PROTOCOL

A Randomized Double-Blind Placebo-Controlled Trial of Ganciclovir/Valganciclovir for Prevention of Cytomegalovirus Reactivation in Acute Injury of the Lung and Respiratory Failure

The GRAIL Study

Clinical Trial Sponsored by the

NHLBI
Bethesda, Maryland, USA

Study Drugs Provided by

Genentech, A member of the Roche Group

IND Exempt #108,748

May 4, 2016

Version 3.4.1

Version 3.4.1 eliminates the use of Valganciclovir, the oral study drug for subjects not already assigned oral study drug. References related to assignment to Valganciclovir have been excluded from the protocol as appropriate.

ABBREVIATIONS

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
CBC	Complete Blood Count
ETT	Endotracheal Aspirate
HSCT	Hematopoietic Stem Cell Transplant
ICAM	Intercellular Adhesion Molecule
ICU	Intensive Care Unit
IL	Interleukin
LOS	Length of Stay
Plt	Platelet
SOT	Solid Organ Transplant
TGF	Tumor Growth Factor
TNF	Tumor Necrosis Factor
WBC	White Blood Cell Count

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1 PROTOCOL SUMMARY

Title

A Randomized Double-Blind Placebo-Controlled Trial of Ganciclovir/Valganciclovir for Prevention of Cytomegalovirus Reactivation in Acute Injury of the Lung and Respiratory Failure (The GRAIL Study)

Study drugs

Ganciclovir sodium: 2-amino-9-76,9-dihydro-3H-purin-6-one. Marketed as Cytovene and Cymevene**.**

Placebo for ganciclovir: [normal saline]

[ONLY for subjects enrolled and assigned oral study drug prior to approval of protocol version 3.4]

Valganciclovir hydrochloride: 2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl) methoxy]-3-hydroxypropyl (2S)-2-amino-3-methylbutanoate. Marketed as Valcyte.

Placebo for valganciclovir: [matching pink-colored tablet]

Patients

Non-immunocompromised, CMV seropositive adults hospitalized with respiratory failure associated with severe sepsis or trauma.

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	80	Ganciclovir 5 mg/kg intravenously	Ganciclovir 5 mg/kg intravenously,
2	80	Normal saline intravenously	Normal saline intravenously,
Total	160		

* “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 reduces plasma IL-6 levels (i.e. reduction between baseline and 14 days post-randomization) in immunocompetent adults with severe sepsis or trauma associated respiratory failure.
Primary hypotheses	In CMV seropositive adults with severe sepsis or trauma, pulmonary and systemic CMV reactivation amplifies and perpetuates both lung and systemic inflammation mediated through specific cytokines, and contributes to pulmonary injury and multiorgan system failure, **AND** Prevention of CMV reactivation with ganciclovir decreases pulmonary and systemic inflammatory cytokines that are important in the pathogenesis of sepsis and trauma related complications.
Study Design	Multicenter randomized placebo-controlled double-blind trial, [randomized in blocks for balance across study sites and genders, with interim analyses of safety].
Study Duration	6 months per patient
Trial Safety Monitoring	Safety Review Team (see Section 14.3) Data Safety Monitoring Board (see Section 14.5)
Study drug provider	Genentech, A member of the Roche Group
Sponsoring Agency	U.S. National Institutes of Health (NIH) National Heart, Lung, & Blood Institute (NHLBI)
Coordinating Center	Fred Hutchinson Cancer Research Center/Vaccine & Infectious Disease Division (VIDD)
Statistical and Data Management	Fred Hutchinson Cancer Research Center/Vaccine & Infectious Disease Division (VIDD), Statistical Center for HIV/AIDS Research & Prevention (SCHARP)
Endpoint Laboratory(ies)	FHCRC/University of Washington
Protocol Leadership Team	Michael Boeckh, MD, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA Ajit Limaye, MD, Dept. of Laboratory Medicine, Univ. of Washington, Seattle, WA Gordon Rubenfeld, MD, MSc Sunnybrook Medical Centre, Univ. of Toronto, Toronto, Canada

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2 BACKGROUND

2.1 Critical illness due to severe sepsis and trauma

Critical illness due to severe sepsis and trauma are major causes of morbidity and mortality, and a substantial economic burden in the United States and worldwide. Despite advances in clinical care, patients with sepsis and trauma-associated respiratory failure represent specific populations with high rates of adverse outcomes. The etiology of respiratory failure in patients with severe sepsis and trauma is multifactorial, but acute lung injury (ALI) is one of the leading causes, and is associated with prolonged ICU and hospital stays, mortality, and long-term sequelae. Other than general supportive care, few specific interventions other than lung protective ventilation have been shown to improve outcomes in such patients. New approaches for understanding the pathogenesis and developing better therapies are urgently needed.

2.2 Acute Lung Injury (ALI)

Acute Lung Injury (ALI) is a syndrome consisting of acute hypoxemic respiratory failure with bilateral pulmonary infiltrates that is associated with both pulmonary and nonpulmonary risk factors (e.g. sepsis, trauma) and that is not due primarily to left atrial hypertension [1]. Although a distinction between ALI and a more severe subtype (termed acute respiratory distress syndrome (ARDS) has been made, the pathogenesis, risk factors, and outcomes appear to be similar [1] and for the purposes of this protocol, the term acute lung injury [ALI] will be used to encompass both entities. Accepted consensus definitions of ALI have been introduced and are now widely used for laboratory and clinical investigations of ALI [2]. Acute Lung Injury (ALI) is defined as:

- PaO_2/FiO_2 <300
- Bilateral pulmonary infiltrates on chest x-ray
- Pulmonary Capillary Wedge Pressure <18mmHg or no clinical evidence of increased left atrial pressure

Although a broad range of risk factors for ALI have been described, those that account for the majority of cases include: sepsis, pneumonia, trauma, and aspiration [1, 3]. It is well established that severe trauma is recognized as a precipitating cause of ALI [3]. Recent studies have demonstrated that the incidence of acute lung injury (ALI) is much higher than previously thought, with an estimated age-adjusted incidence of 86 per 100,000 persons per year, resulting in an estimated ~190,000 cases annually in the US [1]. The clinical and health care system impact of ALI is substantial, with an estimated 2,154,000 intensive care unit (ICU) days, 3,622,000 hospital days, and 75,000 deaths in 2000 [1], and is expected to grow significantly given the marked age-related incidence and the ageing population. Although general improvements in ICU care over the last 2 decades have led to a trend towards lower mortality due to certain ALI-associated risk factors (trauma, aspiration), the most common causes of ALI, sepsis and pneumonia, remain associated with high mortality rates of ~25-35% [4, 5]. Mortality in ALI is most commonly due to secondary infections/sepsis and multiorgan system failure rather than primary respiratory failure due to hypoxemia, highlighting the systemic nature of ALI [4, 6]. Even among initial survivors of ALI, substantial pulmonary and nonpulmonary functional impairment remains for months to years [7, 8]. Specifically, a proportion of those who survive the initial insult are at risk for prolonged mechanical ventilation and ICU/hospital stay, and the risk factors remain poorly defined. It has been hypothesized that a "2^{nd} hit" may predispose certain patients to greater morbidity in this setting. Despite intensive basic and clinical investigation, only a single intervention (low-tidal volume ["lung protective"] ventilation) is generally accepted to decrease mortality in ALI [9], while multiple other strategies have failed to improve survival either in early clinical studies or definitive efficacy trials. Thus, given the high incidence and continued substantial clinical impact of ALI despite improvements in general medical/ICU care, and limited proven options other than lung-protective ventilation, new approaches to understanding the pathophysiology and identifying novel targets for intervention in ALI are a high priority.

Overly intense, persistent and dysregulated pulmonary and systemic inflammation has emerged as the leading hypothesis for the pathogenesis of ALI and its complications, but the contributory factors and mechanisms are incompletely defined [10]. Several carefully-conducted prospective human studies have shown an association between specific inflammatory biomarkers in blood and BALF (both the initial levels at onset and changes over time) and important clinical outcomes in ALI [reviewed in [11, 12]. Animal models have also demonstrated an association between inflammatory cytokines and non-pulmonary organ injury and dysfunction [13, 14] In addition, one of the most important interventions (low-tidal volume [“lung protective”] ventilation) shown to decrease mortality in ALI is associated with reductions in inflammatory cytokines (IL-6, IL-8) in blood and bronchoalveolar lavage fluid [BALF] [9, 15, 16].

2.3 Cytomegalovirus reactivates frequently in adult patients with critical illness and is associated with adverse clinical outcomes

Cytomegalovirus (CMV) is a ubiquitous virus in humans worldwide, and has been linked to adverse clinical outcomes including prolongation of mechanical ventilation, increased length of stay, and mortality in multiple studies of critically-ill, apparently immunocompetent, seropositive adults.

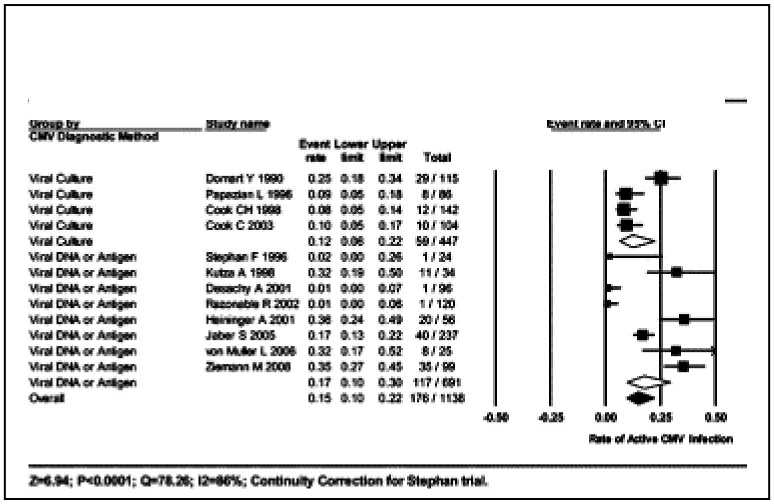
2.4 CMV overview

Cytomegalovirus (CMV) is a human herpesvirus known to infect more than 50-90% of US adults and is known to be a major cause of morbidity and mortality in immunocompromised patients. CMV infection can be acquired through multiple means, including: mother-to-child (in utero, breast milk), infected body fluids (saliva, genital secretions), blood transfusion or organ transplant. The prevalence of CMV infection increases with age throughout life such that by age 90, ~90% of persons will have acquired CMV infection [17]. In immunocompetent persons, following primary infection by any of the routes noted above, CMV is controlled by the immune system and establishes latency (“dormancy”) in multiple organs/cell-types for the life of the host. In particular, the lung represents one of the largest reservoirs of latent CMV in seropositive hosts, and may explain the propensity for CMV-associated pulmonary disease in predisposed hosts [18]. During periods of immunosuppression (or as a result of specific stimuli such as TNF-α, LPS, or catecholamines that are commonly associated with critical illness & sepsis [19], CMV can reactivate from latency (preferentially in the lung) to produce active infection (viral replication). In persons with impaired cellular immunity, reactivation can progress to high-grade CMV replication and commonly leads to tissue injury and 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 [20]. In addition, even low-level, otherwise asymptomatic subclinical CMV reactivation can produce significant biologic effects both in vitro and in vivo, such as inflammation, fibrosis and immunosuppression. Each of these biologic effects of subclinical CMV infection has either previously been demonstrated (inflammation, fibrosis) or could theoretically be important (immunosuppression) in sepsis-associated ALI and its complications. These biological effects of CMV have been shown to occur through various mediators and other indirect means [reviewed in [21]. 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 relatively sensitive means of virus detection such as PCR [22-24].

