rate

will be minimal (~3%), but these will be identified by ELISA and remain eligible as long as they are within the specified enrollment window of 120 hours.

Table 2-2. LFA assay performance characteristics. Results of the novel, point-of-care, rapid CMV IgG LFA test (Qoolabs) compared with the clinically validated CMV IgG assay (Zeus Scientific, Branchburg, NJ) in 200 consecutive serum samples from adults, performed at the CLIA-approved UW Clinical Virology Laboratory. PPV and NPV are calculated based on 60% seroprevalence.

Lateral Flow Assays	Diagnostic Performance (%)			
	Sensitivity	Specificity	PPV	NPV
QNow Automated Reader	99	93	96	98
QNow UV Flashlight[1]	97	97	98	96

[1] only 100 samples were used for the QNow UV Flashlight reader

3 GANCICLOVIR

Ganciclovir [DHPG] is an FDA-approved antiviral agent with potent in vitro and in vivo activity against human cytomegalovirus and has been in widespread use in the United States and worldwide since it was approved in ~1988. More detailed information is contained within the package insert.

3.1 Mode of action

The primary mechanism of action is inhibition of viral DNA polymerase in virally-infected cells. More detailed information is contained within the package insert.

3.2 Clinical use

Ganciclovir is indicated for:

- Sight-threatening CMV retinitis in severely immunocompromised people
- CMV pneumonitis in bone marrow transplant recipients
- Prevention of CMV disease in bone marrow and solid organ transplant recipients
- Confirmed CMV retinitis in people with AIDS (intravitreal implant)

It is also used for acute CMV colitis in HIV/AIDS and CMV pneumonitis in immunosuppressed patients. See the package insert for more information.

3.3 Forms of ganciclovir

Ganciclovir is available in both intravenous (ganciclovir) and oral formulations (valganciclovir) and is proven efficacious for both prevention and treatment of CMV infection and disease in immunocompromised patients (transplant, HIV) and in neonates with congenital CMV infection [51, 52].

3.3.1 Ganciclovir (intravenous formulation)

Ganciclovir is an FDA-approved, commercially-available antiviral medication used to treat or prevent cytomegalovirus (CMV) infections. Generic versions of ganciclovir sodium are marketed by different drug suppliers.

Ganciclovir is a synthetic analogue of 2'-deoxy-guanosine. It is first phosphorylated to a deoxyguanosine triphosphate (dGTP) analogue. This competitively inhibits the incorporation of dGTP by viral DNA polymerase, resulting in the termination of elongation of viral DNA. See the package insert for more information.

3.4 Standard dosing regimens

1. Treatment of active CMV infection (i.e. presence of CMV by culture, PCR, or antigen detection).

   Dosing of intravenous ganciclovir is 10 mg/kg daily, given as 5 mg/kg every 12 hours (adjusted for renal function). A minimum interval of 6 hours is required between the first and second dose.

2. Prevention of CMV reactivation (in CMV seropositive patients with latent CMV infection but without evidence of active CMV infection)

   Dosing of intravenous ganciclovir is 5 mg/kg once daily (adjusted for renal function).

In this protocol we will use an initial 5-day regimen of twice daily dosing intravenous ganciclovir for hospitalized patients, followed by a daily dosing regimen of intravenous ganciclovir. All patients will receive a maximum of 28 days of study drug, provided that they have intravenous access. For patients discharged from the hospital prior to day 28, study drug will be discontinued at the time of hospital discharge or removal of intravenous access, whichever occurs earlier. For patients who remain hospitalized beyond day 28, study drug will be discontinued after day 28. Dose adjustments for reduced renal function will be done according to the package insert.

3.5 Safety profile

It is estimated that tens of thousands of persons have received either intravenous or oral formulation ganciclovir over the last ~30 years since its initial approval. Based on its efficacy and general tolerability, ganciclovir is currently recommended as a first-line agent for prevention & treatment of CMV infection and disease in HIV, solid-organ transplant, and stem cell transplant populations [53, 54]. See the package insert for more information (Appendix I).

3.6 Potential toxicities of ganciclovir

Ganciclovir is generally well-tolerated, with low rates of toxicity when given for less than 28 days (the maximum possible duration of study drug in the present study). The most common adverse effects, which appear to be related to longer duration of exposure and use of concomitant drugs with similar toxicities, are various hematological adverse effects, most commonly leukopenia, neutropenia, and thrombocytopenia, all of which are considered reversible after drug discontinuation. The potential toxicities of ganciclovir have been extensively studied in vitro, in vivo and in placebo-controlled studies in humans. Based on animal and cell culture data ganciclovir is considered a potential human carcinogen, teratogen, and mutagen. It is also considered likely to cause inhibition of spermatogenesis. No human data exist that estimate the actual risk of these effects. Thus, it is used judiciously and handled as a cytotoxic drug in the clinical setting.

3.6.1 Human toxicity data relevant to the proposed trial

In human studies (mostly involving immunocompromised solid-organ or stem-cell transplant recipients), the primary toxicity has been reversible leukopenia or neutropenia and has generally occurred after months of drug exposure and in patients receiving other marrow toxic agents. Baseline leukopenia/neutropenia is an uncommon finding in critically-ill patients with sepsis and acute respiratory failure and is thus not anticipated to be a significant issue but will be closely monitored. For all patients receiving study drug (ganciclovir), routine weekly monitoring (with absolute neutrophil and platelets counts) is recommended and will be performed in the present study. Monitoring will be performed by the research team at each site for all participants, with any reportable AE reporting per the protocol. These research personnel will be blinded. Other potential side effects have generally been similar between ganciclovir and placebo groups in randomized trials.

3.6.1.1 Hematotoxicity

3.6.1.1.1 Platelets

Most placebo-controlled randomized studies, including those in stem cell transplant patients, do not show a difference in the incidence of thrombocytopenia and platelet transfusion requirements [44, 55-59]. However, there are rare anecdotal reports of ganciclovir-related pancytopenia. One study of ganciclovir prophylaxis in hematopoietic cell transplant (HCT) recipients reported delayed platelet engraftment [60]. Overall, the potential to cause thrombocytopenia is considered low.

3.6.1.1.2 Neutropenia

Neutropenia is the principal toxicity of ganciclovir and valganciclovir. The incidence is highest in HCT recipients and HIV-infected individuals, followed by pediatric patients with congenital CMV disease and solid organ transplant (SOT) recipients. Many studies have demonstrated the effect occurs late after drug administration [56, 61, 62]. In fact, several studies in HCT recipients, the most susceptible population for this complication, show that the median time of onset is 5 weeks after start of drug administration. The most relevant data for the proposed study come from a recent randomized trial of valganciclovir prophylaxis in kidney transplant recipients [56]. In that study, the incidence of neutropenia within 28 days (the duration of treatment proposed in the present study) was only 2%. Another recent randomized trial of valganciclovir vs. ganciclovir at treatment doses (900 mg twice daily and 5 mg/kg twice daily, respectively) for CMV disease in SOT recipients showed a neutropenia rate of 1.2% and 0%, respectively, at 21 days of treatment [57].

Ganciclovir-related neutropenia is reversible [56, 57, 61]. The time to recovery can be hastened by administration of G-CSF [54].

3.6.1.2 HIV & hematotoxicity

A trend towards anemia has been shown to occur in HIV-infected patients treated with valganciclovir. However, no strong evidence exists in transplant recipients and other patient populations, suggesting that the effect may be related to concomitant medications specific to the HIV setting. One phase III randomized trial of prolonged valganciclovir prophylaxis in HCT recipients, a population that would be considered at particularly high risk for this complication, did not show an increased rate of anemia or red blood cell transfusion requirements [63]. Other randomized trials also did not show an increased risk of anemia [50, 56, 64].

3.6.1.3 Renal toxicity

Results from randomized trials do not support a role for ganciclovir or valganciclovir as causes of renal toxicity. None of the recently conducted randomized trials shows an increased risk or renal toxicity [50, 56]; however, two earlier trials, one in heart transplant recipients with IV ganciclovir [65, 66] showed increased rates of renal insufficiency. While the potential to cause direct toxicity appears to be low, we will monitor renal function closely and adjust doses according to the creatinine clearance according to the package insert.

3.6.1.4 Neurotoxicity

Rarely observed. Not statistically significant between study arms of most randomized trials except one study in HCT recipients [50]. This effect probably occurs only in a setting of concomitant drugs with neurotoxic potential and high blood levels in the setting of subclinical renal insufficiency.

3.6.1.5 Carcinogenicity

Ganciclovir is considered a potential human carcinogens (see package insert). No studies have been performed to systematically assess this potential in humans. Although tens of thousands of

transplant and HIV infected patients have been treated with these compounds over the past ~20 years, no reports of an increased risk of cancer have been published. However, this does not rule out possible carcinogenic effect.

3.6.1.6 Teratogenicity

There are reports of ganciclovir-associated teratogenicity in humans, and this drug is contraindicated in patients who are or are planning to become pregnant. For the purposes of this study, all patients will be screened and excluded for pregnancy/possible pregnancy. Participants who are women of childbearing potential will be advised to use effective contraception during treatment and for at least 30 days following treatment with study drug. Similarly, participants who are men will be advised to practice barrier contraception during and for at least 90 days following treatment with study drug.

3.6.1.7 Phase 2 RCT results for safety of ganciclovir in critically ill patients.

Ganciclovir was well tolerated in critically ill patients with acute respiratory failure associated with sepsis or trauma in our NHLBI-funded phase 2 double-blind, multicenter GRAIL RCT of 160 patients, of whom 84 received ganciclovir (Table 3-1) [23]. Despite concerns about hematologic toxicity of ganciclovir in other populations (HIV, transplant), no patients in the ganciclovir group developed neutropenia that was felt to be related to study drug. There were also no significant differences in transfusion requirements and other pre-specified adverse effects (use of hematopoietic growth factors, renal insufficiency) associated with study medication. Similarly, valganciclovir was well tolerated in a single-center, open-label RCT in CMV seropositive, critically ill patients [47]. In this trial, oral valganciclovir was used at half the standard dose used for prevention in other populations. No cases of neutropenia were observed in the valganciclovir group, and there were no significant differences were observed in renal insufficiency, platelet transfusions, or mortality between the valganciclovir group and the control group. A recent, separate RCT evaluated pre-emptive ganciclovir for CMV reactivation in 76 immunocompetent ICU patients requiring invasive mechanical ventilation (39 of whom received ganciclovir) [67]. Adverse event rates were comparable for for ganciclovir-treated patients versus placebo recipients, no adverse events related to hematological toxicity were observed, and no leucopenia or thrombocytopenia were reported. Furthermore, creatinine levels, white blood cell and platelet counts, and the percentage of patients requiring renal replacement therapy from randomization to the end of treatment were similar.

Table 3-1. Safety Assessments Among Patients with Critical Illness Receiving Ganciclovir vs Placebo [23].

	Placebo Group, No. (%) (n = 72)	Ganciclovir Group, No. (%) (n = 84)	*P* Value
Patients with ≥1 transfusion	26 (34)	31(37)	.92
Red blood cells	26 (100)	31 (100)	.92
Platelets	7 (27)	1 (3)	.02
Transfusions per patient, median (IQR), No.	1 (1-4)	2 (1-2)	.63
Red blood cell transfusions per patient	1 (1-4)	2 (1-2)	.72
Platelet transfusions per patient	1 (1-2)	1 (1-1)	.49
New tumors at day 180	0	0	
Neutropenia at day 35[a]	0	0	
Granulocyte-colony stimulating factor use	0	0	
Renal insufficiency[b]	41 (57)	36 (43)	.08
Pregnancies	0	1 (<1)	
Patients with ≥1 adverse event[c]	13 (17)	17 (20)	.73
Patients with ≥1 adverse event of grade 3 or more[c]	10 (13)	11 (13)	.88

3.6.1.8 Summary of human toxicity data

Ganciclovir is currently recommended and widely used as a first-line agent for prevention and treatment of CMV infection/disease in patients with HIV and solid-organ or hematopoietic cell transplant [54, 68]. Potential toxicities of ganciclovir are well known, with the primary toxicity being reversible leukopenia/neutropenia [43, 56] that can occur after several weeks of drug exposure, usually in patients receiving other marrow toxic agents. Other important toxicities, per the package insert, include neurotoxicity and thrombocytopenia. As stated above, data from our phase 2 RCT [23] and another trial [47] demonstrate that ganciclovir is well tolerated in immunocompetent patients with critical illness due to sepsis or trauma and has a favorable safety profile. The observed incidence of neutropenia and neurotoxicity in our phase 2 trial was 0%, and there was no significant increase in transfusion requirements and other cytopenias, including thrombocytopenia, in patients receiving ganciclovir versus placebo (**Table 3-1**). These data suggest that severe side effects of ganciclovir are rare in critically ill patients and ganciclovir-related neutropenia occurs very uncommonly in persons without underlying bone marrow dysfunction and generally occurs at a median of 5 weeks after drug exposure (longer than the maximum 28 days in the proposed study).

Anemia has been observed in HIV-infected subjects, but there is no evidence that it is a problem in transplant patients or ICU patients with sepsis, or in the treatment of congenital disease.

There may be some risk of renal toxicity; however, this was not consistently observed across randomized trials. Data from our phase 2 RCT, in otherwise immunocompetent patients with critical illness due to sepsis or trauma, demonstrated no significant increase in renal insufficiency in the ganciclovir group compared with the placebo group [23]. Data from a second RCT had similar findings [47].

Other potential safety issues include teratogenicity and carcinogenicity.

3.6.2 Other recent investigational applications of ganciclovir

A randomized clinical pilot trial of valganciclovir for chronic fatigue syndrome (NCT00478465) has been completed [69]. Valganciclovir was well-tolerated and was not discontinued due to hematologic or hepatic adverse events.

A randomized placebo-controlled pilot trial of valganciclovir has also been completed in patients with glioblastoma multiforme (NCT00400322) [70]. Valganciclovir was safe and well tolerated. Treatment-related adverse events were generally mild to moderate; the most common hematological events were thrombocytopenia and leucopenia.

4 RATIONALE

The study is a phase 3 randomized, double-blind, placebo-controlled multicenter (see Appendix H for a list of the 19 initial clinical sites) clinical trial with respiratory-support-free days (RSFD) as the primary outcome measure. The rationale for key aspects of the study design are summarized in Table 4-1 and described below.

Table 4-1. Rationale for Key Aspects of the Study Design.

Study Population		
Sepsis, Acute Respiratory Failure, and Respiratory Support are defined as in Section 4.1.1 below.		
Prophylaxis vs. Treatment		
	PROS	**CONS**
Prophylaxis	• Conceptually more attractive; prevents all CMV reactivation at any site (including lung) before CMV-associated effects begin • Standard of care for other populations where CMV is a clinical problem (transplantation) • Best experimental/clinical data for preventing CMV indirect effects • Logistically easier (would also be easier to implement clinically)	• Effect "diluted" by high proportion of non-reactivators • Relative "over-treatment" with risk for drug toxicity
Treatment (Preemptive Therapy)	• More targeted approach ("treating a known/diagnosed infection" vs. preventing a theoretical possibility) • Minimizes drug exposure and toxicity by targeting only infected patients	• Logistically complicated • Likely too late to see any benefit of intervention (CMV-mediated effect cascade already initiated) [10] • Less effective in animal models • Plasma CMV PCR is an insensitive marker of CMV reactivation (preferentially local reactivation in lung)
Choice of Drug		
Ganciclovir	• FDA-approved for prophylaxis and treatment of CMV infection and disease • Favorable safety profile in ICU patients • Extensive experience in patients with reduced renal function • Insufficient data for alternative drugs (letermovir, maribavir) • Broad-spectrum antiviral activity (including HHV-6 [71])	
Study drug duration		
Until death, hospital discharge, or 28 days (whichever occurs earliest)	• Incident CMV reactivation occurs in critically ill patients up to 28 days after admission [7, 12, 16]; therefore, discontinuing ganciclovir at ICU discharge would leave CMV seropositive patients at risk of CMV reactivation; • Time-dependent analyses have shown a delay between CMV reactivation and subsequent poor clinical outcomes; thus, early discontinuation of ganciclovir could have a substantial impact on subsequent patient outcomes; • In our phase 2 study, ~8% of days on invasive mechanical ventilation occurred after initial ICU discharge; this figure is likely similar for days on respiratory support.	
Timing of Enrollment		

5-day window from hospital admission	• Early initiation of antiviral therapy (before or soon after CMV reactivation) is important for efficacy • Use of CMV serologic test (FDA has determined that this is a nonsignificant risk device study) allows determination of eligibility within this window • Provides sufficient time to confirm serologic test • Competes less for participants with other RCTs that have very short enrollment windows

4.1 Rationale for study population

4.1.1 Definitions of sepsis, acute respiratory failure, and respiratory support

The study population is sepsis-associated acute respiratory failure.

Sepsis will be defined according to the recent Sepsis-3 consensus definition (outlined briefly below and in more detail in Appendix G) [72]:

- Infection suspected or confirmed by the treating physician and
- Organ dysfunction measured as an acute change in total SOFA score ≥2 points after the infection (baseline SOFA score assumed 0 unless known pre-existing organ dysfunction).

Acute respiratory failure will be defined as the concurrent presence of :

- PaO2/FiO2 ≤ 300mmHg if PaO2 is available. If PaO2 is not available, the SpO2 must be ≤ 96% on any FiO2 setting ≥ 40%,*

  AND

- New, or if no prior imaging, presumed new unilateral or bilateral infiltrates on chest imaging occurring in the setting of sepsis from any source,

  AND

- Requiring **respiratory support for respiratory failure**.

For this study, **respiratory support** includes high flow nasal cannula oxygen with a flow rate ≥ 30 L/min, non-invasive ventilation by mask or helmet with any mode including pressure support, CPAP, or bi-level CPAP (BiPAP) with the equivalent of positive end-expiratory pressure (PEEP) ≥ 5 cmH2O, or invasive mechanical ventilation via endotracheal tube or tracheostomy with PEEP ≥ 5 cmH2O. It does not include respiratory support for sleep apnea or conditions other than acute respiratory failure.

* Although FiO2 is difficult to estimate at lower flow HFNC oxygen, for purposes of this study, the set FiO2 during HFNC oxygen therapy will be used as the study FiO2

Although 67% of these patients meet criteria for the acute respiratory distress syndrome (ARDS), the main difference between the sepsis-associated acute respiratory failure population and ARDS is the chest radiograph. We chose to remove “bilateral radiographic opacities” from our inclusion criteria because: (1) There is no reason to suspect that the deleterious effects of future CMV reactivation differ by the chest radiograph on presentation; and (2) We have recently shown that training clinicians to apply this criterion accurately is ineffective; therefore we do not believe the populations can practically be distinguished [73]. Note that meeting these criteria for acute respiratory failure confers 2 points in SOFA score and qualifies all patients for sepsis if they have clinically suspected/documented infection as indicated by the treating physician and use of antibiotics.

4.1.2 Rationale for focusing on sepsis

The rationale for focusing on sepsis relates to epidemiology and the fundamental biology of CMV reactivation. Sepsis, and particularly sepsis from pneumonia, accounts for 73% of patients with acute respiratory failure [3]. The other 27% of patients include trauma, burn, transfusion, COPD, and acute congestive heart failure. Since cytokine profiles, patient characteristics and outcomes vary considerably by these risk factors, restricting the study to patients with sepsis yields a more homogeneous study population. CMV reactivation also varies by risk factor, with septic patients at highest risk, likely due to sepsis mediated immune dysfunction [8]. Additionally, our phase 2 trial found the greatest signal for prevention in the large subset of patients with sepsis- (rather than trauma-) associated respiratory failure [23].

4.1.3 Rationale for age of study participants

We will include all patients ≥18 years of age who meet all other inclusion criteria. Studies have shown no association between age and CMV reactivation risk in critically ill patients [8, 23, 30].

4.2 Rationale for study intervention

We selected a "prophylactic" approach (in which antiviral therapy will be initiated prior to CMV reactivation in all eligible CMV seropositive patients) over a "treatment" approach (in which antiviral therapy would be started only after CMV reactivation was documented) for this trial (see Table 4-1). Despite potential limitations, use of a prophylactic strategy offers the best opportunity to assess for an effect of ganciclovir with an acceptable likelihood of toxicity. The major weaknesses of a treatment approach are that local CMV reactivation in the lung can occur even in the absence of reactivation in blood [74, 75] and that current methods of CMV measurement in blood (i.e., PCR) are not sensitive enough for detection of all CMV reactivation [76]. Indeed, a recent study showed that patients with sepsis had a much higher proportion of reactive CMV-specific immune response than what would have been expected based on viral load monitoring in the blood [76]; thus reactivation at sites other than the blood (e.g. the lung, salivary gland) is probably more common than viremia. Also, since the kinetics of CMV replication in critically ill patients is so rapid, significant CMV replication and its negative consequences would likely occur before antiviral intervention would be possible. A recent multi-site RCT evaluated a treatment (preemptive therapy) approach, with ganciclovir or placebo administered to mechanically ventilated ICU patients in whom CMV reactivation was detected (by PCR) [67]. The trial was stopped for futility based on an interim analysis by the DSMB, and no significant difference was observed between ganciclovir vs. placebo recipients (n=39 vs. 37, respectively) in ventilator-free days (the primary endpoint), mortality, or duration of hospitalization or ICU stay. These findings lend support to the idea that with a treatment approach, ganciclovir may be administered too late to have clinical benefit. Thus, these data support the prophylactic approach of our study design. A non-controlled study using a test and treat approach (i.e. ganciclovir treatment instituted on the basis of a positive blood test for CMV) failed to demonstrate a clinical benefit [10], probably related to the issues discussed above. Finally, for a treatment strategy to be effective generally, hospitals would need to implement rapid CMV diagnostic techniques that are not available at all centers.

4.3 Rationale for the choice of drug, dose & regimen

4.3.1 Rationale for choice of drug

Among clinically available medications, only ganciclovir and its oral analogue valganciclovir are FDA approved for both the treatment and prevention of CMV infection and disease. To date, insufficient data exists for use of alternative drugs (letermovir, maribavir) in this setting.

Furthermore, ganciclovir offers broad-spectrum activity against other viruses including HHV-6; this may offer added benefit since reactivation of both HHV-6 and CMV is associated with worse outcomes than reactivation of either virus alone in ICU patients [71]. There is extensive experience with ganciclovir due to its widespread clinical use for ~30 years in hundreds of thousands of patients. Its primary mechanism of action is inhibition of viral DNA polymerase and it does not appear to have other significant biologic effects (e.g. anti-inflammatory, antifibrotic). Information on clinical use of ganciclovir, available formulations, standard dosing regimens, safety and toxicity are detailed in Section 3.

As stated above in the Section 2.5, our phase 2 RCT demonstrated that ganciclovir is well tolerated in immunocompetent patients with critical illness due to sepsis or trauma and has a favorable safety profile [23]. These data suggest that severe side effects of ganciclovir are rare in critically ill patients and are likely outweighed by the potential benefit in this high-risk target population. Other potential side effects (e.g., liver toxicity) have generally been similar between ganciclovir and placebo groups in randomized trials [44, 50, 55, 58, 60, 64, 68, 77]. Also of note, there is extensive experience with giving ganciclovir to patients with reduced renal function, including critically ill patients receiving renal replacement therapy.

4.3.2 Rationale for choice of formulation

We chose IV ganciclovir only (rather than also using its oral analogue valganciclovir) due to unclear absorption of enteral medications in critically ill populations. Additionally, data from our phase 2 RCT suggest that oral therapy after hospital discharge occurred in <10% of subjects and (after having in-hospital IV therapy) added no apparent benefit. Participants will receive study drug twice daily for the first 5 days of therapy, then once daily until therapy ends; the initial twice daily dosing will be more effective in disrupting already ongoing reactivation in the small subset of patients that, despite several study design features to minimize this number, are expected to be PCR positive at baseline. Similar regimens have been used successfully in transplant recipients [50, 60].

4.3.3 Rationale for duration of drug

We chose a study drug duration of until death, hospital discharge, or 28 days (whichever occurs earlier), for several reasons:

- Incident CMV reactivation occurs in critically ill patients up to 28 days after admission [8, 15, 23]; therefore, discontinuing ganciclovir at ICU discharge would leave CMV seropositive patients at risk of CMV reactivation;
- Time-dependent analyses have shown a delay between CMV reactivation and subsequent poor clinical outcomes; thus, early discontinuation of ganciclovir could have a substantial impact on subsequent patient outcomes;
- In our phase 2 study, ~8% of days on invasive mechanical ventilation occurred after initial ICU discharge; this figure is likely similar for days on respiratory support.

Administering ganciclovir was highly feasible across sites in our phase 2 study; 92% of patients received all post-ICU discharge doses, and 84% of post-ICU doses were administered successfully.

4.4 Rationale for timing of enrollment

The 5-day enrollment window from the time of initial hospital admission reflects a tradeoff between trial efficacy and feasibility. Initiating prophylaxis before CMV reactivation is important as prophylaxis is less effective once reactivation has occurred. The shortest window would be ideal, and a 5-day window will minimize reactivation; fewer than 8% of patients reactivate CMV

in plasma before day 5 [8]. A shorter enrollment window would reduce the number of eligible patients and contribute to enrollment failure due to missed patients, weekend screening, and delays in consent, affecting feasibility. The 5-day window will allow the PETAL sites included in our trial to enroll patients who are beyond the 24–48 hour enrollment window of planned trials in that Network, and will minimize competition for patients. Finally, this replicates the enrollment window in our positive phase 2 RCT. Enrollment will be defined as occurring at randomization.

4.5 Rationale for choice of endpoints

4.5.1 Justification for Primary Endpoint.

VFDs are a composite outcome of mortality and duration of mechanical ventilation in survivors that assigns zero points to all deaths within a 28-day window and 1 point to every day off the ventilator in 28-day survivors. It was designed, in part, to address the issue of competing mortality on duration of mechanical ventilation. More recently, changes in clinical practice have led to increased use of non-invasive ventilation (NIV) in patients with acute respiratory failure. This change is based on 20 years of accumulating evidence that NIV as initial management in patients with acute hypoxemic respiratory failure is associated with lower mortality and, in some patients, can completely prevent intubation [78]. The COVID-19 pandemic has accelerated the implementation of NIV in acute respiratory failure, and at present, most patients with sepsis associated respiratory failure are managed with NIV initially unless they have a contraindication to its use or need an airway to facilitate transport. While it is impossible to predict every aspect of clinical care 5-7 years in the future, the trend toward non-invasive management of sepsis associated respiratory failure is clear now. Thus, including participants receiving NIV is essential for sufficient enrollment in this trial and applicability of trial results to future clinical practice.

Furthermore, while the mechanisms of sepsis associated respiratory failure are incompletely understood, the risk of CMV reactivation and its attendant harms are likely to be approximately the same whether the patient is managed with invasive versus non-invasive ventilation [8, 79, 80]. In a study of risk factors for CMV reactivation in critically ill patients that included both intubated and non-intubated patients, baseline intubation and mechanical ventilation was not a statistically significant risk factor for CMV reactivation [8].

As such, patients receiving NIV will be included in this trial and respiratory-support-free days (RSFDs) will be the primary endpoint for this trial. The following rationale supports use of RSFD as a primary endpoint, based in part on VFD data as well as available RSFD data.

First, biologically, we believe that CMV prophylaxis is likely to exert its greatest effect on pulmonary related morbidity. Second, VFDs (and more recently, RSFDs or organ support-free days) are frequently used as outcomes in high profile RCTs and influence clinical practice. A recent review of 128 RCTs assessing duration of mechanical ventilation in 10 high-profile general medical and critical care journals found that 43% reported VFDs and 12% used it as the primary outcome variable [81]. The NHLBI ARDS Network FACTT Trial showed that a conservative approach to fluid management increased VFDs by 2.5 with no statistically significant effect on mortality at 60 days [82]. Based primarily on this trial evidence, the Surviving Sepsis Guidelines endorsed this approach with a strong recommendation. Finally, VFDs are the outcome that was significantly improved in our phase 2 GRAIL RCT. Importantly, in recent trials enrolling NIV patients, there has been a change from VFDs to RSFDs and organ support free days in a 28 day window [83-85]. For example, the REMAP-CAP platform trials for COVID and community acquired pneumonia have adopted RSFDs and have determined that 1.5 RSFDs are the minimal clinically important difference [84].

There are several possible limitations to using RSFDs at 28 days as our primary endpoint. As with all composites, patients and clinicians may not value these outcomes in the way they are weighted in the composite. RSFDs will not capture outcomes that go in different directions (e.g., treatments that increase mortality but also reduce duration of respiratory support in survivors). RSFDs can be difficult to interpret as they do not simply reflect either a change in mortality or a difference in duration of respiratory support. Generally, VFDs have a bi-modal distribution and must be analyzed appropriately [81]; we anticipate that the distribution of RSFDs will also have a bi-modal distribution.

RSFDs were selected over several potential alternative approaches, which were deemed less suitable than using RSFDs to study duration of respiratory support with competing mortality. The preliminary data from our observational cohort and our phase 2 trial suggest that the biggest effect of CMV reactivation in the ICU was on duration of mechanical ventilation [8, 23]; in current practice, many of those who received invasive mechanical ventilation in these previous studies would now receive NIV and we anticipate that CMV reactivation would similarly affect duration of respiratory support. Thus, mortality was not selected as the primary outcome for this trial. However, since high mortality is expected in this trial of septic patients requiring respiratory support or mechanical ventilation and there may in fact be an effect on mortality, we deemed it necessary to consider mortality in this trial's outcome. Therefore, we did not choose respiratory support or mechanical ventilation alone as a primary endpoint. While advanced modeling approaches may allow the analysis of one outcome, we are not aware of any that have been used in the primary analysis of a clinical trial to address the effect of competing mortality on duration of mechanical ventilation. The Win-Ratio and the hierarchical outcome approach proposed by Finkelstein and Schoenfeld [86] have been used in trials [87], avoid the weighting issue inherent in VFDs, and are superior to binary composites as they allow time-to-event measures to be compared; however, these were deemed unlikely to offer additional clarity and have not been used extensively to study duration of respiratory support. RSFDs combine two outcomes (mortality and duration of respiratory support in survivors) considered of the same inseparable clinical importance as one endpoint, providing the reader with a single P value. Mortality, duration of respiratory support, and duration of mechanical ventilation will also be included as secondary analyses.

4.5.2 Secondary and Exploratory Endpoints.

Secondary endpoints were selected either because of their known association with clinically significant outcomes in sepsis-associated respiratory failure or because they are clinically relevant themselves as outcomes or safety measures. VFDs will be a key secondary endpoint. Although the study is not specifically powered to detect significant differences in these secondary clinical endpoints, we have provided estimates of the differences that could be detected based on the sample size (see Statistical Considerations).

5 STUDY HYPOTHESES, OBJECTIVES AND ENDPOINTS

5.1 Primary 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 lead to improved clinical outcomes.

5.1.1 Primary Objective

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

5.1.2 Primary Endpoint

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

5.2 Secondary Objectives

The secondary objectives of the study are:

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.

5.2.1 Secondary Endpoints

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 survivors) by day 28.

6. Oxygenation (PaO2/FiO2 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.

5.3 Exploratory Objectives

The exploratory objectives of the study are:

1. To evaluate whether static respiratory system compliance at randomization, day 4 and day 7, is different among ganciclovir recipients relative to placebo recipients.

2. To assess occurrence of invasive bacterial and fungal infections among ganciclovir recipients relative to placebo recipients.

3. To evaluate if organ dysfunction scores (regular SOFA variables including respiratory, coagulation, liver, cardiovascular, CNS and renal; however, SpO2/FiO2 ratio will be used in place of P/F ratio used in regular SOFA) are different among ganciclovir recipients relative to 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 that may associate with CMV reactivation kinetics, including demographics, co-morbidity, 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 of CMV viral load kinetics in blood and lung compartments with RSFDs and VFDs and specific secondary clinical outcomes like oxygenation, static respiratory system compliance, mortality and duration of mechanical ventilation in survivors.

7. To assess performance of rapid lateral flow CMV serostatus assay compared to clinically performed assays for IgG antibodies to CMV, as performed in CLIA-approved labs, throughout the study and at trial completion.

8. To evaluate assays to characterize immunity to CMV (cellular immunity, neutralizing antibodies, antibody epitope expansion, and transcriptional signatures).

9. To determine whether use and duration of ECMO differs among ganciclovir recipients relative to placebo recipients.

10. To assess occurrence of neuromuscular blockade among ganciclovir recipients relative to placebo recipients.

11. To assess the use of prone positioning among ganciclovir recipients relative to placebo recipients.

5.3.1 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
    a. Sex, age, race
    b. Co-morbidity
    c. APACHE III score at baseline
    d. SOFA score and individual components
    e. Lymphocyte count
    f. Time from hospital admission
    g. Viral load prior to randomization
6. Relationship of CMV viral load with RSFDs and VFDs and secondary clinical outcomes:
    a. Viral load: initial, peak, slope, area under the curve (AUC)
    b. Association with RSFD and VFD, day-28 mortality, duration of mechanical ventilation in survivors, static compliance, and PaO2/FiO2.
    c. 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.

5.3.2 Collection and banking of DNA and RNA, and study samples

In order to perform future investigations into the causes of acute respiratory failure and any possible links between acute respiratory failure outcomes and with treatment with ganciclovir, we will collect DNA and RNA samples for gene association and gene expression studies. Other study samples (blood, endotracheal aspirates) as well as left-over material from clinical samples (e.g. endotracheal aspirates, biopsy, autopsy material) will be kept in a repository for future studies of other herpesviruses. IRB approval will be obtained for studies not related to herpesviruses.

5.3.3 Ancillary studies

Cryopreserved samples may be used to perform additional assays to support standardization and validation of laboratory assays, and to evaluate additional endpoints and associations of interest. These assays may include, but are not limited to PCR testing for other pathogens, gene association studies, additional cytokines and chemokines, proteomics, microbiome, gene expression and immune function studies. We will identify specific sites to do certain ancillary pathogenesis studies.

5.4 Post hoc analyses

We will conduct electronic medical record review to systematically capture potential additional adverse effects, that did not meet the protocol defined definitions of AEs and SAEs (e.g. neurologic, cardiac, pancreatic, hepatic, renal events). Additionally, we will extract parameters that are required to analyze population pharmacokinetics of the study product. A systematic review of the medical record according to previously published categorizations to determine the causes of death will be completed.

We will also analyze additional parameters that are already captured in the database, for their association with study groups and outcomes, (e.g. lymphopenia, monocytopenia). Additional post hoc subgroup analyses will be performed to evaluate specific subgroups (e.g. COVID-19, ventilation type, sepsis phenotypes, cause of sepsis, CMV reactivation patterns).

All details of the statistical analyses of these post hoc variables will be outlined in the statistical analysis plan.

Using stored samples obtained during the study, we will conduct the following tests:

- Drug levels (ganciclovir)

- Analysis of inflammatory patterns (e.g. to determine sepsis phenotypes) and pathways using system approaches (e.g. cytokine analyses and proteomic testing)

Using stored study product, we will test for purity and contamination of the study product.

Samples may be sent out to external institutions and testing facilities after removing identifiers. Additional tests may be conducted based on the results.

6 STATISTICAL CONSIDERATIONS

6.1 Power Calculations for Primary and Secondary Hypotheses

6.1.1 Primary Endpoint

The primary endpoint RSFD is defined as follows.

Consistent with use of free-days in other studies, RSFDs will use a "last off" approach. This is the approach that has been used in PETAL Network studies (e.g., NCT02509078), is preferred by FDA as a more durable/sustained recovery landmark, and recommended by our External Advisory Board.

"Last off" means that RSFDs will be counted when a participant gets off and stays off of respiratory support (as defined in Section 4.1.1) to day 28. Days off of respiratory support will be counted back from day 28 to the last day when respiratory support was received. If a participant is still receiving respiratory support on day 28, no RSFDs will be counted, even if they were not receiving support for some number of days before day 28. Following are 3 examples of this calculation:

1. Participant receives respiratory support from days 1-10 and then receives no further respiratory support through day 28. RSFDs=18.

2. Participant receives respiratory support from days 1-10, then again from 16-20, then again from 24-26. No respiratory support is received on days 27 or 28. RSFDs=2.

3. Participant receives respiratory support from days 1-10, then again from 26-28. RSFDs=0.

Participants who do not survive to day 28 are assigned zero respiratory-support-free days [23, 88]. The 28-day landmark is chosen, because

1. Interventional trials in acute respiratory failure typically involve a 28-day treatment or follow-up period after the patient enrolls in the trial; and

2. Most patients with acute respiratory failure have either died or been successfully weaned from respiratory support by day 28 [23, 88].

To estimate the required sample size for the trial with adequate statistical power for the primary endpoint, we used the data of VFDs in the ganciclovir and placebo arms of the GRAIL Phase 2 trial and available RSFD data in the literature [83-85]. The summary statistics of VFDs in the sepsis cohort of GRAIL Phase 2 trial are shown in the table below. Since standard deviation data for RSFD is sparse, we assume that standard deviation for RSFDs is similar to that of VFDs in our sample size calculations as informed by Table 6-1.

Table 6-1. Summary statistics of VFDs in the Sepsis Cohort of GRAIL Phase 2 Trial

	n	Min	Q1	Med	Mean	Q3	Max	StD
Control	66	0	9.0	20	16.33	24	27	9.62
Treatment	71	0	16.5	23	19.07	25	27	9.25
Total	137	0	13.0	23	17.75	25	27	9.50

The sample size of the Phase 3 trial was determined for the primary endpoint, accounting for mortality, realistic and clinically relevant mean difference in RSFD between the arms, and feasibility.

Assume that RSFDs follow a normal distribution within group 1 (ganciclovir) and group 2 (placebo), respectively.

$$RSFD_{1,i} \sim N(\mu_1, \sigma_1^2), \qquad i.i.d., \qquad i = 1, \dots, n_1$$
$$RSFD_{2,j} \sim N(\mu_2, \sigma_2^2), \qquad i.i.d., \qquad j = 1, \dots, n_2$$

The hypotheses are then:

$$H_0: \mu_1 = \mu_2$$
$$H_1: \mu_1 = \mu_2 + d,$$

where d is the difference between the means.