2.4.1 CMV reactivation in non-immunocompromised ICU patients

Reactivation of CMV in apparently immune competent patients with critical illness due to a broad range of causes has been documented in multiple prior studies using a variety of virologic techniques, as summarized in Table 1 [25]. The specific triggers for CMV reactivation from latency have been identified [19, 26] and are known to be elevated in patients with sepsis and acute lung injury [reviewed in [12, 27]. A prospective study in intubated patients with sepsis from Germany reported more than 60% rate of CMV DNA detection in tracheal aspirates [28].

Table 2-1: CMV reactivation in the ICU setting.



In addition to CMV reactivation in sepsis, CMV reactivation has also been demonstrated specifically in lung and blood of patients with acute lung injury.

Retrospectively testing samples collected in a prospective observational cohort study of patients at risk of developing ARDS, CMV reactivation (i.e. CMV DNA by PCR) was detected in BALF and/or plasma of 2/5 [40%] of subjects who developed ARDS, in sequential samples from 7/20 [35%] patients with ARDS, but not in patients at risk but who did not develop ARDS (0/5) [Limaye 2009 unpublished data]. In a separate study, CMV reactivation was retrospectively assessed by PCR in BALF of 88 subjects enrolled in a randomized trial of fish oil for treatment of ALI [29]. Seropositivity at baseline (i.e. evidence of latent CMV infection) in the cohort was 65% (similar to prior age-related estimates), and CMV reactivation (i.e. CMV DNA by PCR) was detected in BALF of 12/57 [21%] patients [Limaye unpublished data 2009].

2.4.2 CMV reactivation in non-immunocompromised adults is associated with adverse clinical outcomes.

Several lines of evidence have linked CMV reactivation with adverse clinical outcomes in non-immunosuppressed adults with critical illness. In a recent meta-analysis, CMV reactivation (compared to no reactivation) was associated with a 2-fold increased odds of mortality in ICU patients (Table 2) [25].

Table 2-2: Metaanalysis of mortality of in patients with CMV reactivation.

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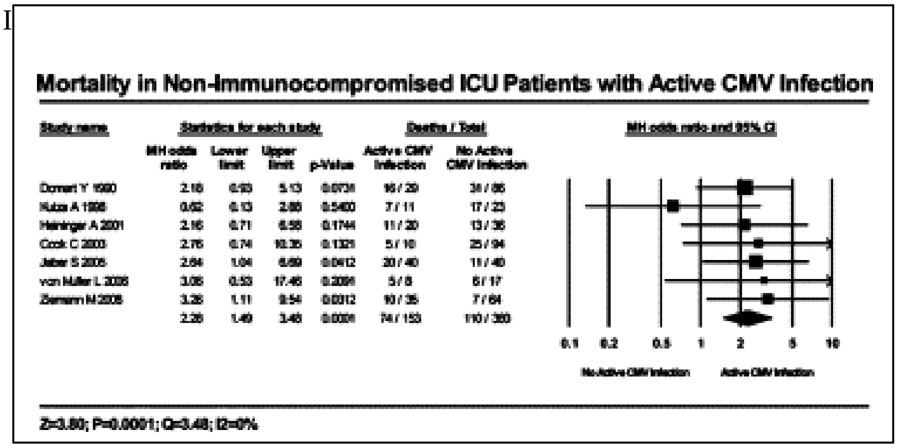


In addition to mortality, recent studies have demonstrated a strong and independent association between CMV reactivation and increased hospital and ICU length of stay [20] and duration of mechanical ventilation [30].

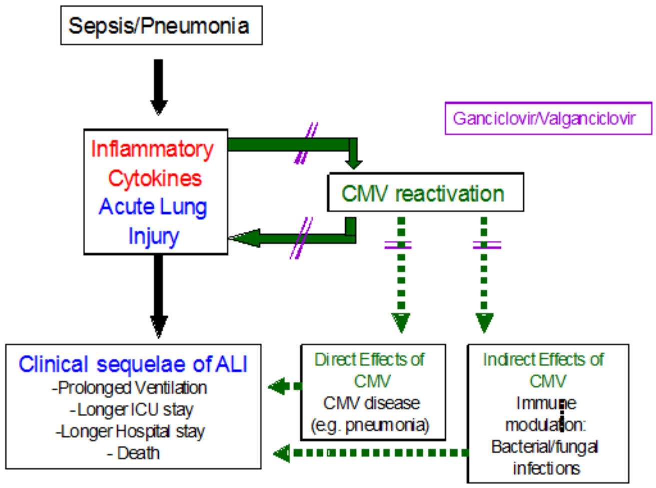
Mechanisms linking CMV reactivation with inflammation and lung injury.

Several of the key inflammatory cytokines hypothesized to be important in the pathogenesis of ALI [12] have been directly linked to CMV infection. Specifically, CMV infection of various human cell types leads to increased production of IL-6 and IL-8 in vitro [31-35]. Elevated levels of these cytokines are found in blood [36, 37] and in the lung [38, 39] of humans with CMV reactivation (as measured by CMV DNA PCR). In an animal model of latently CMV infected mice, sepsis induced by cecal ligation and puncture leads to CMV reactivation and upregulation of proinflammatory cytokines in the lung and resulting lung injury (fibrosis) [19, 40, 41]. Furthermore, in this model, cytokine upregulation and lung injury all are reduced by administration of an antiviral agent (ganciclovir) that prevents CMV reactivation [41].

Thus, these data suggest that CMV reactivation could provide a mechanistic link between ALI and persistent dysregulated inflammation, and provides a novel target for intervention to reduce the morbidity and mortality of sepsis-associated ALI and its complications in adults

The hypothesized causal pathway is as follows: sepsis or pneumonia lead to ALI mediated through a cytokine ‘storm’. The cytokines and other systemic mediators that are upregulated both within the lung and systemically in ALI are known potent stimuli for reactivation of CMV from latency. The resulting CMV reactivation within the lung and systemically then upregulates inflammatory and pro-fibrotic cytokines, thereby amplifying pulmonary and systemic inflammation and lung fibrosis, and ultimately leading to further lung injury, multiple organ dysfunction, prolonged length of stay, and late deaths. Progressively higher levels of CMV reactivation might also lead directly to tissue injury (i.e. CMV pneumonia) through direct CMV lytic effects as has recently been described [42]. And finally, CMV might also produce immunosuppressive effects (as seen in the transplant setting [21] which may predispose to nosocomial bacterial and fungal infections, (Figure 1).

Figure 2-3: Hypothesis: Effects of CMV on the cascade of virus-induced magnification of inflammatory cytokine-mediated lung damage (solid lines) and other possible effects (dotted lines).


Sepsis/Pneumonia
Ganciclovir/Valganciclovir
Inflammatory Cytokines
Acute Lung Injury
CMV reactivation
Clinical sequelae of ALI
-Prolonged Ventilation
-Longer ICU stay
-Longer Hospital stay
- Death
Direct Effects of CMV
CMV disease
(e.g. pneumonia)
Indirect Effects of CMV
Immune modulation:
Bacterial/fungal infections

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 [43, 44].

3.3.1 Ganciclovir (intravenous formulation)

Ganciclovir is an FDA-approved, commercially-available antiviral medication used to treat or prevent cytomegalovirus (CMV) infections. Ganciclovir sodium is marketed under the trade names Cytovene and Cymevene (Genentech, A member of the Roche Group).

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.

3.3.2 Valganciclovir (oral formulation)

NOTE: FOR SUBJECTS ENROLLED AND ASSIGNED ORAL STUDY DRUG PRIOR TO APPROVAL OF PROTOCOL VERSION 3.4

An FDA-approved, commercially-available oral formulation of ganciclovir (a prodrug with good oral bioavailability [valganciclovir]) is also available. Valganciclovir hydrochloride (Valcyte, manufactured by Genentech, A member of the Roche Group), like intravenous ganciclovir, is approved for treatment and prevention of cytomegalovirus infections. As the L-valyl ester of ganciclovir, it is a prodrug of ganciclovir. After oral administration, it is rapidly converted to ganciclovir by intestinal and hepatic esterases. Pharmacokinetic studies in various populations have demonstrated similar systemic ganciclovir exposure (AUC) of intravenous ganciclovir and oral formulations (valganciclovir) [45-48]. Furthermore, clinical studies have demonstrated non-inferiority of oral formulation ganciclovir (valganciclovir) and IV ganciclovir for prevention and/or treatment of CMV disease in various populations [49, 50]. Thus, an oral alternative to intravenous ganciclovir, with similar pharmacokinetics and equivalent clinical efficacy is available, and allows for convenient dosing for patients who are able to tolerate oral medications.

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 or 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 ~20 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 [51, 52]. See the package insert for more information (Appendix H, I).

See the package insert for more information.

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 ALI and is thus not anticipated to be a significant issue but will be closely monitored. For all patients receiving study drug (ganciclovir or valganciclovir), routine weekly monitoring (with absolute neutrophil and platelets counts) is recommended and will be performed in the present study. Other potential side effects have generally been similar between ganciclovir and placebo groups in randomized trials.

3.6.1.1 Hematotoxicity

3.6.1.1.1Platelets

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 [49, 50, 53-56]. However, there are rare anecdotal reports of ganciclovir-related pancytopenia. One study of ganciclovir prophylaxis in HCT recipients reported delayed platelet engraftment [57]. Overall, the potential to cause thrombocytopenia is considered low.

3.6.1.1.2Neutropenia

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 SOT recipients. Many studies have demonstrated the effect occurs late after drug administration [49, 58, 59]. 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 [49]. 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 [50].

Ganciclovir-related neutropenia is reversible [49, 50, 58]. The time to recovery can be hastened by administration of G-CSF [52].

3.6.1.2 HIV & hematotoxicity

Red blood cells: 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 recently completed 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 (Boeckh, 2008 ASBMT abstract). Other recent randomized trials also did not show an increased risk of anemia [49, 60, 61].

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 [49, 60], however, two earlier trials, one in heart transplant recipients with IV ganciclovir [62, 63] 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 trial except one study in HCT recipients [60]. 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 and valganciclovir are considered 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. For the three months following receipt of ganciclovir, abstinence or an effective method of birth control for both partners is recommended.

3.6.1.7 Use of ganciclovir and valganciclovir in immunocompetent subjects

Ganciclovir has been used in a limited number in patients with sepsis and mechanical ventilation [30] and also in a clinical trial of adults with chronic fatigue syndrome [Montoya JG NIH Clinical Trials.gov identifier: INCT00478465].

Numerous case reports have been published on the use of ganciclovir and valganciclovir in individual patients with a variety of manifestations of CMV disease. No assessment can be made on the toxicity of ganciclovir from these reports; however, the drug appeared to be tolerated well, with adverse effects mimicking the spectrum known from immunocompromised patients.