Assume equal sample sizes, the sample size in each randomization group can be calculated using the following formula:

$n = 2\left(t_{1-\frac{\alpha}{2},2n-2} + t_{1-\beta,2n-2}\right)^2 \left(\frac{\sigma_{pooled}}{d}\right)^2$, where

n = estimated sample size for each randomization group$t_{1-\frac{\alpha}{2},2n-2}$ = $\left(1-\frac{\alpha}{2}\right)^{\text{th}}$ quantile $of\ t_{2n-2}$ distribution (with $2n-2$ degrees of freedom)

$t_{1-\beta,2n-2} = (1-\beta)^{\text{th}}$ quantile $of\ t_{2n-2}$ distribution (with $2n-2$ degrees of freedom)

σ^2_{pooled} = pooled variance of both groups

Note that n in the above equation does not have an explicit form, and thus, we use `pwr.t.test` in CRAN library `pwr` to derive sample size for the two-sample t-test here. Although distributional information for RSFD is limited in the literature, based on data that we do have available we anticipate the variance of RSFD to be similar to that of VFD. The pooled variance σ^2_{pooled} was estimated from the GRAIL Phase 2 trial to be 9.4^2, which has been used as an estimate for σ^2_{pooled} in our power calculations. A matrix is given below in Table 6-2. The numbers correspond to the estimated sample sizes required in each arm to achieve 80 - 90% power for various settings for difference in the means (minimum detectable difference in RSFDs between the two arms; 2.5 – 3 days) and the assumption that the pooled standard deviation of

RSFDs is 9.4 days, same as the estimate of σ_{pooled} for VFDs in GRAIL Phase 2, and at a type I error rate of 5%.

Table 6-2. Single arm sample size required to detect a minimum difference of 2.5 – 3 days between the mean of RSFDs in the two arms, with different levels of power (80 – 90%), with the assumptions that pooled standard deviation of RSFDs in the two arms is 9.4 days (same as the estimate of σ_pooled for VFDs in GRAIL Phase 2), and at a type I error rate of 5%.

Single arm Sample Size			
Power	**Detectable difference (d)**		
	2.5 days	**2.74 days**	**3 days**
80%	223	186	156
85%	255	213	178
90%	299	249	208

Note: Although RSFDs (or VFDs) can be considered as the number of event days out of the total 28 days, and conceptually a binomial assumption may be preferred, it is nevertheless not feasible for the RSFDs (or VFDs) given their complex definition involving both mortality and actual respiratory support (ventilator) usage. For the GRAIL Phase 2 Trial, the standard deviation (SD) of VFDs, assuming a binomial distribution, is estimated to be 6.67 days, smaller than the empirical estimate of 9.4 days, which suggests a substantial over-dispersion. Thus, normal assumption with empirical variances (as obtained from the GRAIL Phase 2 Trial and given that we anticipate variance for RSFDs to be similar to that of VFDs) instead appears to be justified for RSFDs. Also note that considering that there is no substantial literature on the distribution of RSFDs, our trial design relies on GRAIL Phase 2 data on VFDs instead and our belief that variability in RSFDs will be similar to that of VFDs. However, to safeguard against the case if variance of RSFDs is substantially larger than that of VFDs, we have planned to conduct a sample size re-estimation analysis after ~50% of the endpoints have been observed (see Section 6.5.3).

In the GRAIL Phase 2 trial, VFDs difference was estimated to be 2.74 days. As shown in Table 6-2, to detect a similar difference (of 2.74 days) in RSFDs between the two arms with 85% statistical power, a sample size of 426 subjects ($213 \times 2 = 426$) total is required in this Phase 3 trial's primary analysis, and that is what we power for in this study. Findings from previous RCTs suggest that approximately 12% of subjects in this Phase 3 RCT will be CMV PCR positive at baseline when rapid lateral flow assay is used to assess serostatus. This 12% estimate is lower than the proportion reported in the GRAIL Phase 2 study due to the use of the rapid lateral flow assay, which we estimate will shorten the time to randomization from ICU admission by approximately 1 day and reduce the baseline PCR positivity rate. Furthermore, we anticipate that the percentage of subjects who are PCR positive at baseline will be lower among individuals on respiratory support (as defined in Section 4.1.1) compared with individuals on mechanical ventilation alone (as in the GRAIL Phase 2 trial) because we will be capturing some eligible participants earlier in the course of their respiratory failure when less time has elapsed to allow for CMV reactivation (both because we will allow enrollment of patients on NIV and HFNC oxygen and because time to obtain consent from participants able to consent for themselves may be shorter than consent through LARs. Since the population for primary analysis includes all patients without regard to PCR positivity at baseline, we inflate the sample size by a factor to

account for the power loss by including these cases in our primary analysis population. We also assume a dropout rate of 1% lost to follow-up for the primary endpoint, based on findings from other large ICU trials, and a dropout rate of 2% for early discontinuation following a negative CLIA CMV ELISA test among randomized participants who have received at least one dose of study drug. Accounting for both of these effects, the sample size for GRAIL Phase 3 trial was increased to 500 subjects in total.

6.1.2 Power Calculations for Secondary Endpoints.

We do not expect this intervention to lead to a statistically significant difference in mortality between treatment groups. Nonetheless, mortality is a common and expected endpoint in critical care clinical trials, and it is a component of our primary outcome RSFDs. As such, we will include mortality as a secondary endpoint in this RCT. The risk of death is typically high (approximately 20%) in the patient population included in our trial. Table 6.3 shows the largest relative risk (for mortality) that we can detect in the treatment versus control group, with a sample size of 500 (including dropouts) and 80% power, and for different assumptions of overall mortality in the control arm (ranging from 10-35%).

Table 6-3. Detectable Relative Risk (Hazard Ratios) under different scenarios of control arm mortality with a two-sided test, 80% power and type I error rate of 5%.

Secondary outcome	Control arm assumption (%)	Hazard Ratio detectable*
Mortality (at 28 days)	35	0.62
	30	0.60
	25	0.56
	20	0.52
	15‡	0.45
	10	0.35

* Assumes control arm mortality rate in table, power = 80%, and 0.05 alpha
‡ From GRAIL Phase 2

Based on this table, with the current sample size of 500 and assuming a control arm mortality of 20% (more realistic assumption of mortality for GRAIL Phase 3), the proposed trial will have 80% power to detect a hazard ratio of 0.52 for mortality between the Ganciclovir arm and the Placebo arm.

6.2 Statistical Analyses for Endpoints.

6.2.1 Primary Endpoint.

We will first compare baseline characteristics of the ganciclovir and placebo groups in assessment of the randomization process. Then to test the primary hypothesis that the mean difference in RSFDs between groups differs significantly from 0, we will use the 2-sample parametric t-test. The t-test is powerful and will be sufficient for our purpose, even if normality assumption for RSFDs fails to hold, as the t-test is robust to non-normality assumption for n>25. However, as a confirmatory test, we will also use the 2-sample permutation t-test. The permutation test does not require any distributional assumption on the data and is valid under the much weaker assumption of exchangeability (which will be satisfied under controlled settings of a trial). As the next step, we will use the semiparametric efficient and robust method of Davidian

et al. [89, 90] to estimate the mean difference in the primary endpoint (intervention vs. control) with a 95% confidence interval, and to test for whether the mean difference differs from 0. This method leverages information in baseline subject characteristics predictive of the primary endpoint to maximize power and precision and is more efficient than a t-test for comparing baseline subtracted levels or analysis of covariance. If subjects are missing a primary endpoint for reasons other than death, then the analysis method will accommodate for it by addressing the missing data mechanism and adjusting for it in the analysis plan (please see Section 6.4.1 for a discussion). Apart from the primary analysis, a classical intent-to-treat (cITT) and two modified intent-to-treat (mITT) analyses will be carried out for the primary endpoint. The primary analysis population will be all patients randomized who receive at least one dose of the assigned study product. The classical ITT population will consider all patients that are randomized, while the two modified intent-to-treat (mITT) populations will be: 1) excluding those patients who were withdrawn from the study for any reason (including initial positive CMV LFA test followed with confirmatory negative CMV ELISA test) after receiving at least one dose of study drug, and 2) only including patients who survived and were followed to day 28.

In the primary analysis (as well as in the secondary and exploratory analyses), gender and race/ethnicity as biological variables will be included and examined.

6.2.2 Secondary endpoints

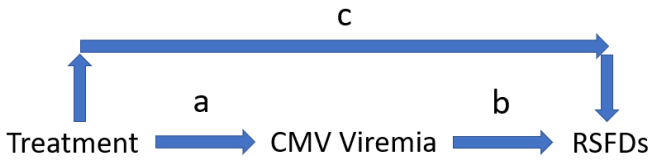
For the quantitative secondary endpoints (including a key secondary endpoint of VFDs or ventilator-free days), the same method as described in the analysis plan for the primary endpoint will be used. For time to event endpoints including CMV reactivation at given thresholds, the Kaplan-Meier method will be used to estimate the probability of not experiencing CMV reactivation by Day 28 for each group. A 95% confidence interval about the group difference in event rates will be computed using the two Kaplan-Meier estimates and the two Greenwood variance estimates. Based on these estimates, a Z-statistic will be used for testing for difference in the group event rates. The multiple testing problem will be handled using a Bonferroni correction, whenever such situations arise. Additionally, multivariate models will be built for the primary endpoint (RSFD) as well as other secondary endpoints, after adjusting for baseline subject characteristics and risk factors for CMV reactivation, using generalized linear models or the semiparametric efficient and robust method of Davidian et al. [89, 90]. The list of baseline characteristics to be considered for adjustment are those listed in (1) under the Exploratory Objectives/Endpoints (Section 5.3). We will repeat the above analyses in several subgroups (separately) as well, including those who are PCR negative at baseline, as well as those that have septic shock, pneumonia, unilateral infiltrates, or lymphopenia at the time of randomization, and by age and sex. In addition, we will repeat the above analyses in the following subgroups as follows: (1) VFDs in those on invasive mechanical ventilation at randomization; (2) RSFDs in those on invasive mechanical ventilation at randomization; (3) RSFDs in those on NIV/high flow at randomization; (4) Intubation after enrollment in those on NIV/HFNC at randomization (i.e. does ganciclovir affect the need for intubation in this subgroup?); (5) RSFDs according to COVID-19 status at randomization; and (6) CMV detection in plasma by day 28 (28 (>0 IU/mL, >1000 IU/mL) in those with invasive mechanical ventilation vs. NIV/high flow at randomization.

We will also compare the duration of mechanical ventilation and respiratory support by day 28 among survivors between the two intervention arms. If the rate of death by day 28 differs between the two groups, then the analysis in survivors may be biased. If there is evidence for a differential death rate, then a sensitivity analysis will be conducted to evaluate how the estimated mean difference changes with a range of assumptions about the degree of possible selection bias. The sensitivity analysis method of Shepherd will be used [91], which was designed to address "truncation by death". A mediation analysis will also be conducted to test if CMV viremia lies in the causal pathway linking the treatment (Ganciclovir) effect with the outcome (RSFDs), that is,

if CMV viremia, which is assumed to be affected by the treatment, itself affects the outcome or not.

This analysis will be conducted in four steps:

1. A regression analysis with Treatment predicting RSFDs to test for path c alone,
2. A regression analysis with Treatment predicting CMV Viremia to test for path a,
3. A regression analysis with CMV Viremia predicting RSFDs to test the significance of path b,
4. A multiple regression analysis with Treatment and CMV Viremia predicting RSFDs.



The purpose of Steps 1-3 is to establish that zero-order relationships among the variables exist. If one or more of these relationships are non-significant, it can be concluded that mediation is not likely. If there are significant relationships in Steps 1 through 3, Step 4 is conducted. In the Step 4 model, some form of mediation is supported if the effect of CMV Viremia (path b) remains significant after controlling for Treatment. If Treatment is no longer significant when CMV Viremia is controlled, the finding supports full mediation. If effect of CMV Viremia is still significant, the finding supports partial mediation. Apart from this, static respiratory system compliance (Crs) will also be used in a separate mediation analysis to understand whether any effect of Ganciclovir is exerted via pulmonary mechanisms.

Alternative approaches to Analyze RSFDs. Our secondary analysis plan also includes a pipeline for exploring alternative approaches to analyze RSFDs. Given that RSFDs are increasingly being used as an outcome in high profile RCTs (as mentioned in Section 4.5.1, RSFDs or organ support-free days have been used as the primary endpoint in several recent high-profile RCTs [83-85]), these proposed statistical considerations can serve as a reference point for RSFD analyses in future clinical trials. Thus, we believe this to be one of the statistical innovations proposed by the Data Coordinating Center (DCC) for this Phase 3 trial.

One important limitation to using RSFDs as an endpoint arises, as previously stated, due to its composite nature. RSFD assigns points based on counting back from day 28 to the last day when respiratory support was received (see Section 6.1.1). The outcome also considers mortality and assigns an overall score of zero points to those who die within those first 28 days. Thus, the composite nature of this endpoint leads to it being a zero-inflated binomial random variable, which has a mixture distribution of a degenerate 0 and a binomial distribution. That is, we can write

$$RSFDs \sim \pi \times 0 + (1 - \pi) \times Bi(28, p)$$

where π is the probability that a death occurs, and $Bi(28, p)$ is a binomial distribution such that p is the probability of a subject experiencing a RSFD while being alive through the specified window, that is desired to be modeled. The zero inflated binomial response is a special instance of over-dispersed responses, and there is considerable literature on the numerous challenges and

statistical issues when modeling such data [92, 93]. Thus, modeling of RSFDs can be handled through a number of approaches:

1. Account for over-dispersion through the zero-inflated binomial model: The zero-inflated binomial model is obtained by mixing a degenerate distribution at zero with a standard binomial distribution [94, 95], and has been subsequently used in epidemiology and health economics to model binomial counts with excessive zeros. The zero-inflated binomial model will be used as an alternative approach to model RSFDs in this analysis.

2. Account for over-dispersion though the beta-binomial model: The beta-binomial distribution is a discrete probability distribution which arises when the probability of success in a fixed number of Bernoulli trials is either unknown or random. It has frequently been used in classical statistics to capture over-dispersion in binomial type distributed data [96, 97], and this will be used as another alternative approach.

3. Separate modeling of composites: In addition to the above approaches, the composite outcome will also be modeled separately for the primary analysis population and considered for assessment. Mortality will be modeled as a time to event outcome, and the Cox model will be used to evaluate whether the treatment (ganciclovir) has any effect on time to death. On the other hand, duration of time off respiratory support (total time in days not spent receiving respiratory support within the first 28 days), regardless of the mortality status, will be analyzed separately in a Generalized Linear Model to evaluate if ganciclovir increases total time off respiratory support within the first 28 days.

6.2.3 Exploratory endpoints

To identify risk factors for CMV reactivation, we will first build univariate generalized linear regression models to test the association between CMV reactivation and different potential risk factors identified in Section 1 C.2. We will also build multivariate Generalized Linear Models for association with CMV reactivation, by including (i) factors found significant at level 0.2 in the first step and (ii) all risk factors, and then using a step-wise backward selection using the Akaike Information Criterion (AIC) or the Bayesian Information Criterion (BIC) for model selection.

We will use descriptive statistics and plots to examine the association of CMV viral load kinetics in blood and lung compartments with RSFDs and VFDs, mortality at day 28 and 180, duration of respiratory support in survivors, PaO2/FiO2, and static respiratory system compliance. We will also build regression models (generalized linear models) to examine these relationships more closely. We will also descriptively determine the sensitivity and specificity of the rapid lateral flow CMV serostatus assay, and assess its concordance with the standard clinical assays, using Cohen's kappa statistic.

6.2.4 Other pre-specified analyses

Primary analysis, cITT, mITT, and survivors

Ideally, any patient randomized would be included in the conventionally defined ITT analysis cohort. Nonetheless in an emergency care setting, such as this study, the following unusual but important scenario will need to be considered: a patient is eligible and randomized but by the time of drug delivery no longer meets eligibility criteria and thus, should have been excluded. Accordingly,it is intended that patients randomized but not having drug/placebo delivered will not be included in the primary analysis cohort. The integrity of randomization shall remain preserved, as the decision to deliver drug/placebo is determined a priori and completely independent of treatment assignment (14). If a participant is withdrawn from the study before a dose of study drug is given, no study procedures will be performed and no study data will be collected, and these participants will be excluded from the primary analysis. However, if a participant is withdrawn after receipt of study drug, safety procedures (e.g. monitoring of

creatinine and CBC with differential) will continue for another 48 hours, no study biosamples will be obtained, and study data in the medical record will continue to be collected per protocol (e.g. respiratory-support-free days), and they will be included in the primary analysis. In addition to the primary analysis, we will also conduct a randomized or classical ITT analysis, which will consider all patients randomized. This will be used as a conservative alternative to our primary comparison. Additionally, we will also perform 2 modified intent-to-treat (mITT) analyses: 1) excluding patients who were withdrawn from the study for any reason (including initial positive CMV LFA test followed with confirmatory negative CMV ELISA test) after receiving at least one dose of study drug, and 2) only including patients who survived and were followed to day 28. We will also test for an interaction of treatment period (pre and post drug use) and the primary treatment differences at the study conclusion. Finally, we will perform subgroup analyses evaluating treatment effect stratified on mode of respiratory support.

6.2.5 Post hoc analyses

Statistical considerations for the post hoc analyses described in Section 5.4 are described in the statistical analysis plan.

6.3 Randomization scheme

The randomization sequence will be uploaded by SCHARP into the Medidata's Randomization and Trial Supply Management (RTSM) system and provided to each site through Medidata. The randomization will be block-randomized by site. At each institution, the pharmacist with primary responsibility for drug dispensing is charged with maintaining the security of the randomization list. A research pharmacy designee may be provided access to the randomization assignment from Medidata RAVE.

6.4 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to treatment arm assignments (e.g., study drug or placebo). Study drug assignments are accessible to those site pharmacists, contract monitors (or the central site IDS pharmacist as backup), and unblinded statisticians who are required to know this information to ensure proper trial conduct. Access to randomization assignment is restricted to unblinded statisticians only. Emergency unblinding if ever needed is managed 24/7 through Medidata RTSM. Any discussion of study drug assignment between the site clinical and pharmacy staff is prohibited. The DSMB members also are unblinded to treatment assignment to conduct review of trial safety. Thus, closed reports for the DSMB will include the treatment indicators of the two participant groups.

Unblinding procedures are discussed in Section 9.15.

6.4.1 Missing data

Every effort will be dedicated to complete, comprehensive, and accurate data collection and data annotation by the DCC. However, issues and challenges arise in every clinical trial. Due to the high proportion of deaths in this patient population, one major challenge in this RCT of which we must be aware and ready to correct is missing data. Missing data can occur to the primary endpoint and key secondary endpoints (e.g., CMV reactivation [missing viral load values] and organ dysfunction or the NHLBI-endorsed acute respiratory failure core outcome measurement set). We have carefully examined sources of missing data from the GRAIL phase 2 trial and adjusted the analysis plan accordingly.

6.4.1.1 Missing Data in Primary Outcome.

It is highly likely that missing data in RSFDs, the primary outcome in our RCT, will be MNAR (missing not at random), as most missingness will result from withdrawal of consent, a rare occurrence that can happen when patients and families are in crisis, and is more likely to occur in sicker patients. However, we only expect <3% missingness in our primary outcome (see Section 2A), and thus this data can be ignored from the primary analysis. However, if for some unforeseen reasons, missingness in RSFDs does increase beyond a set threshold of 5%, we will have to adjust for it in our analysis plan. In that case, we will use baseline patient characteristics identified in (1) under the Exploratory Objectives/Endpoints (Section 5.3) to model the missing data mechanism in RSFDs, which will be used to estimate the treatment effect unbiasedly using maximum likelihood methods, provided the missing data model is correctly specified.

6.4.1.2 Missing Data in some secondary outcomes due to Study Design.

The absence of data pertaining to some secondary endpoints (for example, static respiratory system compliance) may be problematic if participants are discharged from the ICU, are on pressure support after being extubated, or expire prior to our pre-specified time points for endotracheal aspirates and serum collection. For example, if the Ganciclovir intervention reduces duration of respiratory support, it may bias the results because participants cannot undergo endotracheal aspirate if they are extubated. Thus, to minimize missing data and to maximize the CMV detection rate, we have selected multiple time points for the endotracheal aspirate (Day 1 and twice weekly until Day 28). If these issues still occur, we will address them in the analysis phase using data imputation methods, or by acknowledging it as a limitation of any analyses conducted with these variables.

6.4.1.3 Missing Data in covariates.

Missing data can occur in covariates as well, due to factors such as death, withdrawal, or other reasons; this will need to be accounted for in our analyses, if overall missingness (in covariates) increases beyond 5%. Since we expect covariate missingness in this study to be MAR (missing at random), we will use weighting adjustments (for example, weighted generalized estimating equations) or multiple imputation methods [98, 99] to deal with covariate missingness.

6.5 Planned analyses prior to end of study

6.5.1 Safety

The DSMB will have access to unblinded safety data at the time of planned reviews and upon request of the DSMB may review additional analyses. Operating details are specified in the DSMB charter. A scheduled interim safety analysis at midpoint will be performed.

The safety and protocol adherence review team (SPART) will review all clinical and laboratory safety data during the course of the study, at least every 3 months. The site teams are responsible for regularly monitoring all Adverse Events and documenting reportable AEs to the DCC and CCC for review by the SPART. The SPART will include CCC PIs (Drs. Boeckh, Stapleton, Rubenfeld, and Limaye), Clinical Site Manager Sara Ardren, and Clinical Trial Project Manager/Site Monitoring Manager Dr. Louise Kimball. Meetings of the SPART (by videoconference or teleconference) will occur regularly, and will not require attendance of all team members at each meeting. The SPART is responsible for the review of the clinical safety reports, discordant CMV serostatus test results (LFA versus CLIA test), and protocol violations, as well as communication with the IRB, NHLBI executive secretary, and DSMB as outlined in Figure 11.1.

6.5.1.1 Interim safety analysis.

It is expected in this trial that approximately 20% or approximately = 100 of the 500 participants will have death events relative to the safety endpoint. A two-sided interim safety analysis is planned to be performed at the midpoint, either at the 50th event or when 50% of participants (n = 250) are randomized, whichever occurs earlier.

Guidelines for early termination at the interim analysis due to concerns on the safety endpoint should (i) adjust for the nature of interim monitoring that involves repeated testing over time, (ii) reflect particular caution given the relative benefit-to-risk profile of the two arms.

Specifically, a recommendation for stopping will be based on strong evidence for the hazard ratio (treatment/placebo, HR) of death to be less than 1 or greater than 1. The O'Brien-Fleming "upper boundary" will be used to establish if an elevated event rate in the intervention group preserves the (one-sided) 0.025 false positive error rate relative to the hypothesis:

H_0: the event rate for the intervention group relative to control ≤ 1.00, or HR ≤ 1.

The O'Brien-Fleming "lower boundary" will be used to establish if an elevated event rate in the control group preserves the (one-sided) 0.025 false positive error rate relative to the hypothesis:

H_1: the event rate for the control group relative to intervention ≤ 1.00, or HR ≥ 1.

For illustration, Table 6.5.1-1 below presents the O'Brien-Fleming boundaries for the hazard ratio (HR) estimates that would lead to rejection of H_0 at the interim analysis performed when one has observed 50% and 100% of the trial's expected total of 100 death events.

Table 6.5.1-1: Interim analysis assumptions

Information Fraction (% of Total Events)	**Reject H_0: HR ≤ 1.00**	**Nominal one-sided p-values for rejection of H0**	**Reject H_1: HR ≥ 1.00**
50% (50 events)	≥ 2.316	P ≤ 0.0015; Z = 2.97	≤ 0.432
100% (100 events)	≥ 1.480	P ≤ 0.025; Z = 1.96	≤ 0.676

Observe that, for the total of 50 events at the interim analysis, to reach the O'Brien-Fleming boundary for a lower death rate in the intervention group, the control group would need to have at least 20 excess events (15 in intervention group versus 35 in the control group) at the 50% information fraction. Similarly, to reach the O'Brien-Fleming boundary for a lower death rate in the control group for the total of 50 events at the interim analysis, the intervention group would need to have at least 20 excess events (15 in control group versus 35 in the intervention group) at the 50% information fraction. The Lan-DeMets implementation of the O'Brien-Fleming guideline will be used to provide flexibility in the timing and number (in the case of unplanned DSMB meetings) of interim analyses [100].

An efficacy analysis (of the primary endpoint) will also be conducted at the time of interim look. Although no formal stopping rule will be implemented for either efficacy or futility, number of participants (or outcome data) needed to change the direction of the observed treatment effect will be estimated at the time of the interim analysis, which is required for the DSMB's consideration

of the data. Additionally, at the time of the interim analysis, variance of the actual data will be assessed in accordance with the assumptions made in the sample size calculations.

6.5.2 Other endpoint analyses

Distribution will be limited to those with a need to know, for informing future trial-related decisions. To guarantee the unrestricted performance of its task, the DSMB may receive the individual study morbidity and mortality data from an unblinded statistician. The DSMB will have access to unblinded safety data at the time of planned reviews and upon request of the DSMB may review additional analyses. Any analyses conducted prior to the end of the study should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments.

6.5.3 Sample size re-estimation plan

Considering that there is no substantial literature on the distribution of RSFDs, our trial design relies on GRAIL Phase 2 data on VFDs instead and our belief that variability in RSFDs will be similar to that of VFDs. However, in case it happens that variance of RSFDs is substantially larger than that of VFDs (which can happen for a variety of reasons including the fact that RSFDs is expected to be measured on a wider population than those who typically only require ventilation, which may introduce additional sources of variability), the common SD used in the sample size determination (Section 6.1.1) for the study may not reflect the actual value. To ensure the study remains adequately powered, the assumption of variability will be checked when the study is halfway done, ie, when endpoints for $N^* = 250 \sim 50\%$ of total initially planned sample size subjects (500) have been measured. Specifically, total variance will be calculated in a blinded way using the following approach, similar to the one proposed by Kieser and Friede as well as Zucker et al [101, 102].

$$\hat{S}^2 = \frac{1}{N^* - 1}\sum_{j=1}^{N^*}\left(RSFD_j - \overline{RSFD}_{N^*}\right)^2 - \frac{N^*}{4(N^* - 1)}\Delta^2$$

Where N^*: total number of subjects at re-assessment; $= N/2$ where $N = 2n$ is total sample size in the study

$RSFD_j$: RSFDs in patient j in the reassessment.

$\overline{RSFD}_{N^*}$: average of $RSFD_j$, $j = 1, \ldots, N^*$

Δ: expected difference in RSFDs between the arms (based on GRAIL Phase 2 data on VFDs)

Further investigation done by Keiser and Friede[101] indicates that this method has little impact on the type I error of the test for superiority. Therefore, no type I error adjustment needs to be made. The formula for blinded estimation of variance given above depends on the assumed treatment effects. It was noted that if the treatment effect is mis-specified, the estimated variance will be biased and may be under-estimated. However, as pointed by Kieser and Friede, such bias is generally negligible in most clinical trial situations.

If the estimated pooled SD from all available subjects at sample size re-assessment is considerably larger than our assumption of 9.4 days, sample size will be recalculated based on the estimated SD from the re-assessment using a similar approach as described in Section 6.1.1. Table 6.5.3 below provides details on the sample size re-assessment under the same targeted power (85%), the same treatment differences (2.74 RSFDs), and numbers of additional subjects needed for the larger SD values. The blinded variance estimation will be performed by one of the blinded statisticians on the trial. In work by Laterre et al [83], ventilator and vasopressor free days

within 30 days had an estimated SD around 13 and we suspect SD of our RSFD primary endpoint would not go beyond that.

Table 6.5.3: Sample size re-assessment to achieve 85% power

Estimated SD	Sample size per arm	Adjusted Sample size per arm	Total Sample Size	Overall sample size increase
≤ 9.4 days	213	250	500	0
9.8 days	231	271	542	42
10.2 days	250	293	586	86
10.6 days	270	317	634	134
11 days	291	341	682	182
11.4 days	312	366	732	232
11.8 days	334	392	784	284
12.2 days	357	419	838	338
12.6 days	381	447	894	394
13 days	406	476	952	452

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Study population

Five hundred adults will be randomized in a 1:1 ratio to receive either the study drug or placebo. All patients entered into this study will have established respiratory failure associated with sepsis. By virtue of their need for respiratory support (as defined in Section 4.1.1) within an intensive care unit (ICU), all patients will be considered critically ill.

Final eligibility determination will depend on results of laboratory tests, medical history, and physical examinations. Those determined to be eligible, based on the inclusion and exclusion criteria, will be enrolled in the study. Investigators should always use good clinical judgment in considering a subject's overall appropriateness for trial participation. Some subjects may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria because medical, psychiatric, social, or logistic conditions may make evaluation of safety and/or efficacy difficult.

Duration of participation in the study by individual patients will be 180 days.

7.2 Randomization

Patients meeting inclusion and exclusion criteria will be randomized to standard ICU care (including standard lung protective ventilation and weaning protocols, if mechanically ventilated) + intervention or placebo.

7.3 Inclusion criteria

1. Subject/next of kin informed consent
2. Age $\geq$ 18 years
3. CMV IgG seropositive by lateral flow assay (LFA) or standard serologic methods
4. Receiving care from an ICU team/service
5. Acute respiratory failure as defined in Section 4.1.1.
6. Expected to require respiratory support for at least 2 more days after randomization
7. Infection confirmed or suspected by the treating clinician and felt to be causing or contributing to acute respiratory failure (Respiratory failure associated with infection confers at least 2 SOFA points above assumed baseline SOFA score of 0, thereby meeting Sepsis-3 definition).

7.4 Exclusion criteria

1. Known or suspected immunosuppression, including:
    a. HIV+ (i.e. prior positive test or clinical signs of suspicion of HIV/AIDS; a negative HIV test is not required for enrollment)
    b. stem cell transplantation:
        i. within 6 months after autologous transplantation or
        ii. within 1 year after allogeneic transplantation (regardless of immunosuppression)

iii. greater than 1 year after allogeneic transplantation if still taking systemic immunosuppression or prophylactic antibiotics (e.g. for chronic graft versus host disease)

Note: if details of stem cell transplantation are unknown, patients who do not take systemic immunosuppression and do not take anti-infective prophylaxis are acceptable for enrollment and randomization.

c. solid organ transplantation with receipt of systemic immunosuppression (any time)

d. cytotoxic anti-cancer chemotherapy within the past three months (Note: next-of-kin estimate is acceptable)

e. congenital immunodeficiency requiring antimicrobial prophylaxis (e.g. TMP-SMX, dapsone, antifungal drugs, intravenous immunoglobulin)

f. receipt of one or more of the following in the indicated time period (see Appendix C):

i. within 6 months: prednisone, alemtuzumab, antithymocyte/antilymphocyte antibodies, or other immunosuppressive drugs associated with CMV reactivation

Note:

- If no information on these agents is available in the history and no direct or indirect evidence exists from the history that any condition exists that requires treatment with these agents (based on the investigator's assessment), the subject may be enrolled. For all drug information, next-of-kin estimates are acceptable. See Appendix C for commonly prescribed immunosuppressive agents. Information on the use of biologics with moderate immunosuppressive effect but no known effect on CMV are permitted and will be recorded in the CRFs.

- An average of >20mg/day of prednisone for the past 30 days prior to hospital admission. The total prednisone intake should be added and averaged over 30 days. For example, a patient taking 40mg/day for 14 days would not be excluded because (40x14)/30=18.7. Other steroids should be converted into prednisone equivalents. Additionally, any dose of corticosteroids given for any reason on or after hospital admission is acceptable.

2. Expected to survive < 72 hours (in the opinion of the investigator), or not committed to full intensive care support at the time of study enrollment (DNR but otherwise committed to full support is acceptable).

3. Unable to start receiving first dose of study drug within 120 hours after hospitalization (as measured from admission or time of transfer; subjects who are transferred from a chronic care ward, such as a rehabilitation unit, with an acute event are acceptable).

4. Pregnant or breastfeeding (either currently or expected within one month).

Note: for women of childbearing age (18-60 years, unless documentation of surgical sterilization [hysterectomy, tubal ligation, oophorectomy]), if a pregnancy test has not been done as part of initial ICU admission work-up (within 120 hours before enrollment), it will be ordered stat and documented to be negative before randomization. Both urine and blood tests are acceptable.

5. Absolute neutrophil count < 1,000/mm^3 (if no ANC value is available, the WBC must be > 2500/mm^3)

6. Use of anti-CMV drugs (cidofovir, letermovir, foscarnet, valganciclovir, ganciclovir) within seven (7) days of patient randomization.
7. Use of IVIG within four (4) weeks of patient randomization [103].
8. Currently intubated for airway protection only.
9. Currently enrolled in an interventional trial of an investigational therapeutic agent known or suspected to have anti-CMV activity or to be associated with significant known hematologic toxicity (prior approval required).
10. At baseline patients who have a tracheostomy, and have been receiving any positive pressure ventilation through it during the 30-day period prior to ICU admission.
11. Patients with Child Class C Cirrhosis.
12. Patients with severe (requiring home oxygen) pre-existing interstitial lung disease.
13. Allergy to ganciclovir
14. Incarcerated
15. Other, specify (e.g. clinician refusal)

7.5 Subject withdrawal

Under certain circumstances, an individual patient must be terminated from participation in this study. Specific events that will result in early termination include:

- Randomized after positive LFA results but prior to negative CLIA-approved ELISA test result,
- Need for respiratory support ends unexpectedly between time of randomization and first study product administration,
- Unexpected death between time of randomization and first study product administration,
- Subject has been inappropriately enrolled based on inclusion/exclusion criteria (e.g. when information through next of kin was inaccurate),
- Site investigator decides to terminate participation for reasons of patient's safety or to prevent compromising the scientific integrity of the study,
- It is determined that side effects are severe,
- New scientific developments indicate that the treatment is not in the patient's best interest,
- Patient or next of kin refuses further participation,
- Study is terminated.

Patients may be withdrawn at any time once the study team becomes aware that the patient meets one or more criteria for withdrawal. If the participant is withdrawn from the study before a dose of study product is given, no study procedures will be performed, no study data will be collected, and they will not be included in the primary analysis population. If a participant is withdrawn after receipt of study product, safety procedures (e.g. monitoring of creatinine and CBC with differential) will continue for another 48 hours, no further study biosamples will be obtained, study data in the medical record will continue to be collected per protocol and as allowed in the signed consent (e.g. respiratory-support-free days), and they will be included in the primary analysis population.

In the event that a participant does not start receiving first dose of study product within 120 hours after hospitalization, and this is discovered before 5 doses of study drug are administered, they must be withdrawn. In this circumstance, no further study procedures will be performed, no further study data will be collected, and they will not be included in the primary analysis population. If initiation of study drug past the 120-hour window is discovered after 5 or more doses of study drug have been given, the participant may remain in the study.

7.6 Subject replacement

A participant may be replaced in the study under certain circumstances of early withdrawal. The purpose of participant replacement is to compensate for potential data loss. The following circumstances may qualify a participant for replacement:

- Randomized after positive LFA results with subsequent negative CLIA-approved ELISA test result and before administration of study drug,
- Participant did not start receiving first dose of study product within 120 hours after randomization, and was withdrawn,
- Need for respiratory support ends unexpectedly between time of randomization and first study product administration,
- Unexpected death between time of randomization and first study product administration,
- Early termination between time of randomization and first study product administration (e.g., due to patient or next of kin refusing further participation, new scientific developments indicate that the treatment is not in the patient's best interest, or subject has been inappropriately enrolled based on inclusion/exclusion criteria),
- Participant who declines further participation and requests complete deletion of all data after regaining consciousness.

8 STUDY DRUG ACQUISITION, PREPARATION, & ADMINISTRATION

8.1 Study drug & placebo formulation

Intravenous ganciclovir and matching placebo.

8.2 Acquisition of study drugs & placebos

Study drug will be purchased by UW Pharmacy and supplied to study sites. In the event that UW Pharmacy is unable to source sufficient supply or if the drug cannot be directly supplied (e.g. to international sites), study drug from a commercial supplier may be used or purchased directly by study sites (costs to be passed through to the CCC). This provides necessary flexibility to prevent disruption of per-protocol administration of study drug due to global supply chain challenges or drug shortages. The drug supply must be FDA approved commercial ganciclovir sourced from a licensed distributor/manufacturer. The site will be responsible to appropriately document all doses prepared from local drug supply.

8.3 Storage of study drugs & placebos

Study drug will be stored as per manufacturer's recommendations.

8.4 Administration of study drugs & placebos

Ganciclovir (or IV placebo) will be administered via central or peripheral venous access.

Ganciclovir doses must be adjusted according to renal function as per package insert. A subject who is on hemodialysis should continue IV dosing according to the package insert.

8.5 Pharmacy Records

The site pharmacist is required to maintain complete records of all study drugs received from the sponsor or purchased by the site and subsequently dispensed. When using site supplied drug, sites are to maintain a record of manufacturer/lot number used for dose preparations.

9 CLINICAL PROCEDURES

9.1 Patient identification & recruitment

Patients with sepsis-associated acute respiratory failure will be identified via prospective screening of all ICU patients. This process is done by trained and experienced research coordinators who review charts using a standardized screening tool. Additionally, patients may be identified by the attending physician based on eligibility criteria.

9.2 Informed Consent

Informed consent is the essential processes of ensuring that study subjects or legal guardians fully understand what will and may happen to them while participating in a research study. Before any protocol-specific questions are asked or procedures to determine protocol eligibility performed, a screening consent form or protocol-specific consent form (described below) must be obtained. Patients or family members must be provided with a copy of all consent forms that they sign.

Since all potential patients will be intubated and sedated or requiring high levels of respiratory support, initial consent for patients who cannot make decisions for themselves will be from the patients' LAR. Subsequent consent from the patient will be obtained whenever possible. Interested surrogates will be given information about the study, explaining potential risks. They will then undergo informed consent. Consent forms will be approved by the Human Subjects Committee.

Participation in this study is voluntary. The nature of the study will be fully explained to each patient during the informed consent process. If the patient is deemed unable to provide written informed consent, informed consent for the patient's participation must be obtained from a LAR using practices and procedures that are acceptable as defined by local law and the Institutional Review Board. In this situation (the use of surrogate consent), subsequent in-person consent will be obtained from the patient when possible before hospital discharge. In circumstances where the patient has regained the capacity to consent but is discharged before study personnel have an opportunity to schedule an in-person meeting, then a remote consent method of phone/video conference will be used instead. The patient (or authorized representative, when applicable) will have the opportunity to ask questions. The patient (or authorized representative, when applicable) and the individual who performs the consent discussion will sign an informed consent document. The investigator will retain the informed consent document according to Good Clinical Practice. HIPAA authorization will also take place during the informed consent process.