3.6.2 Summary of human toxicity data

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).

In patients without underlying bone marrow dysfunction, two recent trials showed very low rates of neutropenia after 3-4 weeks of ganciclovir at doses similar to those proposed in this protocol (2% within first 4 weeks with prophylaxis of 900 mg VGCV/day [49]; 1.2% at day 21 with 900 mg valganciclovir twice daily; 0% at day 21 with 5 mg/kg ganciclovir twice daily; [50].

There is no convincing evidence that ganciclovir or valganciclovir cause thrombocytopenia.

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

There may be some risk of renal toxicity, however, this was not consistently observed across randomized trials.

Other potential safety issues include teratogenicity and carcinogenicity.

Table 3-1: Ganciclovir and valganciclovir toxicities

Adverse effects	Human data	Documented in randomized trials	Expected incidence increase over placebo
Neutropenia	yes	yes	< 2.0%
Thrombocytopenia	yes	no	no increase
Anemia	yes	some (HIV only)	no increase
Renal insufficiency	yes	no (recent trials)	no increase
GI effects	yes	yes	< 5% (oral phase)
Tumors	no	no	no increase
Birth defects	no	no	no increase (all subjects will use appropriate contraception)

3.7 Other recent investigational applications of ganciclovir

It has been proposed that valganciclovir might have a clinical benefit in the treatment of chronic fatigue syndrome. A clinical pilot trial has been performed (NIH Clinical Trials.gov identifier: INCT00478465) and results are forthcoming.

A randomized placebo-controlled pilot trial of valganciclovir has also been completed in patients with glioblastoma multiforme. Results were not publicly available at the time of protocol [NIH clinical trial.gov identifier: NCT00400322].

4 RATIONALE

The study is a multicenter, double-blind randomized placebo-controlled Phase II test-of-concept trial. The test-of-concept design will provide a preliminary assessment of study drug efficacy, as well as other related information that will be the basis for determining the need for and design of subsequent studies to complete a full evaluation of efficacy of ganciclovir in patients with respiratory failure (including acute lung injury) associated with severe sepsis or trauma.

4.1 Rationale for study intervention

We carefully considered two potential antiviral strategies: a “prophylactic” approach where antiviral therapy would be initiated prior to CMV reactivation in all eligible CMV seropositive patients and a “treatment” approach where antiviral therapy would be started only after CMV reactivation was documented (see Table). 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 [28, 64] and that current methods of CMV measurement in blood (i.e. PCR) are not sensitive enough for detection of all CMV reactivation [65]. 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 [65]; 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 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 [30], 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.

Table 4-1: Antiviral strategies considered for the clinical trial.

	Prophylactic	**Treatment**
Pros	• Conceptually more attractive (prevention rather than treatment) as it prevents all CMV reactivation at any site (including lung) before CMV-associated effects begin • Logistically simpler • Best opportunity to intervene before CMV-associated effects begin • Standard of care for other populations where CMV is a clinical problem • Best experimental and clinical data for preventing CMV effects	• Minimizes drug exposure and toxicity by targeting only patients with documented CMV reactivation
Cons	• Effect “diluted” by high proportion of non-reactivators • Relative “over-treatment” with risk for drug toxicity	• Logistically complicated • May be too late to see any benefit of intervention (CMV-mediated effect cascade already initiated) • Plasma CMV PCR is an insensitive marker of CMV reactivation (preferentially local reactivation in lung)

4.2 Rationale for study population

The primary study population includes patients with respiratory failure (including acute lung injury) associated with severe sepsis or trauma. Such patients continue to have high rates of morbidity and mortality despite general improvements in medical care and in the management of patients with ALI. The inclusion of patients aged 18 years or greater is justified by published rates of CMV seropositivity that increase with age [17]. The 5-day enrollment window from the time of initial hospital admission is justified because CMV reactivation rarely occurs prior to day 4 in this population [20]. The 5-day window also allows for the opportunity to enroll subjects at ARDSNET sites who were not able to be enrolled because of an unavailable surrogate during the 24-48hr window from ALI onset that is typically used for other ARDSNET studies. Patients with immunocompromising conditions known to be associated with a risk for CMV who might be screened or treated for CMV reactivation are excluded. Patients taking medications that might affect the cytokine profiles that are the primary outcome variables of this trial will also be excluded. Other exclusions are designed specifically to minimize the risk for potential ganciclovir-associated toxicities (for example pregnancy, breast feeding, and neutropenia). Because the goal is to study the effects of ganciclovir on CMV reactivation and cytokine profiles in patients associated with severe sepsis or trauma, we will exclude patients at high risk of early death with little chance of observing the primary outcome.

4.3 Rationale for the choice of drug, dose & regimen

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. There is extensive experience with ganciclovir during the ~20 years that it has been in widespread clinical use, and the most common reversible toxicities, leukopenia and neutropenia, are routinely monitored during therapy. Based on data shown in the Background section, the expected risk of neutropenia is estimated to be 2.5%. While other significant toxicities are described in the package insert, these must be carefully balanced against the potential benefit of ganciclovir in the population being studied. Indeed, the 6 month mortality after sepsis associated ARDS approaches 50% is similar to the mortality seen after stem cell transplantation and higher than the mortality after solid organ transplantation–both settings in which ganciclovir and valganciclovir are routinely used. The dosing regimen will consist of initial twice daily dosing for 5 days (adjusted for renal function) to ensure adequate drug exposure during the period when earliest onset of CMV reactivation has been documented [20], followed by a daily dosing. There is significant experience with the use of ganciclovir in critically-ill patients and there are well-established FDA-approved dose adjustments for decreased renal function that will be used as recommended in the ganciclovir package insert. The 28-day total duration of study drug is justified by the period during which CMV reactivation occurs in this population [20].

4.4 Rationale for choice of endpoints

Because of the limited number of treatments shown to reduce mortality in critically ill patients there is a lack of generally accepted Phase II clinical trial endpoints in the field. Valid Phase II endpoints require robust evidence from multiple clinical trials that show that treatments that improve clinically significant outcomes also affect the proposed Phase II endpoint [66]. Unfortunately, there simply is not sufficient evidence from multiple successful clinical trials in ALI to guide the selection of a single Phase II endpoint without being controversial [67].

However, inflammatory cytokines in the blood and BALF of patients with ALI, specifically IL-6 and IL-8, have demonstrated the required criteria for Phase II endpoints [Prentice R Stat Med 1995]. These biomarkers: (1) are reliably associated with mortality and other important clinical outcomes in ALI [12, 27, 68], and (2) are reduced by lung protective ventilation, the one therapy generally accepted to reduce mortality in ALI [9, 15, 16]. There are multiple lines of evidence linking CMV with each of these specific cytokines both in vitro and vivo [31, 32, 34, 37-39]. In this trial, we selected plasma measures rather than BALF because a substantial proportion of patients will have either died, been extubated, or discharged by the follow-up BALF at day 14, making statistical analysis problematic due to missing data. Day 14 was selected as the primary endpoint for measuring the cytokine response because of the known timing of CMV reactivation. We considered and rejected a number of potential primary surrogate endpoints including: ventilator free days (rejected because of the lack of evidence suggesting it is more sensitive than mortality alone or that it always moves with mortality), oxygenation (rejected because of the evidence from clinical trials of PEEP and inhaled nitric oxide that show that it does not move with mortality), and dead space (rejected because the biologic hypothesis of the therapy being tested in this trial is linked to inflammation) [69, 70].

Secondary endpoints were selected either because of their known association with clinically significant outcomes in ALI or because they are clinically relevant themselves as outcomes or safety measures. 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 section).

5 STUDY HYPOTHESES, OBJECTIVES AND ENDPOINTS

5.1 Primary Hypotheses

In CMV seropositive adults with severe sepsis or trauma associated respiratory failure including ALI, pulmonary and systemic CMV reactivation amplifies and perpetuates both lung and systemic inflammation mediated through specific cytokines, and contributes to pulmonary injury and multiorgan system failure,

<u>AND</u>

Prevention of CMV reactivation with ganciclovir decreases pulmonary and systemic inflammatory cytokines that are hypothesized to be important in the pathogenesis of ALI and its complications.

5.1.1 Primary Objective

To evaluate whether administration of ganciclovir reduces plasma IL-6 level (i.e. reduction between baseline and 14 days post-randomization) in immunocompetent patients with respiratory failure, including ALI, associated with severe sepsis or trauma.

5.1.2 Primary Endpoint

Plasma IL-6 level (change between baseline and 14 days post-randomization between placebo & ganciclovir groups).

5.2 Secondary Objectives

The secondary objectives of this study are:

- To evaluate whether ganciclovir affects CMV viral load parameters (i.e. incidence, peak levels, area under the curve) in blood, throat, and ETT aspirates among recipients relative to placebo recipients.
- To assess for differences between Day 0 and Day 7 levels of IL-6, IL-8, IL-10, TGF-β and TNF-α for both groups.
- To assess plasma cytokine levels IL-8, IL-10, TNF-α and sICAM-1 during the first 14 days after randomization.
- To evaluate whether the proportion of organ system failure at by day 14 among ganciclovir recipients is less than the proportion among placebo recipients.
- To evaluate whether the duration of mechanical ventilation and ventilator-free days alive is different among ganciclovir recipients relative to placebo recipients.
- To evaluate whether ganciclovir administration affects safety parameters:
    - ANC < $500/mm^3$,
    - Use of GCSF,
    - Platelet count < 50,000/ml,
    - Hemoglobin < 8 mg/dL
    - Number of red blood cell and platelet products
    - AE > grade 2 (CTC criteria), and
    - We will also assess new tumor diagnoses by day 180 after randomization.

- To evaluate whether length of stay in hospital and/or ICU among ganciclovir recipients relative to placebo recipients is decreased.
- To evaluate mortality at 60 & 180 days among ganciclovir and placebo recipients.
- To assess for occurrence of bacteremia and fungemia among ganciclovir and placebo recipients.
- To evaluate functional assessment at 1 & 180 days among ganciclovir and placebo recipients.

5.2.1 Secondary Endpoints

A. Incidence of CMV reactivation at 28 days (blood, throat, endotracheal aspirate). Specifically, the following virologic parameters will be compared between the groups.

- Time to CMV reactivation at any level
- Time to $\geq$ 1,000 copies per mL
- Time to $\geq$ 10,000 copies per mL
- Area under the curve
- Peak viral load
- Initial viral load

B. Additional cytokines will be compared between the groups. We focused on cytokines with proven association with ALI and CMV (samples will be stored, permitting additional analysis, see Ancillary Studies section below).

- Cytokines IL-6, IL-8, TNF-α & TGF-β will be compared between groups at day 0 and day 7
- Cytokines IL-6, IL-8, IL-10, TNFα & soluble ICAM-1 will be compared during the first 14 days after randomization. Cytokines will be analyzed at each time point as well as over time (area under the curve). We will also compare peak levels between randomization and day 28 (end of treatment).