The determination of appropriate "next-of-kin" will be made in accordance with the standard practices used in provision of medical care. Detailed documentation of all attempts to obtain consent from the patient and/or the patient's next-or-kin will be kept.

9.2.1 Consenting process

Informed consent is not limited to the signing of the consent form; it also includes all written or verbal study information site staff discuss with the patient, before and during the trial. Once an eligible participant is identified, the study team will coordinate a consent conference with the patient or LAR. This can be done in person or via phone/video conference. The patient or LAR will have a copy of the consent form to review during the conference. The patient or LAR will be given ample time to review the consent and ask questions.

When a previously incapacitated patient regains capacity to consent, the study team will schedule a follow up consent conference with the participant and document their willingness to continue participating in the study.

The consenting process for each site will be documented in the individual "Participating Site Application". Participating sites (initial sites listed in Appendix H) may use resources available to their site to conduct eConsent if it meets FDA 21 CFR Part 11 compliance. A template Prescreening consent (for participants where leftover blood sample is not available) and Main consent template will be given to the participating sites to modify.

9.2.2 Consent form

The informed consent form documents that a prospective patient or their agent (1) understands the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in a study.

This study will conduct multi-site research under the regulatory approval of a Single Institutional Review Board (sIRB) in accordance with the NIH Policy on the Use of a Single Institutional Review Board for Multi-Site Research. The Fred Hutchinson Cancer Center IRB (FWA00001920) will serve as the sIRB of record that is responsible for overseeing the conduct of this study. All collaborating sites (initial sites listed in Appendix H) have agreed to rely on the sIRB and any sites added after study initiation will also be required to rely on the sIRB. The Principal Investigator (PI) will disseminate the proposed informed consent to all participating sites and will submit these materials to the sIRB for review and approval. Any subsequent changes will be distributed to sites and submitted to the sIRB as a modification to be approved prior to implementation. The consent form(s) must be developed in accordance with local IRB/IEC requirements and the principles of informed consent as described in Title 45, Code of Federal Regulations (CFR) Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6: Good Clinical Practice: Consolidated Guidance 4.8. It must be approved by all responsible ethical review bodies before any subjects can be deemed to have consented for the study.

9.3 Screening procedures

Screening procedures are done to determine eligibility and to provide a baseline for comparison of data. Baseline data are obtained during screening. All inclusion and exclusion criteria must be assessed within 120 hours before randomization. Importantly, the patient can only be randomized once these test results are available.

Before randomization, the following procedures are performed:

- Clinical laboratory tests as defined in the inclusion and exclusion criteria, including:
    - Serum or urine pregnancy test—the results of this must be negative before proceeding, since ganciclovir is suspected to be teratogenic.
    - CMV serology by lateral flow assay (LFA) or gold standard test. See **Figure 9-1 and Section 9.3.1** for the specific algorithm by which the LFA and confirmatory gold standard test will be used for CMV serology.
        - Leftover serum (preferred) or plasma may be used if available.
        - If leftover material is not available, a small amount of extra blood (0.5-1.5 ml) should be obtained after prescreening consent is administered to permit such testing. Prescreening consent may be obtained verbally or as written consent, as permitted per IRB guidelines.
        - After lateral flow testing is obtained, a portion of the same sample should be sent to a CLIA-approved lab for testing.
    - Absolute neutrophil count/total white blood cell counts

- Collection of medical history
- Collection of samples for CMV PCR in plasma and endotracheal aspirates as well as for storage for subsequent CMV immunity testing, genomic analysis, and ancillary studies
- Assessment of select concomitant medications: steroids, immunosuppressive medications and antivirals
- Net fluid balance at ICU admission
- Obtaining of patient demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001. Available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html

9.3.1 CMV Serology Screening Procedures

CMV serology test results (for rapid CMV serology [LFA] and CLIA test) are reported as a qualitative "Positive" or "Negative", and very rarely "Indeterminate."

Randomization can occur in the follow instances:

- If the Rapid CMV Serology (LFA) comes back positive, randomization can occur prior to the CLIA test.
- If the Rapid CMV Serology (LFA) comes back indeterminate or negative, and then the CLIA test comes back as positive, randomization can occur.
- If sites do not perform a Rapid CMV Serology (LFA) test, and the CLIA test comes back as positive, randomization can occur.

If Rapid CMV Serology (LFA) testing is done, a site progresses to the CLIA test as follows:

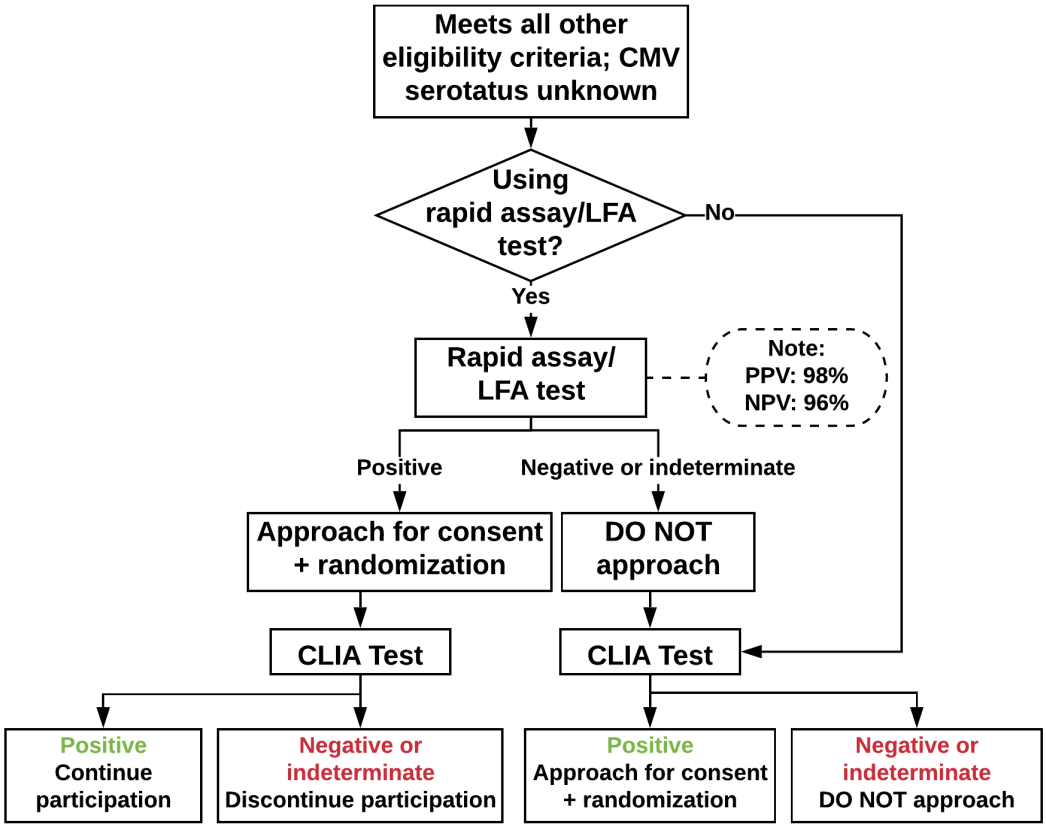
- Regardless of whether the Rapid CMV Serology (LFA) comes back positive, indeterminate, or negative, progress to the CLIA test (using part of the same sample used for the rapid test, as described in section 9.3 above).
- CLIA test result must be recorded as either positive or negative; an indeterminate CLIA test result should be recorded as negative.

Rapid CMV Serology (LFA) is NOT required to progress to CLIA testing:

- Sites can perform only CLIA testing and randomize with a single positive test result.

Discordant results (between the LFA and CLIA test) that lead to administration of study drug will be tracked and reviewed regularly by the Safety and Protocol Adherence Review Team (Section 14.3).

Figure 9-1. Flow diagram of serologic assessment strategy for inclusion using LFA with confirmation via standard serologic assay. PPV and NPV for LFA are calculated based on 60% seroprevalence, using QNow UV Flashlight reader as is planned for this study.



9.4 Patient Registration

Participating sites can verify a patient's consent and eligibility and register the patient into the study. DCC will provide patient accrual reports. Randomization will be through MediData's Balance system, 24/7, with a backup system in place.

9.5 Randomization procedure

Randomization will occur after assessment of positive CMV serostatus as detailed in **Section 9.3.1**, and negative pregnancy test. Randomization and first dose of study drug should occur as close to each other as possible. Randomization and first dose of drug **must** occur within 120 hours of hospital admission **and** on the same calendar day. Patients who are randomized but in whom the need for respiratory support ends, who die, are outside of the 120 hour window, or have consent withdrawn before receiving first dose of study drug should not receive their first dose of drug and are withdrawn from the study (see Section 7.5).

The randomization sequence will be uploaded by SCHARP into the Medidata's Randomization and Trial Supply Management (RTSM) system and provided to each site through Medidata. This

system automatically notifies the site pharmacist of the treatment assignment. The randomization will be block-randomized by site. At each institution, the pharmacist with primary responsibility for drug dispensing is charged with maintaining the security of the randomization list. Patients will be stratified at the time of randomization according to treatment center.

When the patient is randomized, the following information is required by NIH reporting guidelines: date of birth, race/ethnicity, and gender. For the purpose of this study, each patient will be assigned a study number, which will be used for all communications with outside institutions to assure confidentiality.

A CLIA-certified standard serologic assay will be used to confirm CMV positivity, but patients with a positive rapid test result may be randomized prior to the return of the CLIA test results (see **Section 9.3.1**).

Randomization will continue until the target accrual number for participants in the primary analysis are met. The primary analysis population will be all patients randomized who receive at least one dose of the assigned study product.

9.6 First dose of study drug

Study day 1 is defined as the first calendar day on which the patient is randomized and the first dose of study drug is administered. All subsequent study days will start accordingly. At baseline, but before administration of study drug, the following procedures will need to be performed:

- Blood: Creatinine, CMV cell-mediated immunity, platelets, CMV plasma PCR, CBC w/differential, research samples.
- Endotracheal aspirate (ETA). If patient is intubated, collect an ETA specimen at baseline (± 1 day) and twice weekly, at the time that this procedure is routinely performed by respiratory therapy. Specimens will be labeled and stored frozen for subsequent CMV PCR analysis at the coordinating lab at Fred Hutch.
- Clinical Assessments: Apache III, SOFA, COMS survey (see Section 9.10), static respiratory compliance, ventilator parameters, assessment of select concomitant medications (steroids, immunosuppressive medications, neuromuscular blockers, and antivirals), use of ECMO and prone positioning, and bilateral vs. unilateral infiltrates on chest radiograph. See Section 9.11 for details on these assessments.

9.7 Intervention (Study drug administration)

Patients will be randomized in a 1:1 ratio to receive either ganciclovir or placebo. Study drug delivery should begin as soon after randomization as possible; it must occur on the same day as randomization and within 120 hours of hospitalization. The first day of study drug is considered Day 1 of this study.

- Study drug will be administered for a maximum of 28 days. For the initial 5 days of study treatment, the dose will be ganciclovir 5mg/kg or Placebo IV q 12hr.
- If the patient is discharged from the hospital prior to day 28 or when intravenous access is removed, the patient will stop receiving study drug.
- After 5 days, the dose will be reduced to ganciclovir 5mg/kg or placebo IV once daily.
- Ganciclovir doses must be adjusted according to renal function as per package insert.

- Biopsy-proven CMV disease (eg colitis/esophagitis) can occur very infrequently in very sick but immunocompetent patients. In such circumstances, one of the PIs should be contacted and it should be specified that study medication (which could be GCV or placebo) is to be held, and open-label GCV administered. This would likely lead to unblinding since it would be presumed that breakthrough CMV disease is very unlikely to occur while receiving IV GCV at prophylactic doses (those used in this study after day 5). See section 9.15 for more information on unblinding procedures.

9.8 Co-interventions

All patients will receive standard intensive care unit care, which includes respiratory support and ventilator management (standardized lung protective ventilation [Appendix D] and fluid management [Appendix E] protocols will be used at all sites for mechanically ventilated patients, and Appendix I provides recommended approaches for high-flow nasal cannula oxygen and non-invasive ventilation), antimicrobial therapy, blood glucose control, and ICU sedation. Many of these co-interventions occur under local protocols used as a part of routine clinical care at the clinical sites (initial sites listed in Appendix H).

9.9 Specimen collection

Patients will undergo serial blood draws at study entry (± 1 day of randomization) and plasma CMV PCR samples every seventh day while on study. Not more than 200 mL of blood will be collected over the initial 28 days of the study. A substudy of patients will have plasma collected every four days while hospitalized.

Collect an ETA specimen at baseline (± 1 day), and also every fourth day (± 2 days) at intensified monitoring sites, while the patient is intubated, at the time this procedure is routinely performed by respiratory therapy. BAL fluid (from a standard or "mini" bronchoscopy) may be collected in the place of ETA. Specimen will be collected, labeled, and stored frozen for subsequent CMV PCR analysis at the coordinating center lab (Boeckh Lab at Fred Hutch).

After hospital discharge, patients will not be followed daily, but they will be contacted at Day 28 (±4 days) if not hospitalized, and Day 180 for a telephone follow-up to ascertain reportable adverse events, vital status, pregnancy status (at Day 28), and pregnancy within 30 days of study drug (at Day 180).

9.10 Patient-Centered Outcomes Survey (COMS)

Patients will complete a survey at baseline and at 6 months (NHLBI COMS), by previously agreed-upon communication preference. The purpose of this survey is to compare functional assessment and well-being (patient-centered outcomes), at 6 months between ganciclovir and placebo recipients.

At baseline, patients or LARs will complete the Katz Index of Independence in Activities of Daily Living (ADL) and Lawton – Brody Instrumental Activities of Daily Living Scale (IADL), which will take approximately 4 minutes to complete.

At 6 months, patients will be asked to again complete the ADL, IADL, and to complete the Hospital Anxiety and Depression Scale (HADS), EQ-5D-5L, and Impact of Events Scale – Revised (IESR). The survey will take approximately 15 minutes to complete.

9.11 Post-Enrollment Procedures

See the schedule of procedures for specific time points (including permissible windows) in Appendix A. Duration of participation in the study by individual patients will be 180 days.

- Blood:
  - Creatinine, Platelets, CBC w/differential - Days 4, 7, 11, 14, 18, 21, 25, 28 (all ± 1 day); can be obtained from clinical testing results if available
  - CMV PCR (all ± 1 day):
    - At intensified monitoring sites: Days 4, 7, 11, 14, 18, 21, 25, 28
    - At all sites: 7, 14, 21, 28 at all sites
- ETA: At intensified monitoring sites, CMV PCR, twice weekly (± 2 days) while intubated.
- Clinical Assessments:
  - SOFA score daily on Days 1-7, then twice weekly (Day 11, 14, 18, 21, 25, 28) through day 28 if still in the ICU
  - Vital status (collected as ICU and hospital mortality at discharge, and 180-day mortality)
  - Assessment of concomitant antiviral medications daily on Days 1-7, then twice weekly through day 28
  - Ventilator parameters and static respiratory compliance (daily if mechanically ventilated), NIV parameters, and HFNC parameters daily.
  - Net fluid balance at ICU admission and net cumulative fluid balance until ICU discharge
  - Use at any point during study (and if used, duration/number/dose) of ECMO, prone positioning, steroids, neuromuscular blockers; collected at hospital discharge
  - Occurrence of bacteremia and fungemia; collected at discharge
  - Use of antibacterials or antifungals in the event of ventilator-associated pneumonia or nosocomial pneumonia (defined as sputum, BAL or ETA culture with new pathogen associated with new antibiotic +/-48 hours from culture). Any antibacterial used to treat bacteremia or antifungal used to treat fungemia should be reported.
  - Apache III at baseline only
  - COMS survey (see Section 9.10) at Day 180 (± 6 weeks)
  - Bilateral versus unilateral infiltrates on chest radiograph: To be assessed as close as possible to, but not prior to, randomization. Chest radiograph at admission is acceptable if it is the only one available. If the word "bilateral" is **not** on the report of the chest radiograph, then the coordinator will ask the site PI to assign unilateral, bilateral, or "no infiltrates" to the patient's chest radiograph. If the word bilateral is in the report, then the coordinator will assign bilateral.
- For women of childbearing potential, a serum pregnancy test will be performed at day 7 or at the time of hospital discharge if discharged before study day 7.
- In patients discharged from the hospital before day 28, a follow-up call will be completed on day 28 (±4 days) to assess reportable adverse events, vital status, and pregnancy. Vital

status and pregnancy within 30 days of last dose of study drug will also be assessed during the day 180 follow-up phone call.

- Because ganciclovir carries a black box warning for tumors in lab animals, at the Day 180 follow-up call subjects will be asked if there is any known new development of a malignant tumor. If a new tumor is reported, records will be requested from the primary care physician or hospital.

Follow up for this study population has been historically difficult. Despite effort by sites to obtain all study specimens, it is expected that there may be missed blood draws after discharge from the hospital. Because these missed labs are expected, they will not be considered to be unanticipated problems or protocol violations. In the event a patient cannot be reached for the 180 Day follow up, survival data may be determined through death registry records.

9.12 Monitoring of renal function

Renal function will be monitored at least weekly throughout the active study drug dosing period. Monitoring will be performed by the research team at each site for all participants, with any reportable AE reporting per the protocol. These research personnel will be blinded. Study drug dose will be adjusted based on the calculated creatinine clearance according to the package insert.

9.13 Monitoring for and managing neutropenia

Suggested Management of Neutropenia. Short-term neutropenia is a potential reportable adverse event of ganciclovir, although the incidence is projected to be low in the ICU setting and was not reported in the phase 2 ICU study.

1. Neutropenia will be monitored at least weekly in all participants while they are in the hospital. Monitoring will be performed by the research team at each site, who will be blinded.
2. If ANC drops below 1000/mm^3, study drug will be temporarily held.
3. Concomitant drugs should be reviewed and adjusted as feasible.
4. ANC monitoring should continue (i.e. approximately twice a week without G-CSF; once a week with GCSF) until the ANC is > 1000/mm^3.
5. A dose of G-CSF may be administered (5 microgram/kg) at the discretion of the treating physician.
6. If the ANC increases > 1000/mm^3 study drug may be resumed.
7. If the neutropenia recurs at levels of < 1000/mm^3, study drug should be discontinued permanently, but the patient should continue to undergo all other study procedures & be followed for safety & other endpoints.
8. If the duration of neutropenia (ANC <500/mm^3) is ≥ 5 days (with or without GCSF), the event should be reported as a reportable SAE (see SAE reporting section).

9.14 Pregnancy

Participants who are women of childbearing potential will be advised to use effective contraception during treatment and for at least 30 days following treatment with study drug. Similarly, participants who are men will be advised to practice barrier contraception during and for at least 90 days following treatment with study drug.