C. Clinical outcomes

- Organ system failure at 14 and 28 days [Multi-Organ Dysfunction Syndrome, (MODS)]. Proportions will be compared between the groups.
- Duration of mechanical ventilation as assessed by ventilator days and ventilator-free days alive
- Bacteremia and/or fungemia. Culture-proven bloodstream infections will be assessed (all tests done as clinically indicated; no surveillance will be performed)
- Mortality at 60 and 180 days after randomization.
- Composite of survival status, ventilation status and IL-6 levels (ranked)
- Subset analysis of laboratory and clinical outcomes amongst subjects who survive at least 7 days after randomization
- Subset analysis of laboratory and clinical outcomes amongst subjects who are mechanically ventilated for at least 7 through 14 days after randomization

D. Length of stay

- ICU (days alive and not in the ICU by day 28)
- Hospital (days alive and not hospitalized by day 28 and 180)

E. CMV disease (biopsy-proven). For the purpose of this analysis only biopsy-proven CMV disease or CMV retinitis diagnosed by an ophthalmologist will be considered as previously defined [71]. All biopsy samples obtained for clinical reasons will be shipped to the coordinating site in Seattle for analysis. There will be no specific surveillance for CMV disease, only samples obtained for clinical reasons will be examined.

F. Safety. Safety monitoring will be by standard CTC criteria. In addition, specific expected adverse effects will be tracked. Laboratory monitoring will be done for one additional week after discontinuation of study drug (day 35, see below).

- Number and severity of AEs and SAEs as defined in the Adverse Event section of the protocol
- Time to neutropenia (absolute neutrophil count [ANC] < 1000, <500 per mm^3)
- Use of G-CSF
- Time to renal insufficiency (creatinine clearance < 60, < 30 ml/min)
- Time to thrombocytopenia (platelet count < 50,000, < 20,000 per mm^3)
- Number of red cell and platelets products between randomization and day 35 after randomization

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

In order to perform future investigations into the causes of ALI and any possible links between ALI outcomes and with treatment with ganciclovir, we will collect DNA and RNA samples for gene association and gene expression studies. Other study samples (blood, throat, endotracheal aspirates, clinical biopsy samples) 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.

5.2.3 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 and gene expression studies.

6 STATISTICAL CONSIDERATIONS

6.1 Power Calculations for primary hypotheses

6.1.1 Primary Endpoint

The sample size of this phase II study was determined based on sample size calculations for the secondary endpoints, realistic and clinically relevant effect size and feasibility.

To estimate the required sample size for this trial with adequate statistical power for the primary endpoint, we used the rate of change in measured cytokine levels between days 1 and 3 in the 6 ml/kg/min arms of the ARMA and ALVEOLI trials [9, 72]. We used as a benchmark the effect size (measured in percent reduction in mean blood IL-6 levels between days 1 and 3) in a large randomized trial of standard vs. lung protective ventilation [9, 16]. In that trial, a 26% reduction in mean plasma IL-6 levels between enrolment and day 3 was associated with a 22% reduction in mortality between study arms.

The mean and standard deviation in IL-6 and IL-8 levels at day 1 and day 3 was estimated from log-transformed data from the 6ml/kg/min arms of the ARMA and ALVEOLI trials [9, 72]. The rate of decline was assumed to be linear over time. A 10% rate of dropout (deaths, missing data) by day 14 was assumed among the 160 enrolled patients, with a two-sided test and type I error rate of 5%. The standard deviations at different days and the inter-person correlations were used to calculate the standard deviation of the difference between baseline and day 14, using the following table:

Table 6-1: Calculations of the standard deviation of the difference between baseline and day 14.

Cytokine Outcomes	Mean at day 0	Std at day 0	Mean at day 14	Std at day 14	Inter-person correlation	80% power	90% power
						difference	difference
IL6	5.8	1.72	1.09	1.38	0.5	0.74	0.85
					0.6	0.67	0.77
					0.7	0.58	0.67
					0.8	0.49	0.56
IL8	4.19	1.44	2.32	1.18	0.5	0.62	0.72
					0.6	0.56	0.65
					0.7	0.49	0.56
					0.8	0.41	0.47

As shown in the table above, for the primary endpoint of the change in blood IL-6 level between day 1 and day 14, the study will have 80% power to detect a difference between groups of at least 16%.

6.1.2 CMV reactivation

The power to detect differences in rate of CMV reactivation between placebo and ganciclovir/valganciclovir groups is shown in the table below. We estimated several reactivation rates in the placebo group based on published data, ranging from 20% to 30% ($R_{placebo}$). We also assumed several efficacy scenarios for ganciclovir, ranging from 80% (RR 0.2) to 70% (RR 0.3).

Table 6-2: Power to detect the difference in CMV reactivation rate between two treatments with two-sided and type I error rate of 5%. (using Fisher exact test).

$R_{placebo}$	Relative risk ($R_{drug}/R_{placebo}$)	Power (%)
0.2	0.2	85.6
	0.3	69.6
0.25	0.2	93.9
	0.3	82.0
0.3	0.2	97.7
	0.3	90.2

6.1.3 Secondary Clinical Endpoints.

Although the study is not specifically powered to demonstrate differences in clinical endpoints, we also estimated the effect size for the secondary endpoints of length of hospitalization (ICU and total) and ventilation free days (at 28 and 60 days post-enrollment).

Table 6-3 Minimum detectable difference (μ_p-μ_t) and % difference ($\frac{\mu_p - \mu_t}{\mu_p}$*100) between two treatments with 80% or 90% power, two-sided and type I error rate of 5% (n=160).

			80% power		90% power	
Outcomes	μ_p	Std_p	μ_p-μ_t	$\frac{\mu_p - \mu_t}{\mu_p}$ (%)	μ_p-μ_t	$\frac{\mu_p - \mu_t}{\mu_p}$ (%)
Length of hospitalization	30	18	8.0	26.8	9.3	31.0
Length in ICU	19	14	6.2	32.9	7.2	38.0
Ventilation free within 60 days	37	22	9.8	26.5	11.4	30.7
Ventilation free within 28 days	13	10	4.5	34.3	5.2	39.7

For instance, for the length of hospitalization, we will be able to detect a difference of 8 days between the two groups with 80% power and a difference of 9.3 days with 90% power.

6.2 Statistical Analyses for endpoints.

6.2.1 Primary Endpoint.

We will first compare baseline characteristics in assessment of randomization using chi-square or t-tests. To test for whether the mean difference in primary endpoint (intervention vs. control) differs from 0, we will use a 2 sample t-test. In addition, multivariate models will be built including baseline subject characteristics and risk factors for CMV reactivation [20] using linear regression or ANCOVA, or the semiparametric efficient and robust method of Davidian et al. [73].

The primary analysis will evaluate the endpoint in survivors at Day 14. If subjects are missing a primary endpoint for reasons other than death, then the analysis method will accommodate the missing data by assuming endpoints are missing at random, and modeling whether subjects have their primary endpoint observed. If the rate of death by Day 14 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 may be conducted to evaluate how the estimated mean difference changes with a range of assumptions about the degree of possible selection bias or we will estimate survivor average causal effect (SACE) following Hayden et al. [77]. The sensitivity analysis method of Shepherd et al. [74] will be used, which was designed to address “truncation by death.”

6.2.2 Secondary endpoints

For the quantitative secondary endpoints, the same method used for the primary endpoint will be used. For the dichotomous secondary endpoints such as CMV reactivation, the Kaplan-Meier method will be used to estimate, for each group, the probability of not yet experiencing CMV reactivation by Day 28. 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. A Z-statistic based on these estimates will be used for testing for a group difference in event rates. Composite endpoints will be analyzed using a modification of the generalized Wilcoxon test, as proposed by Finkelstein et al. [76].

6.2.3 Other pre-specified analyses

In addition to the intent-to-treat analysis (i.e. all randomized patients), a modified intent-to-treat analysis will be performed (i.e. patients randomized and who have received at least one dose of study drug[primary endpoint]), as well as an analysis of patients who have been ventilated for at least 7 or 14 days. In addition, we will test for an interaction of treatment period (pre and post oral drug use) and the primary treatment differences at the study conclusion. We will analyze subsets of study population (including before and after the 3.4. protocol amendment) on key laboratory and clinical endpoints. Furthermore, association analyses of risk factors for CMV reactivation will be performed [20].

6.3 Randomization scheme

The randomization sequence will be obtained by computer-generated random numbers and provided to each site by the Statistical and Data Management Center (SDMC) at the coordinating center. The randomization will be block-randomized by site. At each institution, the pharmacist with primary responsibility for drug dispensing is charged with maintaining security of the randomization list.

6.4 Blinding

Patients and site staff (except for site pharmacists) will be blinded as to patient treatment arm assignments (e.g., study drug or placebo). Study drug assignments are accessible to those site pharmacists, contract monitors, and SDMC staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study drug assignment between the site clinical and pharmacy staff is prohibited. The DSMB members also are unblinded to treatment assignment in order to conduct review of trial safety.

Unblinding procedures are discussed in Section 9.15.

6.4.1 Missing data

The absence of data pertaining to some of secondary endpoints may be problematic if patients are discharged from the ICU, extubated, or expire prior to our pre-specified time points for endotracheal aspirates and serum collection. If the ganciclovir intervention reduces duration of ventilation, it may bias the results because patients cannot undergo endotracheal aspirate if they are extubated. Since this is a small trial, missing data cannot be imputed and will be dropped from the dataset. To minimize missing data and to maximize CMV detection rate, we have attempted to choose a time point for the endotracheal aspirate (Day 7 ± 1 day) when approximately 40% of patients in the study are expected to be alive and intubated.

6.5 Planned analyses prior to end of study

6.5.1 Safety

During the course of the trial, blinded analyses of safety data will be prepared twice yearly for review by the DSMB. Blinded ad hoc safety reports may also be prepared for DSMB review at the request of the safety review team (see Section 14.3). A scheduled interim safety analysis at midpoint will be performed. The team leadership must approve any other requests for blinded safety data prior to the end of the study. The DSMB decides whether to remain blinded to the treatment assignments at each meeting. Operating details are specified in the DSMB charter.

6.5.1.1 Interim safety analysis.

It is expected in this trial that approximately L = 48 of the 160 participants will have death events relative to the safety endpoint. A safety interim analysis is planned to be performed at the midpoint of the 24th event.
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) should 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 being less than 1 or greater than 1. The single interim analysis will be performed when approximately 50% of the expected total number of primary endpoints has been observed. The Pocock "upper boundary" to establish 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

The Pocock "lower boundary" to establish 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

For illustration, the table below presents the Pocock 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 48 death events.

Table 6-4: Interim analysis assumptions.

Information Fraction (% of Total Events)	Reject H_0 HR ≤ 1.00	Nominal one-sided p-values for rejection of H_0	Reject H_1 HR ≥ 1.00
50% (24 events)	≥ 2.40	P ≤ 0.016; Z = 2.15	≤ 0.42

Observe that, for the total of 24 events at the interim analysis, to reach the Pocock boundary for a lower death rate in the intervention group, the control group would need to have at least 10 excess events (7 in intervention group versus 17 in the control group) at the 50% information fraction.

Observe that, for the total of 24 events at the interim analysis, to reach the Pocock boundary for a lower death rate in the control group, the intervention group would need to have at least 10 excess events (7 in control group versus 17 in the intervention group) at the 50% information fraction.

The Lan-DeMets implementation [74] of the Pocock guideline will be used to provide flexibility in the timing and number (in the case of unplanned DSMB meetings) of interim analyses.

6.5.2 Other endpoint analyses

Distribution will be limited to those with a need to know for the purpose of informing future trial-related decisions. The Protocol Leadership must approve any other requests for prior to the end of the study. 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.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Study population

One hundred sixty 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, including ALI, associated with either severe sepsis or trauma. By virtue of their intubation, all patients requiring mechanical ventilator 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.

7.2 Randomization

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

7.3 Inclusion criteria

1. Subject/next of kin informed consent
2. Age $\geq$ 18 years
3. CMV IgG seropositive. The following tests are acceptable:
    a. FDA licensed test in a local lab approved by the coordinating center (FHCRC, Seattle, WA).
    b. Test in central study lab (ARUP, Salt Lake City, UT)
    c. A report that patient has previously been tested and found to be CMV seropositive at any time (a credible next of kin report is acceptable; confirmatory test will be done but results are not required for randomization)
4. Intubated and requiring mechanical positive pressure ventilation (including Acute Lung Injury/ARDS (EA Consensus Definition))
5. Meets criteria for either:
    a. Severe sepsis criteria (as defined in Appendix G) within a 24-hour time period within the 120 hour window

    Or

    b. Trauma with respiratory failure and an ISS score > 15 within a 24 hour time period, and within the 120 hour window (where mechanical ventilation is not due solely to a head injury)

6. On the day of randomization (by local criteria):
    a. Not eligible for SBT (use of sedation and/or vasopressor does not specifically contraindicate SBT)

    Or

    b. Failed SBT

7.4 Exclusion criteria

1. BMI > 60 (1st weight during hospital admission)
2. 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 years after allogeneic transplantation (regardless of immunosuppression)
        iii. greater than 1 year of 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:
        i. within 6 months: alemtuzumab, antithymocyte/antilymphocyte antibodies
        ii. within 3 months: immunomodulator therapy (TNF-alpha antagonist, rituximab, tocilizumab, IL1 receptor antagonist and other biologics)
        iii. within 30 days:
            1. corticosteroids >10 mg/day (chronic administration, daily average over the time period)
                a. topical steroids are permissible
                b. use of hydrocortisone in "stress doses" up to 100 mg four times a day (400mg/daily) for up to 4 days prior to randomization is permissible
                c. use of temporary short-term (up to 2 weeks) increased doses of systemic steroids (up to 1 mg/kg) for exacerbation of chronic conditions are permissible
            2. methotrexate (> 10.0 mg/week)
            3. azathioprine (>75 mg/day)

    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.

3. Expected to survive < 72 hours (in the opinion of the investigator)
4. Has been hospitalized for > 120 hours (subjects who are transferred from a chronic care ward, such as a rehabilitation unit, with an acute event are acceptable).
5. 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, it will be ordered stat and documented to be negative before randomization. Both urine and blood tests are acceptable.
6. Absolute neutrophil count < 1,000/mm^3 (if no ANC value is available, the WBC must be > 2500/mm^3)
7. Use of cidofovir within seven (7) days of patient randomization. The use of the following antivirals is permitted under the following conditions:
   a. Ganciclovir, foscarnet, high-dose acyclovir, or valacyclovir until the day of randomization
   b. Acyclovir as empiric therapy for central nervous system HSV or VZV infection until the diagnosis can be excluded
   c. For enrolled patients during the active study drug phase, acyclovir, famciclovir, valacyclovir for treatment of HSV or VZV infection as clinically indicated.
8. 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 (Note: confirm eligibility with one of the study medical directors at the coordinating site).
9. At baseline patients who have both a tracheostomy, and have been on continuous 24-hour chronic mechanical ventilation.
10. Patients with Child Class C Cirrhosis.
11. Patients with pre-existing interstitial lung disease.

7.5 Subject withdrawal

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

- 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,
- Subject has been inappropriately enrolled based on inclusion/exclusion criteria (e.g. when information through next of kin was inaccurate); these subjects may be replaced.

- Study is terminated.

If study drug is withdrawn, all safety and follow up procedures will be continued as described in the protocol.

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 provided Genentech, a member of the Roche Group and shipped to the University of Washington Investigational Drug Pharmacy. After expiration of the initial study lot intravenous study drug may also be acquired commercially (placebo infusions will be prepared at the site pharmacies). From there it will be distributed to the study sites.

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. Renal dysfunction and hemodialysis

Ganciclovir doses must be adjusted according to renal function as per package insert (reference pharmacy manual pages 11 and 12). 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 and subsequently dispensed.

9 CLINICAL PROCEDURES

9.1 Patient identification & recruitment

Patients with ALI 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 signed. 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, initial consent will be from the patients' legally authorized representative. 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 legally authorized representative 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 consent will be obtained from the patient when possible. 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.

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.

Each site is responsible for developing a protocol-specific consent form for local use, based on the sample protocol-specific consent form provided along with the protocol. 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. Once the consent form is signed, the patient is considered enrolled in the study. However, in case of provisional enrollment due to pending CMV serology or pregnancy test, the patient can only be randomized once these test results are available.

After the appropriate informed consent has been obtained and 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. CMV serostatus testing may also be done under a waiver of consent if permitted by the study site’s Institutional Review Board (IRB). CMV testing may be performed at the local site using FDA approved kits or at a central reference laboratory. If a waiver of consent for CMV testing using left-over blood has been granted by the local IRB, consent for this testing during the screening process is not required. If a waiver of consent is not permitted, a separate CMV serostatus prescreening consent form will be used.
    - Absolute neutrophil count/total white blood cell counts
- Collection of medical history
- Assessment of concomitant medications
- 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

9.4 Patient Registration

Patients will be registered with the central registration office in Seattle, Washington (SCHARP) via FAX or email.

9.5 Randomization procedure

Randomization will occur after confirmation of positive CMV serostatus and negative pregnancy test. The first dose of study drug should be started within 24 hours of randomization. Randomization will occur via a web-based system at the SDMC, which automatically notifies the site pharmacist of the treatment assignment. 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.

9.6 First dose of study drug

The first dose of study drug is considered study Day 1. The first study day will be defined as the 24 hour period following the first dose of study drug and all subsequent study days will start accordingly. At baseline, but before administration of study drug, the following procedures will need to be performed:

- Blood: Genomic analysis (genetic polymorphisms, gene expression, proteomics), Creatinine, Cytokine, Platelets, CMV PCR, CBC w/differential.
- Endotracheal (ETT) aspirate. Collect an ETT aspirate specimen at baseline (± 1 day) and every fourth day (± 2 days) while the patient remains intubated 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 FHCRC.
- Throat swab: CMV PCR.
- Clinical Assessments: Organ failure score, Vital status, Assessment of concomitant medications

9.7 Intervention (Study drug administration)

Patients will be randomized in a 1:1 ratio to receive either ganciclovir or placebo. Study drug delivery will begin within 24 hours of randomization. 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.

9.8 Co-interventions

All patients will receive standard intensive care unit care, which includes ventilator management (ARDS Network lung protective ventilation protocols will be used at all sites, Appendix D), 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.

9.9 Specimen collection

Patients will undergo serial blood draws at study entry (± 1 day of randomization) and on Day 7±1. Not more than 200 mL of blood will be collected over the initial 35 days of the study. Endotracheal (ETT) aspirates will be collected every four days while intubated.

Collect an ETT aspirate specimen at baseline (± 1 day) and every fourth day (± 2 days) while the patient is intubated every three to four days at the time this procedure is routinely performed by respiratory therapy. Specimen will be collected, labeled, and stored frozen for subsequent CMV PCR analysis at the coordinating lab (Boeckh Lab at FHCRC). After Day 35 or hospital discharge, patients will not be followed daily, but they will be contacted at [Days 60 & 180] for a telephone follow-up to ascertain health status and adverse events.

9.10 Survey study

Patients will complete a survey on Day 1 and at 6 months. The survey will take about 10 minutes to complete and will be faxed via Data Fax. The purpose of this survey is to compare functional assessment at Day 1 and 6 months between ganciclovir and placebo recipients.

9.11 Post-Enrollment Procedures

See the schedule of procedures for specific time points (including permissible windows) in Appendix A.

- Blood:
  - Genomic analysis (gene expression and proteomics) - Day 11 (± 1 day)
  - Creatinine, Cytokine, Platelets, CMV PCR, CBC w/differential- Days 4, 7, 11, 14, 18, 21, 25, 28, 35 (all ± 1 day)
- Endotracheal (ETT) aspirate every fourth day (± 2 days) while intubated. Throat swab: CMV PCR - Days 4, 7, 11, 14, 18, 21, 25, 28, 35 (all ± 1 day)
- Clinical Assessments: Organ failure score, Vital status, Assessment of concomitant medications - Days 4, 7, 11, 14, 18, 21, 25, 28, 35 (all ± 1 , day), Day 60 ± 3 days, Day 180 (± 14 days)
- For women of childbearing potential, a pregnancy test will be performed at the time of hospital discharge
- Because ganciclovir and valganciclovir carry a black box warning for tumors in lab animals (see sections 3.6.1.5 and 3.6.2.), at the 6 months 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. The 6 month time point has been selected in analogy to of the follow-up in a recent randomized trial of valganciclovir given for 6 months (Clinical Trials.gov identifier NCT00478465) in which such assessment was made 6 months after discontinuation of drug administration.

The schedule of post-enrollment procedures will be modified for patients who have been discharged before Day 35.

For all patients, it is <u>critical</u> that the Day 14 laboratory specimens are obtained for primary endpoint analysis. All patients must have Day 21 and Day 28 visits.

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 and or throat swabs 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 reach 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 and for one additional week. Study drug dose will be adjusted based on the calculated creatinine clearance according to the package inserts (Appendix H, I).

9.13 Monitoring for and managing neutropenia

<u>Suggested Management of Neutropenia.</u> Short-term neutropenia is an expected adverse event of ganciclovir/valganciclovir, although the incidence is projected to be low in the ICU setting.

1. Neutropenia will be monitored at least weekly throughout the active study period and for one additional week (day 35 after randomization or one week after hospital discharge for patients discharged prior to day 28).

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 GCSF; 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 > $1500/mm^3$ 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 an SAE (see SAE reporting section).

9.14 Pregnancy

If a patient becomes pregnant during the course of the study, no 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 11.1. 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 women of childbearing potential will also be assessed at the Day 60 and Day 180 follow up phone calls.

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. Boeckh or Limaye). 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. Boeckh or Limaye). Additionally, if a 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. Boeckh or Limaye).

9.15.2 Unblinding procedures

After one of the protocol chairs (Drs. Boeckh or Limaye) 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 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 biomarker studies.
- Endotracheal aspirates at baseline (± 1 day of randomization) and then every fourth day (± 2 days) while the patient is intubated for CMV viral load, cytokine analysis, neutrophil enumeration.
- Aliquot of endotracheal aspirate and/or lung biopsy done for clinical purposes.
- For patients who undergo autopsy, a sample of lung tissue (frozen and paraffin-embedded) is requested.
- Blood samples at baseline, then twice weekly until hospital discharge at least, then one week after discontinuation of study drug.
- For CMV viral load, cytokine analysis, and safety labs (CBC with neutrophil count with platelets, and Creatinine).
- Twice weekly throat swabs CMV DNA PCR while hospitalized, then one week after discontinuation of study drug.
- Bacteremia/fungemia in clinically-performed blood cultures, VAP.