If a patient becomes pregnant during the course of the study, no further administration of study drug should be given but other procedures should be completed unless medically contraindicated. The investigator will submit a pregnancy report form to the coordinating center. The Reporting Plan and timeline is described in the table in Section 11.3. If the subject terminates from the study prior to the pregnancy outcome, the site must keep in touch with the patient in order to ascertain the pregnancy outcome. Pregnancy status for all participants who are women of childbearing potential will also be assessed at the Day 28 (in participants who are no longer hospitalized) follow up phone call, and pregnancy within 30 days after the last dose of study drug will be assessed during the Day 180 follow up phone call.

9.15 Unblinding

9.15.1 Unblinding criteria

Unblinding may be precipitated either by conclusion of the study or an emergency situation, in discussion between the site PI and protocol chair(s) (Drs. Stapleton, Boeckh, Limaye or Rubenfeld). All patients or family members can be informed of their treatment assignment at the conclusion of the study, after all key analyses are complete, and upon written request.

In the event of an emergency situation, patients may be unblinded prematurely. Emergency unblinding decisions will be made by the site PI only after discussion with one of the protocol chairs (Drs. Stapleton, Boeckh, Limaye or Rubenfeld). Additionally, if a reportable serious adverse event (SAE) occurs which qualifies for expedited reporting to one or more regulatory agencies, the patient's treatment assignment will be unblinded, if specifically requested by the regulatory agencies, the institutional review board (IRB), or the DSMB. All cases of unblinding should be discussed with one of the protocol chairs (Drs. Stapleton, Boeckh, Limaye or Rubenfeld).

9.15.2 Unblinding procedures

After one of the protocol chairs (Drs. Stapleton, Boeckh, Limaye or Rubenfeld) agrees with the site PI to unblind the patient's treatment assignment, the protocol chair will request the coordinating site's statistical center (SCHARP) to send a password-protected email to the site PI containing the treatment assignment for the particular patient. The code should not be broken except in an emergency where knowledge of the patient's treatment assignment is absolutely necessary for the further management of the patient, or in the context of review of an expedited reportable adverse event as described in the adverse event section of the protocol. If the treatment assignment is unblinded under any other circumstances, it will be considered a protocol violation. This information should also be recorded in the patient's CRF.

10 LABORATORY PROCEDURES

Routine clinical laboratory tests will be performed through the hospital-based clinical laboratory. In this critically ill population, laboratory tests shall be those deemed necessary based upon clinical indications of the patient; others will be ordered as per protocol.

10.1 Laboratory procedures

Laboratory procedures include but are not limited to:

- Baseline whole blood sample for immunity and biomarker studies.
- ETA at baseline (± 1 day of randomization) and then twice weekly (± 2 days) while the patient is intubated for CMV viral load, inflammatory biomarkers, characterization of cellular content.
- Aliquot of BALF and/or lung biopsy done for clinical purposes. For patients who undergo lung biopsy or autopsy, a sample of lung tissue (frozen or fresh) is requested.
- Blood samples at baseline, then:
    - Weekly (twice weekly in intensified monitoring subset) until day 28 or hospital discharge, whichever happens first.
    - For CMV viral load, cytokine analysis, and safety labs (CBC with neutrophil count and platelets, and creatinine).
    - Samples for studies to assess CMV-specific immunity and transcriptomics will be collected at selected sites that have the capability for sample processing
- Bacteremia/fungemia and nosocomial pneumonia (including ventilator-associated pneumonia) will be assessed by clinical testing.

10.2 Future use of stored specimens

The investigators intend to store specimens from patients. These samples will be used for future testing and research related to furthering the understanding of CMV and other viral infections and immunologic control, which may require additional IRB approval as specified in the informed consent form. Other testing on specimens will only occur after review and approval by the IRB of the researcher requesting the specimens and at the central IRB at the coordinating site.

10.3 Biohazard containment

As the transmission of CMV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC and the NIH or other locally appropriate agencies.

All dangerous goods materials, including Biological Substances, Category A or Category B, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

11 ADVERSE EVENT REPORTING

11.1 Reportable Adverse Events

In this trial, we will not collect data on or report every adverse event (AE) by the commonly used standard definition because critically ill patients have innumerable abnormal signs and symptoms associated with their disease that are expected. Similar to other critical care clinical trials, we will utilize a modified definition of reportable AEs.

For this trial, a *reportable adverse event* is defined as:

1. Any clinically important untoward medical occurrence in a patient receiving study drug or undergoing study procedures which is different from what is expected in the clinical course of a patient with acute respiratory failure/ARDS,

OR,

2. Any clinically important, untoward medical occurrence that is thought to be associated with the study drug or procedures, regardless of the "expectedness" of the event for the course of a patient with acute respiratory failure.

Expected events for patients with acute respiratory failure are clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the day to day care of patients with acute respiratory failure treated in an intensive care unit with respiratory support. Examples of adverse events that are expected in the course of acute respiratory failure include transient hypoxemia, agitation, delirium, nosocomial infections, skin breakdown, and gastrointestinal bleeding. Such events, which are often the focus of prevention efforts as part of usual ICU care, will not be considered reportable adverse events unless the event is considered by the investigator to be associated with the study drug or procedures, or unexpectedly severe or frequent for an individual patient with acute respiratory failure. Examples of unexpectedly frequent adverse events would be repeated episodes of unexplained hypoxemia. This would be in contrast to an isolated episode of transient hypoxemia (e.g. SpO_2 ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency. Expected events for patients with acute respiratory failure will not be or reported in this trial.

The research team at each site will determine if any clinical adverse experiences (while hospitalized) occur during the period from randomization through the last dose of study drug. The site research team will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the course of patients with acute respiratory failure. If reportable adverse events occur, they will be recorded on the reportable adverse event case report form. All reportable AEs, per the modified definition above, will be graded according to CTC guidelines. The severity of each event should be classified into one of five defined categories as follows:

- Grade 1 Mild
- Grade 2 Moderate
- Grade 3 Severe
- Grade 4 Life Threatening or Disabling
- Grade 5 Death

The Safety and Protocol Adherence Review Team (SPART, see section 14.3) will also review laboratory safety and reportable adverse event data reports monthly. The DSMB will review all reportable AEs during their regularly scheduled meetings.

Note: Study drug specific laboratory events (e.g. hematologic values, renal function) will be collected as secondary safety endpoints.

Note: See additional information outlined in Section 5.4 on definitions of AEs for the purpose of post hoc analyses.

11.2 Reportable Serious Adverse Events

In this trial, we will also utilize a modified definition of reportable serious adverse events (SAEs). Investigators will report all events that are **serious AND unexpected AND study-related**, as defined in the reporting guidelines found in the next section, to the Fred Hutch by fax or email within 7 business days of becoming aware of event. Sites must notify their local Institutional Review Board (IRB) in a timely manner, according to local IRB guidelines.

The following will **also** be reported within 7 business days, even if not meeting expedited reportable SAE reporting criteria:

- ANC $< 500/mm^3$ for a period $\geq$ 5 days
- Death in the presence of neutropenia (ANC$< 500/mm^3$ for any duration)

Of note, a large fraction of ICU patients with acute respiratory failure experience other organ failures and/or die, and these events are expected as a result of the usual course of their critical illness. These organ failures and deaths related to acute respiratory failure or the patient's underlying critical condition should not be reported as reportable SAEs *unless they are considered to be study related AND unexpected*.

While not all deaths are reported as SAEs in this trial, **all deaths occurring during this study will be documented in the Study Termination CRF that is completed when any participant leaves the study for any reason**, including death**.** As such, deaths are reported to the central database in a timely fashion and are reported to the DSMB during the regular biannual meetings.

Fred Hutch will report all serious, unexpected, and study-related reportable SAEs to the DSMB and NHLBI by fax or email within 7 business days of being notified of the event. Reportable SAE forms received by Fred Hutch will be sent to participating sites for submission to their respective IRBs, according to their local IRB guidelines. The DSMB will also review all reportable SAEs and all deaths during scheduled interim analyses and at each regularly scheduled 6-month meeting. Fred Hutch will distribute the written summary of the DSMB's periodic review of reportable AEs and SAEs to investigators for submission to their respective Institutional Review Boards in accordance with NIH guidelines.

When a reportable SAE arises, one of the protocol chairs not involved in subject enrollment at the relevant study site will also determine if the reportable SAE is unexpected for ganciclovir. Unexpected for ganciclovir is defined as any event not listed in the package insert.

Investigators must also report Unanticipated Problems, regardless of severity, associated with the study drug or study procedures to Fred Hutch, the DSMB, and NHLBI within 7 business days after becoming aware of the event, and to site IRBs according to local guidelines. An unanticipated problem is defined as follows:

Unanticipated Problem (UP): any incident, experience, or outcome that meets all of the following criteria:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;

- Related or possibly related to participation in the research, in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;

- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Note: See additional information outlined in Section 5.4 on definitions of SAEs for the purpose of post hoc analyses.

11.3 Reporting Adverse Events

1. Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. The site Principal Investigator will evaluate all local reportable adverse events and reportable severe adverse events. The Study Coordinator must view patient records for possible reportable AEs and SAEs throughout the study period. All reportable adverse events occurring within the study period must be reported in the participants' case report forms.

2. Investigators will report all *serious, unexpected, AND study-related* adverse events to the Fred Hutch within 7 business days by fax or email. Sites must notify their local Institutional Review Board in a timely manner, according to local IRB guidelines.

3. Definitions of Adverse Events

    a. A modified definition of a reportable AE (see Section 11.1 above) is being used for this trial.

    b. A *serious* adverse event is any event that is fatal or immediately life threatening, is permanently disabling, or severely incapacitating, or requires or prolongs inpatient hospitalization. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Note that the modified definition of reportable SAE (see Section 11.2 above) is being used for this trial.

        i. Life-threatening means that the patient was, in the view of the investigator, at immediate risk of death from the reaction as it occurred. This definition does not include a reaction that, had it occurred in a more serious form, might have caused death. Assessment of the cause of the event has no bearing on the assessment of the event's severity.

c. An *unexpected* event is any experience not identified by the type, severity, or frequency in the current study protocol or an event that is unexpected in the course of treatment for acute respiratory failure or ARDS.

d. Reportable adverse events will be considered to be study-related if the event follows a reasonable temporal sequence from a study drug or procedure and could readily have been produced by the study drug or procedure.

e. Organ failures or death related to acute respiratory failure or ARDS or the patient's underlying condition that are systematically captured by the protocol should not be reported as reportable SAEs *unless they are considered to be study related and unexpected,* but deaths will be reported to the central database in a timely fashion via the Study Termination CRF.

All reportable SAEs must be reported to the Fred Hutchinson Cancer Center in a timely fashion to allow expedited reporting to the DSMB and other entities (see **Figure 11-1: safety reporting chart**). The **following** table summarizes the reporting timelines:

Type of Event	**Definition of Reportable Event**	**Reporting Plan**	**Reporting Timeline (after becoming aware of event)**
Reportable Serious Adverse Events (SAE)	Any untoward medical event that is: Serious **AND** Unexpected **AND** Related to study drug or procedure	Site to local IRB	According to local IRB guidelines
		Site to coordinating center	Initial report within 7 business days
		Coordinating center to NHLBI executive secretary and DSMB chair DSMB Chair to determine if full meeting is necessary	Within 7 business days of receipt of initial report from site Within 72 hours after Chair receives report from coordinating center
		Coordinating center to NHLBI & participating sites	Within 7 business days of receiving initial report
		Coordinating center to report to FH IRB	Within 7 business days of receipt of initial report from site
Neutropenia	ANC < 500/mm for $\geq$ 5 days	SAME AS ABOVE	SAME AS ABOVE
Death	Death in the presence of neutropenia (ANC< 500/mm^3 for any duration) OR Death that meets the requirements for a reportable SAE as defined above	SAME AS ABOVE	SAME AS ABOVE

	Death not meeting reporting definition above	Site to local IRB	According to local IRB guidelines
		Site to coordinating center	As part of Study Termination CRF
		Coordinating center to NHLBI executive secretary and DSMB	Included in report prepared for each DSMB meeting
		Coordinating center to NHLBI & participating sites	Annually
		Coordinating center to report to FH IRB	Annually

Unanticipated problem	Any untoward event that is: • Unexpected, in terms of nature, severity, or frequency **AND** • Related or possibly related to participation in the research **AND** Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.	Site to local IRB	According to local IRB
		Site to coordinating center	Within 7 business days via memo
		Coordinating center to NHLBI executive secretary and DSMB Chair	Within 7 business days of receipt of information from site
		Coordinating center to NHLBI & participating sites	Within 7 business days of receipt of information from site
		Coordinating center to FH IRB	Within 7 business days of receipt of information from site
Pregnancy	ALL	Site to local IRB	According to local IRB guidelines
		Site to coordinating center	Within 7 business days (via pregnancy report form)
		Coordinating center to NHLBI executive secretary and DSMB	Within 7 business days of receiving pregnancy report form
		Coordinating center to NHLBI & participating sites	Within 7 business days of receiving pregnancy report form
		Coordinating center to FH IRB	Within 7 business days of receiving pregnancy report form
Reportable Adverse Event	Any untoward medical event that is considered by the investigator to be:	Site to local IRB	According to local IRB guidelines
		Site to coordinating center	Reportable AEs reported as required on CRFs. CRFs to be completed on a timely basis.

	• Unexpectedly severe or more frequent than typical course of ALI **OR** • Have any relationship to study drug or procedures	Coordinating center to NHLBI executive secretary and DSMB	Included in report prepared for each DSMB meeting
		Coordinating center to NHLBI & participating sites	Annually - summarized from CRFs in database
		Coordinating center to FH IRB	Annually – summarized from CRFs in database

All sites will be responsible for compliance with local safety reporting guidelines.

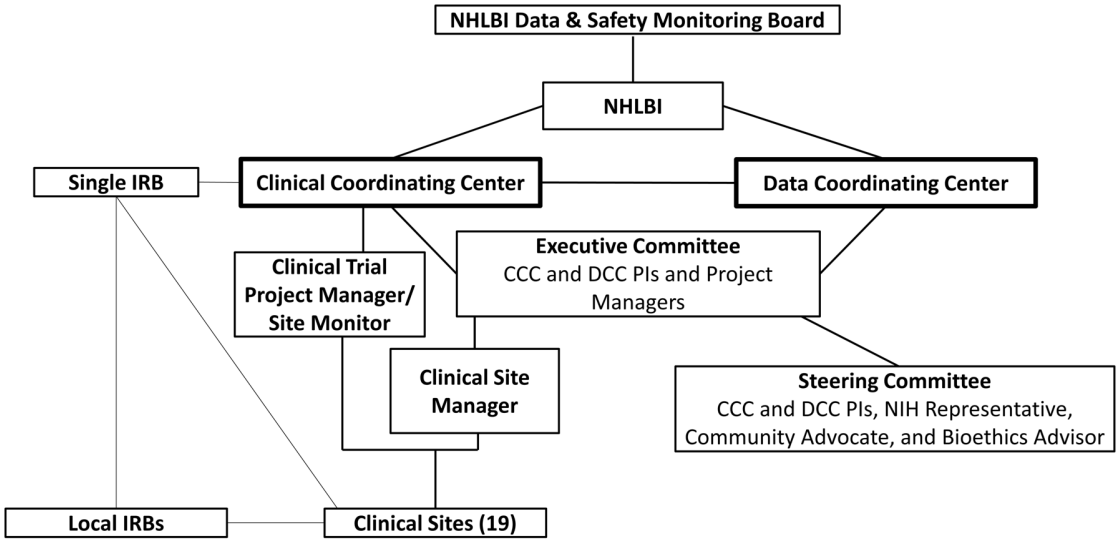


Figure 11-1: Safety reporting chart.

The reportable SAE Report will include the following information (as available):

- Patient ID
- Description of the reportable SAE (onset date, severity, causal relationship)
- Basic demographic information
- Outcomes attributed to the event
- Summary of relevant test results, laboratory data, and other relevant history
- The first and last dates of study drug administration
- Statement whether study drug was discontinued or schedule modified
- Statement whether the event abated after study drug was discontinued/modified

- Statement whether the event recurred after reintroduction of the study drug if it had been discontinued or held

Participating sites will be provided with reportable SAE report forms and contact numbers for transmitting the reports.

11.4 Relationship to study drug

All reportable AEs will have a causality assessment performed at the time of reporting the event to document the Investigator's perception of causality. There is currently no standard international nomenclature to define causality. For the purposes of this study, causality will be assigned using the following criteria:

Definitely related	The event cannot be attributed to the patient's underlying medical condition or other concomitant therapy and there is a compelling temporal relationship between the onset of the events and study drug administration that leads the Investigator to believe that there is a causal relationship.
Probably related	There is a clinically plausible time sequence between the onset of the AE and the study drug administration. The AE is unlikely to be caused by a concurrent/underlying illness, other drugs or procedures.
Possibly related	There is a clinically plausible time sequence between the onset of the AE and study drug administration, but the AE could also be attributed to a concurrent/underlying disease, other drugs, or procedures. "Possibly related" should be used when the study drug administration is one of several biologically plausible causes of the AE.
Not related	The patient's underlying medical condition or concomitant therapy can easily be identified as the cause of the event and there is no temporal relationship between the event and the study drug.

11.5 Pregnancy

A pregnancy is not an adverse event. If a patient becomes pregnant while enrolled in the study following administration of study drug, administration of study drug will be discontinued immediately and the patient will be followed through the outcome of the pregnancy. The investigator will submit a pregnancy report form to the coordinating center. The Reporting Plan and timeline is described in the table in Section 11.3.

11.6 Breaking the blind

The blind will not routinely be broken for reportable SAE's. Decisions on whether or not to break the blind will be made as described in Section 9.15,1, and unblinding will follow procedures outlined in Section 9.15.2.

11.7 Stopping rules

The study may be stopped prematurely if an excess rate of toxicity is observed. The DSMB will monitor throughout the study and there will be scheduled interim analyses for safety (see Statistical section).

12 DATA MANAGEMENT CONSIDERATIONS

12.1 Overview

The Data Coordinating Center will utilize the existing Validation Study Information Management System (VSIMS) to facilitate Trial collaborative activities via the SCHARP Data Management System (CDMS). VSIMS/CDMS provides online, end-to-end data management solutions, including investigator and study coordinator communications, regulatory compliance, remote subject registration, clinical data capture, biospecimen sample management, and document management. VSIMS/CDMS can provide online visibility of analytical datasets for all participating researchers, and statistical and informatics tools relevant to Trial research, and will be specifically configured to support this trial.

12.2 Data Collection

Each patient will be assigned an identification number to be used for all patient data. Links to patient name and identifiers will be maintained and stored in files on computers protected by password and in locked office cabinets. Research staff and physicians will remain blinded until the study is completed. De-identified data collected during this study may be used for future research.

Chart abstraction for demographic, laboratory, and physiologic data will occur at study entry, daily on study days 1-7, then twice weekly through day 28 while the patient is hospitalized, and again at hospital discharge or death. While patient remains hospitalized, review of the hospital record will occur daily throughout the hospitalization (to Day 28) to identify any reportable adverse events.

All information will be in Medidata RAVE.

12.3 Data Management

Participating sites can enter clinical data into VSIMS, in real time. The Data Coordinating Center will assist the Clinical Coordinating Center with monitoring data completion by reporting data entry status. Sites will receive queries to reconcile inconsistencies.