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 to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will only occur after review and approval by the IRB of the researcher requesting the specimens and 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 Adverse Events

Investigators will determine daily (while hospitalized and at study visits after discharge) if any clinical adverse experiences occur during the period from randomization through 7 days after the last dose of study drug. The investigator 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 treatment of patients with ALI. If reportable adverse experiences occur, they will be recorded on the adverse event case report form.

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 ALI/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 ALI.

Expected events for ALI are untoward clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the day to day care of patients with ALI treated in an intensive care unit with mechanical ventilation. Examples of adverse events that are expected in the course of ALI 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 ALI. 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. $Sp0_2$ ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency.

All such reportable AEs 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

These reportable adverse events as defined above will be recorded on the adverse event case report form.

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

11.2 Serious Adverse Events

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 FHCRC 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 SAE reporting criteria:

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

FHCRC will report all serious, unexpected, and study-related adverse events to the DSMB by fax or email within 7 business days of being notified of the event. SAE forms received by FHCRC will be sent to participating sites for submission to their respective IRBs, according to their local IRB guidelines. The DSMB will also review all adverse events during scheduled interim analyses. FHCRC will distribute the written summary of the DSMB's periodic review of adverse events to investigators for submission to their respective Institutional Review Boards in accordance with NIH guidelines.

FHCRC will also determine if the serious adverse event is unexpected for ganciclovir (or valganciclovir for patients assigned to oral study drug prior to approval of protocol version 3.4). Unexpected for ganciclovir/valganciclovir is defined as any event not listed in the Cytovene, Cymevene or Valcyte package insert.

Investigators must also report Unanticipated Problems, regardless of severity, associated with the study drug or study procedures to FHCRC 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.

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 adverse events. The Study Coordinator must view patient records for possible adverse events 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 FHCRC 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 *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.

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.

b. 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 ALI or ARDS.

c. Adverse events will be considered to be study-related if the event follows a reasonable temporal sequence from a study procedure and could readily have been produced by the study procedure.

d. Organ failures or death related to ALI or ARDS or the patient's underlying condition that are systematically captured by the protocol should not be reported as adverse events *unless they are considered to be study related*.

All SAEs must be reported to the Fred Hutchinson Cancer Research Center in a timely fashion to allow expedited report 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)**
Serious Adverse Events (SAE)	Any untoward medical event that is: Serious **AND** Unexpected **AND** Related to study drug	Site to local IRB	According to local IRB guidelines
		Site to coordinating center	Initial report within 7 business days
		Coordinating center to 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 Genentech, A member of the Roche Group	Within 7 business days of receipt of 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 ≥ 5 days	SAME AS ABOVE	SAME AS ABOVE
Death	Death in the presence of neutropenia	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	Annually
		Coordinating center to DSMB	Included in report prepared for each DSMB meeting
		Coordinating center to NHLBI & participating sites	Annually
		Coordinating center to Genentech, A member of the Roche Group	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 **OR** 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 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 Genentech, A member of the Roche Group	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 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 Genentech, A member of the Roche Group	Within 7 business days of receiving pregnancy report form
		Coordinating center to FH IRB	Within 7 business days of receiving pregnancy report form
Adverse Event	Any untoward medical event that is considered by the investigator to be: • Unexpectedly severe or more frequent than typical course of ALI **OR** • Have any relationship to study drug	Site to local IRB	According to local IRB guidelines
		Site to coordinating center	AEs reported as required on CRFs. CRFs to be completed on a timely basis.
		Coordinating center to DSMB	Included in report prepared for each DSMB meeting
		Coordinating center to NHLBI & participating sites	Annually - summarized from CRFs in database
		Coordinating center to Genentech, A member of the Roche Group	Every 6 months – 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.

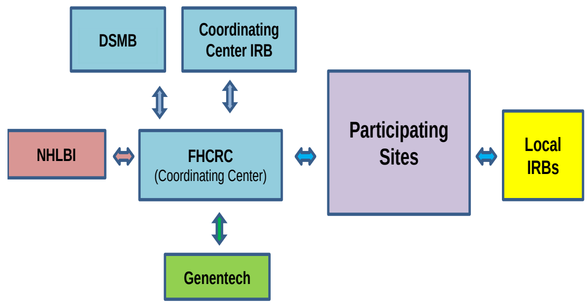


Figure 11-1: Safety reporting chart.

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

- Patient ID
- Description of the 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 SAE report forms and contact numbers for transmitting the reports.

11.4 Relationship to study drug

All 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 pregnancy is described in the table in Section 11.1.

11.6 Breaking the blind

The blind will not routinely be broken for SAE's. If the event is highly unusual or the knowledge of the study arm assignment is critical for optimal management of an individual patient, the case will be referred to the DSMB chair who will make the decision whether or not to break the blind.

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 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.

Chart abstraction for demographic, laboratory, and physiologic data will occur at study entry, daily until the intervention is discontinued, weekly for the remainder of the hospitalization, and again at hospital discharge or death. While patient remains hospitalized, review of the hospital record will occur daily throughout the hospitalization (to Day 35) to identify any adverse events.

All information will be faxed via Data Fax.

12.2 Data Management

Data are entered onto paper case report forms and then faxed into the SDMC via Data Fax. The database has been configured such that missing, extreme, or inconsistent values will be detected at the time of submission. Sites will receive queries to reconcile inconsistencies.

12.3 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, insuring that instructions are followed properly, and maintaining accurate records of study drug dispensing and collection.

12.4 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 with an average of 4 monitoring visits per site. All sites will have a start-up visit by a study monitor, one visit after 3 patients have been enrolled, one visit at approximately 50% of enrollment and one final and close-out visit. Briefly, we will perform 100% monitoring of inclusion and exclusion criteria, SAEs, length of stay, and ventilation data. In addition, every 5th patient will be monitored 100%. A detailed monitoring plan 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 local 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

Common	Valganciclovir: gastrointestinal: diarrhea, nausea, vomiting, abdominal pain.
Less common	Blood: leucopenia, neutropenia, anemia (ganciclovir and valganciclovir)
Uncommon or rare	Ganciclovir and valganciclovir: 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	Ganciclovir and valganciclovir: Cancer

13.3 Risks of Endotracheal Aspirates

ETT 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 Potential benefit of enrollment

ALI, respiratory failure, severe sepsis and trauma carry a high mortality, and consume millions of health care dollars each year. Any treatment that is found to impact outcomes in ALI could have a substantial societal benefit. Ganciclovir is not routinely administered to ALI 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, 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 review team

The safety review team (SRT) will review all clinical and laboratory safety data during the course of the study. The SRT is composed of the following members: protocol chair and co-chair (Drs. Boeckh and Limaye), and the project manager (registered nurse). The clinician members of the SRT are responsible for the review of the clinical safety reports, communication with the DSMB, reporting to IRB and Genentech, a member of the Roche Group as outlined above.

14.4 Data Safety and Monitoring Plan (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 adverse events by the research nurse and principal investigator, with the latter acting as medical monitor.

The investigator is responsible for appropriate medical care of patients during the study. The investigator remains responsible to follow, through an appropriate health care option, 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 of SAEs to the DSMB and 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 may give advice to the study team leadership. The members of the committee are independent of the University of Washington, Fred Hutchinson Cancer Research Center, Genentech, a member of the Roche Group, 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 serious adverse events will be reported to the chairperson of the DSMB. 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 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.

15 REFERENCES

1. Rubenfeld, G.D., et al., *Incidence and outcomes of acute lung injury.* N Engl J Med, 2005. **353**(16): p. 1685-93.
2. Bernard, G.R., et al., *The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination.* Am J Respir Crit Care Med, 1994. **149**(3 Pt 1): p. 818-24.
3. Hudson, L.D., et al., *Clinical risks for development of the acute respiratory distress syndrome.* Am J Respir Crit Care Med, 1995. **151**(2 Pt 1): p. 293-301.
4. Stapleton, R.D., et al., *Causes and timing of death in patients with ARDS.* Chest, 2005. **128**(2): p. 525-32.
5. Erickson, S.E., et al., *Recent trends in acute lung injury mortality: 1996-2005.* Crit Care Med, 2009. **37**(5): p. 1574-9.
6. Montgomery, A.B., et al., *Causes of mortality in patients with the adult respiratory distress syndrome.* Am Rev Respir Dis, 1985. **132**(3): p. 485-9.
7. Herridge, M.S. and D.C. Angus, *Acute lung injury--affecting many lives.* N Engl J Med, 2005. **353**(16): p. 1736-8.
8. Davidson, T.A., et al., *Reduced quality of life in survivors of acute respiratory distress syndrome compared with critically ill control patients.* Jama, 1999. **281**(4): p. 354-60.
9. *Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network.* N Engl J Med, 2000. **342**(18): p. 1301-8.
10. Ware, L.B. and M.A. Matthay, *The acute respiratory distress syndrome.* N Engl J Med, 2000. **342**(18): p. 1334-49.
11. Frank, J.A., P.E. Parsons, and M.A. Matthay, *Pathogenetic significance of biological markers of ventilator-associated lung injury in experimental and clinical studies.* Chest, 2006. **130**(6): p. 1906-14.
12. Levitt, J.E., et al., *Analytic review: the pathogenetic and prognostic value of biologic markers in acute lung injury.* J Intensive Care Med, 2009. **24**(3): p. 151-67.
13. Imai, Y., et al., *Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome.* Jama, 2003. **289**(16): p. 2104-12.
14. Dhanireddy, S., et al., *Mechanical ventilation induces inflammation, lung injury, and extra-pulmonary organ dysfunction in experimental pneumonia.* Lab Invest, 2006. **86**(8): p. 790-9.
15. Ranieri, V.M., et al., *Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial.* Jama, 1999. **282**(1): p. 54-61.
16. Parsons, P.E., et al., *Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury.* Crit Care Med, 2005. **33**(1): p. 1-6; discussion 230-2.
17. Staras, S.A., et al., *Seroprevalence of cytomegalovirus infection in the United States, 1988-1994.* Clin Infect Dis, 2006. **43**(9): p. 1143-51.
18. Balthesen, M., M. Messerle, and M.J. Reddehase, *Lungs are a major organ site of cytomegalovirus latency and recurrence.* J Virol, 1993. **67**(9): p. 5360-6.
19. Cook, C.H., et al., *Lipopolysaccharide, tumor necrosis factor alpha, or interleukin-1beta triggers reactivation of latent cytomegalovirus in immunocompetent mice.* J Virol, 2006. **80**(18): p. 9151-8.
20. Limaye, A.P., et al., *Cytomegalovirus reactivation in critically ill immunocompetent patients.* Jama, 2008. **300**(4): p. 413-22.
21. Fishman, J.A., *Infection in solid-organ transplant recipients.* N Engl J Med, 2007. **357**(25): p. 2601-14.
22. Lowance, D., et al., *Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group [see comments].* N Engl J Med, 1999. **340**(19): p. 1462-70.
23. Sagedal, S., et al., *Impact of early cytomegalovirus infection and disease on long-term recipient and kidney graft survival.* Kidney Int, 2004. **66**(1): p. 329-37.
24. Sagedal, S., et al., *The impact of cytomegalovirus infection and disease on rejection episodes in renal allograft recipients.* Am J Transplant, 2002. **2**(9): p. 850-6.
25. Kalil, A.C., *A silent killer: cytomegalovirus infection in the nonimmunocompromised critically ill patient.* Crit Care Med, 2008. **36**(12): p. 3261-4.

26. Kutza, A.S., et al., *High incidence of active cytomegalovirus infection among septic patients.* Clin Infect Dis, 1998. **26**(5): p. 1076-82.
27. Kellum, J.A., et al., *Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study.* Arch Intern Med, 2007. **167**(15): p. 1655-63.
28. Hamprecht, K., et al., *The Lung as a Central Compartment of Active CMV Infection.* Inflammation Research, 2007. **Supplement 2**(Abstract A383): p. S242.
29. Stapleton, R.D., *A Phase II Randomized Double-Blind, Placebo-controlled Trial of Fish Oil on Lung and Systemic Inflammation in Patients with Acute Lung Injury.* Am J Respir Crit Care Med, 2009. **179**: p. A2169.
30. Chiche, L., et al., *Active cytomegalovirus infection is common in mechanically ventilated medical intensive care unit patients.* Crit Care Med, 2009. **37**(6): p. 1850-7.
31. Carlquist, J.F., et al., *Cytomegalovirus induction of interleukin-6 in lung fibroblasts occurs independently of active infection and involves a G protein and the transcription factor, NF-kappaB.* J Infect Dis, 1999. **179**(5): p. 1094-100.
32. Compton, T., et al., *Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2.* J Virol, 2003. **77**(8): p. 4588-96.
33. Murayama, T., et al., *Human cytomegalovirus induces interleukin-8 production by a human monocytic cell line, THP-1, through acting concurrently on AP-1- and NF-kappaB-binding sites of the interleukin-8 gene.* J Virol, 1997. **71**(7): p. 5692-5.
34. Craigen, J.L., et al., *Human cytomegalovirus infection up-regulates interleukin-8 gene expression and stimulates neutrophil transendothelial migration.* Immunology, 1997. **92**(1): p. 138-45.
35. Iwamoto, G.K. and S.A. Konicek, *Cytomegalovirus immediate early genes upregulate interleukin-6 gene expression.* J Investig Med, 1997. **45**(4): p. 175-82.
36. Tong, C.Y., et al., *Association of tumour necrosis factor alpha and interleukin 6 levels with cytomegalovirus DNA detection and disease after renal transplantation.* J Med Virol, 2001. **64**(1): p. 29-34.
37. Humar, A., et al., *Elevated serum cytokines are associated with cytomegalovirus infection and disease in bone marrow transplant recipients.* J Infect Dis, 1999. **179**(2): p. 484-8.
38. Humbert, M., et al., *In situ production of interleukin-6 within human lung allografts displaying rejection or cytomegalovirus pneumonia.* Transplantation, 1993. **56**(3): p. 623-7.
39. Humbert, M., et al., *Activation of macrophages and cytotoxic cells during cytomegalovirus pneumonia complicating lung transplantations.* Am Rev Respir Dis, 1992. **145**(5): p. 1178-84.
40. Cook, C.H., et al., *Intra-abdominal bacterial infection reactivates latent pulmonary cytomegalovirus in immunocompetent mice.* J Infect Dis, 2002. **185**(10): p. 1395-400.
41. Cook, C.H., et al., *Pulmonary cytomegalovirus reactivation causes pathology in immunocompetent mice.* Crit Care Med, 2006. **34**(3): p. 842-9.
42. Papazian, L., et al., *A contributive result of open-lung biopsy improves survival in acute respiratory distress syndrome patients.* Crit Care Med, 2007. **35**(3): p. 755-62.
43. Puius, Y.A. and D.R. Snydman, *Prophylaxis and treatment of cytomegalovirus disease in recipients of solid organ transplants: current approach and future challenges.* Curr Opin Infect Dis, 2007. **20**(4): p. 419-24.
44. Kimberlin, D.W., et al., *Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial.* J Pediatr, 2003. **143**(1): p. 16-25.
45. Wiltshire, H., et al., *Pharmacokinetic profile of ganciclovir after its oral administration and from its prodrug, valganciclovir, in solid organ transplant recipients.* Clin Pharmacokinet, 2005. **44**(5): p. 495-507.
46. Einsele, H., et al., *Oral valganciclovir leads to higher exposure to ganciclovir than intravenous ganciclovir in patients following allogeneic stem cell transplantation.* Blood, 2006. **107**(7): p. 3002-8.
47. Lim, Z.Y., et al., *Results of a phase I/II British Society of Bone Marrow Transplantation study on PCR-based pre-emptive therapy with valganciclovir or ganciclovir for active CMV infection following alemtuzumab-based reduced intensity allogeneic stem cell transplantation.* Leuk Res, 2009. **33**(2): p. 244-9.
48. Kimberlin, D.W., et al., *Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease.* J Infect Dis, 2008. **197**(6): p. 836-45.

49. Paya, C., et al., *Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients.* Am J Transplant, 2004. **4**(4): p. 611-20.
50. Asberg, A., et al., *Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients.* Am J Transplant, 2007. **7**(9): p. 2106-13.
51. Preiksaitis, J.K., et al., *Canadian society of transplantation consensus workshop on cytomegalovirus management in solid organ transplantation final report.* Am J Transplant, 2005. **5**(2): p. 218-27.
52. Boeckh, M., et al., *Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies.* Biol Blood Marrow Transplant, 2003. **9**(9): p. 543-58.
53. Goodrich, J.M., et al., *Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation.* New England Journal of Medicine, 1991. **325**(23): p. 1601-7.
54. Goodrich, J.M., et al., *Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant.* Annals of Internal Medicine, 1993. **118**(3): p. 173-8.
55. Reusser, P., et al., *Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation.* Blood, 2002. **99**(4): p. 1159-64.
56. Gane, E., et al., *Randomised trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver-transplant recipients. The Oral Ganciclovir International Transplantation Study Group [corrected].* Lancet, 1997. **350**(9093): p. 1729-33.
57. Winston, D.J., et al., *Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients. Results of a placebo-controlled, double-blind trial.* Annals of Internal Medicine, 1993. **118**(3): p. 179-84.
58. Salzberger, B., et al., *Foscarnet and ganciclovir combination therapy for CMV disease in HIV-infected patients.* Infection, 1994. **22**(3): p. 197-200.
59. Boeckh, M., D. Myerson, and R.A. Bowden, *Early detection and treatment of cytomegalovirus infections in marrow transplant patients: methodological aspects and implications for therapeutic interventions.* Bone Marrow Transplantation, 1994. **14**(Suppl 4): p. S66-70.
60. Boeckh, M., et al., *Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study.* Blood, 1996. **88**(10): p. 4063-71.
61. Boeckh, M., et al. *Prevention of Late CMV Disease after HCT: A Randomized Double-Blind Multicenter Trial of Valganciclovir (VGCV) Prophylaxis versus PCR-Guided GCV-VGCV Preemtive Therapy*. in *Annual Meeting of the American Society for Blood and Marrow Transplantation*. 2008. San Diego, CA.
62. Merigan, T.C., et al., *A controlled trial of ganciclovir to prevent cytomegalovirus disease after heart transplantation.* N Engl J Med, 1992. **326**(18): p. 1182-6.
63. Spector, S.A., et al., *Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. Roche Cooperative Oral Ganciclovir Study Group.* New England Journal of Medicine, 1996. **334**(23): p. 1491-7.
64. von Muller, L., et al., *Active cytomegalovirus infection in patients with septic shock.* Emerg Infect Dis, 2006. **12**(10): p. 1517-22.
65. von Muller, L., et al., *Cellular immunity and active human cytomegalovirus infection in patients with septic shock.* J Infect Dis, 2007. **196**(9): p. 1288-95.
66. Bucher, H.C., et al., *Users' guides to the medical literature: XIX. Applying clinical trial results. A. How to use an article measuring the effect of an intervention on surrogate end points. Evidence-Based Medicine Working Group.* Jama, 1999. **282**(8): p. 771-8.
67. Rubenfeld, G.D. and E. Abraham, *When is a negative phase II trial truly negative?* Am J Respir Crit Care Med, 2008. **178**(6): p. 554-5.
68. Meduri, G.U., et al., *Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time.* Chest, 1995. **107**(4): p. 1062-73.
69. Mercat, A., et al., *Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome: a randomized controlled trial.* Jama, 2008. **299**(6): p. 646-55.
70. Willson, D.F., et al., *Effect of exogenous surfactant (calfactant) in pediatric acute lung injury: a randomized controlled trial.* Jama, 2005. **293**(4): p. 470-6.
71. Ljungman, P., P. Griffiths, and C. Paya, *Definitions of cytomegalovirus infection and disease in transplant recipients.* Clin Infect Dis, 2002. **34**(8): p. 1094-7.
72. Brower, R.G., et al., *Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome.* N Engl J Med, 2004. **351**(4): p. 327-36.

73. Davidian, M., A.A. Tsiatis, and S. Leon, *Semiparametric Estimation of Treatment Effect in a Pretest-Posttest Study with Missing Data.* Stat Sci, 2005. **20**(3): p. 261-301.
74. Shepherd, B.E., *Does Finasteride Affect the Severity of Prostate Cancer? A Causal Sensitivity Analysis.* Journal of the American Statistical Association, 2009. **484**: p. 1392-1404.
75. DeMets, D.L. and K.K. Lan, *Interim analysis: the alpha spending function approach.* Stat Med, 1994. **13**(13-14): p. 1341-52; discussion 1353-6.
76. Finkelstein, D.M., and Schoenfeld, D.A., Combining Mortality and Longitudinal Measures in Clinical Trials. Stat Med, 1999, 18: 1341-1354.
77. Hayden, D, Pauler, D. K. and Schoenfeld, D., An Estimator for Treatment Comparisons among Survivors in Randomized Trials. Biometrics, 2005, 61:305-310.

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 by Genentech, a member of the Roche Group will be used solely for the purpose of conducting this study.

____
Principal Investigator (printed name)

____ ____________________
Principal Investigator (Signature) Date

This protocol version 3.4.1 has been approved by the Protocol Leadership Team. The following signatures document this approval.