12.4 Quality Control and Quality Assurance

By signing this protocol, the Investigator/Sponsor agrees to be responsible for implementing and maintaining quality control and quality assurance systems with written Standard Operating Procedures (SOPs) to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules, and regulations relating to the conduct of the clinical study.

By signing this protocol, the investigators agree to conduct the study in an efficient and diligent manner and in conformance with this protocol; to follow generally accepted standards of Good Clinical Practice; and to follow all applicable federal, state, and local laws, rules, and regulations relating to the conduct of the clinical study.

The investigator also agrees to allow monitoring, audits, Institutional Review Board review and regulatory agency inspection of study-related documents and procedures and provide for direct access to all study-related source data and documents.

The investigator shall prepare and maintain complete and accurate study documentation in compliance with Good Clinical Practice standards and applicable federal, state, and local laws, rules, and regulations.

The investigator has the responsibility of explaining the correct use of the study drug to the site personnel, ensuring that instructions are followed properly, and maintaining accurate records of study drug dispensing and collection.

12.5 Study monitoring

Because of the risk profile of the study drug, which carries a black box warning on its package insert and has the potential of hematologic toxicity, we will perform study monitoring of intermediate intensity. Study data and regulatory aspects at study sites will be monitored by a study monitor, managed by the clinical coordinating center. The study monitor will perform monitoring of the first patient at each site as well as a random selection of 10% of participants (to be determined by DCC) across the whole study. The study monitor will conduct remote monitoring via EPIC if feasible, and by uploads into Florence eReg platform if not (with site PI review of charts for unreported AEs), and by Vestigo, according to industry standard (GCP) and as outlined in the monitoring plan. For non-domestic sites, alternative monitoring plans may be designed. The study monitor will create monitoring reports, submit them to site investigators and the Executive Committee, and conduct the necessary follow-up. A detailed monitoring plan, including the scope of monitoring and deadlines for entering the data, is shown in Appendix F.

13 ETHICAL CONSIDERATIONS & HUMAN SUBJECTS PROTECTIONS

13.1 Ethical Review

This study will be conducted in accordance with the ethical principles stated in the Declaration of Helsinki (1996) and applicable guidelines on Good Clinical Practice.

The investigator will obtain approval of the protocol and the informed consent from the single Institutional Review Board before the study may begin. IRB approval will also be obtained locally from each additional clinical site before the study commences at that site. The investigator will supply the following to the Institutional Review Board and Data Safety and Monitoring Board:

- Study protocol and appendices.
- Informed consent document and updates.
- Safety alerts.

This study will be registered with the U.S. NIH's clinical trials registry ClinicalTrials.gov.

13.2 Potential risks of study drugs and procedures

The following table presents common, less common, and uncommon risks based on experience with this drug in humans and animal data. This information will be communicated to patients in the sample informed consent form.

Table 13-1 Summary of potential risks of study medication and administration

Less common	Blood: leucopenia, neutropenia, anemia
Uncommon or rare	Central nervous system: fever, headache, insomnia, paresthesia, and peripheral neuropathy. Ocular: retinal detachment. Effects on the fetus and on pregnancy (which is why pregnant women will be excluded from participating).
Unknown frequency or theoretical risks	Cancer

13.3 Risks of Endotracheal Aspirates

Endotracheal aspiration is routinely performed on intubated patients by respiratory therapy as part of their clinical care routine to help clear respiratory secretions. There are no known risks to this procedure and would be considered inappropriate care if this procedure were not performed.

13.4 Risks of blood collection

Blood collection may cause some discomfort during the insertion of the needle. Bleeding, infection, or hematoma could occur. These risks will be low magnitude, low probability, short duration, and reversible. These risks will be mitigated by using a trained phlebotomist to perform the blood draw.

13.5 Risk of loss of confidentiality

Risk of breach of confidentiality will be minimized by keeping all research forms and electronic databases in locked and protected environments, and through training staff to be especially sensitive to confidentiality whenever interacting with participants.

13.6 Potential benefit of enrollment

Respiratory failure and sepsis carry a high mortality and consume millions of health care dollars each year. Any treatment that is found to impact outcomes in sepsis-associated respiratory failure could have a substantial societal benefit. Ganciclovir is not routinely administered to respiratory failure patients, so individual patients participating in this trial have an opportunity to receive this treatment through the study. If ganciclovir is ultimately found to positively affect outcomes, such as increased respiratory-support-free days, individuals in this study may benefit. It is possible, though, that an individual may not derive any direct benefit from participating in this trial, or even experience toxicities or adverse outcomes.

14 PROTOCOL OVERSIGHT AND GOVERNANCE

14.1 Principal investigator

The PI will adhere to requirements of the Code of Federal Regulations. Additionally, the primary Principal Investigator/Sponsor will sign the final clinical study report for this study, confirming that to the best of her/his knowledge the report accurately describes the conduct and results of the study.

14.2 Protocol Leadership Team

The Protocol Leadership Team will be responsible for administrative oversight of the study, provides the overall operational direction for the trial, and is responsible for the conduct of the trial according to the highest scientific and ethical standards, as well as approving revisions and amendments to the protocol. The Protocol Leadership Team will remain blinded to the treatment group assignment of individual patients during the course of the study.

14.3 Safety and protocol adherence review team

The safety and protocol adherence review team (SPART) will, on a monthly basis, review all clinical and laboratory safety data during the study. The SPART will include CCC PIs (Drs. Boeckh, Stapleton, Rubenfeld, and Limaye), Clinical Site Manager Sara Ardren, and Clinical Trial Project Manager/Site Monitoring Manager Dr. Louise Kimball. Meetings of the SPART (by videoconference or teleconference) will occur regularly and will not require attendance of all team members at each meeting. The SPART is responsible for the review of the clinical safety reports, discordant CMV serostatus test results (LFA versus CLIA test), and protocol violations, as well as communication with the IRB, NHLBI executive secretary, and DSMB, as outlined above.

14.4 Data Safety and Monitoring Plan (outlined briefly below and in more detail in Appendix F)

Investigators are responsible for monitoring the safety of patients who have entered this study. While hospitalized, patients will be assessed daily for evaluation of reportable adverse events by the research nurse/coordinator and principal investigator at study sites. Drs. Rubenfeld, Boeckh, Stapleton and Limaye will act as the investigator on-call for urgent issues and questions; Dr. Rubenfeld or Dr. Boeckh will serve in this role for any questions about eligible patients at Dr. Limaye or Dr. Stapleton's study sites.

The investigator remains responsible to follow, through an appropriate health care option, reportable adverse events (AEs) that are serious, cause the patient to discontinue before completing the study, or are ongoing at the time of study completion. The site investigators will maintain responsibility for forwarding of reportable SAEs to the coordinating center and local Institutional Review Board. The patient will be followed until the event resolves or stabilizes. Frequency of follow-up is left to the discretion of the investigator.

14.5 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be established. This DSMB will assess the effects of the study drug during the trial and is advisory to NHLBI. The members of the committee are independent of the University of Washington, Fred Hutchinson Cancer Center, and clinical investigators participating in this trial, and will not have any other involvement in the study, nor will they have any relation to study subjects.

Prior to beginning patient accrual, the DSMB will review the research protocol and identify any potential problems with randomization and implementation of the protocol. At this early phase, the DSMB will also review plans for data and safety monitoring to ensure that the frequency of monitoring is appropriate for the ganciclovir intervention.

During patient accrual, all reported serious adverse events will be reported according to Figure 11.1. The DSMB may recommend any steps to ensure the safety of study subjects and the integrity of the trial.

The DSMB will be involved with planned interim analyses. The interim monitoring guidelines that the DSMB will follow will be described in the Statistical Analysis Plan. The DSMB minutes will summarize the actions and deliberations of the DSMB and will be made available at the conclusion of the trial. At the time of interim analyses, the DSMB will aid in identifying problems surrounding patient accrual and randomization, data collection, and follow-up. At this time the DSMB will evaluate safety through a comparison of reportable adverse events across study arms.

The DSMB may recommend that specific groups be withdrawn from the study, if any subgroup manifests serious or widespread side effects, or that the trial be terminated altogether. To guarantee the unrestricted performance of its task, the DSMB may receive the individual study morbidity and mortality data from an unblinded statistician.

14.6 Study termination

This study may be terminated by the determination of the US NIH or US Office for Human Research Protections (OHRP). In addition, the conduct of this study at an individual site may be terminated by the determination of the local IRB.

The study may be terminated in the following situations:

- All patients have been accrued and have completed follow-up.
- If the interim analysis conducted by the DSMB at midpoint demonstrates a highly significant difference in treatment groups, as defined above.

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16 INVESTIGATORS STATEMENT/PROTOCOL SIGNATURE PAGE

I have read and understood the contents of this protocol and all study documents, and agree to carry out all of its terms in accordance with Good Clinical Practice.

I agree to permit trial related monitoring, audits, Institutional Review Board review and regulatory agency inspection of study-related documents and procedures, and to provide for direct access to all study-related source data and documents.

I agree that all the test article(s) supplied will be used solely for the purpose of conducting this study.

_____

Principal Investigator (printed name)

_____ _____

Principal Investigator (Signature) Date

This protocol version 5.0 has been approved by the Protocol Leadership Team. The following signatures document this approval.

_____ _____

Signature Date

Michael Boeckh, MD

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APPENDIX A: TIME AND EVENTS SCHEDULE

Visit	Screening	01	02	03	04	05	06	07	08	09	10	Assay Location
Day	-4 to 1	1	4	7	11	14	18	21	25	28	180	
Window (+/- days)	0	1	1	1	1	1	1	1	1	4	42	
Informed consent	X	-	-	-	-	-	-	-	-	-	-	
Administer Study Drug		Patient receives 5 days of ganciclovir (or placebo) intravenously TWICE daily, then up to 23 additional days of ganciclovir (or placebo) intravenously ONCE daily in hospitalized patients.									-	
Blood collection (ml) vol. estimated:												
Pregnancy testing	3[a]			3[b]								L
CMV Serology	3	-	-	-	-	-	-	-	-	-	-	L/A
Genomic analysis sample[c]	-	5	-	-	-	-	-	-	-	-	-	S
T cell immunity samples[c,e,m]	-	(30)	-	-	-	-	-	-	-	-	-	S
Serum Creatinine[d,n]	1	1	1	1	1	1	1	1	1	1	-	L
CMV PCR (plasma)[d,e]	-	5	(5)	5	(5)	5	(5)	5	(5)	5	-	S
CBC, w/diff, platelets[d]	1	3	3	3	3	3	3	3	3	3	-	L
Blood volume (estimate)	**8**	**14 (44)**	**4 (9)**	**9**	**4 (9)**	**9**	**4 (9)**	**9**	**4 (9)**	**9**	-	
Endotracheal aspirate (ETA)												
CMV PCR[e, f]	-	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	-	S
Clinical Assessments:												
SOFA[g] and, fluid balance		Daily on Days 1-7			X	X	X	X	X	X		
Ventilator (including static compliance),[f] NIV, HFNC parameters, and FiO_2		Daily										
Antiviral, antibacterial or antifungal medications[h]		Daily on Days 1-7			X	X	X	X	X	X		
Apache III		X										
AE/SAE monitoring		X	X	X	X	X	X	X	X	X		
ECMO, prone positioning, steroids, immunosuppressive medications, and neuromuscular blockers		X	At any time while on study; collected at discharge									
Vital status			ICU and hospital mortality collected at discharge								X	
Bacteremia, fungemia, nosocomial pneumonia (including ventilator-associated pneumonia)			At any time while on study; collected at discharge									
Contact (e.g. phone)										X[j]	X[k]	
NHLBI COMS survey		X[l]									X[l]	

L = local test; S=Seattle, WA; A= ARUP in Utah

[a] Pregnancy tests (serum or urine) performed within 120 hours before enrollment are acceptable.

[b] At day 7 or discharge, whichever is earlier (if day 7 is missed, at discharge is acceptable). Only serum test is acceptable.

[c] All samples will be stored for analysis at the end of the study
[d] All blood draws will occur only during hospitalization, blood chemistry, CBC and platelet values can be obtained from clinical testing result if available, thereby reducing required blood volume
[e] Time points in ( ) only in patients with intensified monitoring (n=150).
[f] Can only be collected if subject is ventilated
[g] While in the ICU
[h] Use of antibacterials or antifungals will be collected in the event of ventilator-associated pneumonia or nosocomial pneumonia (defined as sputum, BAL or ETA culture with new pathogen associated with new antibiotic +/-48 hours from culture). Any antibacterial used to treat bacteremia or antifungal used to treat fungemia should be reported.
[i] In patients who were discharged before day 28, visit window ± 4 days, to assess reportable adverse events, vital status, and pregnancy within 30 days of study drug (see text).
[j] A reminder contact (e.g. by mail, email, text) will be done ~3 months before the day 180 assessment.
[k] A reminder contact (e.g. by mail, email, text) will be done if the participant does not appear for their day 180 assessment.
[l] Katz ADL and Lawton IADL at baseline and Day 180; HADS, EQ-5D-5L and IESR only at Day 180.
[m] A different window for sample processing may apply; see the MOP for additional information.
[n] Serum Creatinine is not required if the patient is receiving renal replacement therapy

APPENDIX B: NCI COMMON TOXICITY CRITERIA (CTC)

A. The NCI CTC criteria will be used for reportable Adverse Event reporting. The NCI CTC criteria can be downloaded at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50. A hard copy of the NCI CTC can be found in the study reference manual.

B. **For this study the CTC guideline categories have been assigned numbers as follows:**

CATEGORY	CODE
BLOOD AND LYMPHATIC SYSTEM DISORDERS	01
CARDIAC DISORDERS	02
CONGENITAL, FAMILIAL AND GENETIC DISORDERS	03
EAR AND LABYRINTH DISORDERS	04
ENDOCRINE DISORDERS	05
EYE DISORDERS	06
GASTROINTESTINAL DISORDERS	07
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	08
HEPATOBILIARY DISORDERS	09
IMMUNE SYSTEMS DISORDERS	10
INFECTIONS AND INFESTATIONS	11
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	12
INVESTIGATIONS	13
LYMPHATICS	14
METABOLIC AND NUTRITION DISORDERS	15
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	16
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED	17
NERVOUS SYSTEM DISORDERS	18
PSYCHIATRIC DISORDERS	19
RENAL AND URINARY DISORDERS	20
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	21
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	22
SOCIAL CIRCUMSTANCES	23
SURGICAL AND MEDICAL PROCEDURES	24
VASCULAR DISORDERS	25

APPENDIX C: COMMONLY PRESCRIBED IMMUNOSUPPRESSIVE AGENTS WITH KNOWN EFFECT ON CMV REACTIVATION

Generic Name	Trade Name
Antithymocyte Globulin (Equine)	ATG, ATGAM
Antithymocyte Globulin (Rabbit)	Thymoglobulin
Alemtuzumab	Campath
Prednisone[a]	

[a] An average of >20mg/day of prednisone for the past 30 days prior to hospital admission. The total prednisone intake should be added and averaged over 30 days. For example, a patient taking 40mg/day for 14 days would not be excluded because (40x14)/30=18.7. Other steroids should be converted into prednisone equivalents. Additionally, any dose of corticosteroids given for any reason on or after hospital admission is acceptable.

APPENDIX D: LUNG PROTECTIVE VENTILATION PROTOCOL RECOMMENDATIONS

Note: The following are guidelines that are recommended for applicable patients. However, each study site should use their own best judgment, and consult with the coordinating center if there are any questions.

Ventilator Management

A modified, simplified version of the ARDS Network lung protective lower tidal volume strategy will be used in this trial. This strategy, which was associated with low mortality rates in three previous ARDS Network trials (ARMA, ALVEOLI, and FACTT), will ensure that study subjects receive the beneficial effects of lung protection while participating in this trial [104, 105]. ARDS Network personnel have substantial experience in the application of this protocol from the three completed trials noted above.

1. Any mode of ventilation capable of delivering the prescribed tidal volume (V_T, 6ml/kg predicted body weight, +/- 2ml/kg) may be used, provided the V_T target is monitored and adjusted appropriately. If airway pressure release ventilation (APRV) is used, tidal volume is defined as the sum of the volume that results from the ventilator pressure-release and an estimation of the average spontaneous V_T. In the spirit of providing lung protective ventilation, high frequency oscillatory ventilation will also be allowed in this trial.
2. V_T Goal: 6 ml / kg predicted body weight.
3. Predicted body weight (PBW) is calculated from age, gender, and height (heel to crown) according to the following equations:
    a. Males: PBW (kg) = 50 + 2.3 [height (inches) – 60]
    b. Females: PBW (kg) = 45.5 + 2.3 [height (inches) – 60]
4. Measure and record inspiratory plateau pressure (Pplat) according to ICU routine (at least every four hours and after changes in V_T and PEEP recommended)
5. If Pplat > 30 cm H_2O, reduce V_T to 5 ml / kg and then to 4 ml / kg PBW if necessary to decrease Pplat to ≤ 30 cm H_2O.
6. If V_T < 6 ml/kg PBW and Pplat < 25 cm H_2O, raise V_T by 1 ml / kg PBW to a maximum of 6 ml/kg.
7. If “severe dyspnea" (more than 3 double breaths per minute on volume-cycled ventilator or airway pressure remains at or below PEEP level during inspiration), then raise V_T to 7 or 8 ml/kg PBW if Pplat remains below 30 cm H_2O. If Pplat exceeds 30 cm H_2O with V_T of 7 or 8 ml/kg PBW, then revert to lower V_T and consider more sedation.
8. If pH < 7.15, V_T may be raised and Pplat limit suspended (not required).
9. Oxygenation target: 55 mm Hg < PaO_2 < 80 mm Hg or 88% < SpO_2 < 95%. When both PaO_2 and SpO_2 are available simultaneously, the PaO_2 criterion will take precedence.
10. Minimum PEEP = 5 cm H_2O
11. Adjust F_IO_2 or PEEP upward within 5 minutes if there are consistent measurements below the oxygenation target range
12. Adjust F_IO_2 or PEEP downward within 30 minutes if there are consistent measurements above the oxygenation target range.
13. There are no requirements for maintaining a specific PEEP to F_IO_2 ratio. The lower PEEP/higher F_IO_2 table represents a consensus approach developed by ARDS Network investigators in 1995. The higher PEEP/lower F_IO_2 table (ALVEOLI) yielded equivalent results in a randomized trial [105] and would be acceptable and perhaps preferable in patients who appear to respond with a substantial increase in arterial oxygenation in the transition from lower to higher PEEP.

Lower PEEP/Higher F_IO_2 Treatment Group

F_IO_2	.30	.40	.40	.50	.50	.60	.70	.70	.70	.80	.90	.90	.90	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18-24

Higher PEEP/Lower F_IO_2 Study Group

F_IO_2	.30	.30	.30	.30	.30	.40	.40	.50	.50	.50 – .80	.80	.90	1.0	1.0
PEEP	5	8	10	12	14	14	16	16	18	20	22	22	22	24

Note: Levels of PEEP in these F_IO_2/ PEEP tables represent levels set on the ventilator, not levels of total-PEEP, auto-PEEP, or intrinsic-PEEP.