____ ____________________
Signature Date

Michael Boeckh, MD

Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA

____ ____________________
Signature Date

Ajit Limaye, MD

Dept. of Laboratory Medicine, Univ. of Washington, Seattle, WA

____ ____________________
Signature Date

Gordon Rubenfeld, MD, MSc

Sunnybrook Medical Centre, Univ. of Toronto, Toronto, Canada

APPENDIX A: TIME AND EVENTS SCHEDULE

Visit	Screening[a]	01[b]	02	03	04	05	06	07[e]	08	09[e]	10	11	12	Assay Location
Day	-4 to 1	1	4	7	11	14	18	21	25	28	35	60	180	
Window (+/- days)	0	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 1	(+/-) 3	(+/-) 14	
Informed consent	X	-	-	-	-	-	-	-	-	-	-	-	-	
Administer Study Drug	Note: Patient receives 5 days of ganciclovir intravenously TWICE daily, then up to 23 days of ganciclovir ONCE daily intravenously (or matching placebo) in patients that are hospitalized.													
Blood collection (ml) vol. estimated:														
Pregnancy[c]	3					------------------3[c]------------------								L
CMV Serology	3	-	-	-	-	-	-	-	-	-	-	-	-	L/A
Genomic analysis	-	15	-	-	15	-	-	-	-	-	-	-	-	S
Creatinine / T. Bilirubin	1	1	1	1	1	1	1	1	1	1	1	-	-	L/S
Cytokine	-	5	5	5	5	5	5	5	5	5	5	-	-	S
CMV PCR	-	5	5	5	5	5	5	5	5	5	5	-	-	S
CBC, w/differential, platelets	1	3	3	3	3	3	3	3	3	3	3	-	-	L/S
Estimated blood volume	8	29	14	14	29	14	14	14	14	14	14	0	0	Sum: 178
Endotracheal (ETT) aspirate[d]		X[d]												
CMV PCR	-	X	-	X		-	-	-	-	-	-	-	-	S
Cytokine	-	X	-	X		-	-	-	-	-	-	-	-	S
Throat swab:														
CMV PCR	-	X	X	X	X	X	X	X	X	X	X	-	-	S
Clinical Assessments:														
Survey		X											X	
Vital status	-											X	X	

L = local test; S=Seattle, WA; A= ARUP in Utah
[a] Screening must occur during 1st 120 hours of admission to acute care hospital.
[b] Prior to administration of study drug.
[c] A urine pregnancy test is also acceptable. A follow up pregnancy test will be performed at the time of hospital discharge.
[d] Can only be performed while patients are intubated. *ETT Aspiration – If intubated, perform at Day 1 (± 1 day) and every fourth day (± 2 days) on Day 1, 5, 9, 13, 17, 21, 25, and 29.
[e] All patients must have Day 21 & 28 visits. Day 35 visit will only occur in patients when drug is stopped between Days 25-28.

APPENDIX B: NCI COMMON TOXICITY CRITERIA (CTC)

A. **The NCI CTC criteria will be used for Adverse Event reporting. The NCI CTC criteria can be downloaded from the following WEB site: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. 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**
ALLERGY/IMMUNOLOGY	01
AUDITORY/HEARING	02
BLOOD/BONE MARROW	03
CARDIOVASCULAR (ARRHYTHMIA)	04
CARDIOCASCULAR (GENERAL)	05
COAGULATION	06
CONSTITUTIONAL SYMPTOMS	07
DERMATOLOGY	08
ENDOCRINE	09
GASTROINTESTINAL	10
HEMORRHAGE	11
HEPATIC	12
INFECTION/FEBRILE NEUTROPENIA	13
LYMPHATICS	14
METABOLIC/LABORATORY	15
MUSCULOSKELETAL	16
NEUROLOGY	17
OCULAR/VISUAL	18
PAIN	19
PULMONARY	20
RENAL/GENITOURINARY	21
SECONDARY MALIGNANCY	22
SEXUAL/REPRODUCTIVE FUNCTION	23
SYNDROMES 24	

APPENDIX C: COMMONLY PRESCRIBED IMMUNOSUPPRESSIVE AGENTS

Generic Name	Trade Name
Abatacept	Orencia
Adalimumab	Humira
Etanercept	Enbrel
Golimumab	Simponi
Infliximab	Remicaide
Tocilizumab	Actemra

Generic Name	Trade Name
Antithymocyte Globulin (Equine)	ATG, ATGAM
Antithymocyte Globulin (Rabbit)	Thymoglobulin
Azathioprine	Imuran
Anakinra	Kineret
Basiliximab	Simulect
Cyclophophamide	Cytoxan
Cyclosporine	Neoral, Sandimmune
Daclizumab	Zenapax
Everolimus	Afinitor
Methotrexate	Rheumatrex, Trexall
Mycophenolate	Cellcept, MMF, Myfortic
Sirolimus	Rapamune
Tacrolimus	Prograf

Generic Name	Trade Name
Alemtuzumab	Campath
Enteracept	Enbrel
Inflixumab	Remicade
Natalizumab	Tysabri
Rituximab	Rituxan

APPENDIX D: LUNG PROTECTIVE VENTILATION PROTOCOL RECOMMENDATIONS

Note: *The following are guidelines that are recommended for applicable ARDS/ALI 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 [72, 75]. 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 [72] 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 or non-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 (comparing Reference Measurement values, section 6.3)
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
    e. Marked subjective dyspnea

If any of the goals a-e 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 E2) the following day.

Decision to remove ventilator support:

If tolerance criteria for spontaneous breathing trial (a-e 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 E.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 ARDS/ALI 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, 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.

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

§ 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.

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APPENDIX F: DATA AND SAFETY MONITORING PLAN

The purpose of this plan is to describe the oversight and monitoring of the study which is conducted to ensure the safety of study participants and the integrity of data collected as part of the study.

Safety monitoring is carried out by the coordinating center Principal Investigator, site Principal Investigators, an independent safety monitor and an independent Data Safety Monitoring Board.

1. Safety Monitoring

1.1. Monitoring for Safety by Study Sites

Investigators are responsible for monitoring the safety of patients who have entered this study. While hospitalized, subjects will be assessed for adverse events by the research nurse/coordinator and principal investigator or co-investigator(s).

The investigator is responsible for appropriate medical care of patients during the study. The investigator remains responsible to follow, through appropriate health care options, 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 SAEs to the coordinating site and their institutional review board.

1.2. 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:

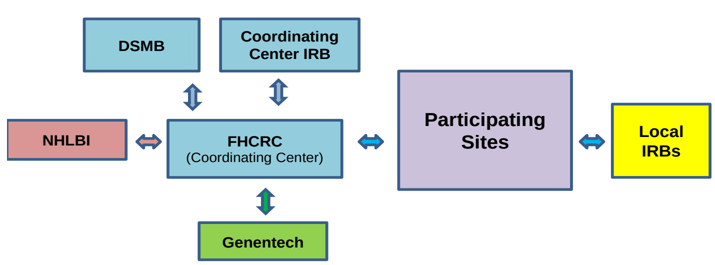
1. Conduct a site initiation visit
2. Perform monitoring visits during the trial
3. Conduct a close-out visit (Note: in selected circumstances [e.g. if there is insufficient enrollment], a site maybe closed out administratively without a visit).

Overall, we expect approximately 4 monitoring visits per site, including a start-up visit, one visit after approximately 2-3 patients have been enrolled, one visit at approximately 50% of enrollment and one final close-out visit.

Study monitoring will consist of:

1. Monitoring of the consent forms, inclusion/exclusion criteria, hematologic safety labs (i.e. neutrophil counts, platelet counts), length-of-stay endpoints, ventilation data and SAEs (100%)
2. Every 5th patients will be monitored 100%.
3. Monitoring of the regulatory binder (at each visit)
4. Monitoring of the investigational drug pharmacy (each visit).

1.3. Organization and Interactions of Parties Involved in Data and Safety Monitoring



The diagram above illustrates the relationship between the study sites and the coordinating site as well as other entities in this study. Communication with the DSMB will be primarily through the coordinating site in Seattle.

1.4. Responsibilities of the DSMB

Data safety monitoring will be performed by the DSMB assembled by the coordinating site and in consultation with NIH NHLBI. This protocol and all SAEs will be forwarded to the DSMB for review. Details of the operating guidelines for the DSMB are summarized in the DSMB charter. Briefly, this DSMB will assess the effects of the study drug during the trial and may give advice to the study team leadership. The members of the committee are independent of the University of Washington, Fred Hutchinson Cancer Research Center, Genentech, a member of the Roche Group, 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 serious adverse events will be reported to the chairperson of the DSMB. 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 the planned interim analysis. 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 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.

1.5 Protection against Risks

Study procedures (blood draw, throat swab, ETT) 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 adverse events resulting from study procedures.

Safety labs will be monitored regularly 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.

1.6 Protecting Confidentiality

Specimens will be coded with unique study identification numbers in order to protect patient confidentiality. No identifying information of any kind may be released to persons or agencies without specific written permission. At the coordinating center, multiple mechanisms have been established to protect the confidentiality of specimens, medical records and data used in this project. All personnel who work on this study have signed or will sign a pledge of confidentiality. Access to the database is controlled through secure password protection, and passwords must be changed at quarterly intervals. Access to the work site is controlled through passkeys and ID badges. Individuals who are not employees must be escorted at all times by an employee. Study sites will employ site-specific confidentiality measures, including electronic and physical barriers.

1.7 Adverse Events and Unanticipated Problems

Please refer to Section 11.0 Adverse Event Reporting.

APPENDIX G: SEVERE SEPSIS CRITERIA

Sepsis is defined as a documented or suspected infection together with at least 2 of the following 4 clinical findings present:

- Temperature greater than 38°C (100.4°F) or less than 36°C (96.8°F)
- Heart rate (HR) greater than 90 beats per minute (bpm)
- Respiratory rate (RR) greater than 20 breaths per minute or arterial carbon dioxide tension ($PaCO_2$) lower than 32 mm Hg
- White blood cell (WBC) count higher than 12,000/µL or lower than 4000/µL, or 10% immature (band) forms

Infection is defined as a documented or a suspected infection. Suspected infection is evidenced by one or more of the following: white cells in a normally sterile body fluid; perforated viscus; radiographic evidence of pneumonia in association with the production of purulent sputum; a syndrome associated with a high risk of infection (e.g., ascending cholangitis) and empiric antibiotic treatment.

Severe sepsis is defined as sepsis (above) associated with organ dysfunction, hypoperfusion or hypotension. Organ dysfunction variables:

Arterial hypoxemia (PaO2/FIO2 <300) if PaO2 is not available then use (SpO2/PaO2<315)
Acute oliguria (urine output <0.5 mL·kg-1·hr-1 or 45 mmol/L for at least 2 hrs)
Creatinine > 2.0 mg/dL in patient without pre-existing renal failure
Coagulation abnormalities (INR >1.5 or a PTT >60 secs)
Thrombocytopenia (platelet count <100,000 µL–1)
Hyperbilirubinemia (plasma total bilirubin > 2.0 mg/dL or 35 mmol/L)

Tissue perfusion variables:
Hyperlactatemia (>2 mmol/L)

Hemodynamic variables:
Arterial hypotension (SBP <90 mm Hg, MAP <70, or SBP decrease >40 mm Hg)
The use of vasopressor medications in an attempt to maintain SBP>90 or MAP>70.

HTTP://WWW.SCCM.ORG/DOCUMENTS/SSC-GUIDELINES.PDF APPENDIX H: GANCICLOVER PACKAGE INSERT

http://www.gene.com/gene/products/information/cytovene/pdf/pi.pdf

APPENDIX I: VALGANCICLOVIR PACKAGE INSERT

http://www.gene.com/gene/products/information/valcyte/pdf/pi.pdf