14. No specific rules for respiratory rate. It is recommended that the respiratory rate be increased in increments to a maximum set rate of 35 if pH < 7.30.
15. No specific rules about I:E. It is recommended that duration of Inspiration be ≤ duration of Expiration.
16. Bicarbonate is allowed (neither encouraged nor discouraged) if pH < 7.30.
17. Changes in more than one ventilator setting driven by measurements of PaO_2, pH, and Pplat may be performed simultaneously, if necessary.

D.2. Weaning

Note: Commencement of Weaning is occurring at the clinician's discretion.

Commencement of Weaning (applicable to patients ventilated invasively)

Patients will be assessed for the following weaning readiness criteria each day between 0600 and 1000. If a patient procedure, test, or other extenuating circumstance prevents assessment for these criteria between 0600 and 1000, then the assessment and initiation of subsequent weaning procedures may be delayed for up to six hours.

1. At least 12 hours since enrollment in the trial
2. $F_IO_2 \leq 0.40$ and PEEP ≤ 8 cm H_2O or $F_IO_2 \leq 0.50$ and PEEP = 5 cm H_2O
3. Values of both PEEP and F_IO_2 ≤ values from previous day
4. Not receiving neuromuscular blocking agents and without neuromuscular blockade
5. Patient exhibiting inspiratory efforts. If no efforts are evident at baseline, ventilator set rate will be decreased to 50% of baseline level for up to 5 minutes to detect inspiratory efforts.
6. Systolic arterial pressure ≥ 90 mm Hg without vasopressor support (≤ 5 mcg/kg/min dopamine or dobutamine will not be considered a vasopressor)

Spontaneous Breathing Trial Procedure and Assessment for Unassisted Breathing

If criteria 1-6 above are met, then initiate a trial of up to 120 minutes of spontaneous breathing with F_IO_2 < 0.5 using any of the following approaches:

1. Pressure support (PS) ≤ 5 cm H_2O, PEEP ≤ 5 cm H_2O
2. CPAP ≤ 5 cm H_2O
3. T-piece
4. Tracheostomy mask

The clinical team may decide to change mode during spontaneous breathing (PS = 5, CPAP, tracheostomy mask, or T-piece) at any time during the spontaneous breathing trial.

Monitor for tolerance using the following:

1. $SpO_2 \geq 90\%$ and / or $PaO_2 \geq 60$ mm Hg
2. Mean spontaneous tidal volume ≥ 4 ml/kg PBW (if measured)
3. Respiratory Rate ≤ 35 / min
4. pH ≥ 7.30 (if measured)
5. No respiratory distress (defined as 2 or more of the following):
    a. Heart rate ≥ 120% of the 0600 rate (≤ 5 min at > 120% may be tolerated)
    b. Marked use of accessory muscles
    c. Abdominal paradox
    d. Diaphoresis
6. Marked subjective dyspnea

If any of the goals 1-6 are not met, revert to previous ventilator settings or to PS greater than or equal to 10 cm H_2O with Positive End-expiratory Pressure and F_IO_2 = previous settings and reassess for weaning the next morning. The patient will be reassessed for weaning (Section D2) the following day.

Decision to remove ventilator support:

If tolerance criteria for spontaneous breathing trial (1-6 above) are met for at least 30 minutes, the clinical team may decide to discontinue mechanical ventilation. However, the spontaneous breathing trial can continue for up to120 minutes if tolerance remains in question.

D.3. Definition of Unassisted Breathing

1. Spontaneously breathing with face mask, nasal prong oxygen, or room air, OR
2. T-tube breathing, OR
3. Tracheostomy mask breathing, OR
4. CPAP ≤ 5 without PS or IMV assistance
5. Use of CPAP or BIPAP solely for sleep apnea management

D.4. Definition of Extubation

1. Removal of an oral or nasotracheal tube
2. If a patient receives a tracheostomy, the time of extubation is defined as the time when the patient achieves unassisted breathing as defined in Section D.3

D.5. Completion of Ventilator Procedures

Patients will be considered to have completed the study ventilator procedures if any of the following conditions occur:

1. Death
2. Hospital discharge
3. Alive 28 days after enrollment

If a patient requires positive pressure ventilation after a period of unassisted breathing, the study ventilator procedures will resume unless the patient was discharged from the hospital or > 28 days elapsed since enrollment.

D.6. Removal from the Ventilator Management Protocol

Patients may be removed from the 6 ml/kg PBW tidal volume ventilation requirement if they develop neurologic conditions where hypercapnia would be contraindicated (e.g., intracranial bleeding, GCS < 8, cerebral edema, mass effect [midline shift on CT scan], papilledema, intracranial pressure monitoring, fixed pupils).

APPENDIX E: CONSERVATIVE FLUID MANAGEMENT

Note: *The following are guidelines that are recommended for applicable patients. However, each study site should use their own best judgment, and consult with the coordinating center if there are any questions.*

This fluid protocol captures the primary positive outcome of the FACTT trial on increasing ventilator free days. This protocol should be initiated within four hours of randomization in enrolled patients when applicable, and continued until UAB or study day 7, whichever occurs first.

1. Discontinue maintenance fluids.
2. Continue medications and nutrition.
3. Manage electrolytes and blood products per usual practice.
4. For shock, use any combination of fluid boluses[#] and vasopressor(s) to achieve MAP ≥ 60 mmHg as fast as possible. Wean vasopressors as quickly as tolerated beginning four hours after blood pressure has stabilized.
5. Withhold diuretic therapy in renal failure § and until 12 hours after last fluid bolus or vasopressor given.

<table>
<tr><th rowspan="2">CVP
(recommended)</th><th rowspan="2">PAOP
(optional)</th><th colspan="2">MAP ≥ 60 mm Hg AND off vasopressors for ≥ 12 hours</th></tr>
<tr><th>Average urine output < 0.5 ml/kg/hr</th><th>Average urine output ≥ 0.5 ml/kh hr</th></tr>
<tr><td>>8</td><td>> 12</td><td>Furosemide*
Reassess in 1 hour</td><td>Furosemide*
Reassess in 4 hours</td></tr>
<tr><td>4-8</td><td>8-12</td><td rowspan="2">Give fluid bolus as fast as possible*
Reassess in 1 hour</td><td rowspan="2">No intervention
Reassess in 4 hours</td></tr>
<tr><td>< 4</td><td>< 8</td></tr>
</table>

§ Renal failure is defined as dialysis dependence, oliguria with serum creatinine > 3mg/dl, or oliguria with serum creatinine 0-3 with urinary indices indicative of acute renal failure.

[#] Recommended fluid bolus = 15 mL / kg crystalloid (round to nearest 250 mL) or 1 Unit packed red cells or 25 grams albumin

*Recommended Furosemide dosing = begin with 20 mg bolus or 3 mg/hr infusion or last known effective dose. Double each subsequent dose until goal achieved (oliguria reversal or intravascular pressure target) or maximum infusion rate of 24 mg or 160 mg bolus reached. Do not exceed 620 mg/day. Also, if patient has heart failure, consider treatment with dobutamine.

NIH ARDS Network

Revision date: March 9, 2009

APPENDIX F: DATA AND SAFETY MONITORING PLAN

Overview Prospective safety monitoring will be performed. Specific emphasis will be on hematotoxic effects as ganciclovir is known to be associated with these effects. The duration of treatment selected in this study is relatively short, thus we do not expect high rates of neutropenia. Relevant studies in this regard in the literature are two comparative studies of valganciclovir or ganciclovir in solid organ transplant recipients. Based on these studies, the rate of neutropenia should be approximately 2% with a 28-day course as proposed in this proposal. Most relevantly, our phase 2 RCT showed no neutropenia events. Numerous randomized trials do not show any evidence of ganciclovir causing thrombocytopenia. Mild anemia has been seen in some studies but the majority of trials did not show an association. A theoretical concern is the carcinogenicity in animal models. This effect has not been reported in humans; however, we have included a late follow-up time point to assess if there are any new diagnoses of tumors. Additional discussion of the safety profile is included in Section 3.

Safety monitoring will be by standard CTC criteria. Also, specific expected adverse effects will be tracked.

- Number and severity of reportable AEs and SAEs
- Time to neutropenia (absolute neutrophil count [ANC] < 1000, <500 per mm3); use of G-CSF
- Time to renal insufficiency (creatinine clearance < 60, < 30 ml/min)
- Time to thrombocytopenia (platelet count < 50,000, < 20,000 per mm3)

Long-term follow-up. The final protocol will include follow-up contact after hospital discharge. This is to assess secondary efficacy endpoints. The subject will be contacted over the phone at days 28 (if discharged from the hospital before this date) and 180 to assess vital status, pregnancy, and reportable adverse events.

1.1. Monitoring for Safety by Study Sites

Investigators are responsible for monitoring the safety of patients who have entered this study. Monitoring of weekly safety labs will be performed by the research team at each site for all participants, while hospitalized, with any reportable AE reporting per the protocol. These research personnel will be blinded.

The investigator remains responsible to follow, through appropriate health care options, reportable adverse events (AEs) that are serious, cause the patient to discontinue before completing the study, or are ongoing at the time of study completion. The investigator will maintain responsibility for forwarding reportable SAEs to the coordinating site and their institutional review board.

1.2. Monitoring by the Safety and Protocol Adherence Review Team

The safety and protocol adherence review team (SPART) will, on a monthly basis, review all clinical and laboratory safety data during the study. The SPART will include CCC PIs (Drs. Boeckh, Stapleton, Rubenfeld, and Limaye), Clinical Site Manager Sara Ardren, and Clinical Trial Project Manager Dr. Louise Kimball. Meetings of the SPART (by videoconference or teleconference) will occur regularly, and will not require attendance of all team members at each meeting. The SPART is responsible for the review of the clinical safety reports, discordant CMV serostatus test results (LFA versus CLIA test), and protocol violations, as well as communication with the IRB, NHLBI executive secretary, and DSMB, as outlined above.

1.3. Monitoring of Safety by an Independent Study Monitor

Study data and regulatory aspects at study sites will be monitored by a study monitor. The study monitor will be managed by Dr. Louise Kimball, and will perform study monitoring remotely and according to industry standard, using EPIC if feasible, and via Florence eReg platform if not, and via Vestigo. The study monitor will perform monitoring of the first patient at each site as well as a random selection of 10% of participants (to be determined by DCC) across the whole study. The scope of monitoring is detailed below. Annually, they will monitor delegation of authority logs and training logs for each site, via Florence. The study monitor will create monitoring reports, submit them to the site investigators and the Executive Committee, and conduct the necessary follow-up. The study monitor will not have a direct reporting

relationship with the DSMB. If an unreported reportable AE or SAE is found during the course of routine study monitoring, the usual processes for reporting the event will occur (see Section 11.3).

Deadlines for data to be put in:

- 4-6 weeks after randomization (except for day 180 data)
- Payments not made until data are in

Scope of Monitoring:

- Informed Consent Form
- Inclusion/Exclusion criteria
- CMV Lateral Flow Assay results and confirmatory IgG ELISA results
- Study drug delivery to confirm intervention accuracy (dose and time of first dose)
- Pharmacy logs to confirm appropriate temperature of drug
- Serious adverse events, with special attention to known complications of ganciclovir
    - Protocol-defined reportable SAEs
    - Hospital discharge or death summary for possible reports of a serious unexplained events that the team associated with study drug administration
- Outcome data, including data that might be used for adjustment in statistical models
    - Respiratory-support-free days (confirm start and stop times, including any separate episodes that might have occurred)
    - Death
    - The following parameters will be monitored randomly **in 5% of subjects** (selected at random from among the 10% of participants selected for monitoring above):
        - Oxygenation (PaO2/FiO2 ratio) on study days 1-7
        - ICU-free days by day (confirm ventilation start and stop times, including any separate episodes of ICU admission that might have occurred)
        - Static respiratory system compliance at randomization, day 4 and day 7
        - Invasive bacterial and fungal infections
        - SOFA variables at baseline, day 4, and day 7
        - The NHLBI-endorsed Acute Respiratory Failure Core Outcome Measurement Set (COMS) in survivors at day 180
        - Risk factors for CMV reactivation
            - Sex
            - Age
            - Race/ethnicity
            - Co-morbidities
            - APACHE III score at baseline
            - SOFA score and individual components
            - Lymphocyte count

- Time of hospital admission

- Use of ECMO; if yes, start and stop time
- Occurrence of neuromuscular blockade; if yes, start and stop time
- Prone positioning; if yes, start and stop time

Protection against Risks

Study procedures (blood draw, ETA) will be conducted in a clinical setting by medical staff trained to perform the various procedures. Medical attention will be promptly provided to patients who experience reportable adverse events resulting from study procedures.

Safety labs will be monitored regularly by the research team at each site for any adverse reactions to study drug. In order to address the black box warning for ganciclovir, we have included an extended follow-up period of six months.

Maintaining confidentiality

Risk of breach of confidentiality will be minimized by keeping all research forms and electronic databases in locked and protected environments, and through training staff to be especially sensitive to confidentiality whenever interacting with participants.

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APPENDIX G: SEPSIS CRITERIA

Sepsis is defined according to the recent Sepsis-3 consensus definition [72], as life-threatening organ dysfunction caused by a dysregulated host response to infection.

Organ dysfunction can be identified as an acute change in total SOFA score ≥2 points consequent to the infection.

- The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction.
- A SOFA score ≥2 reflects an overall mortality risk of approximately 10% in a general hospital population with suspected infection. Even patients presenting with modest dysfunction can deteriorate further, emphasizing the seriousness of this condition and the need for prompt and appropriate intervention, if not already being instituted.

Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.

Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and having a serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation. With these criteria, hospital mortality is in excess of 40%.

Abbreviations: MAP, mean arterial pressure; SOFA: Sequential [Sepsis-related] Organ Failure Assessment.

APPENDIX H: LIST OF INITIALLY SELECTED SITES

Final listing will be on clinicaltrials.gov NCT04706507

Site	PI
Henry Ford Hospital (HFH)	Mayur Ramesh
Ohio State University (OSUMC)	Matthew Exline
Cleveland Clinic (CCF)	Abhijit Duggal
Brigham & Women's Hospital (BWH)	Rebecca Marlene Baron
Harborview & University of Washington Medical Center (UW)	Ajit Limaye
University of Vermont (UVM)	Renee Stapleton
University of Pittsburgh Medical Center (UPMC)	Scott Gunn
University of Maryland (MARYLAND)	Giora Netzer
Washington University, St. Louis (UWSTL)	Christina Vazquez Guillamet
Wake Forest (WAKEHEALTH)	Clark Files
Johns Hopkins University (JHU)	Dale Needham
University of Michigan (UMICH)	Robert Hyzy
University of Colorado Denver (UCDENVER)	Ellen Burnham
University of Cincinnati	Duncan Hite
Duke University (DUKE)	Christopher Cox
Medical University of South Carolina (MUSC)	Nandita Nadig
Intermountain Medical Center	Samuel Brown
Vanderbilt University (VANDERBILT)	Todd Rice
Montefiore Medical Center (MONTEFIORE)	Michelle Gong

APPENDIX I: RECOMMENDATIONS FOR HFNC AND NIV

Excerpt from the HENIVOT protocol (NCT04502576)

Note: *The following are guidelines that are recommended for applicable patients. However, each study site should use their own best judgment, and consult with the coordinating center if there are any questions.*

High-Flow Nasal Cannula (HFNC) Oxygen

HFNC therapy will be delivered with the Optiflow system.

Initial set flow will be ≥ 50 /min, and flows will be decreased in case of intolerance and/or according to patients' requirements: flows ≥30 L/min will be mandatory in all enrolled patients. Humidification chamber (MR860, Fisher and Paykel healthcare, New Zealand) will be set at 37 °C or 34 °C according to patient's comfort [106]. FiO2 will be titrated to obtain an SpO2≥92% and ≤98%.

Weaning the patient from HFNC will be considered only after 48 hours from enrolment.

Weaning from HFNC within the first 2 days of the study will be allowed only whether the patient is considered for ICU discharge, according to the decision of the attending physician.

All enrolled patients will be discharged from the ICU while undergoing low-flow oxygen, according to the prescription of the attending physician and the clinical practice of each participating institution (VenturiMask, nasal prongs, non-rebreathing oxygen mask). As suggested by Maggiore (NCT02107183), weaning from HFNC will be allowed when FiO_2<40% and respiratory rate<25/min. Oxygen flow will be lowered to 10 L/min, keeping FiO_2 unchanged. Weaning from HFNC will be considered successful if the SpO_2 remains between 92% and 98% and the respiratory rate ≤ 25/min with an oxygen flow of 10 L/min. In this case, the HFNC device will be replaced by the low-flow oxygen and oxygen flow or FiO_2 will be set to obtain the same SpO_2 target.

HFNC treatment can be resumed any time if the patient is experiencing respiratory distress and hypoxemia, according to the prescription of the attending physician.

Non-Invasive Ventilation (NIV)

Patients in PSV group will receive continuous helmet pressure support ventilation for at least 16 hours/day the first 2 calendar days. Continuous NIV without interruptions will be strongly encouraged in the first 24 hours of treatment. When NIV is interrupted, patients will receive low flow oxygen therapy or nasal high flow oxygen therapy, according to physician's decision. Dedicated helmets for NIV (Dimar, Italia, or Intersurgical, UK) and size will be chosen according to neck circumference, as suggested by Antonelli et al. [107], or according to manufacturer recommendations, if present.

Table 1. Helmet size according to neck circumference.	
17-27	Extra small
27-34	Small
34-40	Medium
40-47	Large
> 45	Extra large

Each patient will be connected to an ICU compressed gas based ventilator through a bitube circuit with no humidification.

The ventilator will be set in PSV (the choice to use NIV modes will be left to the decision of the physician in charge of the patient), with the following suggested settings [108-112]:

1. initial pressure support≥8-10 cmH2O and adequate to permit of a peak in the inspiratory flow of 100 l/min;
2. positive end-expiratory pressure ≥10 cmH2O and increased to achieve the oxygenation target according to the choice of the attending physician.
3. FiO2 will be titrated to obtain an SpO2≥92% and ≤98%.

4. Inspiratory flow trigger = 1 l/min or according to the practice of each institution;
5. fastest pressurization time;
6. expiratory trigger: 10-50% of the maximum inspiratory flow;
7. maximum inspiratory time 1.2 second.

The use of earplugs to mitigate noise-related discomfort will be allowed according to the decision of the attending physician and will be encouraged especially overnight.

Any modification in the ventilator settings and in the interface set-up to optimize comfort and patient-ventilator interaction will be allowed at the discretion of the attending physicians. However, maintenance of PEEP ≥10 during the treatment is mandatory.

Weaning from NIV will be discouraged within the first 48 hours from enrolment. Weaning from NIV at any time will be attempted only whether FiO_2 ≤40%, respiratory rate ≤25%: to assess the readiness for interrupting NIV, PEEP will be lowered to 8 cmH2O with pressure support=8 cmH2O, keeping FiO_2 unchanged. If the patient maintains SpO_2 ≥92% and respiratory rate ≤25 during the following 30 minutes with these settings, NIV weaning will be considered successful. After weaning from NIV and between two NIV sessions, patients will undergo low-flow, VenturiMask or HFNC, according to the choice of the attending physician: oxygen flow or FiO_2 will be set to obtain the same SpO_2 target.

NIV will be resumed at any time if the respiratory rate is more than 25 breaths per minute and SpO_2 is less than 92% with and/or anytime deemed necessary by the attending physician.